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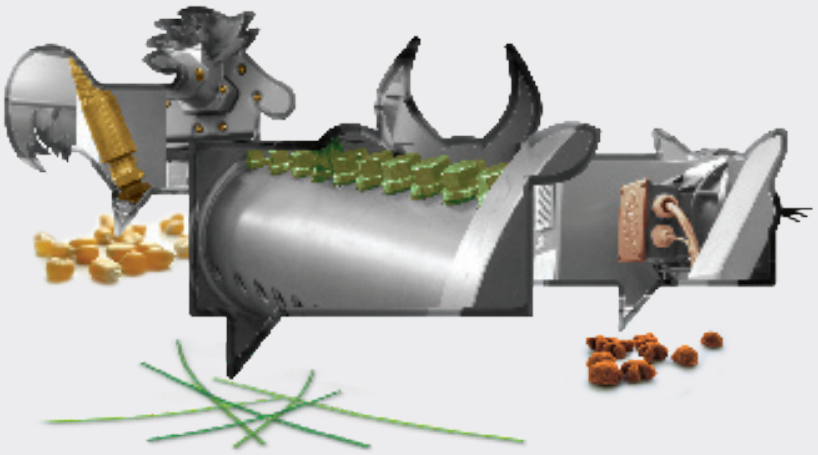

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CONTENT

THE EFFECT OF INCLUDING SELECTED MAIZE VARIETIES IN DIETS FOR RABBITS ON PERFORMANCE AND MEAT QUALITY
Mária Chrenková, Ľubica Chrastinová, Andrea Lauková, Mária Poláčiková, Zuzana Formelova, Viola Strompfova, Ľubomír Ondruška, Vladimír Parkányi, Ján Rafay, Iveta Plachá, Renáta Szabóová **1**

AROMATIC PLANTS AND THEIR IMPLICATIONS IN FEEDS AND PERFORMANCE OF ANIMALS
Ilias Giannenas, Sophia Stavrakakis **9**

EFFECTS OF THE DIETARY ECOLOGIC CAMELINA OIL ON BLOOD PLASMA COMPOSITION IN FINISHING PIGS
Habeanu Mihaela, Hebean Veronica, Taranu Ionelia, Marin Daniela, Lefter Nicoleta **17**

INFLUENCE OF A LIQUID APPLICATION IN THE MAIN MIXER ON MIXTURE HOMOGENEITY OF FEEDING STUFFS
Alexandra Kirchner **25**

AN EMPIRICAL ANALYSIS OF EXPLANATORY VARIABLES AFFECTING *FUSARIUM* AND DEOXYNIVALENOL IN WHEAT FOR FEED PRODUCTION
Sofie Landschoot, Kris Audenaert, Willem Waegeman, Jan Vandepitte, Geert Haesaert, Bernard De Baets **30**

WHEAT-MIDDLINGS IN POULTRY DIET AND ITS EFFECT ON BROILER PERFORMANCE
Vito Laudadio, Girolamo Di Modugno, Luca Laporta, Vincenzo Tufarelli **41**

A REVIEW ON SALMONELLA INHIBITION IN POULTRY AND PIGS THROUGH THE USE OF DIFORMATES
Christian Lückstädt, Peter Theobald **45**

NEW INSIGHTS FOR EFFECTIVE MOULD CONTROL
Maarten van der Heijden, Jaco Eissen, Hans van Dam **54**

DEVELOPMENT OF EUROPEAN PETFOOD MARKET
Leszek Mościcki **59**

ROLE AND SIGNIFICANCE OF ASSOCIATIVE EFFECT IN THE ASSESMENT OF THE NUTRITIVE VALUE OF FEEDS
Mariana Petkova, Jovanka Lević **66**

REGIONAL DIFFERENCES ON THE WORLDWIDE MYCOTOXIN OCCURRENCE
Inês Rodrigues, Katia Pedrosa, Karin Naeherer **74**

CHANGES OF FATTY ACIDS COMPOSITION DURING STORAGE IN CAMELINA SAMPLES OF SEEDS, OIL AND MEAL
Ropota Mariana, Habeanu Mihaela, Voicu Ilie **80**

EFFECTS OF FEEDING COWS WITH SUGARBEET PULP SILAGE AND COMPOUND FEED WITH UREA SUPPLEMENTATION ON MILK PRODUCTION AND QUALITY
Virginijus Uchockis, Saulius Bliznikas, Danguolė Urbsienė, Vytautas Tarvydas, Petras Bendikas **86**

INTERLABORATORY STUDY ON MANGANESE DETERMINATION IN 3 SPECIES OF SPONTANEOUS FLORA USED AS ALTERNATIVE MINERAL SOURCES FOR WEANING PIGLETS (10-30 KG)
Untea Arabela, Criste Rodica Diana, Cornescu Gabriela Maria, Radutoiu Daniel **97**
USE OF ELECTROLYZED WATER FOR INACTIVATION OF ESCHERICHIA COLI, STAPHYLOCOCCUS AUREUS, SALMONELLA TIPHYMURIUM AND PSEUDOMONAS AERUGINOSA FROM POULTRY FARMS
Daniela Vătuiu, Ioana Vătuiu, Laura Anghel, Stelian Matei Petre, Stefana Jurcoane, Ioan Surdu **105**
EFFICIENCY OF USING ENSILED SEMI-LATE CORN HYBRIDS IN DIETS FOR FATTENING STEERS
Ilie Voicu, Dorica Voicu **109**
PERFECTION OF AN ESTIMATION OF PRODUCTION TECHNOLOGY OF HIGHLY STABLE PREMIXES
Bogdan Yegorov, Alla Makarinskaya **116**
RESEARCH AND DEVELOPMENT IN THE FIELD OF FEED TECHNOLOGIES IN UKRAINE
Bogdan Yegorov **122**
EVALUATION OF RADIO FREQUENCY TECHNOLOGY FOR FRESH FILLED PASTA QUALITY
Francesca Alberti, Nicoletta Cristiana Quaglia, Maria Selvaggi, Marco Dario, Luigina Spremulli, Enzo Todaro, Carmela Tamborrino, Maria Palma Celano, Francesco Fasano, Gaetano Vitale Celano **130**
INFLUENCE OF NATURAL ZEOLITE CLINOPTILOLITE ON IMMUNOLOGICAL PARAMETERS IN WEANED PIGLETS
Marcela Šperanda, Hrvoje Valpotić, Tomislav Šperanda, Mislav Đidara, Zvonko Antunović, Željko Mikulec, Boris Habrun, Danijel Špoljarić **137**
APPLICATION OF RAPID TECHNIQUE FOR SCREENING OF MYCOTOXINS IN CEREALS
Menkovska Mirjana, Vesna Levkov, Djorgovska Natasha, Marija Sharic, Vlado Samardjioski, Irina Ushlinovska **144**
TECHNOLOGICAL PROPERTIES AND PREPARATION OF OLIVE CAKE AS A FEED COMPONENT
Tea Brlek, Neven Voća, Tajana Krička, Nikola Bilandžija, Ana Matin, Vanja Jurišić **150**
DEHYDRATED SUGAR BEET MOLASSES AS SUITABLE RAW MATERIAL IN THE PRODUCTION OF ANIMAL FEED
Gordana Koprivica, Nevena Mišljenović, Dragomir Catalin , Ljubinko Lević, Djuro Vukmirović, Dušica Ivanov, Radmilo Čolović **158**
PHYSICAL MEASURES FOR STORAGE INSECTS CONTROL
Petar Kljajić, Goran Andrić **168**
PROTECTIVE EFFECT OF HULLS *Triticum aestivum* ssp. *spelta* AGAINST INSECT INFESTATION DURING STORAGE
Marija Bodroža-Solarov, Radmila Almaši, Danijela Poslončec, Bojana Filipčev, Olivera Šimurina **183**

AMINO ACID PURITY DETERMINATION BY MEASUREMENT OF OPTICAL ROTATION
Željko Mihaljev, Bojana Prunić, Milica Živkov–Baloš **189**
IMPACT OF BENTONITE ON THE QUALITY OF PELLETED FODDER
Milan Adamović, Marina Vukić-Vranješ, Rade Jovanović, Aleksandra Bočarov-Stančić, Mihailo Radivojević, Marija Panić **196**
THE RESPONSIBILITY OF ANIMAL FEED PRODUCERS IN SERBIA IN THE AREA OF ANIMAL PRODUCTION – AT THE ENTRY TO THE WORLD TRADE ORGANIZATION AND EUROPEAN UNION
Jasna Stevanović **203**
POTENTIAL OF PLANTING *AMARANTHUS SP.* IN AGROECOLOGICAL CLIMATE OF VOJVODINA
Marija Bodroža-Solarov, Jovanka Lević, Saulius Bliznikas, Bojana Kokić, Nedeljka Spasevski, Šandor Kormanjoš **209**
THE INFLUENCE OF VARIOUS FACTORS ON THE DEGREE OF NITROGEN MATTER CHANGES IN LEGUME SILAGES
Nenad Đorđević, Goran Grubić, Jovanka Lević, Slavica Sredanović, Bojan Stojanović, Aleksa Božičković, Mladen Lojanica **215**
LABELLING OF LOCAL FOOD: TOWARDS EUROPEAN UNION
Jasmina Živković, Zvonko Nježić, Biljana Cvetković, Milenko Košutić **222**
TABLE QUALITY OF LAYER EGGS FROM VARIOUS PRODUCTION SYSTEMS
Zlatica Pavlovski, Zdenka Škrbić, Miloš Lukić **227**
EFFECT OF THERMAL PROCESSING AND TRYPSIN INHIBITOR EFFECT TO PROTEIN DIGESTIBILITY IN SWINE NUTRITION
Miloš Beuković, Dejan Beuković, Dragan Glamočić, Nenad Đorđević **234**
INTOXICATION OF POULTRY CAUSED BY INADEQUATELY HEAT TREATED SOY PRODUCTS AND THE POSSIBILITY OF PREVENTION
Miloš Kapetanov, Dušan Orlić, Dubravka Potkonjak, Milica Živkov-Baloš, Željko Mihaljev, Bojana Prunić **242**
PRESENCE OF FUNGI IN POULTRY FEED AND EFFECTS OF CONTAMINANTS ON HEALTH STATUS
Dobrila Jakic-Dimic, Ksenija Nesic, Bozidar Savic, Jelena Keckes, Boris Pisinov **248**
MICROBIOLOGICAL DEGRADATION OF THE T-2 TOXIN AND DIACETOXYSCIRPENOL UNDER LABORATORY CONDITIONS
Aleksandra Bočarov-Stančić, Nataša Salma, Jelena Lević, Vladimir Pantić, Slavica Stanković, Saša Barnić **254**
QUALITY OF INEDIBLE BY-PRODUCTS OF PIGS SLAUGHTERING INTENDED FOR INCLUSION IN PET FOOD
Djordje Okanović, Milutin Ristić, Šandor Kormanjoš, Vesna Matekalo-Sverak, Slobodan Lilić, Dragan Palić **262**
MICROSCOPIC EXAMINATION OF FEED FOR THE PRESENCE OF CONSTITUENTS OF ANIMAL ORIGIN
Ksenija Nešić, Nikola Pavlović, Dobrila Jakić-Dimić, Ljiljana Jojić-Maličević **269**

THE USE OF CONCENTRATES IN GAME FEEDING WITH THE PURPOSE TO REDUCE DAMAGES
Nenad Đorđević, Goran Grubić, Zoran Popović, Miloš Beuković, Bojan Stojanović, Aleksa Božičković **273**
EFFECT OF OREGANO ESSENTIAL OIL ON ANTIOXIDATIVE SYSTEM OF BROILER'S BLOOD AND LIVER
Ljiljana Kostadinović, Jovanka Lević, Sava Pavkov, Dragana Kaluđerović **282**
OCCURRENCE OF MYCOTOXINS AND GENETICALLY MODIFIED ORGANISMS (GMO) IN FEED AND FOOD CONTAINING CORN AND SOYBEAN
Jovana Matić, Anamarija Mandić, Jovanka Lević, Gordana Zdjelar, Ivana Vasiljević, Bojana Kokić, Ivana Čabarkapa **288**
PHYTO ADDITIVES (ALLIUM SATIVUM L.) IN THE DIET OF FATTENING CHICKENS
Vidica Stanaćev, Niko Milošević, Nada Plavša, Siniša Bjedov, Vladislav Stanaćev, Nikola Puvača, Željko Arapović **295**
EFFECTS OF TECHNOLOGICAL TREATMENTS IN PREPARING FORAGES ON PROTEIN FRACTIONS AND THEIR RUMINAL DEGRADABILITY
Bojan Stojanović, Goran Grubić, Nenad Đorđević, Aleksa Božičković, Aleksandra Ivetić, Mladen Lojanica **302**
MEDICINAL PLANTS AS POTENTIAL FUNCTIONAL COMPONENTS IN FOOD AND FEED PRODUCTION
Aleksandra Mišan, Neda Mimica-Dukić, Marijana Sakač, Ivana Sedej, Anamarija Mandić, Ivan Milovanović, Đorđe Psodorov **312**
EFFECT OF IRON DEFICIENCY ON HELTH STATUS OF NEONATAL CALVES
Radisa Prodanovic, Dobrila Jakic-Dimic, Vojin Ivetic, Bozidar Savic, Milenko Zutic, Ivan Pavlovic, Branislav Kureljusic **320**
EFFECT OF TEMPERATURE ON RHEOLOGICAL PROPERTIES OF CORN STARCH
Tamara Dapčević, Miroslav Hadnađev **327**
AROMA NEKTAR IN NUTRITION OF PIGLETS
Branislav Živković, Mirko Saftić, Olga Kosovac, Č. Radović, Ibolja Kinčeš **333**
RHEOLOGICAL AND CHEMICAL CHARACTERISTICS OF SUGAR BEET MOLASSES WITH VIEW OF INDUSTRIAL APPLICATION
Rada Jevtić-Mučibabić, Jasna Grbić, Nevena Mišljenović, Gordana Koprivica, Tatjana Kuljanin **339**
EFFECT OF DIETARY SELENIUM ON BODY WEIGHT AND LIVER MASS IN FATTENING POULTRY
Željko Mihaljev, Milica Živkov-Baloš, Željko Ćupić **345**
SYSTEMS AND ECONOMY USE GRAZING IN NUTRITION OF GOATS
Miroslav Žujović, Nurgin Memiši, Zorica Tomić **352**
SPECIFIC NUTRITION OF GOATS IN PRODUCTION CYCLE ON THE BASIS OF BODY CONDITION SCORING
Nurgin Memiši, Miroslav Žujović **360**

NORMATIVE OF PROTEIN FOR GOAT IN INTENSIVE PRODUCTION
Nurgin Memiši, Zorica Tomić **367**
MICROBIOLOGICAL SAFETY OF ANIMAL FEED FROM REGION VOJVODINA IN 2009
Dragana Plavšić, Ivana Čabarkapa, Ljubiša Šarić, Bojana Kokić, Jovanka Lević **374**
STABILITY OF MICROTRACER® IN THERMAL FEED PROCESSING
Dušica Ivanov, Radmilo Čolović, Bojana Kokić, Đuro Vukmirović, Jovanka Lević, Olivera Đuragić, Slavica Sredanović **383**
THE INFLUENCE OF ADDING MULTIENZYME PREPARATION ROVABIO™ ON GROWTH AND INCREASE OF CARP (*Cyprinus carpio*)
Saša Obradović, Marija Vukašinović, Jovanka Lević, Raško Stefanović **392**
MEAT QUALITY OF TWO YEARS-OLD TENCH AND CARP GROWN IN EXTENSIVE CONDITIONS
Miroslav Ćirković, Dejana Trbović, Nikolina Milošević, Vesna Đorđević, Saša Janković, Dragana Ljubojević **400**
INFLUENCE OF TECHNOLOGICAL PROCESS OF PRODUCTION ON MICROBIOLOGICAL SAFETY OF CHEESE
Dragana Plavšić, Jasmina Gubić, Ljubiša Šarić, Ivana Čabarkapa **405**
CONTRIBUTION MARGIN ANALYSIS IN CHICKEN FATTENING
Jonel Subić, Lana Ivanović, Marko Jeločnik **411**
THE SIGNIFICANCE OF THE APPPLICATION OF THE *NIRS* METHOD IN ANIMAL FEED FACTORIES WITH THE GOAL OF IMPROVING QUALITY AND COMPETITIVENESS
Peter F. de Vries, Rade Jovanovic, Milica Pojic, Marijana Maslovaric, Ivan Milenković **418**
THE EFFECT OF A BACTERIAL INOCULANT ON AEROBIC MICROFLORA IN WHOLE CROP MAIZE SILAGE
Ivana Čabarkapa, Dragan Palić, Djuro Vukmirović, Radmilo Čolović, Dragana Plavšić, Dragomir Jeremić **432**
QUALITY OF CARCASS AND BREAST MEAT OF CHICKENS FED WITH EXTRUDED PRODUCTS OF RAPE SEED
Natalija Džinić, Vladimir Tomović, Ljiljana Petrović, Tatjana Tasić, Marija Jokanović, Predrag Ikonić **439**
EFFECT OF DIFFERENT THICKNESS OF DIE ON THE STABILITY OF AMINO ACIDS IN PELLETING PIG FEED
Nedeljka Spasevski, Bojana Kokić, Saulius Bliznikas, Gintautas Švirmickas, Đuro Vukmirović, Radmilo Čolović, Jovanka Lević **447**
INFLUENCE OF DIFFERENT FEEDING AND HOUSING CONDITION ON METABOLIC PROFILES AND MILK PRODUCTION IN ALPINO GOATS
Biljana Radojičić, Jovan Bojkovski, Dragan Bacić, Stanko Boboš **454**
PREDICTION POSSIBILITIES OF AMMONIA PRODUCTION, CONSUMPTION AND UTILIZATION OF OXYGEN IN THE CULTIVATION OF RAINBOW TROUT *Oncorhynchus mykiss (Walbaum)*
Saša Obradović, Marija Vukašinović, Ivanc Aleksandar **458**

TECHNOLOGY FOR ACHIEVING QUALITY PARAMETERS OF CARP FRY FEED
Olivera Đuragić, Rade Jovanović, Slavica Sredanović **471**
THE EFFECTS OF PARTIAL REPLACEMENT OF GRAINS WITH MOLASSES ON RUMINAL MICROBIAL PROTEOSYNTHESIS IN GROWING RAMS
Catalin Dragomir, Andreea Vasilachi, Mihaela Vlassa, Smaranda Pop, Dumitru Drăgotoiu **483**

THE EFFECT OF INCLUDING SELECTED MAIZE VARIETIES IN DIETS FOR RABBITS ON PERFORMANCE AND MEAT QUALITY

Mária Chrenková[1], Ľubica Chrastinová[1], Andrea Lauková[2], Mária Poláčiková[1], Zuzana Formelov[1], Viola Strompfov[2], Ľubomír Ondruška[1], Vladimír Parkányi[1], Ján Rafay[1], Iveta Plachá[2], Renáta Szabóová[2]

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ABSTRACT

The objective of this work was to determine the effect of selected maize varieties in diets on nutrient digestibility and performance, biochemical and mineral parameters in blood, characteristics of meat quality, physical and chemical characteristics of meat in MLD *(musculus longissimus dorsi)* muscle substance of rabbits. Live weight growth, feed conversion and health of rabbits after feeding complete feed mixtures with 12 % proportion of Bt transgenic maize containing the Cryl1A (b) protein (MON 88017) and isogenetic control maize (IC) (DKC 5143) was tested on 48 broiler rabbits (Hycola). Bt transgenic maize deteriorated neither the health in animals nor the production of animal proteins valuable for human nutrition compared with conventional maize.
Keywords*: rabbit; nutrients in meat; biochemical and mineral parameters in blood; Bt transgenic maize*

INTRODUCTION

Maize, cotton, and soybean that have been engineered to resist insect pests and herbicides are now planted crops on almost half of all United States of America. The most commonly introduced genetically engineered (GE) traits allow plants either to produce their own insecticide, reducing crop losses to insect damage, or to resist herbicides, so that herbicides can be used to kill many types of weeds without harming crops. GM crops with the mentioned characteristics are above all of benefit to growers. The next generations of GM crops are expected to have also direct benefits to the consumer - e.g. GM crops with a higher content or better structure of nutrients or GM crops with anti-carcinogenic effects; or of benefit for sectors other than agriculture - e.g. edible vaccines, biodegradable plastics, substitution of fossil fuels, removal of environmental pollution etc.[25]. In 2009, 14 million growers in 25 countries around the world used this technology, 90 % from they were small farmers in landscape [5]. The most frequently cultivated crops are GM varieties of soya, maize, cotton and oil rapeseed. Other GM plants produced in the world are sugar beet, papaya, lucerne, tomato, sweet pepper, poplar, carnation, blue rose, and petunia as 21st country in the world was ranked among growers of GM crops. In the European context, Slovakia ranks

among other 7 EU countries which have practical experience in Bt transgenic maize cultivation; these are: Spain, France, Romania, Portugal, Germany, Czech Republic and Poland [17; 12]. Here, Bt hybrids could represent a possible solution, as in terms of visual assessment of trials, a lower level of infection by fungal diseases was ascertained in comparison with conventional hybrids.

MATERIAL AND METHODS

The effect of complete feed mixtures with 12 % proportion of Bt transgenic maize or isogenic control maize was tested in the experiment on 48 broiler rabbits (Hycola) in metabolic and fattening experiment. In each group were 24 rabbits (males) from weaning (35 days old) to the slaughtering weight 2.5 kg (77 days old). Rabbits were kept in standard cages, two animals per cage.
They were fed *ad libitum* and had free access to drinking water from nipple drinkers during the experiment. The diet formulation (complete granulated mixture, pellets have 3 mm diameter) for all groups is presented in Table 1. Evolution of body weight and feed consumption were registered weekly. In fattening experiment were studied the growth of live weight and consumption of feed mixtures per unit of live weight growth. Between 65 and 70 days of age, 5 rabbits from each group were selected for digestibility tests using the balance method. The digestibility test was performed in accordance with the recommended methodology [18; 22]. The samples of individual feeds are presented in Table 1, and samples of faeces were analyzed for the content of nutrients according to Official Methods of Analysis to STN 46 7092. The content of digestible energy (DE) was calculated by the equation of [26].

Table 1. Ingredients and chemical analysis of the experimental diets (in original matter)

Feed ingredients	in %	Chemical analysis (g. kg^{-1})	Bt transgenic maize	Izogenic control maize
Dehydrated lucerne meal	41.0	Dry matter	901.8	895.8
Dried beet pulp	10.0	Crude protein	172.8	168.1
Rape extr. meal	20.0	Crude fibre	179.2	183.0
Wheat	3.0	Fat	38.4	35.6
Apple pomace	9.0	N free extract	432.2	438.8
Maize	12.0	Organic matter	822.6	821.6
Carob meal	0.4	Starch	154.2	157.6
Minerals &Vitamins*	1.8	Calcium	9.3	6.7
Rape oil	3.0	Phosphorus	6.9	4.1
Limestone, pulverized	5.0	ME (MJ. kg^{-1})	9.42	9.16

*Provided per kg diet: vit. A 12000 IU; vit.D_2 2500 IU; vit. E 20 mg; vit.B_1 1.5 mg; vit. B_2 7.5 mg; vit. B_6 4.5 mg; vit.B_{12} 30 µg; vit.K 3 mg; nicotic acid 45 mg; folic acid 0.8 mg; biotin 0.08 mg ; Choline chloride 450 mg; Premix minerals (per kg diet) ca 9.25 g; P 6.2 g; Na 1.6 g; Mg 1.0 g; K 10.8 g; Fe 327.5 mg; Mn 80 mg; Zn 0.7 mg

Three animals from each group were slaughtered at day 42 by cutting the jugularis and the carotid artery after electro-anaesthesia (90 V for 5sec). The samples of *Musculus longissimus dorsi* (MLD) were collected immediately after death and stored at 5°C for 24 h and physical-chemical analyses (according to STN 57 0185) were made. The content of proteins, fat, ash and water holding capacity were estimated using an INFRATEC 1265 spectroscope and expressed in g/100g original matter from these values). Water holding capacity was determined by compress method at constant pressure [14]. The content of gross energy value of meat in MLD was calculated:
Energy value in kJ/100 g = (16.75x protein content) + (37.68 x fat content). The ash content was determined by mineralisation of the samples at 550°C°according to STN 570185.
The pH 24 hours *post mortem* was measured by portable pH-meter model Radelkis OP-109 with a combined glass-gel electrode penetrating 3 mm into the MLD. Blood samples for biochemical and haematological analyses were obtained from the marginal ear vein (*Vena auricularis*) into dry non-heparinized glass tubes and blood serum was separated by centrifugation at 3000 x G for 10 min. In blood serum were examined levels of proteins and lipids (g/l), cholesterol (mmol/l), glucose (mmol/l), calcium (mmol/l). Biochemical parameters were determined by an enzymatic colorimetric procedure using commercial set of Randox (United Kingdom). The activity of blood glutathione-peroxidase (GSH-Px;U/ml) was determined by a RANSEL standard set from Randox (United Kingdom). The phagocyte activity (PA) was monitored and expressed as percentage of bacteria ingested per phagocyte (100 neutrophils) during a limited period of incubation of particles suspension and phagocytes in serum [16].
The slaughter and digestibility data were processed by analysis of variance. The results were quoted as mean ± standard deviation (SD), statistical evaluation of the results was performed by the one-way ANOVA and Tukey test.

RESULTS AND DISCUSSION

The study was carried out in the Animal Production Research Centre Nitra, Slovak Republic. There were used 48 rabbits in the experiment. They were weaned at 35 days and divided into 2 groups (2 rabbits per cage). Rabbits were fed *ad libitum* and they had free access to drinking water from nipple drinkers during the experiment. After weaning the rabbits were fattened until they were 77 days old. Total process of growth rate of rabbits, average daily weight gain, daily feed intake and feed efficiency is shown in table 2. Experimental results indicate that the hybrid Hycole of rabbits is very fast growing and was relatively homogenous in two groups, in various experimental treatments were not different $P>0.05$. The carcass dressing percentage of each animal was determined (57.3 – 57.4 %); it was similar to those found in the literature [20]. The highest daily body weight gain was registered in rabbits fed the mixture containing Bt transgenic maize (36.95 g) and the best feed utilization (feed/gain) was detected in the group fed the mixture containing isogenic control maize needed 3 g of feed for 1g of live weight gain. The following characteristics were established in Bt transgenic maize and isogenic control maize grain: crude protein (71.5 and 71.2 g/kg), crude fat (33.1 and 36.1g/kg), crude fibre (17.17 and 19.96 g/kg), starch (645.7 and 641.1 g/kg), organic matter (861.1 and 857.7g/kg), essential amino acids (Cys 1.61 and 1.55; Met 1.30 and 1,31; Thr 2.99

and 2.87; Ala 5.3 and 5.15; Val 3.65 and 3.49; Ile 2.47 and 2.36; Leu 9.18 and 8.81; Lys 2.20 and 2.30; Arg 3.54 and 3.53 g/kg respectively). Digestibility test on growing rabbits was carried at the age of 65-70 days (Table 2). Aflatoxin and deoxynivalenol (DON) levels were low in all treatments. The resulting digestibility coefficients for protein fell within the narrow range of 63.97 to 65.72 % which is similar to the data of [3]. Digestibility of fat (78.22 - 72.50%) in diet with isogenic control maize decreased significantly with because of higher content of crude fibre and was also similar to results of [4; 24].

Table 2. Test result of complete feed mixture in feeding and balance experiments with rabbits

Parameters (n=24)	**Experimental group**	
	Bt transgenic maize	**Isogenic control maize**
Live weight in 35th day of life in g	928	926
Live weight in 56th day of life in g	1695	1714
Live weight in 77th day of life in g	2484	2475
Feed consumption per g gain between 35th and 56th day of life in g/g	2.69	2.58
Feed consumption per g gain between 56th and 77th day of life in g /g	3.56	3.51
Feed conversion ratio in g /g	3.08	3.00
Age in days achieving 2500 g l.w.	77.61	77.67
Daily weight gain g/day	36.95	36.88
Carcass yield in %	57.38	57.27
Coefficient of nutrients digestibility (n=5)		
Dry matter	61.42±1.96	60.87±1.61
Crude protein	63.97±3.99	65.72±2.35
Crude fibre	25.29±1.98	25.25±0.56
Fat	78.22±3.93*	72.50±8.45
Ash	53.73±2.03	55.60±2.92
Organic matter	62.16±1.79	61.34±1.54
NDF	40.40±1.72	39.08±2.08
Starch	93.99±0.98	92.64±2.65

*P<0.05 Significant difference

Digestibility of N-free extract, dry matter, NDF and starch tested in measuring time were lower in diet with DKC 5143 isogenic maize; the difference is insignificant (table 2). No significant differences in the body live weight gains were detected between experimental groups. During the trial, there were only two deaths in group 1 and 2 in young rabbits. These results indicate that although low level of fermentable carbohydrate may have a protective effect against rabbit enteritis, it should be coupled with high dietary protein to be effective and that high levels of fermentable polysaccharide in rabbit diets promote

enteritis to a similar extent, irrespective of the dietary protein level. Values of blood parameters were changed in the frame of the physiological level (Post Graduate Committee in Veterinary Science). Some authors presented a wide range of blood parameters, mainly of cholesterol [6]. Increased levels of the biochemical parameters in blood serum could be explained as a result of better resorption and utilization of these nutrients from the gastrointestinal tract that was also described in our previous study [7; 8; 23].

Table 3. Biochemical parameters in blood serum of rabbits

Parameter (n=24)	**Physiological level**	**Experimental group**	
		Bt transgenic maize	**Isogenic control maize**
TP	40-85 (g/l)	61.44± 3.87	54.77± 3.80
CHOL	1-8 (mmol/l)	2.08± 0.24	1.71± 0.29
TRIGS	1.5-9.5 (mmol/l)	1.10± 0.45	0.98± 0.35
GLU	3-8 (mmol/l)	8.10± 0.37	7.96 ± 0.36
ALT	<260 (U/l)*	10.03± 2.78	8.61± 1.47
GHS-Px	GPx (U/g Hb)	125.34± 31.58	121.54±24.89
Ca	2.4-3.4 (mmol/l)	3.24± 0.14	3.07± 0.15
PA	%	43.7±0.50	44.8±0.80

Randox (Crumlin, UK) kits (TP 245, CH 200, AL 100, GL 2623, CA 590) a Bio-La-Test (Lachema Brno) TG L 250 S; * Values from literature (Flecknell, 2000)
TP=Total protein, CHOL= cholesterol, TRIGS = total lipids, GLU = glucose, ALT= alanine aminotransferase, GHS-Px = glutathione-peroxidase, PA = phagocytis activity neutrophils, Hb = haemoglobin

Higher level of total lipids in the group administered Bt transgenic maize at the end of the experiment may indicate a long-term effect on maize lipid resorption from the intestine and/or utilization. The sensitivity to DON varies between species [13;21]. DON is most prevalent in crops used for food and feed production. Its toxicity affects mainly the immune system and the gastrointestinal tract in animals. The toxicity of DON is thought to be due to inhibition of protein synthesis and interference with the metabolism of membrane phospholipids. Activities of antioxidant enzyme glutation peroxidase GPx are examined to find out natural contamination of maize at low level of deoxynivalenol (the concentration of DON was determined by PCLC analyses of two maize varieties; it is within the range of 708±4.7µg/kg to 1009±4.57µg/kg Bt and isogenic maize). The concentration of GSH-Px is often monitored in meat and other organs – liver, kidney in rabbits [11]; studies concerning the serum level of GSH-Px enzyme and health status of rabbits are scarce. Blood parameters give the opportunity to detect conditions of stress; manipulation, environmental and diet changes in rabbits' husbandries often induce physiological or pathological oxidative stress. To avoid these reactions, an antioxidant defence system has been developed in aerobic organisms for free radical elimination, where the glutathione peroxidase (GSH-Px) enzyme family is a prominent member. There are several factors affecting the GSH-Px activity; the nutritional factors are the most essential among them [2; 27]. Measurement of the low

activity of GSH-Px in experimental groups as well as the evidently good health of rabbits indicated that no oxidative stress was evoked during the experiment. From this point of view, our study is a pioneering search.

Table 4. Physico-chemical characteristics of rabbit meat (MLD)

Parameters (g.100g^{-1} MLD)	**Experimental group**	
	Bt transgenic maize	**Isogenic control maize**
Content of water	75.37±0.06	75.6±0.46
Water holding capacity	29.49±6.66	29.30±5.94
Total proteins	21.83±0.15	21.90±0.20
Content of fat	1.80±0.10	1.47±0.31
Energy value (kJ.100g^{-1})	433.53±1.30	423.34±14.90
pH24	5.56±0.02	5.57±0.04

Means within a row followed by the same letter are not significant at α=0.05

The values of selected biochemical parameters in blood ranged within the physiological limits (Table 3). Physico-chemical properties of rabbit meat are presented in Table 4. Addition of maize in complete feed mixtures did not significantly affect the physico-chemical composition of meat; higher values of water holding capacity, fat and energy value were detected compared with rabbit experimental group with isogenic control maize. It is well known that muscle fat content, pH and water holding capacity (WHC) are related. There is a positive correlation between intramuscular fat content and WHC as well as between ultimate pH and WHC [10; 15]. We also confirmed these findings.

CONCLUSIONS

Feeding of complete feed mixtures with 12 % proportion of Bt transgenic maize and isogenic control maize to rabbits did not influence biochemical and zootechnical parameters, as well as it has not negative effect on feed conversion, growth performance and health status of rabbits.

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AROMATIC PLANTS AND THEIR IMPLICATIONS IN FEEDS AND PERFORMANCE OF ANIMALS

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ABSTRACT

The use of in feed antibiotics and chemical anticoccidial drugs has been the main strategy for controlling intestinal diseases in intensively reared productive animals. The last two decades there have been increasing concerns over the routinely use of antimicrobial and antiparasitic pharmaceutical substances in farm animal systems. These concerns arise from: (i) an increasing development of micro- and macro-parasite resistance to these pharmaceuticals: the rate of development of resistance to drugs is much faster than the rate of new antimicrobial drugs reaching the market place; (ii) drug residues potentially present in the food chain and its effect on human health coupled with increased consumer interest in the production of food from animals; and (iii) the consequences of these pharmaceuticals and their metabolites, when excreted in the environment, on wildlife fauna, such as invertebrates. For these reasons the EU has banned the prophylactic use of antimicrobials for farm animals since 2006, and alternatives to control both microbial and parasitic challenges are been sought. Among the alternatives, aromatic plants, herbal extracts and essential oils have a predominant place. Aromatic plants and their extracts are known to exhibit antioxidant, antimicrobial and antiparasitic activity. The knowledge on the effects of consumption of aromatic plants or their extracts in animal nutrition is based mainly on ethnoveterinary sources rather than rigorous scientific investigation and evidence. The aim of this article is the evaluation of aromatic plants and their extracts on animal performance, potential and levels of inclusion, as well as animal health. Successful application of aromatic plants or their extracts in the diets for animals requires an understanding of their modes of action. One of the major challenges is the standardization of the biological multi-component composition derived from herbal sources. New technologies are required to modernize traditional herbs usage into mainstream feed supplements and further *in vivo* studies are necessary to maximize the benefits derived from both animal performance and consumers' health.

Keywords*: aromatic plants, essential oils, natural feed additives, animal performance*

INTRODUCTION

Aromatic plants had been used since ancient times for their preservative and medicinal properties, and to impart aroma and flavour to food. Hippocrates, referred to as the 'father of medicine', used plant extracts and prescribed perfume fumigations. For centuries, aromatic plants, also known as herbs and spices, their essential oils and herbal extracts have been used as natural pharmaceuticals in folk medicine and also to cure

animals. However this use was not justified by extensive investigation from scientific knowledge but stems from ethnoveterinary sources [1]. By the middle of the 20th century, the role of essential oils had been reduced almost entirely to use in perfumes, cosmetics and food flavourings, while their use in pharmaceutical preparations had declined.

For the past 50 years, antibiotics have been supplemented to animal feed to improve growth performance and efficiency and protect animals from adverse effects of pathogenic and non-pathogenic enteric microorganisms. Economically important food producing animals suffer of a number of bacterial and viral infections diseases. The number and frequency of treatments with pharmaceuticals and vaccinations is correspondingly high. The net effect of using antibiotic growth promoters in the poultry industry was estimated by significant improvement in growth and feed efficiency. However, it has been argued that continued, unregulated excessive use of antibiotic growth promoters in animal feeds imposes a selection pressure for bacteria that are resistant to antibiotics. Hence, the use of feed antibiotics as growth promoters has come under increasing scrutiny by some scientists, consumers and government regulators because of the potential development of antibiotic resistant human pathogenic bacteria after prolonged use.

Various trade disputes among the European Union member countries, coupled with rising consumer concern about antibiotic resistance, have driven Scandinavian countries first from all other countries between the years 1994 and 1997, to severely curtail the use of non-therapeutic antibiotics in poultry feed but some coccidiostatic drugs remained as non-prescription feed additives. With the total ban on sub-therapeutic antibiotic usage in European Union countries in 2006, and the potential for a ban in North America, there is an increasing interest in finding alternatives to antibiotics for intensive reared animals; otherwise, this ban will have a strong economic impact on the pig and poultry industry. In organic production systems, particularly, the need for alternative therapeutics is dramatic. It is, therefore, research priority to intensify research into the identification and evaluation of alternatives to traditional antibiotics that would satisfy consumer perceptions and would be closer to environmentally friendly farming practices.

AROMATIC PLANTS (HERBS), HERBAL EXTRACTS AND ESSENTIAL OILS AS FEED SUPPLEMENTS

For centuries, aromatic plants, also known as herbs and spices, their essential oils and herbal extracts have been used as pharmaceuticals in alternative medicine as a natural therapy. Aromatic plants and their extracts have been found to exhibit antimicrobial activity, antiparasitic activity, and possess antiviral and antioxidative properties [2]. Herbs and their secondary metabolites are also said to stimulate the endocrine and immune system. They may promote a higher metabolic and immune status within the animal, as well as enhancing their welfare. Various botanical ingredients have been shown to facilitate beneficial effects on gut environment and microflora. Essential oils are known to stimulate digestive enzymes and may affect lipid metabolism and fat digestibility. There is a lot of evidence from various researchers that aromatic plants have an *in vitro* antimicrobial activity against many bacteria. In this paper we will focus mainly on anticoccidial and antimicrobial activity.

Definition of essential oils and biological effects of aromatic plants (herbs), herbal extracts and essential oils

Herbs and spices are aromatic plants that might be used in animal feeding after drying and grounding. Herbal extracts could be prepared by different extraction methods with various solvents, such as ethanol, methanol, toluene or other organic solvents. An essential oil is a mixture of fragrant, volatile compounds, named after the aromatic characteristics of plant materials from which they can be isolated. The term 'essential' was adapted from the theory of 'quinta essentia' proposed by Paracelsus who believed that this quintessence was the effective element in a medical preparation. Because the term 'essential oil' is a poorly defined concept from medieval pharmacy, the term 'volatile oil' has been proposed to be used instead, however, the term 'essential oil' is used more often. Essential oils are very complex mixtures of compounds and their chemical compositions and concentrations of individual compounds are variable. For example, the concentrations of two predominant components of thyme essential oils, i.e. thymol and carvacrol have been reported to range from as low as 3% to as high as 60% of total essential oils. Similar variation can be found also in the essential oil of oregano which is obtained by steam-distillation of *Origanum vulgare* ssp. *hirtum* plants, and comprises more than 30 ingredients, most of which are phenolic compounds with varying bioactivities. Major components are carvacrol and thymol that constitute about 78-82% of the total oil, with carvacrol to be the main component in most cases, although some times it can be found only in traces. The concentration of other main constituents such as the two monoterpene hydrocarbons, γ-terpinene and p-cymene, that often constitute about 5% and 7% of the total oil, respectively, also vary and the effect of oregano essential oil is often also variable because it depends on all the constituents working together. Cinnamaldehyde, a main principle of cinnamon essential oil, amounts to approximately 60 to 75% of the total oil. This diversity of essential oils would urge future research to select pure principles, i.e. thymol, cinnamaldehyde, beta-ionone and carvacrol, for evaluating their possible role as alternatives to antibiotics in poultry production. The chemical properties and biological activities of these compounds and their combinations should be extensively examined.

Polyphenolic compounds are mainly found in herbs, spices, their extracts and herbal essential oils, and also in fruits and vegetables. Over 8,000 polyphenols have been identified and among them more than 2,000 are found in nature. There is a central role of polyphenolic compounds in plants since are needed for pigmentation, growth, reproduction, resistance to pathogens and fungi and for many other functions. One of the most important groups of polyphenols is flavonoids. They can be divided into the following subgroups: flavones/flavonones, anthocyanins and cathehins/flavonols. In plants, flavonoids usually form complexes with various sugars which are called glycosides. Flavones/ flavonones have been isolated from almost all fruits and vegetables with their highest concentrations being found in the outer layers. Polyphenols have found to possess antimicrobial and antifungal activity and also effective antiparasitic activity against gastrointestinal parasites. Similar activity has been exhibited by saponins and condensed tannins and glycosides. Another study gave evidence that condensed tannins have direct anthelmintic effects towards gastrointestinal nematodes of sheep [3]. The antimicrobial effects of phenols, known for more than a century, are

targeting against the bacterial cell wall and affecting the cell wall structure. Phenols interact with the cytoplasmic membrane by changing its permeability for cations, like H+ and K+. The dissipation of ion gradients leads to impairment of essential processes in the cell, allows leakage of cellular constituents, resulting in water unbalance, collapse of the membrane potential and inhibition of ATP synthesis, and finally cell death.

Mode of action of aromatic plants and their extracts

Aromatic plants and their extracts have been found to exhibit antimicrobial activity, antiparasitic activity, and possess antiviral and antioxidative properties [4]. Herbs and their secondary metabolites are also said to stimulate the endocrine and immune system. They may promote a higher metabolic and immune status within the animal, as well as enhancing their welfare. Various botanical ingredients have been shown to facilitate beneficial effects on gut environment and microflora. Essential oils are known to stimulate digestive enzymes and may affect lipid metabolism and fat digestibility. There is a lot of evidence from various researchers that aromatic plants have an *in vitro* antimicrobial activity against many bacteria.

Anti-microbial activity of herbs and herbal essential oils

The exact anti-microbial mechanism of essential oils is poorly understood. However, it has been suggested that their lipophilic property and chemical structure could play a role. Helander *et al*. [5] investigated how two isomeric phenols, carvacrol and thymol, and the phenylpropanoid, cinnamaldehyde, exert their antibacterial effects on *Escherichia coli* 0157 and *Salmonella typhimurium*. Both carvacrol and thymol, in a similar fashion, disintegrated the membrane of bacteria, leading to the release of membrane-associated material from the cells to the external medium. On the other hand, cinnamaldehyde failed to affect the membrane, but exhibited antibacterial activity, indicating that two molecules have different mechanisms underlying antibacterial activity. It was thus suggested that terpenoids and phenylpropanoids can penetrate the membrane of the bacteria and reach the inner part of the cell because of their lipophilicity but it has also been proposed that structural properties, such as the presence of the functional groups and aromaticity are responsible for the antibacterial activity. It is thought that membrane perforation or binding is the principle mode of action, leading to an increase of permeability and leakage of vital intracellular constituents, resulting in impairment of bacterial enzyme systems. The mechanism of antifungal action of cinnamaldehyde has been investigated and it was proposed that it takes place through the reaction with sulfhydryl groups, which are indispensable for the fungal growth, and that the formation of charge transfer complexes with electron donors in the fungus cell could lead to inhibition of cell division and thus interferes with cell metabolism. It was also reported that cinnamaldehyde inhibits the fungal-cell-wall synthesizing enzymes.
On the basis of their *in vitro* antimicrobial activity, it is logical to consider essential oils application as prophylactic and therapeutic agents in animal production. It would be expected that the intake of essential oils affects the gastrointestinal microflora composition and population. Recently, Waldenstedt *et al*. [6] investigated the possibility of rearing broilers without growth promoters and coccidiostats by incorporating in their

diet oregano essential oil. In this experiment, however, oregano oil was merely examined as an anticoccidial compound but mainly as an antibacterial agent against intestinal colonization by *Clostridium perfringens*. Another field study conducted by Köhler [7] with a commercial preparation of essential oils showed a reduction of colony forming units of *Clostridium perfringens* as compared to the positive control diet containing zinc bacitracin at the level of 20 ppm. The commercial preparation was supplied in a powdered form and added to the diet at the level of 50 ppm. This preparation was also reported to slightly lower ileal ATP concentrations, which is an indicator of microbial activity in broilers. Similarly, a blend of capsicum, cinnamaldehyde and carvacrol lowered the number of *Escherichia coli* and *Clostridium perfringens* in ceca.

Anticoccidial activity of herbs, herbal extracts and essential oils

The ban of anticoccidial drugs, if established, would have a strong economic impact on the poultry industry. There has been, therefore, a growing interest in the identification and evaluation of alternative feed additives and as part of this effort, several natural products have been tested for their potential to provide protection against or modulate the effects of coccidial infections. Allen *et al*. [8] reported that dried leaves of *Artemisia annua* could protect chickens against caecal lesions due to *E. tenella* infection. They investigated *Artemisia annua* as a potential anticoccidial drug in poultry. Pure components of *A. annua*, i.e., artemisinin, 1,8-cineole and camphor at the levels of 17, 119 and 119 ppm, respectively, were fed to chicks from 1-d-old to 3 weeks of age. At 2 wk of age, half of the chicks were inoculated with *E. acervulina* and *E. tenella*. Some prophylactic action against the coccidia challenge was shown in treated chicks, especially in those fed artemisinin. Evans *et al*. [9] investigated whether a mixture of essential oils from clove (1.0%), thyme (0.1%), peppermint (0.1%) and lemon (0.1%) could have effects on coccidia oocyte output and the number of *Clostridium perfringens* in broiler chicks when artificially inoculated. There was no positive control included. Chicks fed the diets containing an essential oil blend showed a reduced oocyte excretion when compared to those fed the non-supplemented diet. Youn and Noh [10] showed that *Sophora flavescens* extracts were more effective than *Artemisia annua* against *E. tenella* infection in chickens. Giannenas *et al*. [11] reported that the essential oil of oregano, an aromatic plant of the *Labiatae* family, exhibited coccidiostatic action against *E. tenella* when incorporated into chicken diets at the level of 300 mg/kg. In another experiment, broiler chickens challenged with *E. tenella*, were administered diets supplemented with ground oregano herb at levels of 2.5, 5.0, 7.5 and 10 g/kg feed. The results suggested that most effective against the infection *E. tenella* was diet supplementation with oregano at 5.0 and 7.5 g/kg of feed. In another study, dietary supplementation with Olympus tea (*Sideritis scardica*) exerted a coccidiostatic effect against *E. tenella* [12]. Similar results were also noticed in a work studied the effect of dietary supplementation with a commercial preparation of herbal extracts from the plants *Agrimonia eupatoria*, *Ehcinacea angustifolia*, *Ribes nigrum* and *Cinchona succirubra* [13].

Antioxidant activity of herbs, herbal extracts and essential oils

Antioxidative properties are well described for herbs and spices [2, 4]. Among a variety of plants bearing antioxidative constituents, the volatile oils from the Labiatae family (mint plants) have been attracting the greatest interest, especially products from rosemary. Their antioxidative activity arises from phenolic terpenes, such as rosmarinic acid and rosmarol. Other Labiatae species with significant antioxidative properties are thyme and oregano, which contain large amounts of the monoterpenes thymol and carvacrol. Plant species from the families of Zingiberaceae (e.g., ginger and curcuma) and Umbelliferae (e.g., anise and coriander), as well as plants rich in flavonoids (e.g., green tea) and anthocyans (e.g., many fruits), are also described as exerting antioxidative properties. Furthermore, pepper (*Piper nigrum*), red pepper (*Capsicum annuum L.*), and chili (*Capsicum frutescene*) contain antioxidative components. In many of these lants, parts of the active substances are highly odorous or may taste hot or pungent, which may restrict their use for animal feeding purposes. The antioxidant property of many phytogenic compounds may be assumed to contribute to protection of feed lipids from oxidative damage, such as the antioxidants usually added to diets (e.g., α-tocopheryl acetate or butylated hydroxytoluene). Although this aspect has not been explicitly investigated for piglet and poultry feeds, there is a wide practice of successfully using essential oils, especially those from the Labiatae plant family, as natural antioxidants in human food, as well as in the feed of companion animals.
The principal potential of feed additives from the Labiatae plant family containing herbal phenolic compounds to improve the oxidative stability of animal derived products has been demonstrated for poultry meat [4], pork, rabbit meat and eggs. Oxidative stability was also shown to be improved with other herbal products. Nevertheless, it remains unclear whether these phytogenic antioxidants are able to replace the antioxidants usually added to the feeds (e.g., α-tocopherols) to a quantitatively relevant extent under conditions of common feeding practice.

USE OF AROMATIC PLANTS AS FEED ADDITIVES

Unlike pharmaceuticals based on a single chemical entity that deal with anomalies in target cells, tissues, or organs, most of the herbal remedies seem to lack scientific foundation and fall more into the realm of myth. In order for them to achieve a sustained growth and become acceptable by the mainstream pharmaceutical industry market, solid scientific evidence is needed to support the functionality claims of many botanical products. However, variable sources of biomass, unknown active ingredients, difficulties in quality control, lack of safety evaluation, unclear mechanism of action, etc., all constitute major challenges in terms of scientific standardization to adhere to the industry norm. The first step is plant identification. The strength of pharmacological effects may vary depending on where the plant was grown, when it was harvested, and how long it was stored. The activity of botanical products can change from year to year due to climatic change and even genetic makeup.
There exists a large gap between indigenous herbal practices and contemporary medical sciences in philosophy, theories and applications. Thus, the opening of opportunities for botanical drugs in western countries must bridge the two. Not until the historical

experience is generally understood by modern medical experts, should the application of botanical products be fully acceptable and become popular in the mainstream pharmaceutical market. Novel methods and technologies are desperately required to facilitate this process. Systems biology is a new field in life sciences that studies the interactions of different components (e.g., molecular pathways and regulatory networks) within an organism to elucidate relevant physiological functions and behaviour. It is based on statistics and suffers the same way as epidemiology in that it only shows association, not causality. Thus, an herbal mixture may display diverse activities by interacting with more than one molecular target. Alternatively, multiple active ingredients in a botanical preparation might be capable of acting on several functionally related systems to produce synergistic effects. Either way will work in coordination to interrupt the pathological processes involved in a disease or symptom. Herbal extracts may become easily popular, because they are not synthetic products. However, they have to be extensively investigated in terms of their mechanisms of action, efficacious level of administration and clinical effects. Issues of safety, toxicity and side effects for medicinal herbs should be standardized and their extraction should also be properly controlled and manufactured before wide use in animal diets.

CONCLUSIONS

The ban on the routinely feeding of antibiotics in Europe and the potential for a reduction or discontinuing of this use in North America, brought about an increasing interest in finding alternatives to antibiotics for animal production. The hypothesis of rearing productive animals without in feed antimicrobials is not new and appears to be of promise for further investigations on large scale experiments. The consumer as end-user expects also safe and healthy products, therefore the substitution of conventional medication by natural products generally recognized as safe, as selected secondary plant products, should be proved and based on the existing but scattered information and knowledge. New therapeutic measurements and (phyto) pharmaceuticals should be developed in co-operation of veterinary medicine, pharmaceutical biology, phytochemistry and the respective industrial partners.

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EFFECTS OF THE DIETARY ECOLOGIC CAMELINA OIL ON BLOOD PLASMA COMPOSITION IN FINISHING PIGS

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ABSTRACT

The purpose of this study is to investigate the influence of camelina oil (ecologic sources of n-3 PUFA) on the biochemical parameters of blood plasma in finishing pigs. At the beginning and the end of feeding test blood samples were aseptically collected by jugular venipuncture from 6 animals of each group in order to determine the chemical composition (glycaemia and lipids, protein, mineral and enzymes profile). Plasma biochemical analyses were performed by Analyser BS -130. Our results showed that the level of cholesterol and triglycerides decreased in camelina oil diet compared with the conventional diet (60.12 *vs* 71.16; <18%), but the differences were not significant (P>0.05). The total protein level was within the range from 7.48 g/dL to 8.82 g/dL in M group and from 7.21 g/dL to 8.08 g/dL in E group, in both cases the values being within physiological limits and not influenced by the camelina oil treatment. There were not significant differences between groups regarding the concentration of minerals in blood plasma (P>0.05). The concentration of glucose, ALT, AST and AP enzymes were slightly higher in the camelina oil group. Most data were within or little higher than the physiological value for this category of domestic pigs, the small differences observed being most likely due to the different methods used. The results obtained could serve for a better understanding of the biochemical processes in finishing pigs for estimating their physiological status.
Keywords**:** *camelina oil, ecological ingredients, plasma composition, finishing pigs*

INTRODUCTION

Camelina (*Camelina sativa)* can be considered a low–input feed ingredient requiring small quantities of water and a lower fertilization than other oleaginous species, representing by its nutritive values an opportunity to nutritional researches and feeding technology. The main characteristic that makes of this raw material a valuable feed ingredient is the high fat content, and its lipid structure. Therefore, camelina has usually a content of 35 – 38% oil, of which at high level are n-3 fatty acids (FA), especially linolenic FA. Based on medical records, Wood et al., (1997) [13], showed that an important target for human health is a dietary lipid ratio of about 0.45% and modern lean pigs meat may provide an adequate structure of lipids benefice for health with the condition of a balanced diet. Changes in FA composition in the human diet can have positive effects on health but, may also influence susceptibility of tissue lipids to free

radicals. Nutritionally, it was proved that we could manipulate through the diet the biochemical metabolic profile of blood, especially lipids composition in order to avoid nutritional and metabolic unbalances. An increased interest in controlling FA composition on animal tissue, is due to consumer awareness of health issues that may be associated with consummation of food of animal origin. It was also stated that the diets are deficient in ω3 and ω6:ω3 ratio (15-20:1 vs. 1:1 of that in wild animals). In the past, more 100 years ago, has been a decrease in this ratio in all European countries. During human evolution, ω3 FA was in all consumed food (meat, wilds plants, eggs, fish, and berries). Moreover, fast dietary changes in short period of time in the last 100-150 years are evolutionary phenomena that remain. The biochemical parameters are influenced by age, sex, nutrition and physical activities [10, 12]. Numerous other factors are also implicated, the stress of sampling being probably the most significant in swine [3].
Pig is used as subject for references biomedical determination concerning biochemical and immunological parameters that define the metabolic profile of blood [2]. The nutritional-metabolic profile of the animals is a valuable indicator of the biological potential of the animals to correlate with the rearing technology [16].
The aim of this study is to asses the effect of camelina oil used as an energy sources rich in n-3 FA especially C18:3 n-3 (FA which have positive effect to human health) on plasma metabolic profile, by biochemical investigation of blood plasma from Large White pigs during the finishing period.
If until recently, oil extraction was done mainly with organic solvents the current trend is to use cold pressing, which is 100% ecological because it uses no chemicals and maintains unaltered the polyunsaturated fatty acids. This is an advantage which allowed us to use camelina oil as alternative sources of omega 3 to linseed or in complement, the production of this first ingredient being higher in many countries (including Romania) than other oleaginous sources. The linseed content in n - 3 fatty acids is most important (more than 50%) but the quantity of this source is limited. The compound feed formula was isoenergetic and isonitrogenous (table 1).

MATERIAL AND METHODS

Animals and diets

The feeding test was conducted on 20 Large White pigs (10 heads / group) for 30 days during the finishing period. The animals were assigned at random in two homogenous groups: the control group (M) received a diet including conventional ingredients. As oleaginous source we used sunflower oil containing predominantly linoleic FA (C18:2n-6 = 64.62%); camelina oil (3%) obtained by cold pressing was used as oleaginous source in the feed for the experimental group (E) with certified ecological ingredients; it has a predominant content of omega 3 FA, especially linolenic FA (C18:3n-3 = 44.45%).

Table 1. Compound feed formulation and index of quality

INGREDIENTS	ECOLOGIC	CONVENTIONAL
	E	M
Barley	37.90	22.85
Wheat	20.00	48.00
Peas	10.00	-
Full fat soy	13.00	-
Soybean meal	5.00	7.00
Sunflower meal	7.00	14.50
Camelina oil	**3.00**	**-**
Sunflower oil	-	3.50
Methionine	-	-
Lysine	0.10	0.30
Calcium carbonate	1.70	1.75
Monocalcium phosphate	1.00	0.80
Salt	0.20	0.20
Choline Premix	0.10	0.10
Premix vitamins - minerals P3+4	1.00	1.00
TOTAL	***100***	***100***
CHEMICAL CHARACTERISTICS		
ME (Kcal /kg)	3192	3025
CP (%)	15.55	15.57
Crude Lys (%)	0.88	0.82
Digestible Lys (%)	0.72	0.69
Crude Met + Cys %)	0.52	0.54
Digestible Met + Cys (%)	0.41	0.45
Calcium (%)	0.92	0.89
Phosphor (%)	0.63	0.60
Crude cellulose (%)	6.42	6.83

Food delivery was "*ad libitum*" with permanent access to water and similar experimental conditions.

Measuring and analyses

Chemical composition and nutritive value of the energy-protein vegetable resources

The crude protein was determined using a semiautomatic classical Kjeldahl method using a Kjeltek auto 1030 – Tecator. The fat was extracted using an improved version of the classical method by continuous extraction in solvent, followed by fat measurement with Soxhlet after solvent removal. The crude fibre was determined with a classical semiautomatic Fibertec-Tecator method and the ash by calcinations' at 5500 until constant mass [14]. The nitrogen-free extractives (NFE) were calculated from the formula: NFE = DM - (CP + EE + CF + Ash). The metabolisable energy (ME) was

calculated with regression equations developed by the „Oskar Kellner" Institute of animal nutrition: ME = 5.01 × DP + 8.93 EE + 3.44 CF + 4.08 DNFE [15].
At the beginning and the end of the experiment blood samples were aseptically collected by jugular venipuncture from all animals (heparinized Vacutainer tubes, Vacutest®, Arzergrande, Italy) in order to determine glycol –lipids composition (glucose, cholesterol and triglycerides), proteins (total protein, albumin, creatinine and urea), minerals (phosphorus, calcium, magnesium and iron), enzymes (ALT, *alanine aminotransferase;* AST, *aspartate aminotransferase* and AP, *alkaline phosphatase*). BS-130 Chemistry analyzer (Bio-Medical Electronics Co., LTD, China) was use for determine biochemical parameters.
The camelina seeds were obtain from ecological culture. Cold pressing was used as ecologic method for oil extraction. The oils extracted with this method are better in terms of content and they preserve unaltered the nutritive quality of he seeds. The yield is 38-45% oil.

Statistical analysis

The results were expressed as mean values ± SEM. The data were submitted to an analysis of variance using SPSS statistical software, (ANOVA linear model) and the averages were compared using the Student test at 10%, 5% and 1% significance level. The experimental design consisted of 2 treatments with diet as factors of influence. At 10% we consider there are a tendency to be influenced by the diet the results.

RESULTS AND DISCUSION

The biochemical parameters of plasma are important markers to evaluate the subclinical health as a valuable tool in modern preventive medicine. Several biochemical variables are strongly influenced by the chronic disease and nutritional deficiencies [6]. The performance of animals is influenced by the metabolic efficiency and by maintaining a dynamic equilibrium between the extent of anabolic and catabolic changes in the body [5]. The intensity of metabolic changes mainly of protein is also reflected in the concentration of other biochemical indicators of blood such as total protein urea (ALT) aspartate aminotransferase or (AST) alanine aminotransferase [5]. In the present study we noticed a significant influence of camelina oil on glucose concentration (86.95 vs 72.90) (Table 2). Our results showed also a decreased tendency in the level of cholesterol of pigs fed camelina oil diet compared to the pig control fed the conventional diet (60.12 vs. 71.16; <18%), but the differences were not significant (P>0.05). The high level of PUFA in the camelina oil could have an influence in the level of cholesterol. The concentration of triglycerides was also lower in the camelina oil group than in the control group (30.10 mg/dL vs. 49.82 mg/dL; < 65%) (Table 2).

Table 2. Glucose, cholesterol and triglycerides level on blood plasma in finishing pigs

Variable	Groups*								SEM	P**
	Control				Camelina oil					
	Minim	*Maxim*	Mean	*CV %*	*Minim*	*Maxim*	Mean	*CV %*		
Glucose (mg/dL)	62.80	95.10	**72.90^{b}**	*15.99*	79.00	99.70	**86.95^{a}**	*8.56*	*3.43*	*0.03*
Cholesterol (mg/dL)	50.20	96.50	**71.16**	*25.27*	55.90	65.70	**60.12**	*5.98*	*3.94*	*0.17*
Triglycerides (mg/dL)	21.00	56.40	**49.82**	*41.00*	22.70	43.20	**30.10**	*23.1*	*9.19*	*0.31*

** Normal values: glycaemia – 55 – 115 mg/dL; cholesterol – 78 – 200 mg/dL; triglyceride – 33-50 mg/dL (Friendship, 1984)*
*** a,b – different letters = significant differences between groups (P<0.05).*

Many literature data indicate that the levels of lipids and cholesterol depend on the breed of pigs, their genotype in relation to lipoproteins, sex and the type of feed given [11, 9, 7]. Over the years, numerous feeding studies corroborated the blood cholesterol predictive equations and showed that saturated fatty acids were hypercholesterolemic and that polyunsaturated fatty acids lowered blood cholesterol concentrations [4].

Table 3. Protein profile on blood plasma in finishing pigs

Variable	Groups*								SEM	P**
	Control				Camelina oil					
	Minim	*Maxim*	Mean	*CV%*	*Minim*	*Maxim*	Mean	*CV %*		
Total protein (g/ dL)	7.48	8.82	**7.98**	*6.94*	7.21	8.08	**7.66**	*4.52*	*0.13*	*0.25*
Albumin (g/ dL)	3.53	3.89	**3.69**	*3.97*	3.28	3.99	**3.69**	*3.97*	*0.05*	*0.56*
Creatinine (mg/dL)	1.70	1.86	**1.80^{a}**	*3.30*	1.52	1.80	**1.65^{b}**	*6.93*	*0.03*	*0.02*
Urea (mg / dL)	32.00	59.00	**44.33^{T}**	*20.8*	27.00	44.00	**34.33^{T}**	*19.8*	*2.69*	*0.06*

** Normal value: total protein – 6.8 – 8.0 g/dL; albumin – 3.74 – 4.40 g/dL; creatinine – 0.5 -2.3 mg/dL; urea – 8-25 mg/dL, [6]*
*** a,b – different letters = significant differences between groups (P<0.05),*
T - P=0.06 – there are a tendency to be influenced.

Table 3 shows the protein profile in plasma of pig fed experimental and control diets and no differences were noticed between the two groups. The total protein concentration was within the range of 7.48 g/dL to 8.82 g/dL in control group and from 7.21 g/dL to 8.08 g/dL in the camelina oil group in both cases the value being within the physiological limits. The data recorded for albumin were slightly lower than normal values and for creatinine they were within the recommended limit and once again no effect of camelina oil on these two biochemical parameters was noticed. Mean urea value in our samples was higher than the limit recommended in both groups but there is a wide variability. Urea is a waste product of protein metabolism, in the blood. Urea is formed by the liver and carried by the blood to the kidneys for excretion. Because urea is cleared from the bloodstream by the kidneys, a test measuring how much urea nitrogen remains in the blood can be used as a test of the renal function. We remarked a decrease almost

significant (P=0.06) in the concentration of urea in plasma of pigs receiving camelina oil and the urea to creatinine ratio was lower in the camelina oil group (urea:creatinine = 20). The plasma concentration of minerals and enzymes is shown in tables 4 and 5.

Table 4. Mineral profile on blood plasma in finishing pigs

Variable	Groups*								*SEM*	*P*
	Control				Camelina oil					
	Minim	*Maxim*	Mean	*CV%*	*Minim*	*Maxim*	Mean	*CV %*		
Phosphorus (mg /dL)	0.22	1.25	**0.66**	*58.78*	0.15	0.71	**0.55**	*56.92*	*0.09*	*0.30*
Calcium (mg /dL)	7.20	10.04	**8.16**	*13.45*	7.38	8.40	**7.80**	*5.92*	*0.23*	*0.47*
Magnesium (mg /dL)	0.89	1.41	**1.09**	*20.82*	0.89	1.44	**1.05**	*18.93*	*0.05*	*0.74*
Iron µg / dL	79.40	173.40	112.25	*30.07*	33.20	133.30	84.77	*41.03*	*10.3*	*0.19*

**Normal value: P – 2.5 -9.3 mg/dL; Ca– 7.6 - 11.9 mg/dL; Mg – 1.3 -2.58 mg/dL; Fe – 22 -135 ug / dL,*

Table 5. Enzyme profile on blood plasma of finishing pigs

Variable	Groups*								*SEM*	*P*
	Control				Camelina oil					
	Minim	*Maxim*	Mean	*CV%*	*Minim*	*Maxim*	Mean	*CV %*		
ALT (U/L)	27.80	66.00	**46.12**	*31.26*	33.20	92.60	**47.03**	*48.02*	*5.21*	*0.93*
AST (U/L)	27.00	74.20	**44.90**	*37.19*	33.50	196.50	**64.08**	*101.52*	*13.3*	*0.50*
AP (U/L)	41.20	145.9	**84.23**	*45.46*	52.40	138.40	**93.23**	*34.27*	*9.80*	*0.66*

** Normal value: ALT (alanine aminotransferase) - 9-43 U/L; AST (aspartate aminotransferase) – 10-31 U/L; AP (alkaline phosphatase) – 35-130 U/L*

P level decreased in the plasma of camelina oil group by 1.5 factor and Ca by 1.04 factor, but the differences against control group were not significant (P>0.05). The coefficient of variability is higher for all minerals especially in control group, so we had a wide interval between the minimum and maximum level (Table 4). AP activity is a potential marker used as a supportive measure of Ca and P adequacy [1]. The changes in this enzyme activities lead to inadequate level of minerals. The values recorded for both groups were within the recommended limit and a slightly higher in plasma of pigs fed camelina, but the difference was not significant, (P>0.05). As can be seen in Table 5, the camelina treatment determined an increase in the concentration of aminotransferases enzyme, markers of liver functionality. The activity of these enzymes is very important because they act as a catalyst in connection with the metabolism of amino acids and carbohydrates. Changes in their activity in the blood can be a consequence of their increased activity in the cells, or cell structure damage [8]. In our study, AST was the hepatic enzyme the most influenced by the dietary camelina oil, 64.08 in experimental group *vs* 44.90 in the control group, the upper limit for AST being 74.2 U/L in control diet and higher in the camelina oil group, 196.5 U/L. The upper limit for ALT was 66 U/L in the control group and 92.60 UI in the camelina oil group, the difference between the mean values of the two groups being lower than in the case of ALT and AP.

CONCLUSION

Most of the data obtained in this study are within or little higher than the physiological values for this category of domestic pigs, the small differences observed being most likely due to the different methods used.The higher content of n-3 PUFA in the camelina oil influenced particularly the lipid composition. The concentration of cholesterol and triglycerides was decreased probably due to the content in n-3 FA. The addition of camelina oil in the diet of finishing pigs determined also an increase in the glucose and enzyme concentration (ALT, AST, AP). The results obtained could serve for a better understanding of the biochemical processes in finishing pigs for estimating their physiological status.

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INFLUENCE OF A LIQUID APPLICATION IN THE MAIN MIXER ON MIXTURE HOMOGENEITY OF FEEDING STUFFS

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ABSTRACT

Technological properties of feeding stuff powders e.g. miscibility and segregation are influenced by the properties of their components like particle size distribution and density. For the improvement of desired product attributes of feeding stuffs, an adjusting of the particle size distribution is possible within technological limits and under consideration of nutritional aspects. Adding small amounts of liquids (e.g. oil, molasses) into the main mixer shall especially avoid segregation of macro and micro components by creating bonds between wetted neighbouring particles. In this regard an inhomogeneous distribution of liquids in the bulk material influences the free movement of particles and consequently proper mixing.
***Keywords**: liquid application, mixture homogeneity*

INTRODUCTION

Common feeding stuffs are composed of rough and fine organic and mineral particulate components. Their composition depends on the animal species as well as the period of growth and is adjusted e.g. due to the availability of raw materials. An adequate feeding of animals requires besides grain-based feeding stuffs an addition of essential mineral components (e.g. salt, limestone), trace elements and additives, whereby the use of high potential additives is lawfully regulated. Ensuring equitable feeding of all animals in stock, animal health as well as quality demands of food from animal origin, micro components in ppm amounts must be mixed homogeneously with main mesh particles. A high degree of fineness of micro components is necessary to reach an adequate distribution due to a high number of particles, thus particulate macro and micro components show considerable differences in their particle size distribution, material density as well as flowability and dusting behaviour which complicate the production of homogenous mixtures with high mixing stability from feed mill up to trough [1].

Well-directed measures are necessary to ensure the compliance with product demands like accurate mixing and mixture stability. Adjusting the particle size distribution of powders is possible within technological limits and under consideration of nutritional aspects [2]. Adding small amounts of liquids – 1 to 2 % – like molasses into the main mixer has the benefit of stabilizing the mixture, thus preventing segregation and reducing the dusting potential, leading to a higher animal's acceptance. To overcome problems of undesired agglomeration it is common practice to introduce a dry mixing cycle, during which solids are mixed without liquid addition interfering. Anyhow, depending on the process and equipment design as well as the amount of liquids added

negative effects on product quality – like undesired agglomeration of additives, therewith inaccurate mixing or caking as source of cross contamination – are likely to occur.
In summary, the state of knowledge of the process design, the amounts of liquids added as well as their relevant material properties considering the mixing progress in discontinuous main mixers while producing feeding stuffs are not sufficiently characterized until now [4]

The aim of an experimental study at the IFF Research Institute was to mark up relevant material and process parameters for an optimization of the mixing process when small amounts of macro liquids are added. Special consideration was given to feed quality.

MATERIAL AND METHODS

Partial experimental investigations were carried out with an intensive mixer in pilot scale (45 l volume, Gustav Eirich Maschinenfabrik, Hardheim, Germany) equipped with a simple pipe for liquid application. Aim of comparative investigations was to create findings on the influence of the addition of

- different liquids with
- different liquid properties (e.g. viscosity, surface tension) and
- different common amounts of liquids (1 and 3 %)

on the mixture quality of feeding stuffs. The impact of an introducing dry mixing and a subsequent mixing cycle at the end of the liquid application on mixture homogeneity were determined, too.
Commercially available grain-based feed mixtures for pigs (finely structurized) and layers (roughly structurized) as well as mineral feeding stuffs for pigs (finely structurized) and cattle (roughly structurized) were used as test materials (without previous liquid application).
Sugar beet molasses with a sugar content of 42 % (feed quality) and soy oil were chosen as liquid test materials. Due to the depending on temperature of molasses' viscosity, it was heated up to approx. 30 °C.
For the characterization of the mixture homogeneity a particulate organic colourant was used as additives' indicator. The colourant can be detected photometrically with a low analytical error. It was added in a ratio of 1:100,000 or 1:50,000.
The liquids were marked with the colourant for characterization of their distribution in the bulk material. The evaluation was done indirectly by analyzing the concentration of the colourant in single samples. Analyzing the change in moisture content directly appeared not to be adequate because of the small amounts of liquids added and therewith an expected high analytical error.
The mixing process of feeding stuffs and colourant was started with an introducing dry mixing cycle of 30 sec as well as without. Then liquids were added (application period: 1 % = 30 sec, 3 % = 60 sec). The mixing process was stopped directly at the end of the liquid application as well as at the end of an additional subsequent mixing cycle of 30 sec. Samples were taken (10 single samples of 20 g), the concentration of the indicator was analyzed and statistically evaluated by the coefficient of variation (CV).

Recommendation for good mixture homogeneity for grain-based feeding stuffs is CV ≤ 0.07, for mineral feeding stuff ≤ 0.1 [3]. For comparison and evaluation of the variation of mixture quality when adding liquids, dry mixtures of test feeding stuffs and colourant were produced and analyzed in the mentioned way.

RESULTS AND DISCUSSION

The results show that the quality of a mixture of organic or mineral feeding stuffs, a micro component (colourant) and liquid macro components (sugar beet molasses, soy oil) is mainly influenced by the distribution of the liquids in the bulk materials. The liquid concentration in single samples is directly correlated to the concentration of the particulate colourant in single samples taken from a parallel trial, as exemplary shown in *Figure 1* with cattle mineral feed when 3 % of soy oil was added.

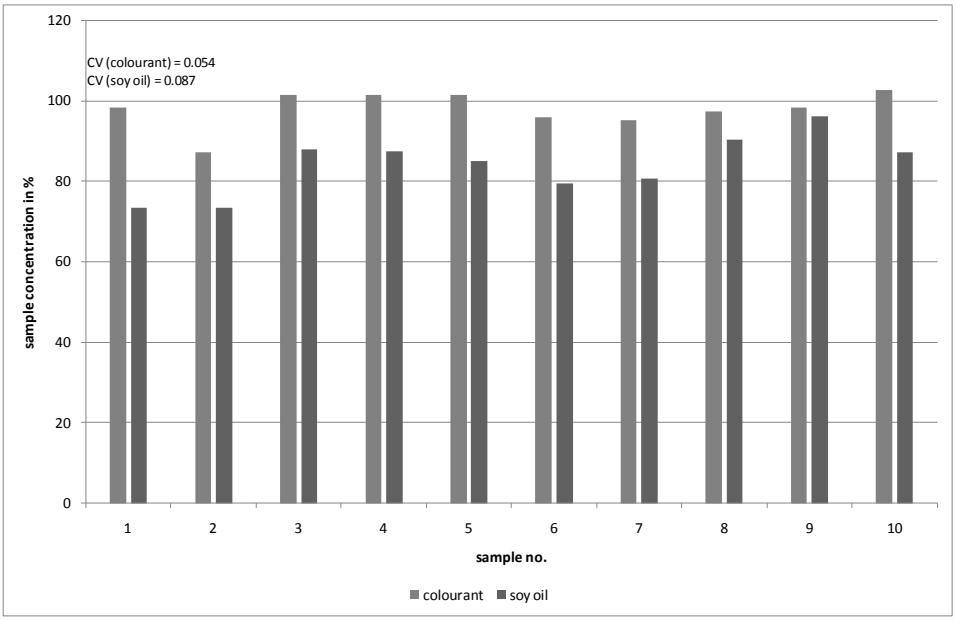

Fig. 1. Concentration of soy oil (marked with colourant) and particulate colourant when soy oil was added to cattle mineral feed

The liquid distribution in the mesh depends on the liquid application system used as well as on the liquid properties (viscosity, dry matter, surface tension, wettability) and the mixing performance of the mixer. As expected, the distribution of soy oil in mesh feed is better than of molasses due to specific viscosity and surface tension.
Interpreting the results needs to consider that the used intensive mixer is characterized by a high mixing performance which is not directly comparable with other common solid mixer types.

The results also show that there seems to be a feed-specific optimum in the amount of liquid which enables an equally liquid distribution in the mesh, thus better mixture homogeneity. For the investigated combinations of mesh feeding stuffs and liquids, in nearly most cases, the addition of approx. 3 % of liquids achieved better mixing results compared with 1 %. This finding is contrary to current conclusions of feed producers. Reasonable effect may be an equal wetting of all macro particles – wherefore a minimum amount of liquid is needed – and the agglomeration of fine and coarse particles in special consideration of the high mixing performance of the mixer. An example is given in *Figure 2*, showing the comparison of mixture homogeneity of the dry mixture and the mixture when 1 and 3 % of soy oil were added. Furthermore the effect of a subsequent mixing cycle at the end of the liquid application is illustrated. To evaluate the results, the area of good mixture homogeneity (CV ≤ 0.1) for mineral feeding stuffs is marked.

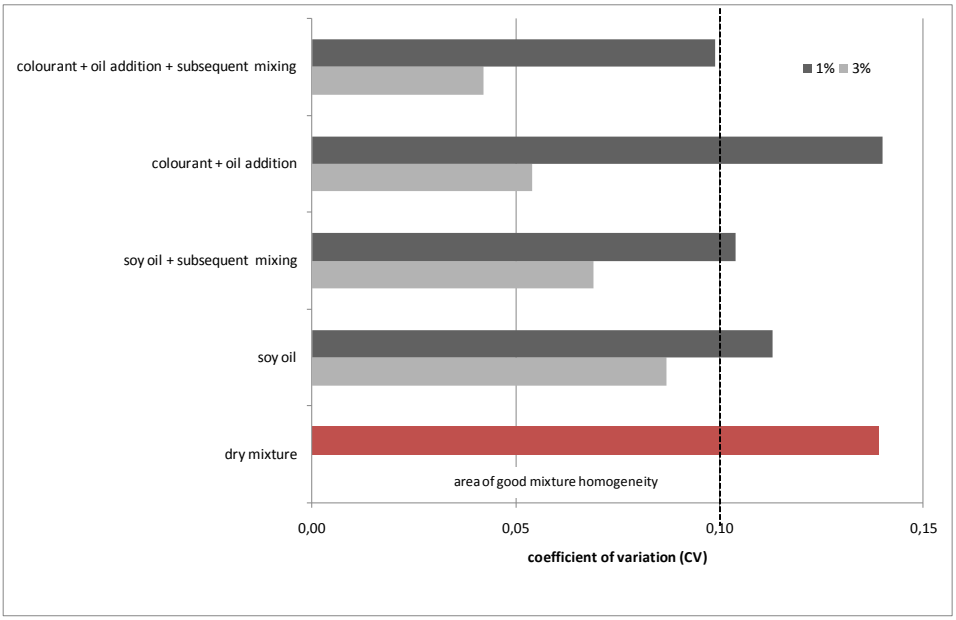

Fig. 2. Mixture qualities of cattle mineral feed, colourant and different amounts of soy oil (with and without subsequent mixing)

In comparison with the dry mixture, mixture homogeneity can be significantly improved when 3 % of soy oil were added and distributed evenly in the mesh. The addition of 1 % of soy oil seems not to be sufficient for a homogeneous wetting. Therefore a subsequent mixing cycle effects a further improvement of mixture quality.
An introducing dry mixing cycle do not effect an improvement of mixture quality. These results confirm the findings of former investigations of the IFF Research Institute on the topic [4].

CONCLUSIONS

The results of the experimental investigations show that the quality of a mixture of organic or mineral feeding stuffs, a micro component and liquid macro components is mainly influenced by the distribution of the liquids in the bulk materials. Mixture homogeneity can be significantly improved in comparison with dry mixtures by adding and evenly distributing a material specific optimum in the amount of liquid whereby general conclusion seems not to be possible at the moment. Depending on the liquid distribution correlating with the mixing performance of the mixer a dry mixing cycle is not needed in the case that up to 3 % of oil or molasses were added to feeding stuffs.

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AN EMPIRICAL ANALYSIS OF EXPLANATORY VARIABLES AFFECTING *FUSARIUM* AND DEOXYNIVALENOL IN WHEAT FOR FEED PRODUCTION

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ABSTRACT

Models for predicting *Fusarium* Head Blight (FHB) and deoxynivalenol (DON) contamination in winter wheat have shown to provide farmers with an adequate tool for preventing a direct reduction of grain yield, the production of mycotoxins and an increased grain cleaning cost. Due to the complex nature of FHB, such models typically consider several types of variables as input, including weather conditions, field-specific variables and crop characteristics. Yet a complete picture of all factors influencing FHB remains to be revealed. Instead of developing new prediction models, we present a thorough statistical analysis of weather, crop and field variables that might affect FHB, using an extensive and unique database of field observations covering eight different years and more than fifteen locations in Belgium. The results confirm several hypotheses that have recently been put forward by other authors without clear empirical evidence, but they also unravel new variables that play a more important role than expected beforehand, such as the underestimated effects of species interactions, preceding crop and early-stage weather conditions. An analysis of the species composition and DON level showed that the presence of F. *graminearum* and/or F. *culmorum* not automatically results in elevated DON levels. Conversely, wheat following maize in rotation leads to higher DON levels. Moreover, a correlation analysis revealed that weather conditions early in the growing season have a significant effect on DON accumulation. Apart from obvious associations during the last phase of the growing season, monthly temperature from November till May had a positive correlation with DON level and FHB occurrence, whereas monthly relative humidity and rainfall were negatively correlated in that period. Furthermore, the prediction of FHB occurrence or DON contamination should be seen as two distinct goals; it turns out that most explanatory variables give evidence of higher statistical associations with FHB.

Keywords: *crop rotation, deoxynivalenol, disease index, Fusarium head blight, weather*

INTRODUCTION

Fusarium Head Blight (FHB) or scab, caused by several *Fusarium* species, is a destructive fungal disease of wheat and other small grain cereals. It has become an important issue for two reasons. First, the incidence and severity of FHB has increased worldwide and this increase has resulted in significant yield losses. Second, in addition to causing yield losses, FHB is of greater significance due to the ability of several *Fusarium* species to synthesize a range of mycotoxins. The most important *Fusarium* mycotoxin worldwide is the trichothecene deoxynivalenol (DON). In Europe, *F. culmorum* and *F. graminearum* are the key fungal species responsible for cereal grain contamination with DON. Grain contaminated with DON is unsuitable for both human and animal consumption because of adverse health effects of such toxins. Growing wheat is, therefore, faced with the challenge of keeping contamination with *Fusarium* spp. and related mycotoxins at the minimum (Van Der Fels-Klerx et al., 2010). The relationship between *Fusarium* disease intensity and DON content in harvested wheat grain has been the subject of discussion among FHB researchers for years. Results from individual studies have led researchers to conclusions ranging from a total lack of significant association (Liu et al., 1997; Mesterhazy et al., 1999) to very strong positive relationships (Bai et al., 2001) between FHB and DON. However, a quantitative synthesis of the findings from individual studies of association between DON and disease intensity previously showed that overall there was a positive relationship (based on the Pearson product moment correlation coefficient) between DON and all commonly used field measures of *Fusarium* head blight intensity. Nevertheless, caution should be taken when using the disease index (DI) as predictor of DON values, due to the fact that DON can accumulate in the absence of visual symptoms (Paul et al., 2006). As a result of the poor relationship between disease incidence and DON, there have been two main approaches in *Fusarium* prediction, one based on predicting disease symptoms and another on predicting DON content in grain at harvest. However, accurate predictions of mycotoxins from *Fusarium* fungi are more useful to reduce their impact in the food and feed chain than predicting the visual presence of the disease itself in wheat, corn, and other grain crops (Schaafsma and Hooker, 2007). A thorough statistical study, presented below, indeed supports the hypothesis that a distinction should be made between visual symptoms of FHB and DON production. In addition, we will also claim that the relationship between both strongly depends on the types of species causing infected fields. The large efforts invested worldwide to determine the main factors responsible for DON accumulations in cereal crops, food and feed provides the impetus for the development of predictive models. Schaafsma et al. (2001) showed that the effect of year was the primary contributing factor to DON content at harvest. They speculate that these relatively high changes in toxin concentration from one year to the next, or within the same year among distant geographical areas, were primarily due to differences in weather. For FHB, the most susceptible stage appears to be anthesis, although considerable infection is still possible at the milky stage. Usually, inoculum strength, which is dependent on rainfall, relative humidity (RH), temperature and disease carry-over, etc. is very difficult to estimate. However, regardless of the level of inoculum available, favorable weather conditions are critical for infection to occur in wheat heads (Hooker et al., 2002). Crop residues on the soil surface provide the substrate for

ascospore production in conditions of warm weather and high humidity or precipitation. Therefore previous cropping history and residue management practices also affect the incidence of FHB, but to a lesser extent than weather variables (Schaafsma and Hooker, 2007). The use of maize in a rotation is to be avoided as maize is also susceptible to *Fusarium* infection and can lead to carry-over onto wheat via stubble/crop residues (Aldred and Magan, 2004). In the survey of Cromey and Shorter (2002), almost all crops with high levels of FHB and mycotoxins followed maize crops.

In order to determine the factors that influence FHB epidemics and DON contamination, it is helpful to consider the *Fusarium* disease cycle. Central to the cycle is the initial source of *Fusarium* inoculum from the soil, which survives either as saprophytic mycelium or as thick-walled resting spores (chlamydospores), depending on the *Fusarium* species. Warm and dry soil conditions during the early part of the growing season promote the development of *Fusarium* foot rot and the production of inoculum on stem bases. All of the *Fusarium* species that infect cereals are also capable of surviving saprophytically on crop residues and alternative hosts. For example, broadleaved weeds may provide an important source of inoculum. Probably the most obvious source of inoculum for the development of FHB epidemics arises from *Fusarium* foot rot in a growing cereal crop. Later in the growing season, air-borne inoculum, usually in the form of conidia or ascospores, may infect the ears of plants, resulting in FHB. Intense rainfall during the period of anthesis can effectively disperse *Fusarium* inoculum to ears when they are most susceptible to infection. Prolonged periods of warm humid conditions are conducive to the infection of cereal ears by *Fusarium* species (Parry et al., 1995).

Predictive modeling can be subdivided into three main steps: data collection, model development and model validation. Collecting and combining information from different sources in step one forms the core of model development, starting with the analysis and preprocessing of all collected variables. By putting together all variables that play a role, one obtains a simulation model for predicting fungal development in step two. The third and final step then consists of model validation and evaluation, before building up a final model that can be used in practice (Prandini et al., 2009). This study particularly concerns the first step; it is investigated to what extent a large number of explanatory variables can affect DON and FHB. Notwithstanding that most previous studies already focused on the effect of weather conditions and agronomic variables on FHB, this study tries to include all factors that could have an influence. Several of these variables, like early-stage weather variables, preceding crop information and species interactions, have not been investigated in detail before. All claims that we make are supported by statistically relevant interpretations, made after a thorough analysis on an extensive and unique database containing field observations, agronomic variables and year-round time series of local weather conditions. From 2002 to 2009, FHB incidence, *Fusarium* species and DON level were determined on several sites in Belgium. Weather variables from nearby local weather stations, such as daily rainfall, air temperature, relative humidity, air pressure, wind and leaf wetness duration were obtained for each individual field. From these variables, a lot of summary statistics such as monthly averages, minima, maxima, and percentiles were computed to extract the most relevant information. Using this database, several important research questions for FHB can be answered. First, the

relationship between visual estimates of FHB incidence (measured in terms of the disease index) and DON accumulation in harvested grain is analyzed. Second, we investigate to what extent DON levels and the disease index are influenced by the interaction between species. Third, the influence of agronomic factors such as wheat cultivars and preceding crops is studied, and last but not least, the impact of weather conditions is verified. While previous studies mainly focus on the weather conditions around heading, this study investigates the influence of weather conditions during the whole growing season and agricultural factors both on DON and FHB. So, all possible factors that can have an influence on DON or FHB are taken into account.

MATERIAL AND METHODS

The information from the experiments from 2002 to 2009 together with the weather data is stored in a relational database management system. An overview of the different variables in the database is given in Figure 1. The rectangle in the middle represents one field trial with different wheat varieties sown in three replications. Our database contains information from 67 field trials, which results in 1647 experimental results from the different varieties and parallels. The rectangles on the left top and bottom in Figure 1 show the weather variables and agronomic variables in the database. Wheat varieties are divided into five classes according to their susceptibility for DON and disease (rectangle on the right). Experimental results: FHB symptoms, species composition and DON levels are the final part of the database.

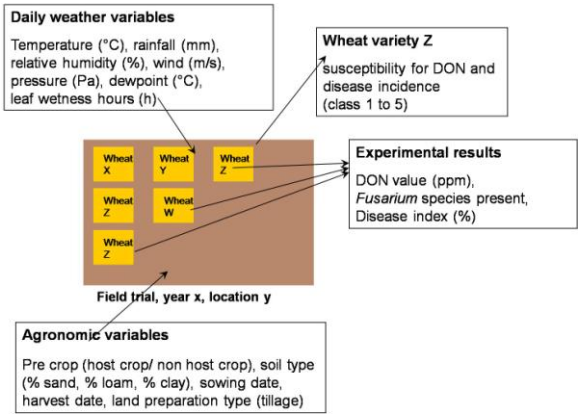

Figure 1: Schematic representation of the database. Variables on the left side of the experimental field trial influence FHB incidence, while variables on the right side are the experimental results obtained from each variety and parallel of the field trial.

Statistical analysis: For statistical evaluation, the R software package version 2.10.1 was used. Data were presented as box plots showing the median value, 5/95 (dots), 10/90 (whiskers), and 25/75 (box) percentiles. Differences between groups of data were tested for signifcance using a nonparametric Kruskall-Wallis test with a sequential Bonferroni correction for multiple comparisons, at P = 0:05=(n - 1) with n the number of pairwise

comparisons. Relationships between the weather data, DON levels and disease incidence were investigated using the Pearson product moment correlation at P = 0:05. Below, a letter code on the x-axis of the box plots distinguishes groups for which significant differences are observed.

RESULTS AND DISCUSSION

Figure 2 shows the pairwise correlations between the percentage of ears in disease class 1 till 5, the disease index (DI), the species composition and DON level. Correlations lower than -0.33 and higher than 0.10 were significant at P = 0:05. The number of ears in class 1 has a negative correlation with the other variables, ears in class 1 appeared healthy and were thus probably not infected by *Fusarium* species. The high positive correlations between the DI and disease classes 2, 3, 4 and 5 are obviously due to the fact that the DI was calculated as in (1). The correlations of DON with the presence of the different *Fusarium* species are rather low. The correlations between DON and non-DON producers *F. avenaceum* and *M. nivale* are insignificant (at P = 0:05). Interestingly, the correlation between DON and the DON producer *F. culmorum* is also not significant, while the highest correlation was found between DON and *F. poae*, which does not produces DON. This can be due to the fact that F. *poae* was in association with a DON producer, e.g. *F. graminearum* or *F. pseudograminearum* (not included in this study). Regarding the correlations between two different *Fusarium* species, all positive correlations between two species are significant, while the negative correlations are not significant (at P = 0:05). *F. poae* exhibited the highest positive correlations with other species, except with *F. culmorum*. Correlations between F. *culmorum* and the other species were all insignificant. Several factors may have resulted in these observed positive correlations between species. First, it is generally accepted that FHB is most severe in cereals where warm and wet conditions occur during the anthesis period though the exact relationship between disease development and environmental conditions may differ between FEB pathogens. Thus, provided that inoculum is present at a given site, conditions conducive to one FHB pathogen are also likely to favor others. Second, current varieties may have similar resistance/susceptibility to a pair of pathogens. Finally, fungicides used in previous seasons may also have similar activity on a pair of species. Negative correlations may arise from competitions between species (Xu et al., 2005). The positive correlation between *F. poae* and the other species can be explained by the fact that F. *poae* merely acts as a secondary invader, colonizing the weakened ears already infected by other more aggressive FHB pathogens such as F. *graminearum* (Xu et al., 2005; Audenaert et al., 2009). In the study conducted by Klix et al. (2008), the combination of F. *poae* and F. *avenaceum* was the second most important, whereas in our study this combination is most important. Negative correlations between species can be due to the fact their mycotoxins have an adverse effect on other species (Klix et al., 2008).

Figure 3 illustrates that samples with no *Fusarium* species contained significantly lower DON levels than samples containing one or more species. A comparison of the DON levels in samples with a single species and samples with two species did not result in statistically significant p-values, but in samples with three different species DON levels were significantly higher. Xu et al. (2007) drew similar conclusions in previous work:

the total mycotoxin production in mixed inoculation may decrease, increase or remain at a similar level, compared to single-isolate inoculation, depending on the fungal species concerned and environmental conditions. Furthermore, mycotoxin accumulation was greater following co-inoculations, compared to the single-species inoculations.

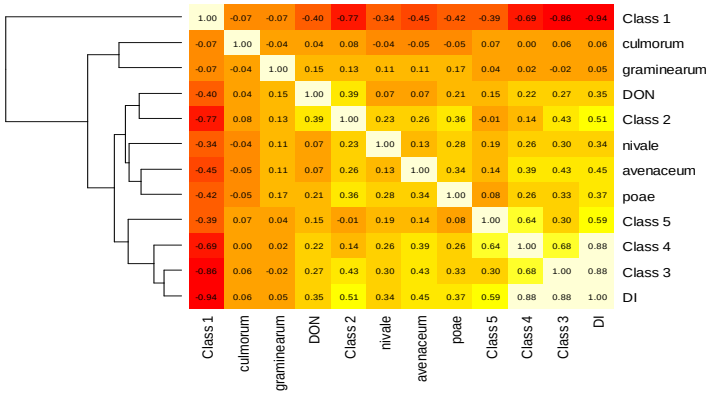

Figure 2: Heatmap of correlations between DON, disease index and Fusarium species present. Correlations lower than -0.33 and higher than 0.10 were significant at P = 0:05.

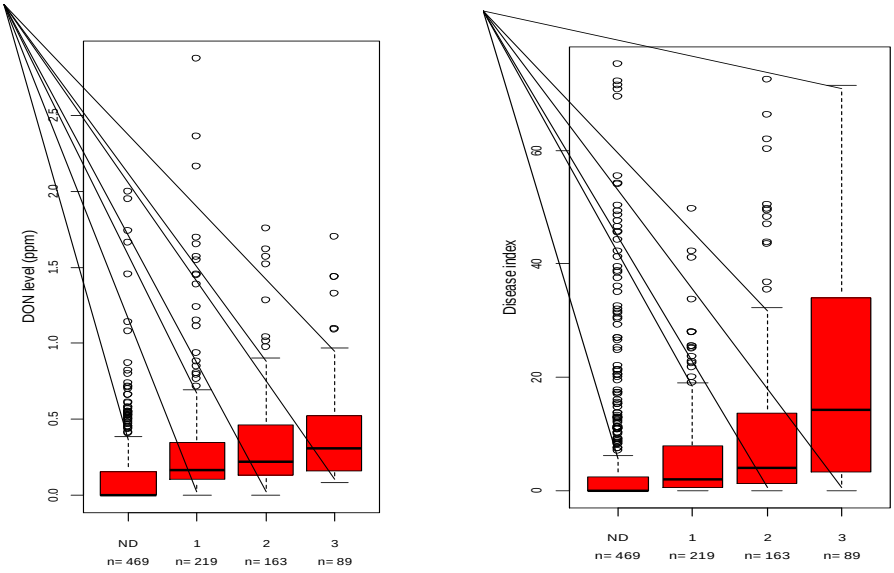

Figure 3: Effect of number of species on DI and DON content

This apparent synergistic effect on mycotoxin production may result from competition between species for resources; toxigenic fungi may produce more toxins under stress. However, the increase in toxin production was not universal, particularly for inoculations with isolates of different chemotypes (DON-producers versus NIV-producers). Thus, mixed inoculation gives evidence of increased production of toxins, but the exact composition of the toxins depends on the relative competitiveness and the toxin-producing ability of the isolates. Figure 4 shows the DON levels with host crops and non-host crops as preceding crop. One can see that DON levels tend to increase for fields with a host crop as preceding crop. If the comparison is made for every year individually, the host crops dominate the non-host crops in terms of DON for four (2002, 2003, 2004 and 2009) of the five years (in 2005, 2006 and 2007, wheat was sown after non-host crops). The differences turned out to be only statistically significant for these four years. Our observations are in line with the conclusion drawn by Schaafsma et al. (2001).

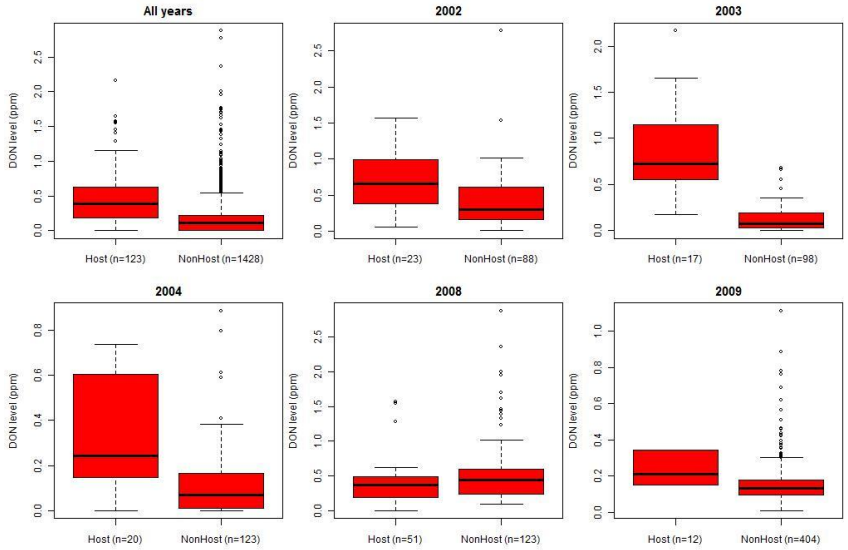

Figure 4. Influence of preceding crop on DON level in winter wheat grain.

Regarding weather variables, information from previous research efforts was used to construct predictor variables possibly useful during the modeling (Moschini et al., 2001; Hooker et al., 2002; Schaafsma and Hooker, 2007). We calculated the monthly average, the median, the 10th, the 25th, the 75th and 90th percentiles of all weather variables that were collected: daily minimum, maximum and average temperature, relative humidity (RH), precipitation, air pressure and wind. In addition, we also calculated the number of freezing days per month in winter, the number of rainy days per month, and the number of days per month with RH >80%. From mid April until June, we computed in addition five-day averages, median and percentiles, because two important periods for FHB infection can be expected during spring time. The production and dispersal of conidia and ascospores of F. *graminearum* happens in the first period, which includes the early

spring period up to anthesis initiation. The second period is shorter and covers the time of flowering (Klem et al., 2007). Surprisingly, in the period before infection, from November till April, temperature seems to play an important role. Early in the season from sowing to around GS31, RH and rainfall are negatively correlated with the DON level, while temperature obtains a positive correlation (Table 1). We have the following explanation for this phenomenon: early in the season, weather conditions influence the build-up of inoculum, which is favored by warm and dry weather. In this period, soil moisture and soil temperature can affect the rate of residue decomposition and the subsequent survival of the pathogens. Little decomposition occurred during winter, when soil temperature limited microbial degradation of residues. In addition, these conditions also may favor micro-organisms that compete with F. *graminearum*. Soil moisture and temperature have been reported to alter the inoculum potential of F. *graminearum* and the populations of fungal species common in wheat residues (Pereyra et al., 2004). The period starting in May plays a key role for spike infection, *Fusarium* development and subsequent mycotoxin production, so we also expected beforehand high associations with DON and FHB in this period. Nevertheless, the monthly aggregates of weather variables here tend to manifest lower correlations with DON and FHB. The correlation between monthly weather and DI tends to be higher than between weather and DON, but the variables with the highest correlation coefficients are more or less the same. Early in the growing season temperature has a positive correlation with DI and from May RH and rainfall are negatively correlated with DI. The higher correlation between weather and DI can be due to the fact that toxin production in the field seems to be of a complex nature, influenced by many factors including host resistance, chemotype and aggressiveness of the prevalent fungal species. Mesterhazy et al. (1999) found that DON contamination changes more extensively than FHB symptoms or kernel infection due to the year it is tested. Also the infection period plays an important role in the accumulation of DON, Del Ponte et al. (2007) detected higher toxin levels in kernels from inoculations at watery ripe or early milk stages than toxin levels in kernels from earlier (anthesis) or later (late milk) inoculations, while visual symptoms remained almost the same.

Table 1: Highest correlation coefficients for weather variables and DON level in grain, 10%P, 25%P,75%P, 90%P respectively mean 10%, 25%, 75%, 90% percentiles

Month	Variable	negative	Variable	positive
November	25%PRH	-0.25	AverageTemperature	0.29
December	25%PRH	-0.20	MedianPressure	0.40
January	Days Frost	-0.35	AverageTemperature	0.39
February	RH>80%	-0.33	AverageTemperature	0.46
March	Average RH	-0.17	AverageTemperature	0.41
April	Rainfall	-0.29	90%PTemperature	0.16
May	MedianPressure	-0.33	25%PTemperature	0.29
June	AveragePressure	-0.43	75%PRH	0.36
July	MedianTemperature	-0.38	10%PRH	0.32

In conclusion, this article described a thorough empirical analysis of explanatory variables causing visual symptoms of FHB and DON accumulation in wheat. In this

study, all possible factors that can have an influence are taken into account. In a nutshell, several important conclusions can be drawn. First, the results indicate a positive relationship between DON and the disease index, but the strength of the association varied substantially over years. This variation can be due to environmental and climatic conditions, which affect the toxin production capability of the *Fusarium* spp. Second, an analysis of the species composition and DON level showed that the presence of F. *graminearum* and/or F. *culmorum* not automatically results in elevated DON levels. No clear relationship could be detected between the DI and the profile of the *Fusarium* species causing FHB. It seems that the number of different species is more important; samples with three different species resulted in higher DON levels and more disease symptoms. As a third conclusion, wheat varieties and the preceding crop play a key role in FHB occurrence and DON production. Only in 2008, almost no difference between host and non-host crops could be observed. Finally, as a fourth conclusion, a correlation analysis between DON and the DI on the one hand and weather conditions on the other hand led to somewhat surprising observations. Weather conditions, early in the growing season, have a significant effect on DON accumulation and the DI. Since lower correlations were observed for weather conditions later on in the growing phase, this give hope for building predictive models that can warn farmers in an early phase. From November till May, monthly temperature had a positive correlation with DON level, while negative correlations were observed with DON for monthly relative humidity and rainfall. In June and July, the correlation between DON and relative humidity was positive, while in July, the temperature was negatively correlated with DON. From November till March, the correlation between monthly temperature and the DI was positive. In April, pressure seems to have the highest positive correlation with the DI, whereas in May and June the correlation between the DI and pressure was negative. From May till July the RH was positively correlated with the DI. In the period around flowering, rainfall had a correlation of 0.35 with DON and relative humidity 0.40. Overall, the correlation coefficients between the DI and weather variables were higher than those between DON and weather variables. Predictive models for DON will hence obtain a lower accuracy than models based on visual symptoms.

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WHEAT-MIDDLINGS IN POULTRY DIET AND ITS EFFECT ON BROILER PERFORMANCE

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ABSTRACT

A feeding trial was carried out to evaluate the dietary substitution of corn with wheat-middlings, a by-product of wheat, in rations for broiler chicks. Two-hundred Hubbard strain broiler chicks were used. Two dietary treatments based on corn-soybean (C-SBM, control diet) or wheat-middlings-soybean meal (WM-SBM, experimental diet) were formulated to meet the nutrients requirements of birds. The diets were isocaloric and isonitrogenous. The experiment was a completely randomize design. Each treatment was replicated ten times, with 10 birds/replicate. No obvious health problems were encountered during the experiment and broilers' mortality were recorded during the feeding trial. Parameters measured were feed intake, live body weight and gain, feed conversion ratio. Results show that dietary inclusion of wheat-middlings has not worsened feed intake, live body weight, gain and feed conversion ratio of broilers. In conclusion, data obtained support the total substitution of corn with wheat-middlings in diets for broiler chicks with no negative effects on growth performance.
***Keywords**: wheat-middlings, broiler, growth performance*

INTRODUCTION

In the last decade, wheat by-products have shown a steady increase with a good amount for export. The durum wheat division results strategic in the EU agro industry picture because it is the basis of milling industry [1].
Wheat middlings are a by-product of the wheat milling industry and these by-products have the potential to reduce livestock feeding costs. Middlings consist of coarse and fine particles of bran, shorts, germ, flour, and the offal from the tail of the mill. This product contains the offal and approximately 8–9% crude fibre, 30–44% neutral detergent fibre (NDF) [2], and due to the mixture of coarse and fine particles, and highly fermentable carbohydrates, must therefore be evenly mixed in diets [3].
The gross nutrient profile of wheat middlings indicates that it has a feeding value similar to that of cereal grains (with the exception of high fibre), and therefore may be used either in whole or in part to replace the grain component in ruminant rations. Wheat middlings have been fed to livestock species such as dairy cow and beef cattle [4-5].
However, research is not available on the value of wheat middlings inclusion for poultry. Therefore, wheat middlings have the potential to replace the grain component, such as corn, in broiler chick rations. Thus, it is necessary to study the inclusion of this by-product to provide alternative feeding strategies for poultry producers.

The objectives of this study were to provide an alternative feeding strategies and to compare the feeding value of diets based on wheat middlings to conventional corn-based diets, and to assess its effect on growth performance of broilers.

MATERIAL AND METHODS

A trial with 200 day-old female Hubbard strain broiler chicks was conducted from 14 to 49 d of age and involving two dietary treatments. Broilers, from a commercial hatchery, were raised in a conventional environment and fed *ad libitum* a common starter diet until 14 d of age. On day 14, birds were individually weighed and randomly divided among 20 pens in a commercial poultry facility located in Province of Bari, Italy. Each diet (treatment) was replicated ten times, with each replicate comprising one pen of ten birds. From 14 d to slaughtering age (49 d) birds were fed two diets containing corn-soybean meal (SB, 48% CP) or corn-wheat-middlings (WM, obtained from durum wheat, *Triticum durum* Desf. cv. Appulo). The WM was previously sieved to separate the fibrous component to obtain a raw material with a crude fiber content of less than 3%. Feed (pelleted form) and water were provided *ad libitum*. Randomized samples from each pelleted diets were collected for proximate analysis by the procedure described by AOAC [6].
Body weight (BW) and feed intake (FI) were measured every week. Body weight gain was calculated on weekly basis throughout the experimental period of 49 d of age. The consumed amounts of feed were recorded every week and daily feed intake was mined calculated at the end of the experiment. Feed to gain ratio was calculated using the following ratio FI:BW. Ingredient and chemical composition of the diets are shown in Table 1.
A completely randomized design was used with two treatments and ten replicates (pens) per treatment. Data were statistically analyzed by the GLM procedure of SAS [7] and means were compared by the Student-Newman-Keuls method when appropriate.

RESULTS AND DISCUSSION

The effect of experimental diets on live body weight, weight gain, feed intake and the feed conversion ratio are reported in Table 2. The birds' mortality during the whole experiment was very low (1%), and it was not related to the experimental diets.
Live body weight of broiler chicks at the end of trial (49 days of age) was not significantly influenced by substitution of corn with wheat-middlings in ration ($P>0.05$). The level of wheat by-product (724 g/kg of diet) did not show negative effects on birds' weight gain among groups. Wheat-middlings at the our level of inclusion led to improved the feed intake of broilers during the experimental period (from 15 to 49 days of age). In fact, the experimental treatment containing wheat-middlings did not reduce feed intake in comparison to the control group diet containing corn (107 vs. 108 g/day). As consequence, the feed conversion ratio of broilers resulted not significantly affected ($P>0.05$) by any of the experimental treatments for the whole period of trial (2.08 vs. 2.12, $P>0.05$). Our findings indicate furthermore that replacing corn with durum wheat-middlings reduces the use of soybean meal up 50% in diets for broiler chickens, reducing also the cost of feed production in countries where wheat production is more favorable .

Table 1. Ingredients and chemical analysis of experimental diets

	Experimental diets	
Ingredients, g/kg	**C-SBM**	**WM-SBM**
Corn	614.0	-
Wheat middlings[1]	-	723.7
Soybean meal (48% CP)	335.0	210.0
Soybean oil	5.0	15.0
Calcium carbonate	11.5	11.0
Dicalcium phosphate	12.0	12.0
Monocalcium phosphate	10.0	10.0
Sodium chloride	2.5	2.5
Sodium bicarbonate	2.0	2.0
Vitamin-mineral premix	5.0	5.0
L-Lys HCl	0.5	4.0
DL-Met	2.0	2.5
Thr	-	1.8
Choline chloride	0.5	0.5
Chemical analysis, %		
Dry matter	89.88	90.01
Crude protein	20.57	20.97
Crude fibre	3.01	3.21
Crude fat	3.82	3.91
Ash	6.36	6.25
Calculated analysis		
ME (kcal/kg of diet)	2,905	2,901
Lys, %	1.17	1.14
Calcium, %	1.03	1.02
Thr, %	0.80	0.79
Met + Cys, %	0.91	0.89
Available P, %	0.49	0.52

[1]Wheat-middlings obtained from durum wheat (*Triticum durum* Desf. cv. Appulo);

Table 2. Growth performance of broiler chickens (at 49 d of age)

	Experimental diets			
Item	**C-SBM**	**WM-SBM**	**SEM**	***P*-value**
BW, g/bird	2,539	2,549	15.72	0.163
BW gain, g/bird/d	50.9	51.4	0.31	0.212
Feed intake, g/bird/d	107.0	108.1	0.55	0.088
Feed to gain ratio, g/g	2.08	2.12	0.03	0.076
Mortality, %	1.0	1.1	-	0.809

CONCLUSIONS

Data obtained in this experiment is supportive of the utilization of wheat-middlings in poultry rations. The use of these alternative wheat by-product in diet, without any negative effects in growth performance, offers a viable option to help counteract the current constraints of corn.

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A REVIEW ON SALMONELLA INHIBITION IN POULTRY AND PIGS THROUGH THE USE OF DIFORMATES

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ABSTRACT

Salmonella control has a high priority in European pork and poultry production. It is a significant cause of human salmonellosis and causes major economic losses in the pork/poultry production chain, through reduced productivity, increased veterinary and hygiene control costs. Preventing the spread of salmonella to the consumer requires special control measures during slaughter and processing. The extra cost of these controls is increasingly being transferred back to the producer in the form of financial penalties or the loss of the market for contaminated pigs and birds. Gut health is increasingly being shown to be effective against intestinal pathogens, a strategy that has only really been made possible through the removal of antibiotic growth promoters in feed. Creating and maintaining a healthy intestinal environment has become essential to productivity and food safety programmes alike.
While biosecurity and hygiene in the feed mill and on farm are essential, the acidification of feed ingredients or finished feeds with organic acids and their salts also offers considerable benefits to Salmonella control. Feed acidification is not only effective within the feed; possibly its biggest benefit occurs within the animal itself.
A rather new and successful concept is the application of salts or double salts of the formic acid (diformate) into both industries. Such trials will be reviewed.
***Keywords**: Salmonella control, acidifier, diformate, gut health*

INTRODUCTION

Both the feed industry and the food production sector still suffer from losses due to the contamination of feed with pathogenic bacteria and their resultant impacts in the animal, such as lower weight gains and increased mortality. Banning the use of in-feed antibiotics (AGPs) in livestock, as happened in the EU as well as in parts of Asia, puts more pressure on animal producers and feed millers. It also poses an important challenge to innovative animal nutritionists. Addressing these problems in a suitable manner can help the industry regain the trust of consumers and NGO's, concerned about the safety of food. Now, alternative feed ingredients are being adopted in order to fill the gap left by removing AGPs from the food chain.
Organic acids have long been used to counteract gram-negative pathogenic bacteria in animal feed, mainly in pig production. This approach is currently being further investigated for poultry nutrition – especially to combat Salmonella.
Contamination with pathogenic bacteria like Salmonella creates an enormous social and economic burden world-wide. The annual cost of Salmonella to the UK economy for instance exceeds 76 million USD; and is estimated to be around 4.1 billion USD across the EU. Human cases of salmonellosis have been widely reported. The latest figures

from the EU (2007) mention that 152,000 people were directly affected by Salmonella in that year.
Combating Salmonella is therefore a matter of some urgency, with management and dietary strategies in pig and poultry production.
The potential of single organic acids in feed preservation lies in their ability to protect feed from microbial and fungal destruction. Their effects on stomach pH and gut flora have also been known for decades and proven in many laboratory and field trials (Eidelsburger et al., 1992; Eidelsburger and Kirchgessner, 1994; Freitag, 2007). Acidifiers act as performance promoters by lowering the pH in the gut (mainly upper intestinal tract), inhibiting the proliferation of unfavourable microorganisms. Gut acidification stimulates enzyme activity and thus optimises digestion and the absorption of nutrients and minerals. Un-dissociated forms of organic acids penetrate the lipid membrane of bacterial cells and dissociate into anions and protons. After entering the neutral pH of the cell's cytoplasm, organic acids inhibit bacterial growth by interrupting oxidative phosphorylation and inhibiting adenosine triphosphate-inorganic phosphate interactions.
Waldroup *et al.* (1995) studied the effect of supplementing citric acid at 1% inclusion in broiler feed, and observed that the number of birds contaminated with *Salmonella spp.* was increased when compared to the control group. The same researchers found that fumaric acid (0.5, 1.0 and 2.0%) in broiler diets was not sufficient to control caecal *Salmonella typhimurium* colonization or carcass contamination. Likewise, lactic acid (0.25, 0.5, 1.0 and 2.0%) fed to broilers, as a supplement also did not control caecal *Salmonella typhimurium* colonization or carcass contamination. In other trials, Jørgensen *et al*. (2001) reported that a dietary inclusion of 2.8% of lactic acid reduced the number of salmonella-positive faecal samples in weaned piglets. When added to drinking water before slaughter, lactic acid at 0.45 - 0.5% was effective in reducing Salmonella populations in broilers (Byrd *et al*., 2001). Izat et al. (1990a) studied formic acid and calcium formate in broiler feed. Adding 0.36% calcium formate and 0.25% formic acid significantly reduced levels of *Salmonella typhimurium* in poultry carcasses. Caecal salmonella counts were reduced when 0.36% calcium formate or 0.5% formic acid were added in the diet. Izat et al. (1990b) also examined the effects of buffered propionic acid, associated with glycol. They observed that when 0.4% of this mixture (corresponding to 0.2% of propionic acid) was added to the feed, it reduced the population of *Salmonella typhimurium* in broiler carcasses after slaughter. Kovarik and Lojda (2000) report that formic acid at 0.5% in the diet can be successfully used on farms to reduce salmonella contamination in feed, excretion of *Salmonella spp.* and re-infection of chicken populations. Byrd *et al*. (2001) demonstrated that 0.5% of formic acid added to drinking water pre-slaughter could also control salmonella populations in broilers.
Al Tarazi and Alshawabkeh (2003) reported that a mixture of dietary formic and propionic acids (total concentration 2% or more in the diet) for newly hatched infected layer chicks significantly decreased the crop and caecal population of *Salmonella pullorum*; and reduced mortality. Walsh et al. (2003a, 2003b) observed that a combination of organic acids at 0.4% of the diet, or a mix of different organic and inorganic acids at 0.2 or 0.4%, can successfully reduce the salmonella shedding in faecal samples from piglets, whereas addition of 0.2% of inorganic acid did not have any impact. The use of pure formic acid in breeder diets reduced the contamination of tray

liners and hatchery waste with *S. enteritidis* drastically (Humphrey and Lanning, 1988). Hinton and Linton (1988) examined how salmonella infections could be controlled in broiler chickens, using a mixture of formic and propionic acids. They demonstrated that under experimental conditions, 0.6% of this organic acid blend was effective in preventing intestinal colonization with *Salmonella spp.* from naturally or artificially contaminated feed.
Improving hygienic conditions in pig and poultry with the aid of organic acids has been reported by many sources, as mentioned above. An important limitation, however, is that organic acids are rapidly metabolised in the fore-gut (till stomach) of animals, which will reduce their impact on bacterial inhibition. ADDCON's patented double-salt technology (diformate) has been proven to be effective against pathogenic bacteria along the whole gastro-intestinal tract, including Salmonella and Campylobacter.

DIFORMATE IN POULTRY

The objective of this trial was to evaluate the effect of sodium diformate (0.3% and 0.6%) in starter and grower diets for broilers. The product's ability to control bacterial contamination in the digestive tract was tested against a negative control under hot conditions (Spain). 1750 one-day old broilers were used, distributed between 14 batches of 125 animals each (5 batches per treatment; excluding control with 4 batches only). The birds were fed a starter diet for 21 days; a grower diet for 18 days and a finisher diet for 3 days. After 39 days of treatment, prior to the supply of finisher feed, 10 birds from each of the 3 treatments were taken for further microbial analysis.
Sodium diformate (NDF) was only given in the starter and grower diets. The finisher feed (last 3 days) did not contain NDF and was the same in all 14 groups.
The collected data were analysed with ANOVA by the StatisticsXL program. A $P<0.05$ value was considered to be a significant result.
Results clearly showed the beneficial effect of sodium diformate against pathogenic bacteria in broiler chickens. No positive samples were found for Salmonella in the crop ($P=0.15$) or intestine ($P=0.15$) in all treated groups. Campylobacter counts were also significantly reduced in the crop ($P<0.01$) and intestine ($P<0.001$) with both treatment doses. Lower Enterobacter numbers in the crop ($P<0.001$) and intestine ($P=0.10$) for the 0.6% treatment as well as higher numbers of Lactobacilli ($P<0.01$) and Bifidobacteria ($P<0.05$) with the same dosage were found. Moreover, the 0.3% dosage of the NDF tended to increase the Lactobacilli count in the small intestine ($P=0.07$).
The results achieved are shown in the following 3 tables (Lückstädt and Theobald, 2009).

Table 1. Results of various sodium diformate (NDF) dosages on Campylobacter inhibition (% positive samples)

	Control	**FORMI NDF 0.3%**	**FORMI NDF 0.6%**
Crop (microbiol.)	60	0	0
Intestine (microbiol.)	80	20	0
Meat (serol.)	80	0	0

Table 2. Results of various sodium diformate (NDF) dosages on Salmonella inhibition (% positive samples)

	Control	**NDF 0.3%**	**NDF 0.6%**
Crop (microbiol.)	20	0	0
Intestine (microbiol.)	20	0	0
Faeces (microbiol.)	25	0	0
Meat (serol.)	0	0	0

Table 3. Results of microbiological investigation of the intestine (CFU/g)

	Control	**NDF 0.6%**
Enterobacteria	10^7	10^5
Lactobacilli	10^7	10^8
Bifidobacteria	10^5	10^6

More recent investigation on the impact of diformates against necrotic entiritis (Mikkelsen et al., 2009) proved the beneficial impact of the additive. Diformates at 0.45% dosage significantly reduced mortality caused by necrotic entirits (*Clostridium perfringens*). It is noteworthy that the same dosage of NDF positively influenced the final weight of challenged birds (+3.2%). After the necrotic enteritis outbreak (day 35 of the trial period), diformate significantly reduced the number of *Clostridium perfringens* in the jejunum, in agreement with results showing that formic acid inhibits growth of *Clostridium perfringens* (Mroz, 2005) in vitro.

Reducing the impact of pathogenic bacteria on broilers, together with the improved gut microflora, leading to a state of eubiosis in treated chickens, suggests that including NDF will also result in improved bird performance. This hypothesis formed the impetus for a further broiler trial.

A scientific trial with diformate was conducted at the research farm of the All-Russian Poultry Institute in Moscow, Russia. Each of five groups, 0.1% diformate, 0.3% diformate, 0.5% diformate, 0.3% acid blend (powdered formic acid – lactic acid mixture as a positive control) and a negative control, consisted of 35 one day old Cobb broilers, which were raised till 38 days on a commercial wheat-corn-soy diet (Lückstädt and Theobald, 2010).

Diformate addition was found to enhance individual live weights with increasing dosage. By the end of the experiment, diformate treatment improved broiler weight gain by 6.5% to 10.3% compared to the negative control. Based on the Empirical Rule all diformate

treated groups differed statistically from the negative control ($\mu \pm 3\sigma$). Diformate furthermore improved feed conversion ratio by 7.6% (dosage: 0.1%), 12.0% (0.3%) and 11.4% (0.5%) compared to the negative control group. The positive control on the other hand achieved intermediary results in this respect and did not differ, for instance, from the 0.1% diformate inclusion.
These findings lead to the conclusion that addition of diformate considerably improves poultry performance by increasing live weight and reducing feed consumption and thus, feed conversion compared to a negative control. The best improvements in respect of these parameters, calculated as European Broiler Index (EBI), were obtained for the dosages of 0.3% and 0.5% diformate. The EBI for 0.3% diformate inclusion improved by more than 31% compared to the negative control group; and was still noticeably enhanced compared to the positive control by 11%.
The mode of action of the acidifier in poultry is mainly due to its antimicrobial action, unlike in pigs where a key activity is the reduction of stomach pH (Desai et al., 2007). In the trial discussed above, the final body weight of the broiler chickens fed acidified diets was increased. Average daily weight gain was higher in the acidifier group, and FCR was reduced, resulting in an improved European Broiler Index. Additionally, other trials have shown improved health status in chickens, as demonstrated by a significantly improved gut microflora – reflected in lower Enterobacter numbers and high Lactobacilli and Bifidobacteria counts.
Using acidification in broiler diets is a valuable strategy in the producer's armoury against productivity losses caused by pathogenic bacteria. Combined strategies, including coarse feed particle size along with the use of organic acid salts like diformate can have further beneficial impact in the fight against Salmonella (Visscher et al., 2009)

DIFORMATE IN PIG

Gut health is increasingly being shown to be effective against intestinal pathogens, a strategy that has only really been made possible through the removal of antibiotic growth promoters in feed. On the other hand, pathogens are still widely found in pig units.
S. enteritica typhimurium is the predominant serotype found in pig carcasses in Europe, accounting for around 71% of cases. Several serotypes are resistant to antibiotics, putting increasing pressure on producers to prevent contamination. While Salmonella cannot be eradicated in pig units, it can be controlled to minimise the risk to consumers. Biosecurity plays a significant role in Salmonella control. In feed compounding, although heat treatment is effective in reducing contamination of feed leaving the feed mill, this effect does not persist during transport, storage and subsequent outfeeding. When conditions within the feed are less conducive to bacterial infection, Salmonella contamination can be reduced. The next critical control point is within the pig's gut itself, where conditions for bacterial growth may once again be optimal. Salmonella growth requires warmth (35-37°C is optimal), a moisture content greater than 12% and a pH between 4.5 - 9.0. It is no coincidence that the pig gut can provide Salmonella everything it needs to thrive.
While biosecurity and hygiene in the feed mill and on farm are essential, the acidification of feed ingredients or finished feeds with organic acids also offers considerable benefits to Salmonella control. Feed acidification is not only effective

within the feed; possibly its biggest benefit occurs within the pig itself. Research trials in the UK, France and Ireland with 0.6% potassium diformate (KDF) feed additive, showed significantly reduced Salmonella count in the feed as well as in the gut of pigs. This effect is particularly well illustrated by data collected on 12 farms in Ireland (Lynch et al., 2007). The main objective of this investigation was to evaluate the efficacy of Salmonella control measures on highly infected farms. Salmonella control has been compulsory under Irish law since 2002 and farm status is categorised by the percentage of positive pigs in a herd according to the Danish mix-ELISA test. Category 3 (>50% positive) farrow-to-finish farms and their associated fattening units were selected for the study. All the farms that were treated with KDF alone; or a combination of KDF with improved hygiene and biosecurity measures had notable improvements in both bacteriological and serological prevalence of Salmonella spp. All but one farm in which KDF was used ended the trial with a much improved Salmonella status, with bacteriological prevalence also low on most farms. Using improved hygiene and biosecurity measures alone also improved Salmonella status, but to a much lesser extent. The reduction in prevalence obtained by KDF alone, compared to the two farms which also implemented additional hygiene and biosecurity, demonstrates the additive's efficacy (Table 4).

Table 4. Bacteriological and serological prevalence of Salmonella spp. in finishing pigs on 7 farms highly infected with Salmonella spp. before implementation of control measures and following implementation of control measures for approximately 24 months (Percentages are given in brackets)

Farm and Group[1]	***Salmonella status before controls***		***Salmonella status after the use of 0.6% potassium diformate***	
	Bacteriological prevalence	***Serological prevalence***	***Bacteriological prevalence***	***Serological prevalence***
Farm F	12/12 (100)	21/24 (88)	0/20 (0)	12/24 (50)
Farm G	27/35 (77)	26/70 (63)	1/30 (3)	0/24 (0)
Farm H	8/25 (32)	4/24 (17)	1/31 (3)	1/24 (4)
Farm I	25/35 (71)	13/24 (54)	1/27 (4)	1/24 (4)
Farm J	6/40 (15)	10/24 (42)	7/48 (15)	0/24 (0)
Farm K	11/30 (37)	23/24 (96)	3/24 (14)	1/24 (4)
Farm L	2/45 (4)	23/24 (96)	0/39 (0)	0/24 (0)

These findings are not unique, however. Studies by Dennis and Blanchard (2004) in the UK as well as most recently in France (Correge et al. 2010) concluded potassium diformate, to be an effective tool in a salmonella control strategy in commercial farms, reducing the percentage of salmonella positive pigs by 50% and in pork meat juice ELISA scores by 46%, respectively in grower finisher pigs. The UK trial also showed an improvement in daily gain of 7.7%, reduced mortality and a reduction in medicinal intervention compared to the rolling average for that unit. The economic benefit of implementing salmonella control was also evaluated.

CONCLUSIONS

The results described above prove irrefutably how both, a healthy gut with inhibited growth of pathogens and food safety can be achieved by dietary means. Additionally, a balanced acidifier, such as diformate, increases the performance of broiler and pigs and is furthermore a sustainable option for maintaining or improving animal growth and efficiency, without resorting to supplementation with an AGP.

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NEW INSIGHTS FOR EFFECTIVE MOULD CONTROL

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ABSTRACT

Three different types of propionate products were tested by using two in vitro tests mimicking storage conditions of raw materials and compound feed.
We hypothesize an improved mould control by using activated ammonium propionates (Fylax Forte) compared to the ammonium propionate and commercial propionate products. Activation of ammonium propionates during the production process of Fylax Forte resulted in 50% improvement of minimal inhibitory concentrations (MIC) values and an improved shelf life efficacy of 79 to 87% compared to ammonium propionate and commercial products.
Keywords: *mould control, propionates, shelf life, feed conservation*

INTRODUCTION

Moulds are micro organisms that produce thousands of tiny particles called spores as part of their reproductive cycle. Actively growing mould colonies are usually visible as colorful “woolly” or “slimy” growths. They can be virtually and color red, blue, brown, green, white or black. There are thousands of known species of moulds worldwide. The presence of mould in feeds is a persistent problem. The need to control moulds is that they consume the main ingredients. This results in a deteriorated nutritional quality like a reduced starch and protein content, and a poorer palatability. Moreover, moulds also produce mycotoxins which pose a serious threat to animal and human health and even low levels can result in sub-clinical health problems and reduced productivity. Already was indicated that mould growth is likely the most important microbiological activity with both nutritional activity with both nutritional and toxicological implications that should be controlled from a commercial animal nutrition point of view (4).
Moulds need a couple of drivers determining mould growth in raw materials and animal feeds like specific nutrients, right temperature and conditions, and free available oxygen and water as described (1; 4). An efficient control program is complex with several factors involved in the growth of moulds. Besides, the quality of mould control measures in previous tiers of the raw material and fed chain cannot always be ensured. This is reflected in scientific reports that state 15 % of pig diets and 28% of poultry diets exceed acceptable mould levels (6) with 25,4% of corn samples being mould positive (5). As a consequence, mould inhibitors are used in commercial practice as a strategy to maintain the nutritional quality and safety and extend the shelf life.
Mould inhibitors should offer an effective broad spectrum killing of vegetative moulds with a long lasting protective effect against germination of existing mould spores and/or recontamination with moulds. In practice, mould inhibitors usually contain various levels and combinations of organic acids with propionic acid being the principle ingredient. Propionic acid is regarded as the most cost effective anti mould product for us in animal

feed. The exact mode of action remains to be elucidated but the helical structure and lipophylic characteristics of propionic acid seems to play a crucial role. Still, there are articles reporting a certain tolerance to propionic acid and some species may even use propionic acid as a substrate (7; 8). An effective broad spectrum killing of vegetative moulds with a long lasting protective effect against germination of existing mould spores and/or further could be strengthened by 'activating' propionates during the production process. The formation of activated propionates takes place in a neutralization reaction with ammonium hydroxide combined with a micelles stimulating technology. The result is an activated ammonium propionates bound in micelles (Figure 1). These micelles increase the porosity of the cell wall and destabilize the cell membrane of the mould, leading to an increased openness for and influx of organic acids. Inside the mould, organic acids dissociate decreasing the intracellular pH. This will inhibit glycolysis and ultimately kill the mould (9). This activation of moulds may eliminate the previously noted disadvantage of Propionic acid.

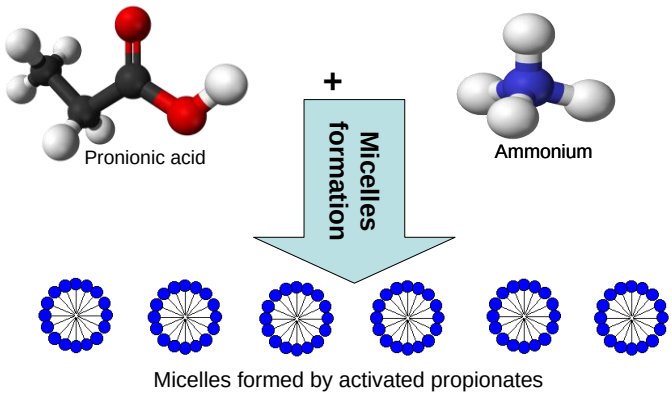


Figure 1. Formation of activated propionates

In this study, we tested three types of propionate products by using two in vitro tests (MIC test and a stress test). We hypothesized improved anti-mould effects regarding shelf life and MIC values of activated propionates compared to the other products.

MATERIAL AND METHODS

Three types of products were compared in a laboratory study using two *in vitro* models. The three types of products were:

- Ammonium propionate as reference for industrial standard (Amm. Propionate)
- Three commercially available mould inhibitors based on a high level of propionates (Product 1, 2 and 3).
- Mould inhibitor based on a high level of propionates that were activated during the production process (Fylax Forte).

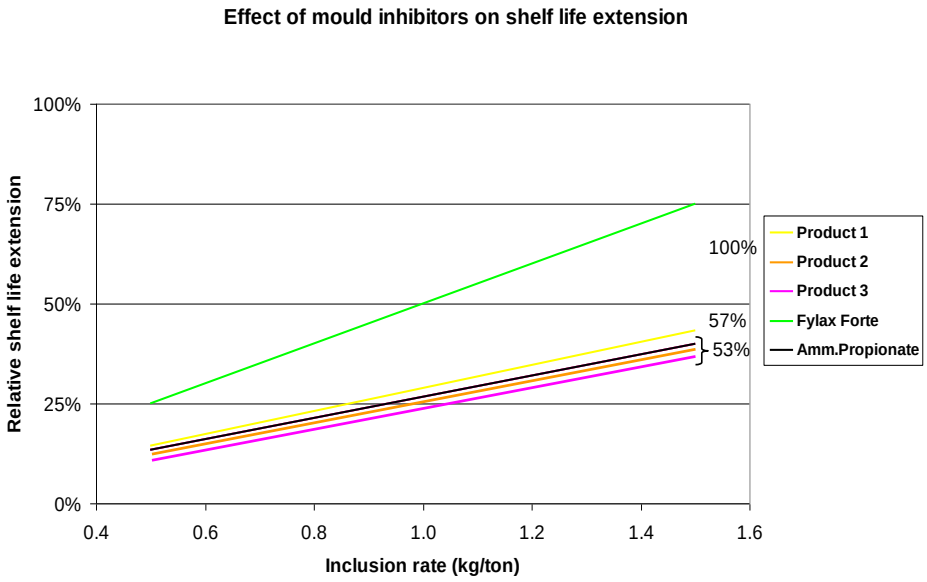
All three product types were tested in a mixed mould culture containing the species *Aspergillus*, *Penicillium*, *Fusarium* and *Zygomycetes*. Two different test methods were developed to mimic in a lab environment the commercial storage conditions of feed raw materials and compound feeds. These were a tube MIC test (Minimal Inhibitory Concentration (2;3) and an accelerated shelf life test, also called 'stress test'.

RESULTS AND DISCUSSION

The use of activated propionates improved the anti-mould working a great deal. The results of all three commercial products were similar to ammonium propionate, each showing a decent anti-mould function with stronger effects at higher dosages. The activation of ammonium propionate during the production process resulted in a 50% improvement of MIC values (Table 1). In line, an improvement in efficacy of 79 to 87% was achieved in the shelf life test compared to ammonium propionate and commercial products (Graph 1 and Table 2). This matches with a strong reduction in needed dosage to arrive at equal shelf life extension as normal propionates. These results indicate that a propionic acid based mould inhibitor with a production process activating the ammonium propionates is a strong combination.

Table 1. Effect of mould inhibitor products on MIC values

Products	Dosage					
	1.0%	0.75%	0.5%	0.375%	0.25%	**MIC**
Product 1	-	+	+	+	+	**1%**
Product 2	-	+	+	+	+	**1%**
Product 3	-	+	+	+	+	**1%**
Ammonium propionate	-	+	+	+	+	**1%**
Fylax Forte	-	-	-	+	+	**0.5%**



Graph 1. Effect of mould inhibitor products on shelf life extension

Table 2. Slope and relative anti-mould strength of various products

Product	Slope of the line	Relative anti-mould strength (As % of Ammonium propionate
Product 1	0.29	108%
Product 2	0.27	100%
Product 3	0.27	100%
Amm. Propionate	0.26	100%
Fylax Forte	0.50	187%

CONCLUSIONS

Mould inhibitor based on high level of activated ammonium propionates during production process of Fylax Forte product showed a strong shelf life extension compared to other propionate products. A lower dosage of Fylax Forte can be used to get the same mould inhibition effect. For mould control, this means a more cost effective strategy to maintain the nutritional quality and safety of raw materials and feeds.

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DEVELOPMENT OF EUROPEAN PETFOOD MARKET

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ABSTRACT

Development of the European petfood market in many aspects is discussed in the paper. Pets population, social factors and consumer expectations determine development of this specific market now and in years to come.
***Keywords**: petfood, market development, pets population, premium feed, extrusion*

INTRODUCTION

The European pet food market has continuously expanded during recent decades. This can be explained by a long lasting growth in pet ownership, by the decreasing use of table scraps and the general trend toward convenience foods. In the past years the situation in Western Europe has changed and the growth rate of the market has slowed down in many countries so that a mature market situation has developed [5]. In contrary very spectacular development has been observed in Central and Eastern Europe after economical and political changes happen in 1990, where Poland can be used as the best example for (see Fig. 1). In the European countries exist considerable differences: in the standard of living and there are obvious differences in the habits of keeping pets and the traditions of pet feeding. All of that affect the market. The European countries differ in population density of dogs and cats, the calorie coverage by commercial pet foods and the balance between commercial foods and the traditional ways to feed pets with table scraps.

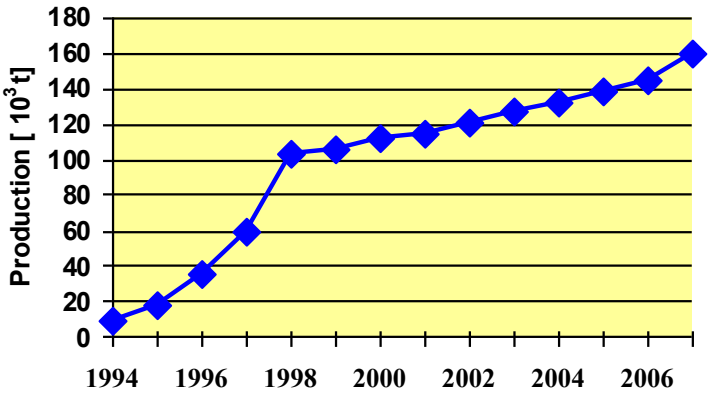

Fig. 1. Petfood production in Poland [3]

THE EUROPEAN PET POPULATION

The European Pet Food Industry Federation (FEDIAF) estimates the number of households with pets to 62 million in the Western European countries [4]. In my opinion talking about whole Europe we have to at least double this number. Cats population is bit higher than the population of dogs. FEDIAF estimates that 60 million cats and 56 million dogs are kept in the Western European countries. A population of 35 million pet birds, 9 million aquaria and 40 million other pets, mainly rodents and small mammals, but also reptiles, must be considered as another important segment of the market [4]. In the Figures 2 - 5 have been shown the European dogs and cats population, their location and feeding situation.

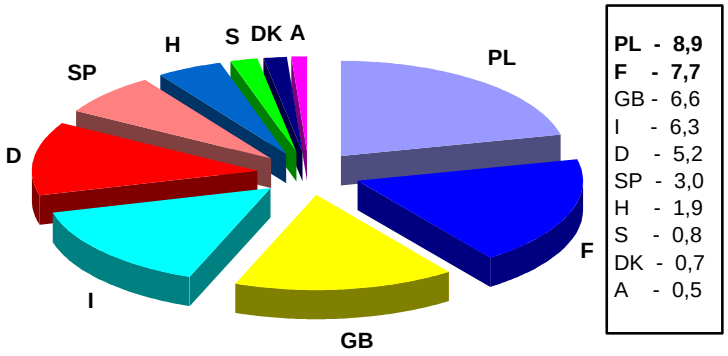

Fig. 2. Dog population in Europe (x10^6) [3]

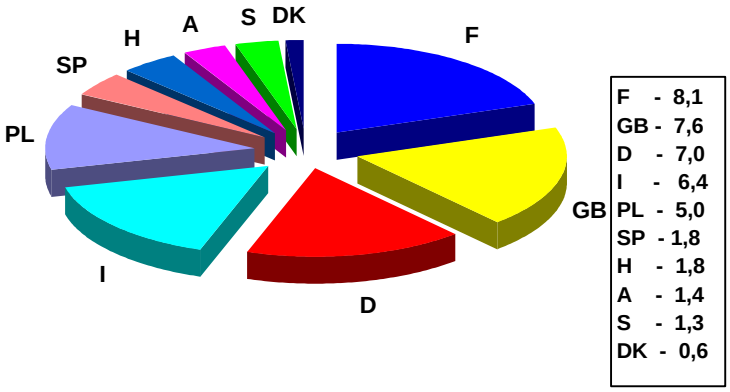

Fig. 3. Cat population in Europe (x10^6) [3]

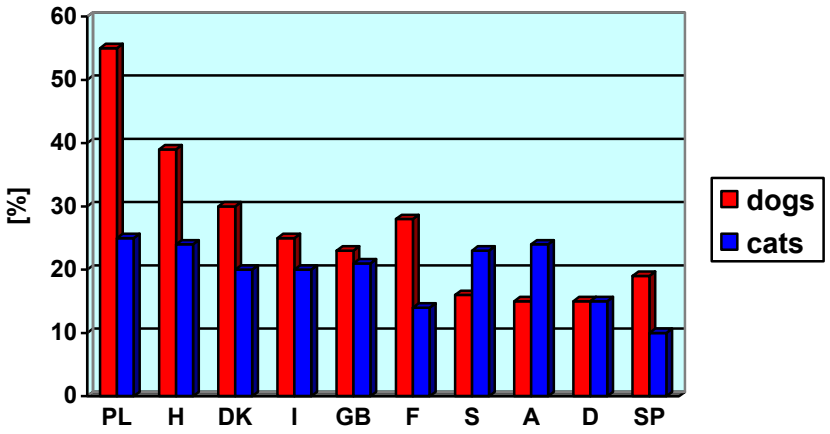

Fig. 4. The European families with pets [3]

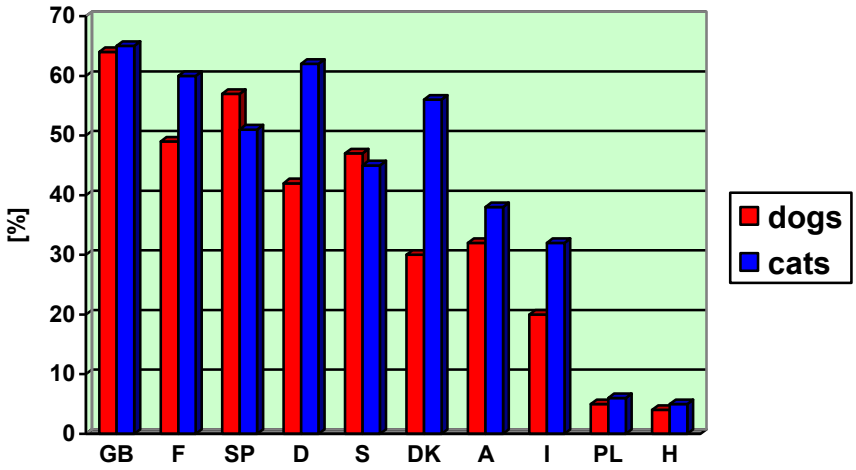

Fig. 5. Commercial petfood in the pets daily diet [3]

An average number of 1.1 dogs and 1.4 cats per family keeping pets are reported [4, 5]. The number of cats has increased and the trend to keep more than one cat per household is observed in many countries. Why? Because cats are easier for the owners on one hand and for another it's recommended to facilitate socialization by keeping more than one cat, especially in households where cats must stay alone over longer times during the day. Nowadays the public opinion become obvious that keeping dogs as pets is a very sensitive hygienic problem in urban environments. The stable or negative trend in the dog population is also observed with regard to dog breed and dog size preferences. In Europe the number of small sized dogs (<10 kg BW) was estimated to be 34%, the medium and large sized dogs make up 37 and 29% of the total population [5,6]. The southern countries have a certain preference for smaller dogs, while other - especially the northern - countries seem to prefer larger breeds.

SOCIAL FACTORS

Nowadays we can observe negative trends in population dynamics in Europe. In many countries higher numbers of households and lower numbers of persons per household is noted. Moreover, individualization becomes very important for many young people, who prefer to spend time out of home during the day. Statistical data are showing how its result in pets ownership. Most pets are kept by owners aged 35-49 years (39% of all pets) with a clear relation to the number of family members. Pets are often kept in families with at least one child (46% of all pets), lower frequencies are found in single households (22%) and with families without children (32%) [5]. In Germany according to the investigation overtaken by ZZF [6] couples without children and singles represent

on a household basis 60% of cat owners, 59% of dog owners, 58% of the pet bird owners and 48% of the aquarium owners. Singles seem to prefer cats as pets, dogs are only second choice. This fact is important, because in EU the sociologists estimate that the number of households, either singles or couples without children, will increase systematically in years to come. Changing lifestyles of pet owners have other effects on the development of pet markets. Humanization of pets is seen because they play a specific role as social partners. This development is known by petfood producers. Pets become so important for many owners that they are willing to spend more time and money with them, looking for extraordinary goods and food.

MARKET EXPECTATIONS

FEDIAF estimates the total number of petfood companies in Europe at 450 and the current volume of all pet food is estimated over 5 million tons with an estimated sales value of 8,5 billion € [4]. 21 000 of employees are directly and more than 30 000 indirectly involved in petfood production in Europe. The yearly purchase of agricultural by-products in the European Union is 2,75 million tons. The distribution and retail channels have changed significantly in the past years. Petfood is sold mainly in hypermarkets and small shops. However small shops have lost significant market share all over Europe due to the competitive price situation, start to specialize in higher priced products and with products that need more explanation for the consumers. Many of European market players are saying that nowadays the total volume of petfood has reached a highest figure. Production and consumption potential is different in the European Union. In the Western European countries customer demands are increasingly orientated toward premium products, treats, and in the segment of complete diets toward dry food in dogs and cats. New member states still are mainly busy with development of basic pet food production, which has big room enough to expand. However personal income is still much lower in those countries pets owners found great opportunity in application of industrial petfood and are willing to spend more and more money for convenience and handy food. New product trends is a top global factor. Of the 12 broad factors covered in the survey reported by Lummis [2], new product trends is number one overall, with two-thirds (66.7%) of respondents considering it very important to the development of the global petfood industry during the next five years. The most important components of this factor are:

- functional/condition-specific/novel ingredient foods (lifestage, weight loss, breed-specific, therapeutic);
- high-growth segments such as treats;
- hyperpremium products;
- and Human-grade ingredients.

More than three-quarters (75.8%) of petfood manufacturers cite this factor as very important, making it the top-ranked among other questions. For retailers, the factor tops out at 85%. Among all respondent roles, new product trends ranks first except for veterinarians and respondents holding university researcher/scientist positions [2]. Generally pet owners expect high quality standards in each price segment. Petfood

should be safety, nutritive and healthy. Nowadays many of them are prepared to pay higher prices for premium products. There are pet owners who don't pay great attention to healthy aspects but they expect that the pet enjoys eating a specific product or that they themselves are enjoying feeding the specific product to their pet. A third group of pet owners is mainly interested in getting access to reasonable products at economic prices. All will expect not only high nutritional value and adequacy, but also high palatability and digestibility of the food [5].
Petfood market development in whole European Union will stabilize in coming 5 years. Most probably new member states will rich consumption level of 20 – 25 % of a daily diet. A growing part of the consumers will demand premium quality and will be prepared to pay adequate prices. Quality, safety and nutritional adequacy will be an ongoing issue for this group. Functionality of pet food will result in new products, functional supplements for pets start to play an important role and products of natural origin. Most of dry and semi-moist petfood is extrusion-cooked and extrudates will be the most popular products for many years to come, due to great potential and effectiveness of this technique. Fully automated production lines in the factories assure optimum precision in the dosing of ingredients, eliminate all risk of human error and avoid any physical contact of the operators with the materials and food.
The industry goes a step further than the official veterinary controls. Manufacturers have their own control systems whereby samples are analyzed every hour on the production line.

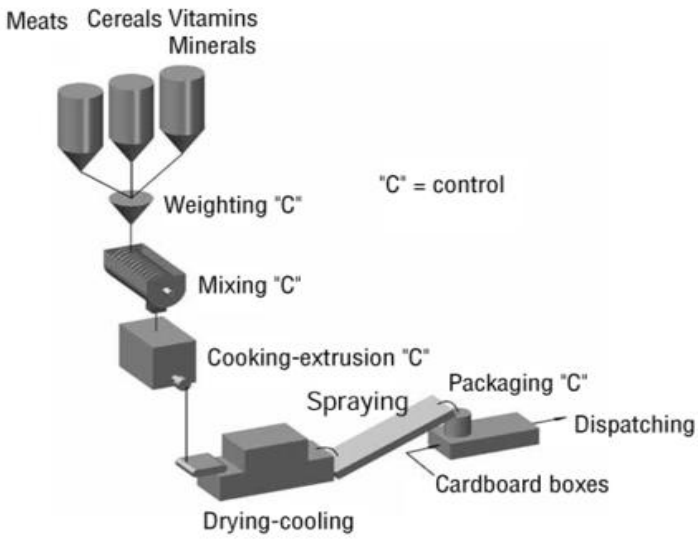

Fig. 5. Set-up of dry petfood production [4]

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ROLE AND SIGNIFICANCE OF ASSOCIATIVE EFFECT IN THE ASSESMENT OF THE NUTRITIVE VALUE OF FEEDS

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ABSTRACT

Sixteen feed mixture, formulated with seven single feeds, were used to study the influence of associative effects on rumen degradability of both dry matter (RDDM) and protein (RDP) of feed mixtures. The *in sacco* incubation technique was used for RDDM and RDP determination. It was found that there is very good correspondence between experimental and theoretical calculated values of RDP for concentrate feeds. It was found also that the presence of bulky feeds cause significantly discrepancy between these values. It was concluded that there exist significant associative effect of feed mixture with bulky feeds on rumen degradability. It is necessary to characterize the degree of influence of additivity factor for bulky feeds and in any conditions and the limits of its manifestation.
***Keywords**: in sacco, RDDM, RDP, associative effect, feeds, ruminants*

INTRODUCTION

Associative effect of feeds is a long time well - known. It was Kellner (1912) who first noted the nonlinearity between efficiency and fiber content and his evaluation of the more fibrous feeds in calculated starch equivalents was not complimentary. Already there are a lot of definitions (Brandt and Klopfenstein, 1986; Hart, 1986; Robinson, 2009), but we accept that, according to which this is the effect of one feed on the utilization of another given with it (Fuller, 2004). The classical view of associative effect is the change in digestibility of feed A attributable to the presence of feed B. It is generally assumed that the associative effect is interaction among feeds.
Associative effect occurs in all animal species and there are many evidence of their role on level of the metabolizable energy of mixed diets (Hong, 2002; Freer, 2007), feed intake and digestion in ruminants (Champion et al., 2004; Cortes et al., 2006; Niderkorn and Baumont, 2009), ruminal kinetics and particle passage in beef cattle (Bhatti et al., 2008), quality of the forage (Galyean and Goetsch, 1993; Adesogan, 2002). There are mathematical models for the innovative interpretation of the associative effect (Fancy and Acciaioli, 1997).
The use of mixtures of forages may positively (Van Soest, 1994) or negatively (Dixon and Stockdale, 1999) affect the various components of the feeding value.
Measuring interactions between feeds requires them to be tested both singly and in one or more generally binary combinations (Van Soest, 1994). Associative effects occur when digestion or intake of one feed is not independent of the other. They can be detected when the combination show a non-linear response. For these studies, *in vitro*

and *in vivo* experiments can be carried out alternatively or simultaneously (Zhao et al., 2005; Niderkorn and Baumont, 2009). Feed interaction termed associative effect reflects the level-of- intake problem. The addition of concentrate to forage increases efficiency partly through the depression in methane, reduced rumination, and lowered heat increment. Associative effects occur to varying degrees depending on the combination of feeds and depend on a variety of factors.

True associative effect of feeds is not easy to estimate. It could say the same for the quantitative definition of the associative effect. It can only be recommended that an associative effect be borne in mind as a possible source of error in ration formulation and the prediction of animal performance (Freer, 2007). Although associative effect occurs in all species, it is most important in ruminants given concentrates and roughage together. There is one more very important cause of associative effect in ruminants. In modern scientific concepts for assessing feed protein for ruminants there is a particular attention to the metabolic changes in the digestive tract as a whole or its individual sectors. Using the degradation of protein in the rumen (RDP), rating systems create precondition for use of two protein components in the duodenum: undegraded feed protein (UDP) and / or synthesized microbial protein (MP). The quantities of these components determine the amount of recovery of protein rations. Experimental results obtained by Voigt and Piatkovski (1983) and Voigt (1990) first pay attention to one crucial fact - offered by different systems RDP values are defined by individual feed. When used, however, mixed rations, i.e. combination of feeds, values of RDP called into question. In this connection research group from Rostock, Germany, makes a proposal (Voigt and Piatkovski, 1987), which does not use RDP, and reported the recovery of protein in rations values reaching duodenum. Sandev (1991), referring mainly to the developments cited in Rostock, offer a critical evaluation of new protein systems. The author suggested that the presence of associative effects on feed significantly alter their RDP. Published experimental results (Sandev et al., 1994; Petkova, 2001) show the correctness of the assumption: for incubation in the rumen of a combination of feed with the same physical form, for example only concentrated components, there is good correlation between actual and theoretical values, it is mean that associative effect is absent. The same does not apply to cases where the roughage components (e.g. silage, fodder beet) involved in combination of feeds for incubation.

The purpose of this study was to examine the influence of basic ration on *in sacco* rumen degradability of dry matter and protein of individual feeds and their combinations.

MATERIAL AND METHODS

Animals and nutrition. The survey was conducted by three non-lactating and non-pregnant cows with rumen fistula. Nutrition was conducted in duplicate and two rations A and B (table 1). The composition of the daily ration A consisted of meadow hay (30%, wheat straw (30) and concentrate mixture (40% o barley (23%), maize (23%), wheat (23%), expeller (27%) and mineral supplements (4,0%) and that of ration B - corn silage (71%), grass hay (21%) and concentrate mixture (8%) of corn (70%), sunflower meal (10%) and mineral supplements (20%).

Table 1. Experimental design

Items	**Ration A**	**Ration B**
Components of the rations	Wheat straw Meadow hay Concentrate mixture	Corn silage Meadow hay Concentrate mixture
Daily intake, DM, kg/cow	5,2	5,9
CP, kg DM/cow	1,9	2,05
NE, MJ/kg DM/cow	127,6	135,45
In sacco incubations		
Individual feeds	A_1, barley	-
	A_3, corn	A_2, corn
	A_5, wheat	-
	O_1, expeller	-
	B_1, meadow hay	B_2, meadow hay
	S_1, corn silage	S_2, corn silage
	-	E_2, fodder beet
Mixture feeds	A_1A_3	S_2A_2
2 feeds	A_1A_5	B_2S_2
	O_1A_1	B_2A_2
	O_1A_3	E_2B_2
	B_1A_1	-
	B_1A_3	-
	B_1O_1	-
	S_1A_3	-
3 feeds	$A_1A_3A_5$	$E_2B_2A_2$
	$O_1A_1B_1$	-
	$O_1A_3B_1$	-
	$B_1O_1A_1$	-
Incubation time, h	24	24

That composition of the rations provided a daily consumption of one animal to 5,2 and 5,9 kg DM for rations A and B respectively, and NE 135 and 128 MJ / kg DM respectively and protein 1,9 and 2,05 kg respectively. Energy value of the rations was calculated by OE after GfE (1998) and NE after DLG (1997). The chemical composition of all feeds was determined by Weende (AOAC, 1980)

In sacco incubation. Rumen degradability (of DM and protein) was determined by the *in sacco* procedure. Each ration was fed for 14 days, followed by incubations in the rumen of nylon bags. In general, we keep the methodological guidance of Oldham (1987). We made six incubations of individual feeds (barley, corn, wheat, expeller, and meadow hay and corn silage) and 11 variants of mixed samples (seven options with a mixture of 2 feeds and four options with a mixture of 3 feeds) for ration A. For ration B we made four incubations of individual feeds (corn, corn silage, and meadow hay and fodder beet) and five variants of mixed samples (four options with mixture of 2 feeds and one variant with a mixture of 3 feeds). Disappearance of the dry matter and protein from the nylon

bags were made after rumen incubation for 24 hours. The number (n) of bags from each variant was 12 (2 repeats of experiments x 2 days of incubation x 3 bags).
Theoretical values for the rumen degradability (RD) for mixed samples were calculated based on data for individual feeds involved in feed mixtures: weight presence, protein content and RD in fact.
Statistical methods: The obtained experimental data were analyzed by method of variance analysis and Tukey test (Stat.Soft, Inc. STATISTICA, version 6).

RESULTS AND DISCUSSION

Table 2 reflects the values obtained for the degradation of individual feed after 24 h of incubation in the rumen. These values are the background for calculating the theoretical values for mixed samples. Three individual feed components, corn, meadow hay and corn silage incubated in both basic rations for cows (A_3 and A_2, B_1 and B_2, and S_1 and S_2) indicate values of RDP, which are indicative of stimulating effect of ration B.

Table 2. RDDM and RDP of individual feeds for 24 hours

Incubations	**Ration A**		**Ration B**	
	RDDM	**RDP**	**RDDM**	**RDP**
A_1, barley	89,66	90,4	-	-
A_3, corn	77,58	50,9	-	-
A_5, wheat	90,52	93,8	-	-
O_1, expeller	75,52	95,4	-	-
B_1, meadow hay	36,56	43,5	-	-
S_1, corn silage	66,00	92,5	-	-
A_2, corn	-	-	84,92	75,1
B_2, meadow hay	-	-	47,26	52,8
S_2, corn silage	-	-	63,79	92,9
E_2, fodder beet	-	-	46,96	96,7

Ration B has a similar effect on the RDP and in the mixed samples of a pair of feeds (Table 3) variations in corn and corn silage (S_1 A_3 and S_2 $A_{2)}$ and grass hay and corn (B_1A_3 and B_2A_2). A comparison of the experimentally established and theoretically calculated values for the RDP (Table 5) indicates that a combination between the concentrate feeds in terms of both rations were good correspondence with each other. Even with some combinations of concentrate feed the correlation between these two values is too large ($A_1A_{3;}$ $A_1A_{5;}$ $O_1A_{1;}$ $O_1A_{3;}$ $B_2A_{2;}$ $A_1A_{3}A_{5;}$ $O_1A_1B_{1).}$ Combination of corn silage only with the concentrate and corn grain difference between the two values for degradability of protein – experimental identified and theoretical calculated - as demonstrated again and it significantly. In ration B, a combination between bulky juicy

Table 3. RDDM and RDP of double mixture of feeds for 24 hours

Incubations	Ration A		Ration B	
	RDDM	RDP	RDDM	RDP
A_1A_3	84,06	73,2	-	-
A_1A_5	91,18	91,6	-	-
O_1A_1	80,49	93,9	-	-
O_1A_3	79,30	83,2	-	-
B_1A_1	49,74	56,3	-	-
B_1A_3	45,27	44,1	-	-
S_1A_3	78,04	84,8	-	-
S_2A_2	-	-	76,80	87,8
B_2S_2	-	-	46,88	83,0
B_2A_2	-	-	65,71	64,1
E_2B_2	-	-	47,86	88,0

Table 4. RDDM and RDP of three mixture of feeds for 24 hours

Incubations	Ration A		Ration B	
	RDDM	RDP	RDDM	RDP
$A_1A_3A_5$	86,63	80,7	-	-
$O_1A_1B_1$	67,87	87,7	-	-
$O_1A_3B_1$	67,25	82,4	-	-
$B_1O_1A_1$	60,17	79,5	-	-
$E_2B_2A_2$	-	-	64,17	87,5

fodder and other is more practiced and clearly demonstrated difference between the observed and calculated values. In the implementation of systems for assessing protein in ruminants in practice should be used absolute rather than relative values. Too good correlation between experimental and theoretical values found in the concentrated feed probably creates confidence in the reliability of absolute values. There are few studies on combination between bulky feed, but they could be used to support received from us results, although their authors have not used them for that purpose. Obtained results confirm the postulate that the associative effect of feeds is essential for assessment of nutrition value especially for their joint use in the diet, mainly involving the succulent feeds. This leads to the relative nature of tabular data on degradability of protein which was raised from Stern and Satter (1984). This is consistent with the conclusion of Susmel et al (1989) that the most important factor determining the degradation of protein in the rumen is the composition of the basic ration. In conclusion it must be underlined that obtained in this study results show significant associative effect on protein degradation in the rumen. The extent of this impact can be so high that it to eliminates the effect of protein degradation in different feed. Application of evaluation systems of the protein, as we saw, it was complicated, irrespective of the correctness of the theoretical formulation.

Table 5. Experimental and theoretical values of RDP

Incubations	Ration A		Ration B	
	Experimental	Theoretical	Experimental	Theoretical
A_1A_3	73,2	71,4	-	-
A_1A_5	91,6	91,9	-	-
O_1A_1	93,9	92,8	-	-
O_1A_3	83,2	85,9	-	-
B_1A_1	56,3	60,9	-	-
B_1A_3	44,1	46,1	-	-
S_1A_3	**84,8**	**68,0**	-	-
S_2A_2	-	-	**87,8**	**82,1**
B_2S_2	-	-	**83,0**	**70,3**
B_2A_2	-	-	64,1	65,0
E_2B_2	-	-	**88,0**	**62,0**
$A_1A_3A_5$	80,7	80,2	-	-
$O_1A_1B_1$	87,7	87,6	-	-
$O_1A_3B_1$	82,4	80,9	-	-
$B_1O_1A_1$	79,5	82,4	-	-
$E_2B_2A_2$	-	-	**87,5**	**71,5**

CONCLUSIONS

Degradability of protein in the rumen *in sacco* in mixture of feed with bulky feeds - fodder beet, silage - change significantly compared with theoretically calculated value of the data for degradation of protein in individual feeds.
Experimental obtained values for RDP for combination of concentrates did not differ from the estimated value of the data for single feeds.
It is necessary to characterize the degree of influence of additivity factor for bulky feeds and in any conditions and the limits of its manifestation.

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REGIONAL DIFFERENCES ON THE WORLDWIDE MYCOTOXIN OCCURRENCE

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ABSTRACT

From the cereal trade companies up until feedmillers and animal producers, all those involved in the long chain of food production are at risk of being impacted by the effects of some invisible, odorless and tasteless substances called mycotoxins. Mycotoxin contamination often begins in the field and continues throughout harvest, transportation and storage, depending on the activity and colonization levels of fungi which are in turn determined by the prevailing environmental conditions and the nutritional components of the food matrix.
What is of interest for all participants in the "farm to fork" chain is the prevalence and concentration of these substances in feed intended for animal consumption. The objective of this work is to show the size and importance of the mycotoxin problem to all players of this industry. A pioneer activity has been conducted for the last years by Biomin GmbH in order to measure the impact of the diverse mycotoxins worldwide. The results show the contamination of feedstuffs and feed not only qualitatively, but also quantitatively. From January 2010 until June 2010, a total of 1211 worldwide samples were analysed for the most important mycotoxins in terms of agriculture and animal production – aflatoxins (Afla), zearalenone (ZON), deoxynivalenol (DON), fumonisins (FUM) and ochratoxin A (OTA).
***Keywords**: mycotoxin, occurrence, aflatoxins, zearalenone, deoxynivalenol, fumonisins and ochratoxin A.*

INTRODUCTION

The term mycotoxin derives from the Greek word "mycos" and the Latin "toxicum" and refers to the secondary highly toxic substances that are produced by fungi mainly belonging to *Fusarium*, *Aspergillus* and *Penicillium* species under a wide variety of environmental conditions in the field and during harvest and storage. Especially in critical weather conditions, namely intensive rain or drought, these fungi produce mycotoxins with the group of trichothecenes (e.g., deoxynivalenol, T-2 toxin), zearalenone, ochratoxins, aflatoxins and fumonisins being the most prevalent.
Worldwide surveys on the occurrence of mycotoxins are scarce. Binder *et al.* (2007), published the results of a comprehensive 2-year survey program which evaluated the incidence of mycotoxins in different feed and feed raw materials sourced from Europe and Asia. More recently, Griessler *et al.* (2010) shared the results of a 2.5 year study on the occurrence of mycotoxins in commodities and feeds from Southern Europe. Other existing reports limit their scope to a single commodity and/or country or to the analysis of fungal presence. For example, Bankole *et al.* (2004) studied the occurrence of

aflatoxins and fumonisins in preharvest maize in south-western Nigeria. Berghofer et al. (2003) examined the microbiology of wheat and flour milling in Australia. As for Asia, Charoenpornsook and Kavisarasai (2006) reported the occurrence of mycotoxins in animal feedstuffs of Thailand. Likewise, Gonzalez et al. (2008) determined the mycobiota and mycotoxins in pig feed in central Argentina.
In total, 326 samples were analyzed from Central Europe, Southern Europe and Northern Europe in collaboration with Romer Labs Diagnostic GmbH (Austria). 482 samples were analyzed from North Asia, South East Asia, South Asia and Oceania in collaboration with Romer Labs Singapore Pte Ltd.. 403 samples were analyzed from North and South America in collaboration with ROMER Labs Inc (USA) and SAMITEC (Brazil). Samples tested were diverse, ranging from cereals such as corn, wheat and barley to other fodder such as silage and finished feed.

MATERIAL AND METHODS

All samples were analysed by accredited labs specialized in food and feed analysis. According to the type feed samples were analysed either by high performance liquid chromatography (HPLC) or Enzyme-Linked Immunosorbent Assay (ELISA). Commodities and grains were preferably analysed by ELISA whereas compound feed and feed premixes were analysed by HPLC.
For the purpose of data analysis, non-detect levels are based on the quantification limits (LOQ) of the test method for each toxin: deoxynivalenol and acetyldeoxynivalenol 50 µg/kg (HPLC) or 250 µg/kg (ELISA), respectively; T-2 toxin and HT-2 toxin 30 µg/kg (HPLC) or 75 µg/kg (ELISA), respectively; zearalenone 10 µg/kg (HPLC) or 40 µg/kg (ELISA), respectively; total fumonisins 25 µg/kg (HPLC) or 250 µg/kg (ELISA), respectively; total aflatoxins 0.3 µg/kg (HPLC) or 1 µg/kg (ELISA) and ochratoxin A 0.2 µg/kg (HPLC) or 2 µg/kg (ELISA), respectively.

RESULTS AND DISCUSSION

From all European surveyed samples 18%, 15%, 55%, 31% and 26% tested positive for AfB1, ZON, DON, FUM and OTA, respectively (Table 1). In regards to Northern Europe, DON is the most abundant mycotoxin representing 68% of the analysed samples. 5% and 14% tested positive for ZON and OTA, respectively. In Central Europe it was verified that DON is the mycotoxin of higher concern, representing 54% of the analysed samples. Afla, ZON, FUM and OTA were found in 12%, 16%, 29% and 29% of analysed samples. DON was the most abundant mycotoxin in the Southern Europe, present in 47% of the analyzed samples. Afla, ZON, FUM and OTA were present in 38%, 26%, 38% and 29% of the analyzed samples, respectively.

Table 1. Occurrence of mycotoxins in the European region

Europe Total	**Afla**	**ZON**	**DON**	**FUM**	**OTA**
number samples tested	28	241	313	42	42
% Positive	18%	15%	55%	31%	26%
average of positive (ppb)	7	109	962	2894	34
maximum (ppb)	18	655	26121	7260	331
Northern Europe	**Afla**	**ZON**	**DON**	**FUM**	**OTA**
number samples tested	3	42	40	0	7
% Positive	0%	5%	68%	-	14%
average of positive (ppb)	-	50	704	-	6
maximum (ppb)	0	65	3474	0	6
Central Europe	**Afla**	**ZON**	**DON**	**FUM**	**OTA**
number samples tested	17	176	239	34	28
% Positive	12%	16%	54%	29%	29%
average of positive (ppb)	1	120	1049	2449	45
maximum (ppb)	1	655	26121	6770	331
Southern Europe	**Afla**	**ZON**	**DON**	**FUM**	**OTA**
number samples tested	8	23	34	8	7
% Positive	38%	26%	47%	38%	29%
average of positive (ppb)	11	80	687	4379	1
maximum (ppb)	18	112	2160	7260	1

From all Asian surveyed samples 37%, 43%, 45%, 42% and 31% tested positive for AfB1, ZON, DON, FUM and OTA, respectively (Table 2). Afla was the most abundant mycotoxin in the South-Eastern area of Asia, present in 67% of analyzed samples. ZON, DON, FUM and OTA were present in 60%, 42%, 62% and 35% of the analyzed samples, respectively. Afla and OTA are a great concern in the Southern Asian region, as 86% and 73% of the samples tested positive for these mycotoxins. ZON, DON, and FUM were found in 43%, 39% and 55% of analyzed samples. In regards to Northern Asia region, DON is the mycotoxin of higher incidence representing 61% of positive samples. In Oceania, 13%, 22%, 30%, 9% and 15% of the samples were contaminated with Afla, ZON, DON, FUM and OTA, respectively.

Table 2. Occurrence of mycotoxins in the Asian region

Asia Total	**Afla**	**ZON**	**DON**	**FUM**	**OTA**
number samples tested	482	454	454	478	453
% Positive	37%	43%	45%	42%	31%
average of positive (ppb)	52	282	610	1486	9
maximum (ppb)	726	16712	6965	13862	174
North Asia	**Afla**	**ZON**	**DON**	**FUM**	**OTA**
number samples tested	176	148	148	176	149
% Positive	7%	37%	61%	37%	22%
average of positive (ppb)	9	730	953	2728	6
maximum (ppb)	51	16712	6965	13862	52,8
South-East Asia	**Afla**	**ZON**	**DON**	**FUM**	**OTA**
number samples tested	164	164	164	164	164
% Positive	67%	60%	42%	62%	35%
average of positive (ppb)	39	90	402	953	4
maximum (ppb)	726	2601	4805	6196	29,8
South Asia	**Afla**	**ZON**	**DON**	**FUM**	**OTA**
number samples tested	49	49	49	49	49
% Positive	86%	43%	39%	55%	73%
average of positive (ppb)	110	52	156	541	17
maximum (ppb)	593	297	556	1476	174
Oceania	**Afla**	**ZON**	**DON**	**FUM**	**OTA**
number samples tested	93	93	93	89	91
% Positive	13%	22%	30%	9%	15%
average of positive (ppb)	19	237	328	1306	17
maximum (ppb)	51	926	1360	3229	111

Finally, from all American surveyed samples Afla, ZON, DON, FUM and OTA were present in 29%, 57%, 64%, 71% and 9%, respectively (Table 1). In North America, DON is the mycotoxin of higher concern representing 85% of positive samples. 6%, 52%, 48% and 9% tested positive for Afla, ZON, FUM and OTA, respectively. Regarding South America, the most abundant mycotoxin found is FUM representing 89% of the analysed samples. 34%, 67%, 13% and 10% of the samples were contaminated with Afla, ZON, DON and OTA, respectively.

Table 3. Occurrence of mycotoxins in the American region

America Total	**Afla**	**ZON**	**DON**	**FUM**	**OTA**
number samples tested	252	272	253	374	64
% Positive	29%	57%	64%	71%	9%
average of positive (ppb)	3	249	1222	3174	4
maximum (ppb)	23	5930	10100	53700	13,9
North America	**Afla**	**ZON**	**DON**	**FUM**	**OTA**
number samples tested	49	178	178	166	44
% Positive	6%	52%	85%	48%	9%
average of positive (ppb)	2	213	1271	1352	5
maximum (ppb)	2	2593	10100	22260	13,9
South America	**Afla**	**ZON**	**DON**	**FUM**	**OTA**
number samples tested	203	94	75	208	20
% Positive	34%	67%	13%	89%	10%
average of positive (ppb)	3	300	480	3952	1
maximum (ppb)	23	5930	2520	53700	1

CONCLUSIONS

With basis on these irrefutable results and on the known negative impacts mycotoxins cause in animals, these substances should be considered with precaution by professionals on the agricultural, animal production and feed sectors. Analyzing commodities and feed is crucial to monitor mycotoxins' presence, as these substances are invisible, odourless and tasteless. The use of mycotoxin deactivating products to counteract the hazardous impacts of these toxins on animal health and performance should be considered as a preventive measure.

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CHANGES OF FATTY ACIDS COMPOSITION DURING STORAGE IN CAMELINA SAMPLES OF SEEDS, OIL AND MEAL

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ABSTRACT

The purpose of this study was to determine the fatty acids composition of various *Camelina sativa* samples (seeds, oil, meal) at two days interval for the seeds, oil, and 10 days for the meal, in order to determine the possible changes of structure. We conducted 6 assays for each working samples of seeds and oil, for 2 weeks, while the 3 samples of Camelina meal were analysed, also with 6 assays, at 10 days intervals, for one month. The detailed FA composition of the Camelina derivates was determined by gas chromatography (GC) which presumes the previous transformation of them into methyl esters, followed by their separation on the capillary chromatograph column, with high polarity stationary phase. The determinations revealed changes over time in FA composition, particularly of the polyunsaturated FA which undergo oxidation and hydrogenation (γ linolenic acid from 27.9% to 26.16% and α linolenic acid from 12.21% to 10.11%, while the palmitic acid, from 7.19% to 8.64% and the stearic acid from 2.00% to 2.42%) irrespective of the physical form of Camelina. The change in fatty acid composition and shortcomings of storage should be avoided by reducing the storage period as much as possible in order to protect the lipid profile in the obtained products (meat, milk, eggs).
Keywords: *seeds, oil, Camelina meal, fatty acids, storage*

INTRODUCTION

The scientific research of the recent years generally focus on the use of feed ingredients which to manipulate the fatty acids profile in animal products. Starting from premises, several oleaginous ingredients were analysed chemically with the view to identify those ingredients which have an adequate level of n-3 family polyunsaturated fatty acids. Thus, animal feeding can be modified with the purpose of improving the feeding and organoleptic quality of the obtained products, aiming particularly the modulation of lipid, and fatty aids, quantity and structure. These studies were aimed to improve the content of FA and their long chain derivatives (EPA, DHE) in animal products (Woods, 2009). In their study, Mach et al., 2006, have shown that muscle n–3 PUFA increases linearly with the level of n-6/n-3 ratio, which can be improved by decreasing the intake of dietary n-6 FA. Furthermore, Riediger et al., 2009, Mahecha et al, 2009, acknowledge the particularly important role of the dietary FA structure and amount in maintaining the healthy status.

The knowledge on the use of Camelina in animal feeding is scarce, even this plant was known in the Bronze era when it was cultivated for the first time in Europe. The Camelina oil was used as feed ingredient, for medicines and as lamp oil. This feed source has a high feeding potential being characterized by a high content of n-3 fatty acids, of linolenic FA particularly (Dubois, 2007, Habeanu et al, 2009). Further arguments in support of using Camelina as forage plant are its resistance to cold weather, to scarce rainfall, its good adaptation to the temperate climate. Camelina is about to become a widely cultivated species, of economic importance.
On this basis, we undertook to determine at specific time spans, by gas chromatography, the fatty acids composition of Camelina in various forms (seeds, cold pressing oil and meal).

MATERIAL AND METHODS

The chemical analyses were conducted on Camelina seeds, meal and oil, at various time intervals. Thus, we conducted determinations on Camelina seeds and oil for two weeks, at 2-days interval, and at 10-days interval, for one month, for the Camelina meal. We collected 6 samples from the Camelina seeds and oil and 3 samples of Camelina meal. Six subsamples were thereafter collected from each sample.
FA composition was determined by gas chromatography. After lipid extraction from the samples, the FA were transformed tomethyl esters by transmethylation, and the components were separated on the capillary chromatograph column. The fatty acids were identified by using internal standards of FAME mixture and quantified as percent per 100g of fat. We used SUPELCO 37 Component FAME Mix; 10 mg/mL as standard solution of methylated fatty acids; we also used Soybean Oil and Sunflower Oil; SUPELCO, as reference material. We used a Perkin Elmer-Clarus 500 gas chromatograph fitted with a system of injection into the capillary column (splitting ratio about 1:100), with programmed chromatographic column oven heating; the system was fitted with flame ionization detector (FID) and column of high polarity stationary capillary separation (SGE forte GC Capillary Column BPX70, 60m L; 0.25mm inner diameter, 0.25um.gros.film). We used hydrogen as carrier gas and the air oxygen as burning gas.
The methylated fatty acids from the sample were separated according to chain length, to the level of unsaturation and to the geometry of the double bonds.
A control sample (n-hexane) and a reference sample (CRM) were analysed in parallel with the analysed sample (or batch of samples).

Statistical analysis

The results were expressed in average values and mean standard deviation (SEM). The values for fatty acids are expressed as percent of the total FA. The data were submitted to variance analysis by SPSS (General linear model – repeated measurements) at a significance level of 10%, 5%, 1% and 0.01%. If the model indicated significant differences between the samples, we used the „t" test to determine the moment when the differences in composition appeared.

RESULTS AND DISCUSSIONS

Table 1 shows the FA composition of the Camelina seeds.

Table 1. Centesimal fatty acids composition of the Camelina seeds at various time intervals

Fatty acids	Camelina seeds							P	SEM
	T_1	T_2	T_3	T_4	T_5	T_6	General mean		
	N = 6	N = 6	N = 6	N = 6	N = 6	N = 6			
C14:0	0.07	0.09	0.08	0.09	0.08	0.10	0.09	*0.29*	*0.02*
C16:0	6.40	6.43	6.45	6.58	6.71	6.75	6.43	*0.11*	*0.39*
C18:0	1.91	1.98	1.96	2.00	2.02	2.05	2.02	*<0.0001*	*0.08*
C18:1n-9	15.05	15.03	14.94	14.92	14.89	14.84	14.92	*<0.001*	*0.17*
C18:2n-6	18.19	18.18	18.12	18.11	18.01	17.95	18.01	*0.0008*	*0.02*
C18:3n-6 γ Linolenic	39.28	38.81	38.42	38.34	38.37	37.86	38.37	*<0.0001*	*0.08*
CLA	1.65	1.52	1.60	1.59	1.60	1.49	1.52	*0.73*	*0.28*
C18:3n-3 α Linolenic	12.73	12.73	12.68	12.41	12.14	12.09	12.73	*<0.0001*	*0.06*
C22:1n-9	1.63	1.72	1.70	1.66	1.63	1.73	1.72	*0.0003*	*0.01*
C20:4n-6	1.24	1.18	1.23	1.22	1.23	1.20	1.18	*0.055*	*0.00*

* T = time of determination
** P<0.05 – significant differences; P<0.0001 very significant differences, P>0.05 to 0.10 = trend to influence.

We can thus observe the existence of a high content of γ linolenic and α linolenic (~39%, and 12.73%) fatty acids, which makes Camelina an ingredient with valuable nutritional characteristics, similar to the linseed, which has over 50% linolenic FA. Another important FA, the linoleic FA had an average content of ~18%, with C18:2(n-6)/C18:3(n-3) ratio of 1.41, which is beneficial to human health (Wiseman, 2006). The data also suggest a depreciation in time, particularly of the polyunsaturated fatty acids, due to peroxidation and transformation into other FA by hydrogenation, especially into stearic acid (neutral in terms of influence on human health). As the storage time increases, the differences of FA concentration became very significant (P<0.0001) as it was the situation with the γ and α linolenic, linoleic, oleic, stearic and erucic FA. A trend of change (P = 0.055) has also been noticed for the arachidonic acid. CLA, another FA with proven anti-carcinogenic, anti-adipogenic, anti-inflammatory and anti-diabetic properties, and with capacity to alleviate atherosclerosis recently proven (Pariza et al., 2001; Wahle et al., 2004; Tricon and Yagoob, 2006), was identified at an average level of 1.52%.

The Camelina oil, obtained by the ecological cold pressing, is another feed ingredient. Table 2 shows its centesimal FA composition.

Table 2. Centesimal fatty acids composition of the Camelina oil at various time intervals

Fatty acids	Camelina oil							P	SEM
	T_1	T_2	T_3	T_4	T_5	T_6	General mean		
	N = 6	N = 6	N = 6	N = 6	N = 6	N = 6			
C14:0	0,08	0,09	0,10	0,09	0,10	0,10	0,09	*0.5670*	*0.00*
C16:0	6,58	6,30	6,37	6,51	6,71	7,35	6,64	*0.4086*	*0.08*
C18:0	2,43	2,46	2,56	2,54	2,59	2,61	2,53	*<0.0001*	*0.02*
C18:1n-9	14,34	14,28	14,53	14,53	14,47	14,43	14,43	*0.0213*	*0.10*
C18:2n-6	20,15	20,01	19,50	19,97	19,91	19,56	19,85	*0.0016*	*0.08*
C18:3n-6 γ Linolenic	36,90	36,62	36,26	36,11	35,37	35,07	36,05	*<0.0001*	*0.12*
CLA	1,49	1,31	1,41	1,38	1,35	1,39	1,39	*0.0412*	*0.02*
C18:3n-3 α Linolenic	13,63	13,59	13,11	12,98	12,93	12,72	13,16	*0.0053*	*0.08*
C22:1n-9	1,55	1,65	1,63	1,68	1,66	1,73	1,65	*0.2537*	*0.03*
C20:4n-6	1,19	1,05	1,33	1,18	1,19	1,45	1,23	*0.0008*	*0.06*

* T = time of determination
** P<0.05 – significant differences; P<0.0001 very significant differences, P>0.05 to 0.10 = trend to influence.

The obtained values are close to the values determined for the Camelina seeds. As with the seeds, FA stability is affected by the time factor, particularly in the case of the polyunsaturated fatty acids (P<0.0001) and the saturated stearic acid, accountable by the possible transformation on the polyunsaturated fatty acids in this FA. The analysed Camelina oil had an average content of 13.16% α linolenic FA, 36.05% γ linolenic FA, 1.39% CLA, 19.89% linoleic FA. The high PUFA concentration of this ingredient and its high energy value recommend it for animal feeding, irrespective of the species; it can also be used to balance the energy content of the compound feeds.

Table 3. shows the fatty acids composition of the Camelina meal.

Concerning the Camelina meal, the transformation of the polyunsaturated fatty acids into monounsaturated fatty acids or saturated fatty acids and the significant differences that were noticed, can be attributed mainly to the determinations that were performed over a longer period of time.

Table 3. Centesimal fatty acids composition of the Camelina meal at various time intervals

Fatty acids	Camelina meal				P	SEM
	T_1	T_2	T_3	General mean		
	N = 6	N = 6	N = 6			
C14:0	0,11	0,10	0,13	0,11	*0.0263*	*0.00*
C16:0	7,19	7,39	8,64	7,74	*<0.0001*	*0.15*
C18:0	2,00	2,07	2,42	2,16	*<0.0001*	*0.04*
C18:1n-9	19,48	18,76	18,04	18,76	*<0.0001*	*0.59*
C18:2n-6	26,14	23,81	23,59	24,51	*<0.0001*	*0.28*
C18:3n-6 γ Linolenic	27,90	27,17	26,17	27,08	*<0.0001*	*0.17*
CLA	1,53	1,33	1,12	1,33	*0.0001*	*0.04*
C18:3n-3 α Linolenic	12,21	11,92	10,11	11,41	*<0.0001*	*0.22*
C22:1n-9	1,26	1,24	1,16	1,22	*<0.0001*	*0.01*
C20:4n-6	0,73	0,87	0,61	0,74	*0.0044*	*0.03*

* T = time of determination

** P<0.05 – significant differences; P<0.0001 very significant differences, P>0.05 to 0.10 = trend to influence.

CONCLUSIONS

Camelina is a valuable oleaginous feed ingredient irrespective of its physical form. Its value is given by the high content of polyunsaturated fatty acids, particularly α and γ linolenic acid. The C18:2(n-6)/C18:3(n-3) ratio close to 1.00 recommends it as a feed ingredient, with a beneficial impact on the quality of animal products and on the human health, implicitly.

The longer the storage time, the poorer FA quality, particularly that of the polyunsaturated fatty acids which are affected by peroxidation and hydrogenation. An optimal shelf life has to be determined and more analyses must be performed on Camelina samples.

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EFFECTS OF FEEDING COWS WITH SUGARBEET PULP SILAGE AND COMPOUND FEED WITH UREA SUPPLEMENTATION ON MILK PRODUCTION AND QUALITY

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ABSTRACT

Afeeding trial with Lithuanian Black-and-White cows of 5000 kg 4.3 % fat and 3.3 % protein milk productivity in the previous lactation was carried out at the Institute of Animal Science of Lithuanian Veterinary Academy. Two analogous groups of 6 cows each were used in the trial. The cows were offered diets containing 31.4 – 31.5 % sugarbeet pulp silage on a dry matter (DM) basis. Soybean and sunflower oil meals and rapeseed cake were used as a protein source in the compound feed given to the control group of cows. Correspondingly, in the compound feed for the experimental cows soybean and sunflower oil meals were replaced with rapeseed cake supplemented with urea (0.8 % by weight). The study indicated that dried sugarbeet pulp silage made in a trench silo sealed with plastic contained 23.3 % dry matter with 11.56 MJ metabolizable energy and 118 g crude protein per kg. Sugarbeet pulp silage that accounted for 31.5 % of the diet on a dry matter basis and compound feeds with different protein resources including 0.8 % urea supplementation had no significant influence on the digestion processes in the rumen of cows. Different diets also had not affected milk yield and its quality. The milk had its usual chemical composition and good technological properties suitable for butter and cheese production.The use of rapeseed cake with urea supplementation in place of soybean and sunflower oil meals resulted in reduced price of the compound feed. Consequently, sugarbeet pulp silage based diet was 23.2 % cheaper and the expenses for the production of kilogram 4 % fat – corrected milk were 21.8 % lower.

Keywords: *compound feed, urea, lactating cows, rumen, milk production, milk quality*

INTRODUCTION

Food industry development results in increasing amounts of by-products of a certain feeding value. Rational use of these products might increase feed resources, lower the utilization costs and, consequently, reduce environment pollution.

Sugarbeet factories after processing 1000 kg sugarbeet produce about 540 kg of sugarbeet pulp with approximately 13 % dry matter (DM) content (Jeroch at al. 1999). For more effective feeding of animals, the DM content is increased to about 22 % by squeezing the pulp.

Sugarbeet pulp remains fresh only for several days, therefore, its ensilage is suggested (Beckhoff 1980). Pulp ensilage in trench silos or clamps with plastic sealing helps to

preserve the nutrients and their quality for a longer time and is considered a beneficial technique not requiring larger capital investment.
Ensilage of squeezed sugarbeet pulp containing 20 – 25 % DM differs from that of other feeds due to high pectin and low sugar contents in it. Pectin is easily degradable when affected by enzymes and the sugar content is sufficient for the inducement of active fermentation of thermo resistant lactic acid bacteria (Kubadinow et al. 1984; Rombouts et al.1984).
It is best to make silage from warm (about 50 ^{o}C) sugarbeet pulp because the temperature affects the fermentation of the pulp structural elements what is beneficial to fibre availability (Braunsteiner et al. 1983; Courtin and Spoelstram 1989).
Due to high content of pectin and peculiarities of its degradation in the digestive tract of cattle, sugarbeet pulp is valuable as an energy source. Breakdown of pectin in the rumen is slower compared with sugar or starch and, therefore, the pH value of the rumen contents remains more constant and favourable for the activities of fibre degrading micro flora (Van Soest et al. 1991).
Inclusion of sugarbeet pulp silage in the diets of cows allows to reduce the level of compound feeds (Tarvydas et al. 2004). Feeding of cows with high levels of concentrates results in lower fat content of milk (Bhattacharya and Lubbadan 1971), while partial replacement of concentrates with sugarbeet pulp silage allows to avoid this reduction. Sugarbeet pulp silage in the diets of lactating cows could constitute up to 30 % of the allowance on DM basis (Hemingway et al. 1986; Jeroch et al. 1999).
The protein content of sugarbeet pulp is low and amounts to on the average 117.8 – 124.9 g/kg DM (Bliznikas et al. 2003; Tarvydas et al. 2004). Thus, diets containing higher amounts of sugarbeet pulp silage should be supplemented with protein rich compound feeds. Consequently, more expensive protein materials – oil meals, cakes, etc. – should be used in the production of compound feeds.
Alongside with natural plants, nitrogenous non-protein materials such as urea may be used to increase the protein content of compound feeds for cattle. The nitrogen from these materials is used for the synthesis of microbial protein in the rumen, digestion of which provides the animal with valuable amino acids. Urea or other nitrogenous non-protein matter may constitute as equivalent by nitrogen up to 3 % of crude protein content in the compound feeds for cows (Weinreich et al. 1992).
Substantial feeding of cows with sugarbeet pulp silage which is characterized by peculiar physicochemical traits may influence not only digestion processes or nutrient availability but also animal performance and milk quality. High quality silages have no negative influence on milk composition and quality provided the permissible content of butyric acid in silages is not exceeded (Weisbach and Köller 1989).
This study was designed to investigate the effects of sugarbeet pulp silage and urea containing compound feed on the digestion processes in the rumen of cows, milk production and quality.

MATERIAL AND METHODS

Diets. Sugarbeet pulp was delivered from joint-stock company Danisco Sugar Panevėžys. The pulp was ensiled at temperatures of approx. 50 ^{o}C in the trench silo. The trench was sealed with RANI plastic (Rani Plast, Finland) of 0.15 mm thickness. After

the loading and pressing with a tractor had been completed, the plastic was covered with a 15-20 cm thick layer of straw. The trench silo was filled on the same day. The samples of pulp silage were first taken for analysis 30-35 days after the ensilage and subsequently analyzed once a month. Sugarbeet pulp silage contained on the average 23.3% DM, and there were on the average 118.2 g crude protein (CP) and 11.56 MJ metabolizable energy (ME) per kg DM.

Two formulas of compound feed were used. Barley meal was the basic component in both feeds, the respective amounts being 68.0 and 73.0 %. Protein sources were, respectively, soybean (10.0 %) and sunflower (10.0 %) oil meals and rapeseed cake (8.0 %) for compound feed No.1 and only rapeseed cake (23.0 %) for compound feed No. 2. This feed also contained 0.8 % urea. There were 12.51 and 12.24 MJ ME and 214.9 and 231.0 g CP per kg DM in each of the two compound feeds, respectively.

Other ingredients used for cow feeding were perennial grass hay and grass silage containing, respectively, 8.24 and 9.03 MJ ME and 79.2 and 127.7 g CP per kg DM.

Animals. The feeding trial was carried out with Lithuanian Black-and –White cows. Two groups of cows analogous by age, milk production in the previous lactation, calving time and milk production at the time of group formation of 6 newly calved cows each were used in the trial. The average productivity of the cows in the previous lactation was approx. 5000 kg 4.30 % fat and 3.30 % protein content milk.

The trial consisted of two – pre-experimental (20 days) and experimental (72 days) – periods. During the experimental period both groups of cows received the same sugarbeet pulp silage based diets. The control group of cows was fed compound feed No.1 and the experimental group received compound feed No.2 containing urea in place of part protein feed.

The animals in both groups were healthy and had the same housing conditions. The cows were tethered, automatically watered and milked twice daily.

Measurements and laboratory analyses. During the experiment, feed intakes were recorded by weighing feeds and feed remains per animal each week. The chemical composition of feeds and faeces (dry matter, crude protein, crude fat, crude fiber, crude ash, calcium, phosphorus) was determined using standard methods (AOAC 1995). The organic acid (acetic, butyric and lactic) contents in silages were determined by the method of N. Kubadinow (1982), pH value - by a glass electrode.

Rumen fluid was analysed once during the pre-experimental and three times during the experimental period. Samples of the rumen fluid were collected from four analogous cows in each group in 1.5 – 2 hours after the a.m. compound feeding using a pharynx probe with a steel tip. The DM content in the rumen fluid was determined by drying them at 105 ^{o}C to obtain a constant mass. Nitrogen and ammonia contents in ruminal fluid were determined with conventional methods using Tecator (Foss-Tecator, Hoganas, Sweden) equipment; the pH value by a glass electrode. Volatile fatty acids (VFA) were determined on a Shimadzu GC-2010 (Shimadzu corporation, Kyoto, Japan) gas chromatograph following preparation of acidified ruminal fluid by the method of Erwin et al. (1961). In this case 0.25 mm ID GC capillary column of 25 m length filled with AT ^{TM}- 1000 phase (Alltech Associates, USA) was used.

Every cow had its control milking once a week. Every individual milk sample was analysed for fat, total protein content and lactose by the analyser Milko-Scan 133 B (A/S N. Foss Electric, Hillerod, Denmark); casein, whey protein (α-lactoalbumin, β-

laktoglobulin) by the analyser Promilk MK II (A/S N. Foss Electric, Hillerod, Denmark). The DM content in milk was determined by drying at 105 °C to obtain a constant mass, ash by gravimetric method that was proceeded by sample mineralization with dry burning at 400-500 °C temperature; calcium by atomic absorption spectrophotometer Perkin-Elmer 603 (Perkin-Elmer, Norwalk, Connecticut, USA); phosphorus by photometric method with molibdovanadat reagent; urea - by photometric method with dimethylamine-4-benzaldehide (Tondu 1986); total acidity by titration with NaOH; rennet clotting time and firmness of the curd by microbiological control instructions for milk processing plants (Pustovoi 1978).

Fat was obtained from combined milk samples taken proportionally to the daily milk yield of a separate group of cows. Milk fat was analysed for fatty acid composition (Christopherson and Glass 1969, Pustovoi 1978) by the gas chromatograph Shimadzu GC-2010 (Shimadzu corporation, Kyoto, Japan). In this case 0.25 mm ID GC capillary column of 30 m length filled with AT ™- FAME phase (Alltech Associates, USA) was used.

Economic efficiency. Economic efficiency of different cow feeding was determined taking into consideration factual feed consumption, price of feeds and milk yield. The price of feed allowances for separate groups of cows and feed costs per kg of 4 % fat corrected milk have been estimated.

Statistical analyses. The data were processed statistically. The arithmetic average values (x), standard errors of the mean (SEM), standard deviation (SD) were calculated for all data. The significance of differences between the average values was determined according to Snedecor and Cochran (1989). $P < 0.05$ was an indicator of the data significance.

RESULTS AND DISCUSSION

Feed intake. Recording of the amounts of feeds consumed did not show any significant differences between the groups. During the treatment control and experimental groups of cows consumed daily on the average the same amount of feeds and received the same amount of energy and nutrients (Table 1).

Table 1. Average composition of dairy cow diets on as-fed basis

Feedstuff	Group of cows	
	Control	Experimental
Hay, kg	2.0	2.0
Perennial grass silage, kg	20.6	21.0
Sugarbeet pulp silage, kg	25.0	25.0
Compound feed No.1, kg	7.5	-
Compound feed No.2, kg	-	7.5
Analytical data (intake from feeds):		
Dry matter, kg	18.57	18.54
Metabolizable energy , MJ	203.73	201.33
Crude protein , g	2803.0	2778.0
Crude fiber, g	4441	4471
Calcium, g	185	154
Phosphorus, g	77	86

Less conventional feed – sugarbeet pulp silage - was eaten willingly by both groups of animals. The level of DM consumed with this feed accounted for 31.4 – 31.5 % of DM content in the diet and corresponded to the optimum recommended sugarbeet pulp silage intake (Heller and Potthast 1990).
Different protein sources used and urea supplementation of the diet had no influence on the intake of compound feed.
Fermentation of nitrogenous matter and carbohydrates in the rumen. No significant differences between the groups were found after the analysis of the rumen contents (see Table 2). Many of the differences determined during the treatment were also registered at the pre-experimental period. Some of the parameters between the groups were highly different, but due to high data variation within the group these differences were not statistically significant.

Table 2. Biochemical indicators of the rumen contents

Item	Group	During the pre-experimental period *x ± SEM*	Average during the treatment *x ± SEM*
Infusoria count in 1000/mL	C*	599.60±72.86	522.20±53.62
	E	464.00±45.91	430.36±52.97
pH value	C	7.14±0.16	7.04±0.08
	E	7.14±0.08	6.89±0.13
Ammonia nitrogen, mg/100mL	C	17.56±0.68	17.14±1.02
	E	16.17±1.19	17.49±0.77
Total nitrogen, mg/100 mL	C	70.79±1.26	74.13±1.12
	E	65.14±4.65	71.98±2.18
VFA content , mmol/100 mL	C	9.04±0.70	9.12±0.53
	E	9.62±0.36	9.33±0.23
VFA ratio in %:			
Acetic acid	C	70.14±0.33	68.70±1.48
	E	66.74±1.64	65.29±2.31
Propionic acid	C	13.99±0.80	16.12±0.67
	E	17.04±0.98	18.26±0.75
Butyric acid	C	10.41±0.58	10.66±1.00
	E	11.69±0.69	11.60±1.16

* C – Control group; E – Experimental group.

During the pre-experimental period the infusoria count was 29.22 % higher in the rumen fluid of the control cows. This indicator of rumen microflora activity and development remained higher (on average 21.34 %) in this group at the experimental period, too. In this case it is quite probable that more favourable conditions should be formed for the development of these protozoa and more intensive nutrient breakdown in the rumen of cows fed control compound feed. However, the fermentation data for nitrogenous matter and carbohydrates presented in Table 2 do not support this tendency. Both groups could be characterized by high variations for the infusoria count within the groups though during the whole experiment this indicator was more stable in the rumen fluid of

experimental cows. Thus, it has been concluded that the differences between the groups in the experimental period were predetermined by the individual traits of cows and not by the composition of the compound feed.

During the pre-experimental period the pH value of the rumen contents was the same (7.14) in both groups of cows. The tendency towards higher acidity of the rumen contents was observed in both groups during the treatment. The pH values of the rumen contents in the control and experimental groups were on the average 0.1 and 0.25 unit lower, respectively, compared with the pre-experimental period. The difference in the pH value at the experimental period amounted to on average 0.15 unit, but due to sufficiently high variations within both groups, the difference was also not statistically significant.

The difference of the protein feed composition in the compound feed had no influence on the nitrogenous matter degradation in the rumen. This was supposed due to the fact that there were no changes in the ammonia nitrogen concentration in the rumen contents of both groups of cows at the pre-experimental and experimental periods. It is also supposed that urea nitrogen present in the compound feed for the experimental cows was fully employed for the synthesis of microbial protein though there are opinions that urea is efficient only in the diets for lower productivity or dry cows (Heller and Potthast 1990).

Carbohydrate fermentation during the trial did not undergo any visible changes. VFA concentration in the rumen contents of both groups of cows was within the physiological norm limits and there were no differences between the groups. The analysis of the research data showed a tendency common to both groups: during the trial VFA composition in percentage had somewhat changed in the rumen of both groups of cows. In the rumen fluid of control and of experimental cows the part of acetic acid had decreased by, respectively, 1.44 and 1.45 % and that of propionic acid increased by, respectively, 2.13 and 1.22 %. The percentage of butyric acid was almost the same during the whole experiment.

It is considered that the changes of the rumen biochemical indicators were influenced by the natural changes of the feed quality in the course of the trial.

Milk production. Different feeding of cows had no significant influence on the milk production of cows (see Table 3). In the course of the trial, daily milk yield was 3.84 kg higher for the control group of cows and 5.11 kg higher for the experimental group of cows, but the increase in the milk fat in the urea (experimental) group was somewhat lower. Consequently, the production of 4 % fat corrected milk was almost similar in both groups. Thus, it may be concluded that feeding of cows with sugarbeet pulp silage and compound feed containing 0.8 % urea had no negative effect on the production of newly calved cows.

Table 3. Milk production of cows

Group	Period		In comparison with the pre-experimental period ±
	During the pre-experimental period *x* ± *SEM*	During the treatment *x* ± *SEM*	
Whole milk, kg per day			
Control	21.65±1.21	25.49±2.86	+3.84
Experimental	21.58±2.22	26.69±4.00	+5.11
4% Fat corrected milk, kg per day			
Control	18.93±0.33	24.54±2.81	+5.61
Experimental	19.83±2.23	25.09±3.67	+5.26

Milk quality. During the trial, there were changes in most of the chemical composition indicators of milk in both groups, and from the nutritional viewpoint chemical composition of milk during the treatment was better compared with the pre-experimental period (see Table 4). The differences in the total protein, casein and whey protein contents during the treatment including the changes since the start of the trial accounted for 0.01 – 0.17 % and were insignificant. Also insignificant differences between the groups were found for milk fat, mineral matter (ash, calcium and phosphorus) changes in milk. The content of lactose in the milk of experimental cows changed and was 0.12 % ($p<0.05$) higher than that of control cows. This might have been influenced by insignificantly different carbohydrate fermentation in the rumen that had slightly altered VFA ratio. The content of urea in the milk of both groups of cows was normal (15 – 30 mg/100 ml) and indicated that different feeding factors had no influence on the protein metabolism in the animal body.

Table 4. Milk quality

Item	Group	During pre-experimental period *x±SEM*	During the treatment *x±SEM*
Dry matter, %	C*	11.54±0.30	12.62±0.61
	E	11.96±0.52	12.57±0.71
Fat , %	C	3.17±0.25	3.76±0.45
	E	3.46±0.30	3.62±0.48
Protein , %	C	2.89±0.21	3.10±0.21
	E	3.04±0.37	3.08±0.20
Including			
Casein , %	C	2.44±0.24	2.60±0.18
	E	2.54±0.36	2.57±0.21
Soluble protein , %	C	0.45±0.08	0.50±0.09
	E	0.50±0.04	0.51±0.11
Lactose , %	C	4.73±0.17	4.82±0.14
	E	4.74±0.10	4.94±0.18 **
Ash ,%	C	0.74±0.13	0.74±0.04
	E	0.71±0.01	0.73±0.02
Calcium ,%	C	0.098±0.004	0.099 ±0.005
	E	0.093±0.061	0.096±0.005
Phosphorus ,%	C	0.088±0.014	0.089±0.013
	E	0.085±0.017	0.088±0.011
Urea , mg/100mL	C	16.07±4.07	15.56±3.27
	E	14.25±0.92	16.33±5.11
Acidity , °T	C	14.37±1.87	16.92±1.05
	E	15.50±1.51	17.07±1.16
Coagulation time, minutes	C	47.5± 13.8	24.9±6.4
	E	30.2±6.49 **	22.6±7.6

* C – Control group; E – Experimental group; ** $p<0.05$.

There were almost no differences between the groups for the milk quality indicators (protein and their different fraction contents, protein: fat ratio) that are important for cheese production and indicated good technological properties of milk. Milk acidity was relatively lower in the experimental group compared with the control group but corresponded to the properties of good whole milk (16 – 18 T^o). Rennet clotting time (between 15 – 45 min.) was significantly shorter for both groups of cows during the treatment compared with the pre-experimental period and corresponded to the requirements for cheese production.

Milk fat quality analysis showed that the content of saturated fatty acids was 6.24 % ($p<0.05$) lower and that of unsaturated fatty acids 6.25 % ($p<0.05$) higher in the milk fat of experimental cows compared with the control (see Table 5). Correspondingly, the ratio of saturated and unsaturated fatty acids in the milk fat of experimental cows was by 0.89 parts lower ($p<0.05$) and the milk was more suitable for production of good quality

butter. Moreover, the biological value of the experimental milk was significantly higher because the content of polyunsaturated fatty acids was 0.18 % higher in the milk fat of experimental cows. These differences as well as the above mentioned difference between the groups for the lactose content in milk might have been influenced by insignificant but better ratio of carbohydrates and nitrogenous matter in the experimental diet that better corresponded to the physiological needs of cows.

Table 5. Milk fat composition

Item	Group	During pre-experimental period *x±SEM*	During the treatment *x±SEM*
Saturated fatty acids , %	C*	71.95±3.96	79.42±0.40
	E	70.13±2.86	73.18±0.99 **
Including			
Volatile	C	6.77±1.12	7.72±1.14
	E	7.01±0.60	7.05±0.51
Non volatile	C	65.18±5.09	71.70±1.08
	E	63.12±2.26	66.13±0.62 **
Unsaturated fatty acids , %	C	28.05±3.96	20.57±0.40
	E	29.87±2.86	26.82±0.99 **
Including			
Monounsaturated	C	26.01±3.92	18.41±2.84
	E	27.69±0.29	24.47±0.91 **
Polyunsaturated	C	2.04±0.04	2.17±0.18
	E	2.18±0.02	2.35±0.12
Saturated and unsaturated acid ratio	C	2.60±0.51	3.86±0.09
	E	2.36±0.32	2.73±0.14 **

* C – Control group; E – Experimental group; **p < 0.05

Economic efficiency. The price of feeds for the average experimental diet was 23.2 % lower than that of control diet and, therefore, the expenses for production of 1 kg 4 % fat corrected milk were 21.8 % lower because the compound feed in the diet of experimental cows was cheaper due to soybean and sunflower oil meals replacement with cheaper local rapeseed cake and usage of urea.

CONCLUSIONS

Silage made from squeezed sugarbeet pulp in a trench silo sealed with plastic was of high feeding value. The DM content in it was 23.3 % and there were 11.56 MJ ME and 118.2 g CP per kg DM.

Sugarbeet pulp silage that accounted for 31.5 % of DM in the diet and compound feed supplemented with 0.8 % urea had no significant effects on the fermentation of nitrogenous matter and carbohydrates in the rumen, production and quality of milk.

There were no differences in the chemical composition of milk, it was characterized by good technological properties that are important for the production of butter and cheese. Soybean and sunflower oil meals replacement with rapeseed cake and urea reduced the price of compound feed and, therefore, sugarbeet pulp silage based diet was 23.2 % cheaper and expenses for production of 1 kg of 4 % fat corrected milk were 21.8 % lower.

Abbreviations

CP	crude protein	SD	standard deviation
DM	dry matter	SEM	standard error of the mean
ME	metabolizable energy	VFA	volatile fatty acids
p	level of significance		

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INTERLABORATORY STUDY ON MANGANESE DETERMINATION IN 3 SPECIES OF SPONTANEOUS FLORA USED AS ALTERNATIVE MINERAL SOURCES FOR WEANING PIGLETS (10-30 KG)

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ABSTRACT

Three medicinal herbs (*Origanum vulgare; Vaccinium myrtillus L.; Tribulus terestris*) were used in a nutrition experiment with the main purpose to enhance the mineral status of weaned piglets. The three medicinal herbs used in our study, were grown in Oltenia County, Romania. A homogeneity test was applied to the samples and they were distributed to each participant on sealed plastic bags. All laboratories accomplished ten measurements for each sample. The formulation of the experimental diets and the inclusion rate of the dietary supplements were done on the basis of the chemical composition of the wild plants. An interlaboratory study involving 7 participants was set up in order to obtain an accurate evaluation of the reference values for Mn content of these wild herbs. We calculated the reference values from the mean of the laboratory results after exclusion of outliers and using robust statistics (Algorithm A). The method of determination set by the protocol was the flame atomic absorption spectrometry. The appearance frequency of the results was determined by Kernel distribution plot and the removal of the doubtful results was done with the Algorithm A method. The performance of the participants was evaluated by Z score and Zeta score. The reference values determined during the interlaboratory study were: *Vaccinium myrtillus* L. (81.11 ppm Fe); *Origanum Vulgare* (1873.98 ppm Fe); *Tribulus Terrestris* (278.25ppm Fe).
Keywords*: manganese, wild herbs, FAAS, assigned value, interlaboratory study*

INTRODUCTION

The increased concern for bacterial resistance in humans (Lalles, 2007) and in animals and the higher risks of environmental pollution with animal manure which is rich in trace elements (Nicholson, 2008) led to the complete ban on antibiotics and to the emergence of a demand for lower dietary minerals in pig diets. The *in vivo* research on the use of dietary phytogenic plants/additives (individual or in mixtures) produced contradictory results, although the *in vitro* studies showed their antibacterial action (Lalles, 2008). The use of plants and plant extracts (such as essential oils) is an increasingly used alternative to the control of diarrhoea and other gastro-intestinal disfunctionalities (Han, 2006). The question is whether these plants can also be a natural source of trace elements (Rozica, 2005), bioavailable at the intestinal level, so that the excreted minerals are diminished.

Origanum vulgare, Vaccinium myrtillus L. and *Tribulus terestris* are among the wild plants having therapeutic action. *Origanum vulgare* is a plant from the mint family, with antibacterial properties and prevents the digestive tractul misbalances (De Konig, 1993). The bilberry has been included in mixtures of herbs studied as antioxidants (Szentmihalyi, 2005) and was studied as diet ingredient decreasing the blood glucoses and tryglicerides (Petlevski, 2001). *Tribulus terestris* is a known diuretic and it has recently been proved (Sengul, 2009) to have antioxidant and antimicrobial properties.
Therefore, testing the above wild plants in a feeding experiment aiming to improve the Mn status in weaned piglets requires the accurate determination of these elements. One of the key problems with wild plants utilization is the fluctuating concentration of the active substances and thus the trace elements content is rather unknown. One way to assess the exact content of active substances is through interlaboratory studies.
An interlaboratory study has been undertaken in order to assay the Fe and Zn concentration in oregano (*Origanum vulgare*), bilberries (*Vaccinium myrtillus L.*) and puncture vine (*Tribulus terestris*) in view of using them as natural sources of minerals for piglets. The study was joined by seven laboratories, both governmental and commercial, with experience in minerals assays in foods and feeds. The method used by all participants was flame atomic absorption spectrometry (AAS) after wet digestion, using a microwave oven technique.
The objectives of this study were, a) to perform a comparative determination of Mn concentration in *Origanum vulgare, Vaccinium myrtillus* L. and *Tribulus Terrestris* with the purpose to evaluate their potential as natural sources of trace elements in animal diets and, b) to evaluate the capacity of some laboratories working in the field of food and feed quality to determine trace elements in wild herbs.

MATERIAL AND METHOD

The three plants used in our study, were cropped in Oltenia county, Romania.

- *Origanum vulgare* - Family LAMIACEAE was harvested from the wild flora, at anthesis. It was collected from northern Oltenia, Romania (lat-45°4'0" N, long. 24°8'0" E, altitude 400 m) in the morning. The plants were dried in thin layers spread on wooden frames.
- *Vaccinium myrtillus* - Family ERICACEAE was harvested from the mountain area in northern Oltenia, Romania (lat-45°17′29″N, long- 23°41′20″E, altitude 1600 m). The fruits were collected at physiological maturity, in August. They were dried on wooden frames with galvanized wire mesh, in aerated rooms.
- *Tribulus terestris* - Family ZYGOPHYLLACEAE was harvested at anthesis. It was collected from Craiova, Romania (lat- 44°20' N, long-23°49' E, altitude 75-116 m). We collected mainly the fruits, and seldom the aerial parts of the plant. Drying was done under shadow, in thin layers, in places properly aerated.

Seven laboratories participated at interlaboratory study for determination of copper. The method used by all participants was flame atomic absorption spectrometry after wet digestion, using a microwave oven technique. The institutions are: National Research Development Institute for Biology and Animal Nutrition; Research Development

Institute for Nonferrous and Rare Metals; Institute for Food Bioresources; Institute for Food Chemistry; Institute for Hygiene and Veterinary Public Health; Research Institute for Soil Science and Agrochemistry.
The samples were conditioned by organizer (National Research Development Institute for Biology and Animal Nutrition) and distributed to participant. Plants were dried and grinded.

Chemicals and Reagents

- Concentrated nitric acid 65%, (Merck, Germany).
- Hydrogen peroxide 30%, analytical quality.
- Ultrapure water (Milli-Q Millipore, 18,2 MΩ/cm)
- Zinc stock solution.-1000 mg/L $Zn(NO_3)_2$ in HNO_3 0.5 mol / L
- Iron stock solution.-1000 mg/L $Fe(NO_3)_2$ in HNO_3 0.5 mol / L

Apparatus

- Analytical balance
- Stove
- Laboratory microwave oven
- Flame atomic absorption spectrometer with deuterium lamp

Statistical parameters
The assigned value, its incertitude and the statistical methods for calculating the consensus mean and the standard deviation was determined using the consensus values between the participants in the test (Koch, 2009).
The population mean and the standard deviation were established using robust means, using the Huber estimation (algorithm A).
Using algorithm A, we consider our set of data characterized by a robust mean X and a robust std dev S. As initial values for X we took the median, and for S we calculated the median of the absolute deviation (S = 1,483 x med(Xi – X)).
The limits of the range are X - δ and X + δ; δ = 1,5 x S
With these data we calculated a new X* and a new S*.

$$S^* = 1{,}134\sqrt{\frac{(X_i^* - X^*)^2}{p-1}} \qquad (1)$$

For the statistical performance we applied Z, Z' and Zeta scores

Z score: $$Z = \frac{x-\mu}{S} \qquad (2)$$

x = laboratory mean
μ = assigned value
S = standard deviation

Z' score: $$Z' = \frac{(x-\mu)}{\sqrt{S^2 + u_x^{\,2}}} \qquad (3)$$

u_x = laboratory uncertainty
u_X = uncertainty of assigned value

Zeta score: $$\xi = \frac{(x-\mu)}{\sqrt{u_x{}^2 + u_X{}^2}} \quad (4)$$

- $|z| \leq 2$, satisfactory, probability - 95,46%;
- $2 < |z| < 3$, questionable,
- $|z| \geq 3$, unsatisfactory,

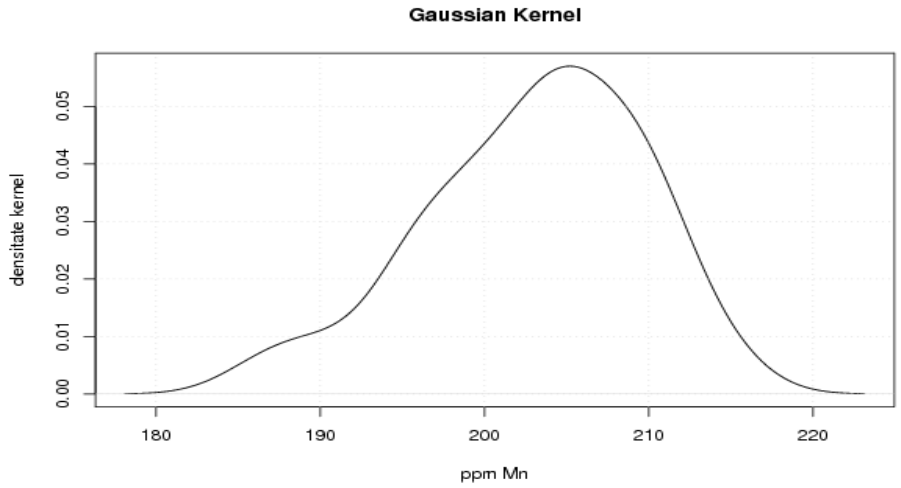
RESULTS AND DISCUSSIONS

Table 1 shows the analytical results reported by each laboratory participating in the study.

Table 1. Average means and standard deviations reported by each participant

Mn	***Vaccinium myrtillus* L.**	***Origanum vulgareL.***	***Tribulus Terrestris***
Lab 1	184.18 ± 4.95	74.42 ± 2.78	41.40 ± 1.46
Lab 2	204.12 ± 2.37	74.92 ± 11.46	46.74 ± 0.90
Lab 3	214.48 ± 7.35	84.21 ± 4.46	49.28 ± 2.12
Lab 4	199.90 ± 3.96	75.00 ± 2.40	43.80 ± 2.15
Lab 5	206.70 ± 4.35	80.50 ± 2.27	50.20 ± 1.32
Lab 6	219.54 ± 13.81	81.04 ± 3.76	50.68 ± 3.29
Lab 7	201.60 ± 9.48	67.40 ± 4.79	40.40 ± 3.10

The results reported by the participants form data strings which cover a quite wide range of values. Given the specificity of the data strings (Table 1), the fact that they are contaminated with values which obviously do not belong to the same family, we need a calculation algorithm to determine the robust mean (Thomson, 2006). On the other hand, the large variation of the date in the string shows the usefulness of an interlaboratory study, particularly under the conditions in which the analysed samples are from the wild flora.
Consensus from all participant laboratories is the most common method for establish the assigned value.
The estimation of the density functions for the considered data strings (Table 1) was done using the Kernel density function (fig 1). For our data strings we have chosen the Kernel density in order to avoid the functional shapes with different weights (Chu, 1991).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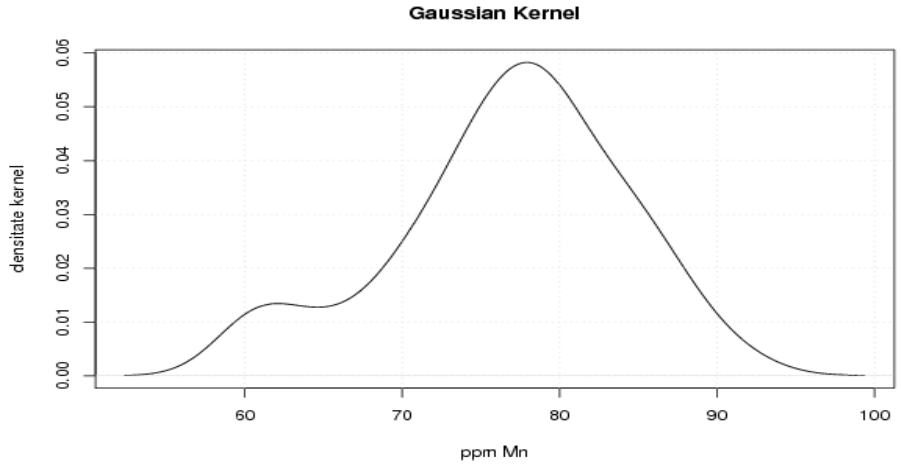
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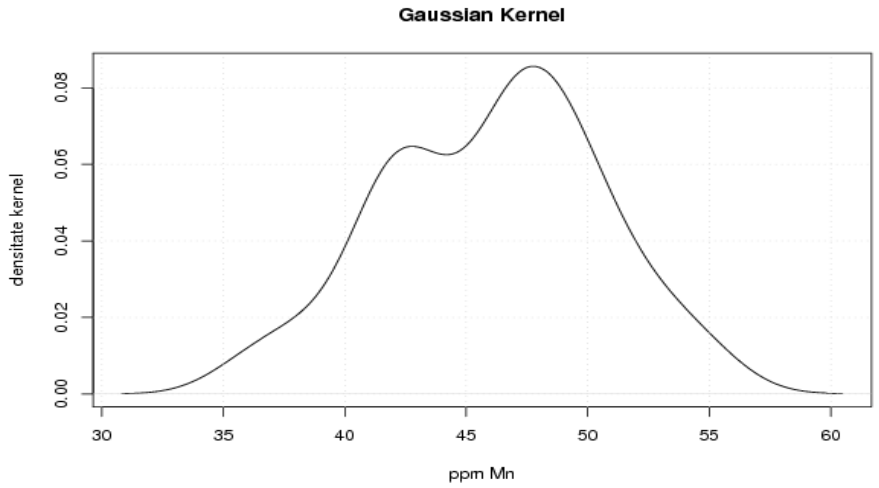

Fig 1. Kernel density for calculating the central tendency of results of Mn determination for: Vaccinium myrtillus L.; Origanum vulgare and Tribulus Terrestris

As expected, given the range of values reported by the laboratories, (table 1), the Kernel density for Mn determination shows a non homogenous set of results for the three studied herbs. Therefore we used another test to determine the assigned value.
The population means and standard deviations were calculated using Algorithm A and the assigned values are presented in table 2.

Table 2. Population means and standard deviations

		***Vaccinium myrtillus* L.**	***Origanum Vulgare L.***	***Tribulus terrestris***
Mn	**Mean (mg/kg)**	204.10	77.17	45.97
	Std. Dev.	11.86	7.13	4.89
	Uncertainty	2.71	1.63	1.12

The results displayed in Table 2 show that all three studied plants have sizeable concentrations of Mn. Using these natural sources of minerals in pig diets (1 or 3% inclusion rate) will meet animal requirements for Mn (3 ppm, NRC 1998) while decreasing or even excluding the premix from the feed. The efficiency of these wild plants can only be assayed in studies of mineral bioavailability.

Statistical performance

The analytical capacity of the participating laboratories was evaluated using Z, Z' and Zeta scores. The results are shown in table 3.

Table 3. Results of scoring

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7
	Vaccinium myrtillus L. - Mn						
Z score	-1.68	0.00	0.88	-0.35	0.22	1.30	-0.21
Z' score	-0.88	0.00	0.24	-0.35	0.20	0.88	-0.26
Zeta score	-1.02	0.00	0.24	-0.34	0.20	0.87	-0.25
	Origanum vulgare - Mn						
Z score	-0.39	-0.32	0.99	-0.30	0.47	0.54	-1.37
Z' score	-0.26	-0.18	0.41	-0.31	0.48	0.60	-0.26
Zeta score	-0.35	-0.18	0.41	-0.30	0.46	0.58	-1.00
	Tribulus terrestris - Mn						
Z score	-0.94	0.16	0.68	-0.44	0.87	0.96	-1.14
Z' score	-0.50	0.06	0.19	-0.31	0.60	0.73	-0.23
Zeta score	-0.58	0.06	0.19	-0.31	0.60	0.72	-0.99

Data obtained showed satisfactory results of all three parameters studied for laboratories performances (2 < parameter < 3).

CONCLUSIONS

Because the samples were wild plants, we calculated reference values from the analytical results (consensus value) of all the laboratories. We used the robust mean to estimate the mean population of values, using the Huber estimate (algorithm A). The reference values calculated with algorithm A were: for *Vaccinium myrtillus* L. 204.10 ppm Mn; for *Origanum vulgare* 77.17 ppm Mn; for *Tribulus Terrestris* 45.97 ppm Mn.

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USE OF ELECTROLYZED WATER FOR INACTIVATION OF ESCHERICHIA COLI, STAPHYLOCOCCUS AUREUS, SALMONELLA TIPHYMURIUM AND PSEUDOMONAS AERUGINOSA FROM POULTRY FARMS

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ABSTRACT

This paper reports the effect of electrolyzed water on different microorganisms present in poultry farms on the surface of the eggs**.** The encountered pathogens in poultry in order of their frequency are: *Salmonella tiphymurium, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*. The electrolyzed water is produced by passing an electric current through a dilute solution of salts in water. Anolit neutral electrolyzed water was produced using a generator EL400 (provided by I. Envirolyte Td.).
The experimental results showed that electrolyzed water (ANK) has a bactericidal role on some microorganisms, being an effective bactericidal agent which can be used in poultry farms.
Keywords*: electrolyzed water, anolit neutral electrolyzed water (ANK), bactericidal agent*

INTRODUCTION

The use of electrolyzed water in food industry has become of major interest in USA, due to its role in controlling microorganisms. Birds are exposed to numerous opportunities for contamination and infection from the egg incubator, floor, leather, bird feces, food, drinking water, etc.
Electrolyzed water use has proved effective in poultry farms by destroying the pathogenic bacteria present on the surface of eggs (*Salmonella tiphymurium, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli*). The encountered pathogens in poultry in order of their frequency are: *Salmonella tiphymurium , Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa.* A study of *Escherichia coli* and *Listeria monocytogenes* present on plastic panels and on bird halls showed that spraying the surfaces with electrolyzed water resulted in inactivation of pathogens [1].
Birds contaminated with pathogens are transmitting them to other birds or carcasses by faeces, nasal secretions, during housing in open spaces, transport and processing.
Using electrolyzed water in bird halls by electrostatic spraying proved to be effective for eliminating pathogenic bacteria from the surface of eggs [2].

The carried out experiments were intended to study the effect of electrolyzed water (ANK 200 mg active chlorine) on some pathogens [3].

MATERIALS AND METHODS

To evaluate the effect of ANK on pathogens a working protocol was established, as follows:

a) Three areas were selected for experiments: ceramic, steel and plastic;
b) The micro-organisms used to prepare the Control strains: *Stafilococcus Aureus* - ATCC 2 5923, *Salmonella tiphymurium* ATCC, *Escherichia coli* -25922, *Pseudomonas aeruginosa* ATCC-27853 [4].
c) Use of ANK (32, 50, 100, 200 si 400 mg active chlorine)

The electrolyzed water is produced by passing an electric current through a dilute solution of salts in water. Anolit neutral electrolyzed water was produced using a generator EL400 (provided by I. Envirolyte Td.) [5, 6].
A salt solution with 25% concentration and drinking water were pumped in generator achieving anolit neutral electrolyzed water with the following characteristics: pH =7,8; ORP (redox potential) = 743 mV; active chlorine = 12mg/l.
Procedure:
Areas selected for experiments were inoculated with microorganisms and taken as trial witnesses while other surfaces were inoculated with these strains + ANK; contact time 15 minutes; samples were sown on those specific areas, were selected and analyzed after the incubation period of 48 hours at 37^{o}C [4].

RESULTS AND DISCUSSIONS

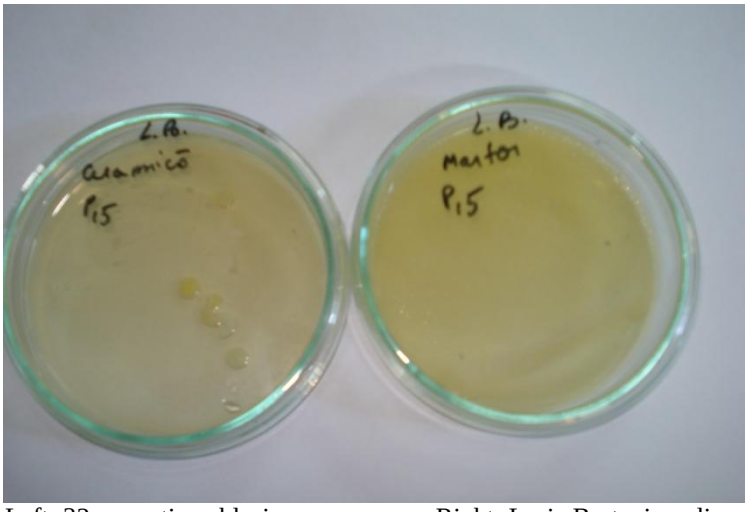
Detailed results of the screening tests are presented in the table 1**.**

Table 1. Effect of electrolyzed water on the tested microorganisms

Nr. Crt.	**Microorganisms**	**Selected areas**	**Results (colony number)**					
			Control sample	**ANK 32mg**	**ANK 50mg**	**ANK 100 mg**	**ANK 200 mg**	**ANK 400 mg**
1	*Stafilococcus aureus*	Ceramic Steel Plastic	>1500	22 15 25	0	0	0	0
2	*Escherichia coli*	Ceramic Steel Plastic	>1500	549 434 657	281 234 322	7 5 9	0	0
3	*Salmonella tiphymurium*	Ceramic Steel Plastic	163	72 67 81	12 6 8	0	0	0
4	*Pseudomonas aeruginosa*	Ceramic Steel Plastic	>1500	9 5 2	0	0	0	0

The analysis of the table shows that on the surfaces contaminated with strain + ANK:

- *Staphylococcus aureus* was 87% inhibited at concentration of 32 mg active chlorine, but for the other concentrations it did not develop;
- *Escherichia coli* was 50% inhibited at concentration of 32 mg active chlorine, 75% at concentration of 50 mg active chlorine, and did not develop at 200 and 400 mg concentration of active chlorine;
- *Salmonella tiphymurium* was 50% inhibited at concentration of 32 mg active chlorine, 95% at 50 mg active chlorine and did not develop for the other concentrations;
- *Pseudomonas aeruginosa* was inhibited in proportion of 90% at the concentration of 32 mg active chlorine and did not develop for the other concentrations (fig. 1).



Left: 32 mg active chlorine Right: Luria Bertani medium

Fig. 1. Aspect of Pseudomonas aeruginosa

CONCLUSIONS

Starting with 50 mg of active chlorine, the electrolyzed water has a total bactericidal effect on *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

For *Escherichia coli* and *Salmonella tiphymurium* the electrolyzed water has a total bactericidal effect starting with 100 mg of active chlorine.

We may conclude that the electrolyzed water (ANK) has a bactericidal role on some microorganisms, being an effective bactericidal agent which can be used in poultry farms.

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EFFICIENCY OF USING ENSILED SEMI-LATE CORN HYBRIDS IN DIETS FOR FATTENING STEERS

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ABSTRACT

The purpose of the experiment was to compare the productive potential of the silages made of various semi-late corn hybrids: F 365, F 376 and Olt, in terms of their potential and influence on steer performance. We used diets consisting of corn silage as bulk forage supplemented with a concentrate based on barley and sunflower meal. The test used 3 groups of 8 Romanian spotted fattening steers each weighing in average 279 to 330 kg in the beginning of the experiment. Hybrid F 376 was shown to produce the best animal performance, with 1304 g daily weight gain, compared to 1226 g in F 365 and 1126 g in Olt. The differences were statistically significant ($P \leq 0.001$) between experiments 2 (F 376) and 1 (F 365), significant ($P \leq 0.05$) between experiments 3 (Olt) and 2 (F 376) and not significant ($P \geq 0.05$) between experiments 3 (Olt) and 1 (F 365). Organic matter digestibility revealed the same order: 73% in F 376 compared to 71% in F 365 and 69% in Olt, while the gross efficiency of the dietary energy utilization for gain (RE/ME) was 22% in F 376, compared to 21% and 17% in F 365 and Olt, which shows a better quality and a better utilization of the semi-late hybrid F 376.
Keywords: *steers, corn hybrids, silage, diet efficiency*

INTRODUCTION

A lot of research was undertaken in Romania over the past two decades on all forage crops for ruminants, which yielded new hybrids and cultivars with a higher production potential.

Among the main forage plants, with multiple uses and with valuable biological traits, the corn is grown on the largest areas. Under the coil and climate conditions of Romania, the corn for silage, next to the alfalfa under different forms of preservation, is the core of ruminant diets during stabulation and during the periods when other forage sources are not available. In many European countries with advanced animal husbandry, corn represents 25-30% of he feed resources ext to pastures (40%), grass silages (20%) and hay (15%), supplemented with concentrate feeds. The success of the corn crops depends largely on the cultivated hybrid (Moga, 1996). However, no country uses yet only corn hybrids specialised for the production of silages, although efforts are done (Barrière, 1997). Such hybrids should have, besides a strong cob, highly digestible stalks and leaves with high levels of protein and readily fermentescible sugars.

In the lack of such hybrids, hybrids for the production of ear corn are used, and among them are preferred the hybrids with deep roots, large leaves producing a lot of vegetal mass consisting of cobs and leaves (at least 60% on DM basis), which still have green leaves when the wax stage starts. Most semi-late and semi-early hybrids meet these requirements; the early hybrids have shallow roots and less developed leaves.

Émile et al., 1995, confirmed and stressed the importance of selecting the corn hybrid and the harvesting time to produce high quality forages for ruminants, as also mentioned by Rankin, 2003. Other researchers (Undersander et al., 2005; Schroeder, 2004) show that the corn for silage cultivated later may influence plant maturity and the productive potential.
In our attempt to increase the efficiency of corn silage conversion into animal products, we undertook to evaluate the productive potential of silages made of various semi-late corn hybrids: F 365, F 376 and Olt, given to fattening steers.

MATERIAL AND METHOD

The experiments set up for each corn hybrid preserved as silage used three groups of 8 Romanian Spotted steers each fed on complex diets formulated to produce daily weight gains of 1300 g. The average initial weight of the steers ranged between 279 and 330 kg.
The basic diets consisted of the studied corn hybrids supplemented with compound feeds with barley, sunflower meal, feed-grade limestone, salt and remixes adequate to the category of fattening steers. Each of the three diets had on of the evaluated corn silages:

- diet 1 – with hybrid F 365 (semi-late);
- diet 2 - with hybrid F 376 (semi-late);
- diet 3 - with hybrid F Olt (semi-late).

Organic matter, and organic matter components digestibility and energy digestibility were determined during two-week series of balances. This presumed measuring feed intake, the leftovers, and the manure using digestibility stands. The ratio of the digested elements to the ingested one produced the digestibility coefficients (%).
The energy and protein value of the three corn silages, given in net energy (NE) for meat and milk production, and in IDP (intestinally digestible protein), was calculated using the mathematical model for energy and protein metabolism simulation in ruminants (Burlacu 2002) based on equations adapted from INRA (Vermorel et al.,1987):
NEmp = ME x Kmp (q=ME/GE); Animal production level = 1.5), for meat production
NEmilk = ME x Km, for milk production
For protein: (Vérité R et col 1987)
IDPN=CP(1-Dg)xdr+0,576xCPxDg
IDPE=CP(1-Dg)xdr+0,093xFOM
IDPN is the degradable nitrogen-based intestinally digestible protein
IDPE is the fermentescible energy matter-based intestinally digestible protein
Dg=degradability (Alderman G, 1993); dr=true digestibility ; FOM=fermentescible organic matter.
The feeds and excreta were analysed chemically DM-gravimetric meth; ISO6496:2001; CP KJELDHAL SR13325-1995; TF organic solvent method iso6492:2001; CF gravimetric meth.STAS 9597/5-77) and calorimetrically (adiabatic calorimeter). The retained energy and nitrogen were determined by measurements after the animals were slaughtered (two steers in the beginning and end of the experiment). Differences between results were determined using the Student test (Sandu, 1995; Cucu, 2005)

RESULTS AND DISCUSSIONS

The comparative analysis of the chemical composition (Table 1) showed that the crude protein (CP) content of hybrid F 365 was 82 g and the crude fibre (CF) content was 229 g; the corresponding values for hybrid F 376 were 77 g and 240 g (similar to those obtained by Voicu et al., 2004), and 78 g and 234 g in Olt hybrid.
The protein and fibre level of the compound feeds were within the normal range, 344 and 256 g/kg DM, respectively for the sunflower meal and 115 g and 54 g/kg DM for barley. The nature of the fermentation products (VFA) and their pH were also considered during these analyses.

Table 1. Chemical composition of the dietary ingredients (g/kg feed/1000g DM)

Item	DM	OM	CP	CF	GE(MJ)
Corn silage, F 365	328 1000	304 927	27 82	75 229	6.08 18.54
Corn silage, F 376	269 1000	250 929	21 78	63 234	4.96 18.44
Corn silage, Olt	287 1000	266 927	22 77	69 240	5.21 18.15
Sunflower meal	845 1000	785 929	291 344	216 256	15.80 18.69
Barley	861 1000	840 976	99 115	46 54	16.00 18.58

Table 2 shows nutrient and energy digestibility of the 3 studied semi-late hybrids. The data show a clearly higher digestibility of the organic matter (73%) from the silage made of hybrid F 376, which consequently produced a different nutritive value. Crude protein digestibility of hybrid F 365 was slightly higher in hybrid 365 compared to Olt and F 376, 56%, 53% and 54%, respectively.

Table 2. Nutrient and energy digestibility in the silages made of semi-late corn hybrids (%) (average determined values)

Item	OM	CP	CF	DE
Experiment I F 365	71 ± 0.83	56 ± 1.15	63 ± 1.71	69±1.23
Experiment II F 376	73 ± 0.83	54 ± 3.37	66 ± 1.83	71±1.85
Experiment III Olt	69 ± 0.85	53 ± 1.18	63 ± 1.29	68±1.37

The nutritive value of the ensiled silages was expressed in FUmilk, FUmeat, IDPN, IDPE, per kg dry matter (DM); the nutritive value for energy and protein generally range with the literature (Table 3).
FUmilk = feed units milk = 6.07 MJ NE
FUmeat = feed units meat = 6.16 MJ NE

Table 3. Determined nutritive value of the ensiled silages (g/kg DM)

Item	DM	FUmilk	FUmeat	IDPN	IDPE	Ca	P
Corn silage F 365	328	1.04	1.02	49.06	65.81	3.8	2.7
Corn silage F 376	287	1.07	1.06	46.07	63.47	3.9	2.7
Corn silage F Olt	269	1.02	1.01	46.67	63.92	3.6	2.5

Table 4 shows the evolution of the average body weight for the three experiments: the initial body weight ranged between 279 and 330 kg, while the average final body weight ranged between 529 and 450 kg. This shows that the lowest average daily weight gain was produced by Olt hybrid (1126 g), preceded by hybrid F 376 (1304 g). The best average daily weight gain was produced by hybrid F 365 (1226 g).
The average daily weight gains were distinctly ($P \leq 0.001$) statistically different between experiments 2 (F 376) and 1 (F 365) and significantly ($P \leq 0.05$) different between experiments 3 (Olt) and 2 (F 376). The differences between experiments 3 (Olt) and 1 (F 365) were not statistically different ($P \geq 0.05$).

Table 4. Animal performance (average values)

Experiment	Exp. days	Weight				
		Initial (kg)	Final (kg)	Total gain (kg)	Average daily gain (g)	Significance of ADWG differences
Experiment I, hybrid F 365	106	321 ±8.00	451 ±9.25	130 ±6.75	1226.38 ±63.72	(XX) | (X) | (NS)
Experiment II, hybrid F 376	115	279 ±10.50	429 ±12.00	150 ±7.25	1304.25 ±63.00	
Experiment III, hybrid Olt	95	330 ±6.50	437 ±8.75	107 ±8.00	1126.38 ±84.22	

XX – distinctly significant differences ($P \leq 0.001$);
X - significant differences ($P \leq 0.05$);
NS – not significant differences ($P \geq 0.05$).

Table 5 shows feed conversion ratio for the dry matter, energy and protein for the three experiments. The lowest values were recorded for experiment II (F 376) which shows a better use of the dietary energy and protein.

Table 5. Feed conversion ratio for the dry matter, net energy and digestible protein (average values)

Item	Feed conversion ratio		
	DM kg/kg gain	FUmeat/kg gain	IDP g/kg gain
Experiment I (F 365)	6.224±0.314	6.24±0.32	527.07±26.58
Experiment II (F 376)	5.859±0.283	5.93±0.29	489.16±23.60
Experiment III (Olt)	6.247±0.445	6.45±0.46	566.10±40.35

These values are comparable with the results reported by Haurez et al., 1995, who used two hybrids with precocity close to our hybrids, to feed fattening Charolais cattle.
Table 6 shows the use of the dietary energy for various processes including weight gain. The average value of the ingested energy was 140740 KJ for experiment I (F 365), 145800 KJ for experiment II (F 376) ad 142492 KJ for experiment III (Olt). Energy digestibility (DE) was 70%, 69% and 69%, respectively. Energy distribution correlated with animal performance, as shown by the average gross daily gain of 1226 g for hybrid F 365, 1306 g for hybrid F 376 and 1126 g/animal/day for hybrid Olt.
The ratio of the metabolisable energy to the ingested energy had quite similar values ($P \geq 0.05$) in all experiments: 58.85% in experiment 1 (F 365), 59.81% in experiment 2 (F 376) and 59% in experiment 3 (Olt), as also reported by Kirkland et al., 2005 in a paper on the influence of corn and grass silage quality on the feed intake and ME concentration in finishing fattening steers.
The proportion of retained energy (RE) within the total metabolisable energy (ME), which represents the gross efficiency of the dietary energy utilization, was 21%, 22% and 17%, respectively.

Table 6. Energy balance – average values (KJ/steer/day)

Item	GE	DE	ME	RE
Experiment I (F 365)	140740± 12067.50	98550± 10032.63	82826± 8317.86	17393± 748.84
Experiment II (F 376)	145800± 6793	100440± 2306.13	87203± 2496.69	19185± 607.5
Experiment III (Olt)	142492± 7495	98710± 3380.94	84082± 1745.50	14026± 1051.25

GE = ingested gross energy ME = metabolisable energy
DE =digestible energy RE = retained energy

GE, FE (faeces energy), DE, UE (urine energy) were determined.
Methane E = % CH4/DE, was calculated on the basis of the dietary percentage of gross fibre (Schiemann et al., 1976)
RE was determined based on the data from the comparative slaughtering.
RE = Lr + Pr (Lr = retained lipids, Pr = retained protein)
Table 7 shows the use of protein within nitrogen balance. The ingested nitrogen was 156 g in experiment I, 174.95 g in experiment II and 145.17 g in experiment III. The nitrogen retained in the body of the steers after the losses through faces and urine was 29.98 g in experiment I, 38.71 g in experiment II and 28.39 g in experiment III, which means 19.11%; 21.64%, and 19.48%, respectively.

Table 7. Nitrogen balance – average values

Item	Ingested N (g)	N. faces		N. digested		N. urine		N. retained	
		(g)	%	(g)	%	(g)	%	(g)	%
Exp. I (F 365)	156.0± 2.23	47.88± 1.89	30.58± 1.12	108.70± 2.60	69.42± 1.12	78.71± 5.86	50.31± 3.98	29.98± 6.92	19.11± 4.33
Exp. II (F 376)	174.9± 5.38	57.54± 3.75	32.38± 2.76	120.53± 7.27	67.62± 2.76	81.82± 2.51	45.98± 1.71	38.71± 6.36	21.64± 3.09
Exp. III (F Olt)	145.1± 3.38	48.91± 1.85	33.72± 1.68	96.26± 3.65	66.28± 1.68	67.88± 1.54	46.80± 1.82	28.39± 4.36	19.48± 2.65

CONCLUSIONS

- the energy potential expressed in feed units for milk or meat production was the highest in the semi-late hybrid F 376 with 1.07 FUmilk and 1.06 FUmeat;
- although the IDP potential expressed as IDPN and IDPE was highest in hybrid F 365, with values of 49.06 g and 65.92 g, it didn't have a positive influence on animal performance;
- organic matter digestibility was 73% in F 376, compared to 71% and 69% in the other two hybrids;
- the use of the dietary energy correlated with the nutritive value of the dietary ingredients and implicitly with organic matter digestibility produced daily gross weight gains of 1226 g for hybrid F 365, 1306 g for hybrid F 376 and 1126 g/steer/day for hybrid Olt;
- the use of protein, as determined by the nitrogen balance, produced higher values in experiment II (hybrid F 376), 38.71 g, compared with the other two experiments, 29.98 and 28.39 g respectively;
- the nutritive value, steer performance and the efficiency of the dietary energy utilization showed that the ensiled semi-late corn hybrid F 376 produced the best results when fed to fattening steers.

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PERFECTION OF AN ESTIMATION OF PRODUCTION TECHNOLOGY OF HIGHLY STABLE PREMIXES

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ABSTRACT

Modern food enterprises are complex technological objects, so the estimation of the efficiency of their functioning should be based on the system analysis and synthesis. The quality of the finished product and its competitiveness in the market depends on the effective functioning of all technological systems.

The development of the methods for estimating the efficiency of the technological systems (production technology of highly stable premixes) functioning should be regarded in accordance with the main principles of the system analysis and synthesis. The level of the system`s efficiency is a rule estimated by certain groups of parameters. But those groups do not always give the objective picture of the system`s state. That`s why these groups of parameters should be changed depending on the conditions under which the system is exploited. Offered to consider the parameter of the system`s efficiency as a functional which should take into account both the characteristics of the system`s functioning and the characteristics of its interaction with the environment or to apply the parameter of reliability, quality of operation and stability of the system in estimation of the system`s efficiency, evaluating each of them as a difference in the parameters of the system`s functioning under normal conditions and when the system`s functioning is influenced by outer and inner factors.

When the methods of the quality control HACCP having been introduced at food enterprises the fluctuations in the quantitative – qualitative characteristics of technological systems have been registered. The methods of quantitative appraisal of stability for technological system (S_t, H) are characterized by various methods of approaches and results that are not equal in strength.

According to a law of probability and mathematical statistics the normal distribution law allows to evaluate dissipations of variable value X, applying a mean absolute deviation, a dispersion D [X] and mean square deviation σ [X]. However, desperation D[X] has advantages in comparison with other indexes; so it is possible to give an estimation of stability of operating process or system of production technology of highly stable premixes.

As the dispersion of dispersing any index of technological process characterizes its capacity to get set range of dispersing and set absolute value X_i , it is possible to evaluate stability of technological systems in food process and estimation of production technology of highly stable premixes. Thus, the application of this approach allows to control technological processes in food enterprises and estimation of production technology of highly stable premixes not only by means of methods HACCP, but also to estimate reproduction of dispersion of estimated quality indexes.

Keywords*: technology, estimation, stability, system`s, premixes, quality*

INTRODUCTION

Modern food enterprises are complex technological objects, so the estimation of the efficiency of their functioning should be based on the system analysis and synthesis. The quality of the finished product and its competitiveness in the market depends on the effective functioning of all technological systems.
Generalizing the works of such well – known scientists as V. Afanasyev, N. Buslenko, I. Zhelesnov, V. Kafarov, V. Panfilov, A. Orlov, S. Sarkisyan, V. Khubka and others it is possible to formulate the following definition: " The technological system represents the organized combination of functionally interacted elements with stable and targeted quantitative – qualitative connection between them". If to accept this definition as a basis then the development of the methods for estimating the efficiency of the technological systems (production technology of highly stable premixes) functioning should be regarded in accordance with the main principles of the system analysis and synthesis.

MATERIAL AND METHODS

In work analytical and mathematic-statistical methods have been used.
In 1984 I. Zhelesnov offered to perceive the efficiency of a technological system under conditions of production exploitation as its capacity to operate with assigned accuracy and reliability [1]. The level of the system`s efficiency is a rule estimated by certain groups of parameters. But those groups do not always give the objective picture of the system`s state. That`s why these groups of parameters should be changed depending on the conditions under which the system is exploited.
V. Kafarov and the co – authors offered to consider the parameter of the system`s efficiency as a functional which should take into account both the characteristics of the system`s functioning and the characteristics of its interaction with the environment [2]. V. Khoubka offered to represent the parameter of the system`s efficiency as a functional [3]. N. Buslenko offered to apply the parameter of reliability, quality of operation and stability of the system in estimation of the system`s efficiency, evaluating each of them as a difference in the parameters of the system`s functioning under normal conditions and when the system`s functioning is influenced by outer and inner factors [4]. S. Sarkisyan and the co-authors offered a comprising parameter for the estimation of the efficiency of the system`s functioning, but it cannot estimate the system`s behavior as to reproductiveness of the results of functioning and the stability of functioning [5].

RESULTS AND DISCUSSION

Table 1 presents the estimation of the efficiency of the functioning of the technological systems.
When the methods of the quality control HACCP having been introduced at food enterprises the fluctuations in the quantitative – qualitative characteristics of technological systems have been registered. In this connection it is essential to consider the fundamentals of the estimation of the stability of the technological system`s functioning, which is submitted in the Table 2.

Table 1. Estimation of efficiency of operation of technological systems production technology of highly stable premixes

The writer, source	Parameter of estimation
V.Kafarov etc. [2]	a functional: $R = R(\bar{P}, \bar{W})$, (1) where $\bar{P} = /P_1, P_2, \ldots P_n/$ - vector of the parameters of members of the technological system; $\bar{W} = /W_1, W_2, \ldots W_m/$ - vector of the outer influence parameters.
V.Khubka [3]	a functional: $E = E(T_e, E_e, C_e)$, (2) where T_e,- technological efficiency; E_e - economic efficiency; C_e - consumer efficiency.
N.Buslenko etc. [4]	a parameter of reliability, quality of control and stability of the system: $\Delta R = R^o - R^*$ (3) where Ro - parameter(index) of efficiency in the normal conditions of functioning $R^o = R(\alpha_1^o, \alpha_2^o \ldots \alpha_n^o; \beta_1^o, \beta_2^o \ldots \beta_m^o)$; R* - parameter of efficiency in conditions of the outer and inner influences of the control action $R^* = R(\alpha_1^*, \alpha_2^* \ldots \alpha_n^*; \beta_1^*, \beta_2^* \ldots \beta_m^*)$, α_i^o, α_1^* - parameters of the system functioning β_1^o, β_1^* - parameters of the outer influence
S.Sarkisyan etc. [5]	$K = F[[S_{ij}][C_n][K_o][P_{or}]] \le f[[R][\tau]]$ (4) where S_{ij} - strategy of behavior of the system of i-type in technological operation of j-type; C_n - matrix, showing the expenditure used for the operation fulfillment; K_o –parameter showing the potential of the successive technological operations; R - resource limitations; τ - limitations of time.

Table 2. About stability and steadiness of operation of technological systems definition

The author source	Definition
V.E.Vlasov and others. [6]	Stability of technological process or system as a whole means its capacity to keep the riched accuracy in time.
A.A.Voronov and others. [7]	Stability of any phenomenon is its capacity to keep the forms of its existence, without of which the phenomenon ceases to be itself, and to keep them for a long time and with accuracy.
A.M.Tsirlin and others. [8]	Steadiness (stability) is a term for which the following statement is right. When conditions of problem change a little, the solution will change a little too.
V.A.Panfilov and others. [9]	Stability is a property of technological process of keeping accuracy of quality indexes for products for some time and stability as an index of quantitative and qualitative variability of technological process.
The GOST 15895-77	Stability is a property of technological process, which causes its steadiness in probability distribution of its parameters during some time period without outside intervention.

Analyzing these definitions with reference to exploited technological systems of food enterprises and production technology of highly stable premixes, one can see that the concepts of stability and steadiness of technological process differ only in origin. The definition of stability according to V.A.Panfilov corresponds to the essence of phenomenon. Nevertheless, the methods of quantitative appraisal of stability for technological system are characterized by various methods of approaches and results that are not equal in strength.

According to V.A.Panfilov, stability of operation of any technological process is determined by means of the formula:

$$\boldsymbol{St} = 1 - \frac{\boldsymbol{H}}{\boldsymbol{H}_{\max}}, \qquad (5)$$

Where H – is the entropy, that corresponds to the given distribution of analyzed index quantity.

H max - is the most possible entropy, that corresponds to a normal distribution law.

V.A.Panfilov offered to determine entropy quantitatively for the experience using two possible results (where analyzed index can coincide or cannot coincide with area of set value) by means of formula :

$$H = P\ log2P\ -\ (1 - P)\ log2(1 - P), \qquad (6)$$

Where P – probability of satisfactory result experience in measurement.

According to a law of probability and mathematical statistics the normal distribution law allows to evaluate dissipations of variable value X, applying a mean absolute deviation, a dispersion D [X] and mean square deviation σ [X]. However, desperation D[X] has advantages in comparison with other indexes; so it is possible to give an estimation of stability of operating process or system of production technology of highly stable premixes.

A way, that V.Panfilof offered to determine stability is the most precise undoubtedly when estimating technological systems in the food enterprises. But quantitative determination of entropy by means of formula (6) causes ambiguous results because some values of probability correspond with several values of an entropy.

In response that it is necessary to esteem other approaches to definition of stability, as one of integral indexes of efficiency in operation of modern technological systems. Especially it is important when introducting the new methods that control the quality of raw materials, semi-finished, highly stable premixes and finished products (for example HACCP, etc.). The proposal by S.Ahnazarova and V.Kafarova about the definition of dispersion as characteristic of efficiency of operating technological systems corresponds very well with the concept of stability [11]:

$$S[X] = \sum_{i=1}^{n} (X_i - m_x)2\ p_i \qquad (7)$$

Where m_x is mathematical expectation of variable value *Xi*.

As the dispersion of dispersing any index of technological process characterizes its capacity to get set range of dispersing and set absolute value X_i , it is possible to evaluate stability of technological systems in food process and estimation of production technology of highly stable premixes by means of formula [12, 13]

$$St = 1 - \frac{D[x_i]_{\max} - D[x_i]_{\min}}{D[x_i]_{\max}} = \frac{D[x_i]_{\min}}{D[x_i]_{\max}},$$

Where D [Xi] max, D [Xi] min - maximum and minimum dispersion of distribution of a random variable X i, as parameter of an estimation of stability of operation of a technological system, the parameter of which is measured during time period permitting to get right dates of measuring.

CONCLUSIONS

The given expression is right if the structure of technological process during the period of time τ between two measurements does not change. In this case deflection for estimation of a dispersion will be connected to disturbing effects both concerning uniformity of raw material and constancy of values for structurally – technological factors.
Thus, the application of this approach allows to control technological processes in food enterprises and estimation of production technology of highly stable premixes not only by means of methods HACCP, but also to estimate reproduction of dispersion of estimated quality indexes.

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RESEARCH AND DEVELOPMENT IN THE FIELD OF FEED TECHNOLOGIES IN UKRAINE

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ABSTRACT

Ukraine has become a member of WOT, develops market economy and has proclaimed agroindustrial sector of economy as one of the basic directions of development and applies for continental leadership of manufacture of organic foodstuff. Manufacture of cattle-breeding production in such conditions demands introduction of fair, transparent and, mostiy, effective rules of their manufacture and quality formation. Manufacture of cattle-breeding production in modern competitive conditions is based on use of high-efficiency breeds, hybrids and cross-countries of animals and agricultural birds and also on use of highly effective mixed fodders. Manufacture of mixed fodders grows in Ukraine every year and on the average makes about 5 million т in a year. Today mixed fodders are developed mainly on modern mixed fodder enterprises focused on those consumers who develop poultry-farming and cattle-breeding production on industrial basis.

Quality and cost of mixed fodders production depend on the level of development of technological system of their manufacture. Evolution of mixed fodders technological systems (MTS) for all period of development can be divided in three generations.

In the structure of mixed fodders made in Ukraine the greatest segment is necessary on mixed fodders for agricultural birds and makes 47 % that is connected with conditions of conversion and the least expenses of forages. 19 % are on mixed fodders for pigs, 11 % - are for large horned livestock and 23 % - are for mixed fodders for other kinds of animals and birds.

For the last five years maintenance of from requirements of the population with meat and meat products has increased in Ukraine 1,9 to 2,3 mln.t, and is provided at the expense of own manufacture (1,5-2 mln.t) and import (0,24-0,5 mln.t). Today in Ukraine provision meat of on 1 person in a year for the last five years has increased from 40 to 50 kg among which on a share of beef and veal meat on the average it is necessary 11,5 kg, pork - 14,98 kg, fowl has increased 13,9 to 22 kg, other kinds of meat - 0,94 kg.

According to Goskomstat of Ukraine and Department of statistics of agriculture and environment, Ukraine, since 2005, has been included into ten countries-leaders on manufacture of eggs in the world. In 2009 in comparison with 2008 manufacture of eggs has increased by 6 % - to 15mln 856,8 thousand t. Volumes of manufacture of domestic cattle-breeding production entirely depend on number of a livestock of agricultural animals, birds and fish. According to the data of June, 1st, 2010 in comparison with the past year the livestock of the cattle was reduced by 3,3 % - to 5 million 534,2 thousand heads, including cows - by 4 % - to 2 million 767,8 thousand heads. At the same time, the livestock of pigs has increased by 15,0 % - to 7 million 952,8 thousand heads; by 5,5 % of sheep and goats - to 2 million 277,1 thousand heads; by 5,9 % of birds of all kinds.

Keywords*: technology, feed mix, production, meat, eggs.*

INTRODUCTION

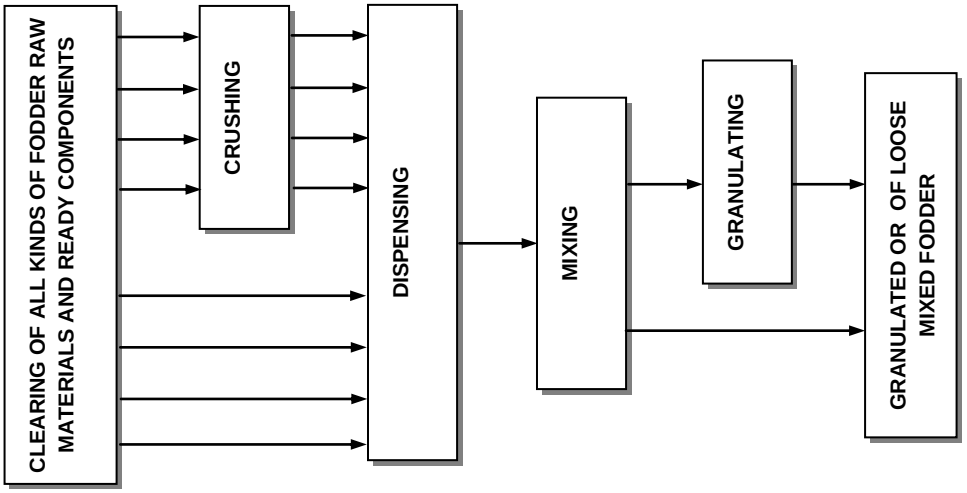
Ukraine has become a member of WOT, develops market economy and has proclaimed agroindustrial sector of economy as one of the basic directions of development and applies for continental leadership of manufacture of organic foodstuff. Manufacture of cattle-breeding production in such conditions demands introduction of fair, transparent and, mostiy, effective rules of their manufacture and quality formation. Manufacture of cattle-breeding production in modern competitive conditions is based on use of high-efficiency breeds, hybrids and cross-countries of animals and agricultural birds and also on use of highly effective mixed fodders. Manufacture of mixed fodders grows in Ukraine every year and on the average makes about 5 million т in a year. Today mixed fodders are developed mainly on modern mixed fodder enterprises focused on those consumers who develop poultry-farming and cattle-breeding production on industrial basis.

MATERIALS AND METHODS

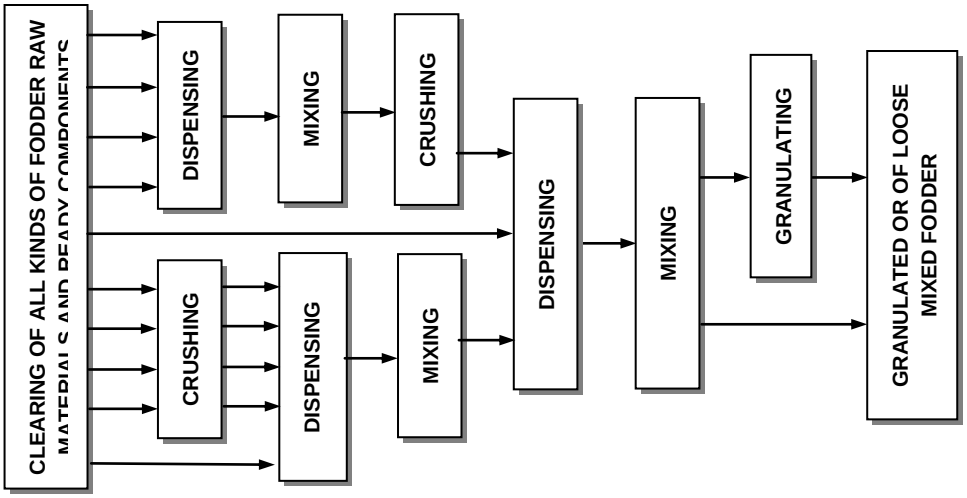
Analytical and mathematic-statistical methods have been used in the work. Initial objects of work "know-how" of mixed fodders, data about number of a livestock of agricultural animals and birds, manufacture volumes production of mixed fodders and cattle-breeding production have been chosen.

RESULTS AND DISCUSSION

Quality and cost of mixed fodders production directly depend on the level of development of technological system of their manufacture. Evolution of mixed fodders technological systems (MTS) for all period of development allows to divide them into three generations. It is possible to consider MTS, the structure of which assumes line preparation of all components of mixed fodders before receiving of loose granulated mixed fodder, to belong to the I-st generation (Graph 1).
It is possible to consider MTS in which structure there were technological systems of preliminary dispensing and mixing, called to raise quality of made production generation, to belong to the II-nd generation (Graph 2).
The most advanced technological systems based on the principle possible of portion dispensing and mixing can be considered to belong to the III rd generations (Graph 3).

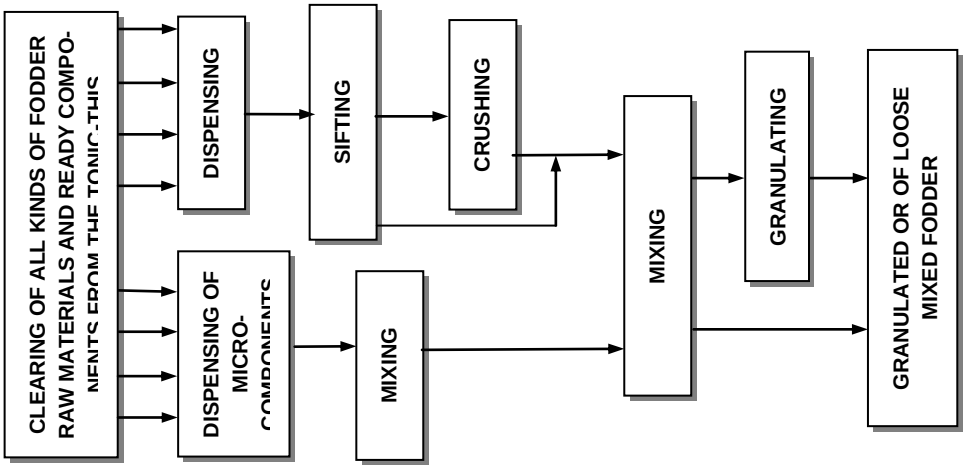


Graph 1. Structure mixed fodders technological systems of I th generation

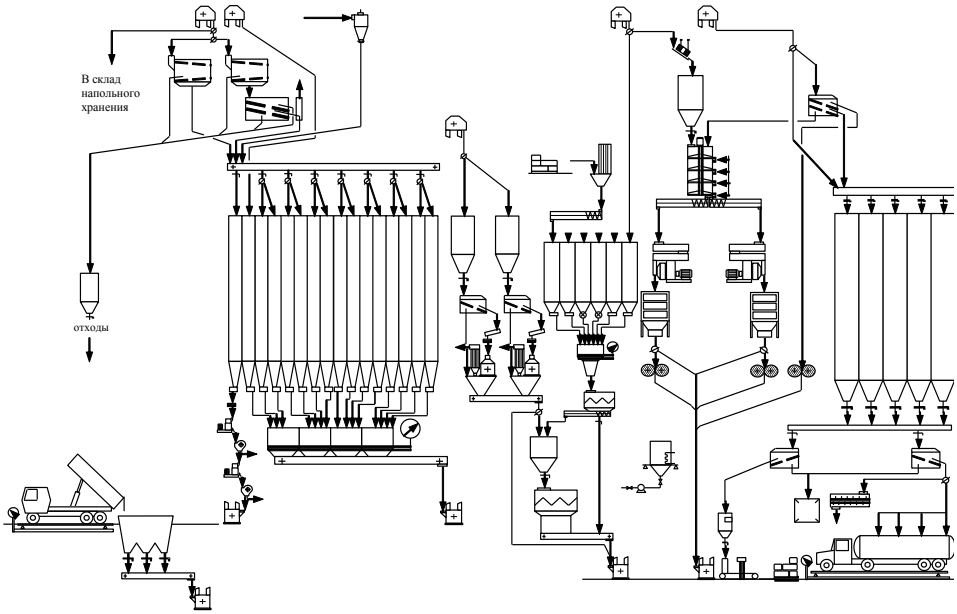


Graph 2. Structure mixed fodders technological systems of II th generation

Considering that fact, that at consecutive connection of elements of the system, stability of its functioning always is less, than stability of functioning of separate elements, further development of MTS was characterised by reduction of number of consecutive technological operations. And thanks to that stability of functioning of in parallel connected elements of system is always higher, than stability of functioning of separate elements, the wide principle of modular designing of MTS has received application. So, for example, MTS of the 2 nd generation material capacity of all the system and high specific power inputs on production were replaced by low-expensed technologies of the III-rd generation based on portion dispensing (Graph 4).



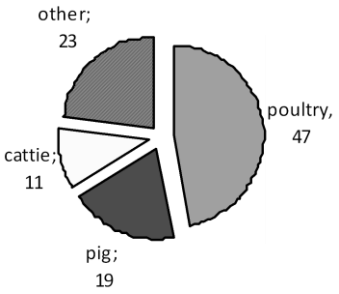
Graph 3. Structure mixed fodders technological systems of III th generation



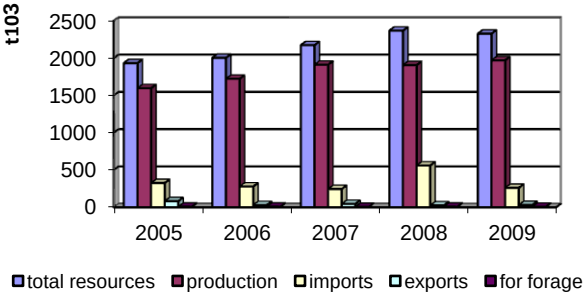
Graph 4. The basic scheme of technological process portion manufactures of mixed fodders (VanVyne, the Netherlands))

In the structure of mixed fodders made in Ukraine the greatest segment is necessary on mixed fodders for agricultural birds and makes 47 % that is connected with conditions of conversion and the least expenses of forages. 19 % are on mixed fodders for pigs, 11 % - are for large horned livestock and 23 % - are for mixed fodders for other kinds of animals and birds (Graph 5).

For the last five years maintenance of from requirements of the population with meat and meat products has increased in Ukraine 1,9 to 2,3 mln.t, and is provided at the expense of own manufacture (1,5-2 mln.t) and import (0,24-0,5 mln.t) (Graph 6).

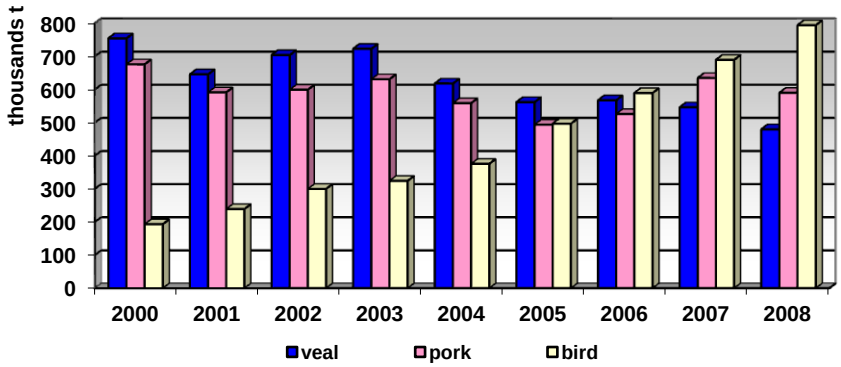


Graph 5. Structure mixed fodders in Ukraine



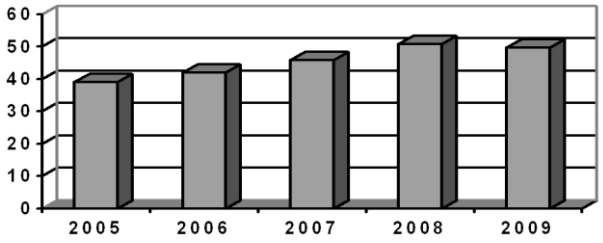
Graph 6. Balance of meat and meat products

In Ukraine since 2000 meat manufacture in slaughtering weight fluctuates from 1,5 to 2 million т. In 2009 manufacture of meat of all kinds (in live weight) in comparison with 2008 has increased, and makes 2 million 728,4 thousand т. In general dynamics of manufacture of meat on different categories in 2000 manufacture of meat of (beef and pork) prevailed, since 2006 fowl, mainly the chicken meat, the volumes of which in 2008 made about 0,8 million t, have prevailed (Graph 7).



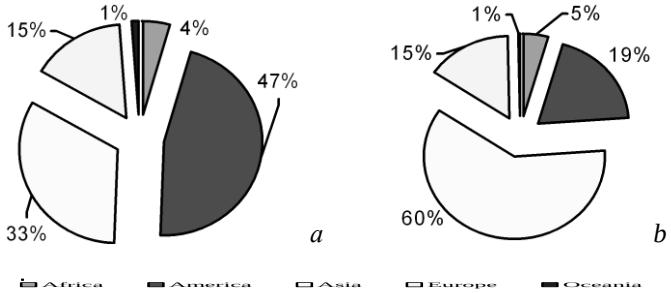
Graph 7. Dynamics of manufacture of meat in Ukraine

Today in Ukraine provision meat of on 1 person in a year for the last five years has increased from 40 to 50 kg among which on a share of beef and veal meat on the average it is necessary 11,5 kg, pork - 14,98 kg, fowl has increased 13,9 to 22 kg, other kinds of meat - 0,94 kg (Graph 8).

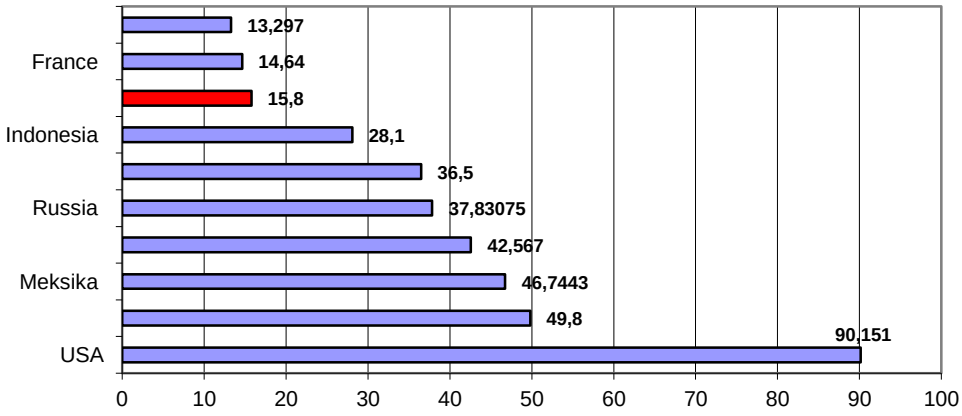


Graph 8. Meat manufacture in Ukraine counting on 1 person in a year, kg

At the given stage of development of stock-raising in the leading positions both in the world, and in Ukraine are observed in the direction of cultivation of agricultural birds (laying hens), broilers, turkey-cocks, ducks, geese). The greatest share of manufacture of fowl in the world (chicken meat, turkey-cock meat) is concentrated in the USA, eggs - in Asia (Graph 9).

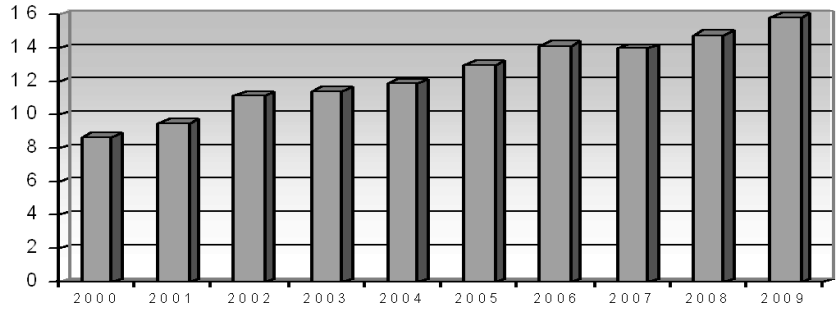


Graph 9. A share of manufacture a – meat, b - eggs of hens in the world



Graph 10. Ten leaders-manufacturers of eggs in the world, billion pieces

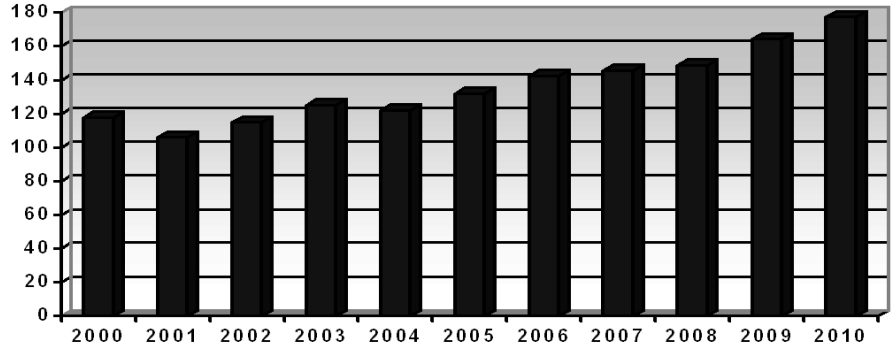
Recently manufacture of eggs of birds of all kinds in the world promptly increases. The leader of manufacture of eggs in the world is China (in 2009 manufacture volumes have made about 455 billion pieces of eggs). According to Goskomstat of Ukraine and Department of statistics of agriculture and environment, Ukraine, since 2005, has been included into ten countries-leaders on manufacture of eggs in the world (Graph 10) (data for 2008 without China). In 2009 in comparison with 2008 manufacture of eggs has increased by 6 % - to 15mln 856,8 thousand t. (Graph 11).



Graph 11. Manufacture of eggs in Ukraine, billion pieces

Volumes of manufacture of domestic cattle-breeding production entirely depend on number of a livestock of agricultural animals, birds and fish. According to the data of June, 1st, 2010 in comparison with the past year the livestock of the cattle was reduced by 3,3 % - to 5 million 534,2 thousand heads, including cows - by 4 % - to 2 million 767,8 thousand heads. At the same time, the livestock of pigs has increased by 15,0 % - to 7 million 952,8 thousand heads; by 5,5 % of sheep and goats - to 2 million 277,1 thousand heads; by 5,9 % of birds of all kinds.

The most developed and constantly growing remains poultry farming sector (Graph 12).



Graph 12. Dynamics of a livestock of hens (million goals) in Ukraine

CONCLUSIONS

Thus, the analysis of capacities of operating mixed fodders factories of Ukraine and new factories, which are under construction, testifies the possibility to satisfy requirements of cattle-breeding sector in full. By introduction and realization of programs of development of mixed fodder branches, stock-breeding and support of agrarian sector at the nation-wide level, an adoption of law of Ukraine "About fodders", it is possible to state with assurance that today Ukraine has considerable potential for creation and maintenance qualitative domestic cattle-breeding production for the Ukrainian population.

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EVALUATION OF RADIO FREQUENCY TECHNOLOGY FOR FRESH FILLED PASTA QUALITY

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ABSTRACT

The aim of this work is to evaluate the effects of radio frequency (RF) treatment on the quality characteristics of fresh filled pasta. Experiments were carried out on two different types of fresh filled pasta: *tortellini* with meat and *cappelletti* with ham. The filled pasta was pasteurized and dried through a radiofrequency continuous plant. The RF plant consists of a single steel tunnel, in which electrodes are placed and teed at 27.12 MHz, where simultaneously pasteurization and pre-drying of fresh pasta occur. Chemical, physical and microbiological parameters of pasta were evaluated immediately after RF treatment and during refrigeration storage. Organoleptic characteristics and cooking quality were tested by determination of weight gain during cooking and overcooking, optimal cooking time and loss matter. During storage, two different types of fresh filled pasta samples showed no significant changes in microbiological parameters examined. The cooking test showed high cooking firmness and low stickiness and adhesiveness of the treated fresh pasta compared to conventionally treated pasta.
Keywords: *fresh filled pasta, radio frequency technology, pasteurization*

INTRODUCTION

In recent years, interests in application of food heating by RF have been increased, because it is associated with a rapid and uniform heat distribution, large penetration depth and lower energy consumption. Currently this technology is successfully applied to drying, cooking and defrosting processes of food [4]. In the process of RF heating, the electromagnetic field is applied which generates heating within the food. The generation of heat can be explained due two main mechanism, ionic polarization and dipole rotation, and the heating is largely depended on the dielectric properties of food [5]. The frequency or rate of oscillation within the range is about 3 Hz and 30 GHz, invisible and completely undetectable to humans.
In conventional processes, fresh pasta is treated in six different phases: pasteurization with steam, drying with hot air, cooling, packaging, second pasteurization and final cooling of the package. The RF technology used in this work is able to simultaneously

pasteurize and dry unpacked fresh pasta reducing the productive cycle to only three phases: pasteurization and contemporary drying of the pasta, aseptic cooling and packaging, with consequent reduction costs a simplified productive process, and improvement of qualitative characteristics products.
This technology has already been applied with success for pasteurization and pre-drying of durum semolina fresh pasta in industrial plants, therefore the aim of this study was to evaluate the RF system on fresh filled pasta quality.

MATERIALS AND METHODS

The RF plant (STC Science Technology & Consulting, patent BR2006A000008) located in Soave factory, used for the pasteurization and pre-drying test is formed by a steel tunnel including some electrodes for the RF voltage application. The tunnel contains two continues modules where pre-drying and pasteurization simultaneously occur. Two different types of fresh filled pasta, *cappelletti* with ham and *tortellini* with meat, have been prepared for treatment in the RF plant.
The fresh pasta was obtained by mixing durum wheat semolina, flour and pasteurized fresh egg. The pasta was extruded by continuous press with a bronze. The pasta was shaped by a single sheet shaping unit for *cappelletti* and *tortellini* equipped with a rod system for filling distribution. Afterwards, each kind of pasta has been treated by radio frequency at 27.12 MHz with 3 kV of power for the duration of 10-12 min in every treatment, and the temperature in the module was 90°C. The filled pasta temperature have been risen over 80°C, with P value equal to 62 min (Trif. 70°C e Z 7,5)(Fig.1). In Fig. 2 is reported the flow chart of the fresh filled pasta process.

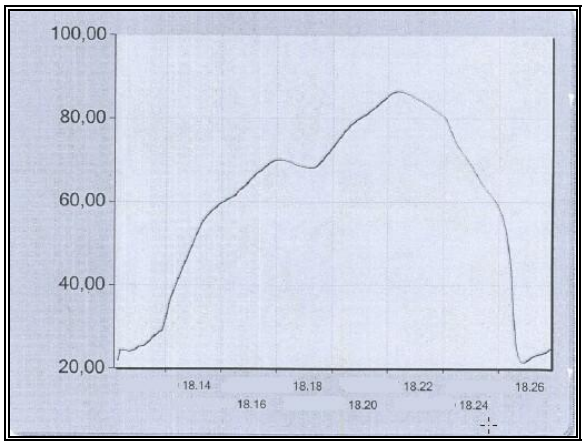

Fig. 1 Heating penetration curve

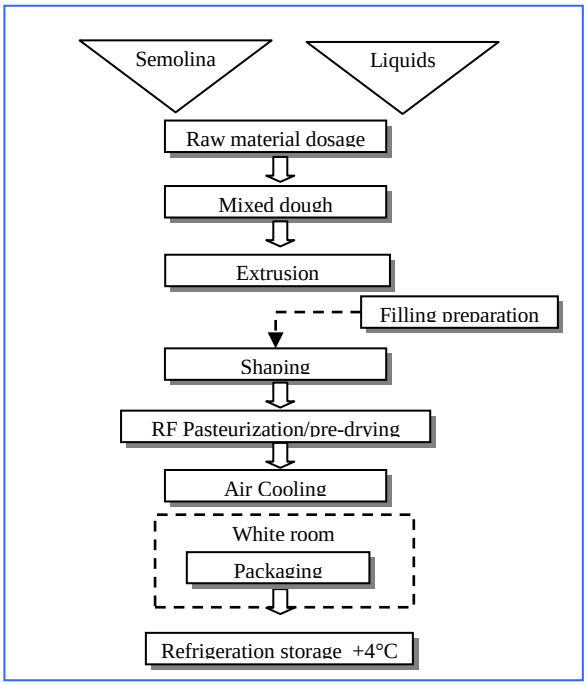

Fig. 2 Flow chart of the fresh filled pasta process

After the RF treatment, the fresh filled pasta samples, packaged in modified atmosphere, have been stored at 4 °C for 45 days and analyzed every 15 days for microbiological parameters, pasta color water activity, moisture, pH. Microbiological analysis are made by using Tempo® System (bioMériux, France), based on the most-probable-number (MPN) determination to evaluate total aerobic mesophilic plate count (TEMPO® TVC, bioMériux, France), total coliform (TEMPO® TC, bioMériux, France), coagulase-positive staphylococci (TEMPO® STA, bioMériux, France), Yeast and Moulds (TEMPO® YM, bioMériux, France), *Escherichia coli* (TEMPO® EC, bioMériux, France), *Enterobacteriaceae* (TEMPO® EB, bioMériux, France) and lactic acid bacteria (TEMPO® LAB, bioMériux, France), results were expressed as colony forming unit/g (CFU/g).
Sample colour was measured using a Chromameter-2 Reflectance (Minolta, Osaka, Japan). The colour differences were recorded as CIELab a* (redness–greenness), and b* (yellowness–blueness), chroma and Hue Angle values. Moisture content was determined by thermo-balance (Kern MLB-N). Water activity was measured by an electronic hygrometer (Aqua Lab. CX-2 – Decagon Devices, Pullman, USA), and pH by PH metro (Krison, 507).
Cooking studies were carried in order to determine optimum cooking time, weight gain during cooking and overcooking, loss matter and organoleptic properties before and after the cooking test.

Determination of cooking time was performed by subjecting samples of pasta cooked in distilled water (water/pasta ratio, 20/1 w/w), the optimal cooking time as the time required to observe the disappearance of the white central of the pasta samples manually squeezed between two thin slides. Weight gains were monitored during cooking and overcooking at given time each min until 12 min, the results were expressed as the ratio between the change in weight at time and the initial weight ($\Delta W/W_0$) [2].
Matter loss of the pasta during cooking was evaluated by determining the dry matter content of the cooking water, dry matter was determined in cooking water, previously evaporated and then desiccated to constant weight in an oven at 105 ◦C. Results were expressed as grams of matter loss/100 g pasta dry matter [1].

RESULTS AND DISCUSSION

In Tab. 1 and 2 the microbiological parameters are reported for pasteurized fresh filled pasta samples after RF treatment and during storage. The evaluated microbiological parameters were not significantly changed during storage for the two pasta types, except only a slight increase of about 1 Log of total aerobic mesophilic plate count after 45 days storage in *cappelletti* samples.
Tab. 3 and 4 report the chemical and physical value of treated filled fresh pasta, immediately after the radiofrequency treatment and during storage. The meat filled *tortellini* and ham filled *cappelletti* treated with RF did not change noticeably their initial chemical and physical characteristics during the storage. The water activity values and humidity remained nearly unchanged, consequence of an effective barrier effect of the packaging to water vapour. Also, the protective atmosphere in the packaging remained constant up to 45 days of storage at 4°C. The hue angle and chroma values did not change noticeably, however, they tend to increase and lead therefore to a saturation of the yellow colour typical for pasta.
The pasta treated with radio frequency showed in general to have better organoleptic characteristics compared to pasta treated with traditional thermal methods. Particularly the flavour and colour resulted to be more intensive and closer to fresh pasta.
Furthermore, the stickiness of the pasta has been reduced due to minor starch gelatinization in respect to traditionally treated pasta. [3].
The optimal cooking times were 5 min for *cappelletti* filled with ham and 6 min for *tortellini* filled with meat.
Fig. 3 shows results in terms of weight gain during cooking and overcooking. Weight gain at optimal cooking time was slightly higher for *cappelletti* samples.
Matter loss is an indicator of quality pasta and was similar for the two types of filled fresh pasta tested: 2.5 g/100g d.m for *cappelletti* and 2.3 g/100g d.m. for *tortellini.*

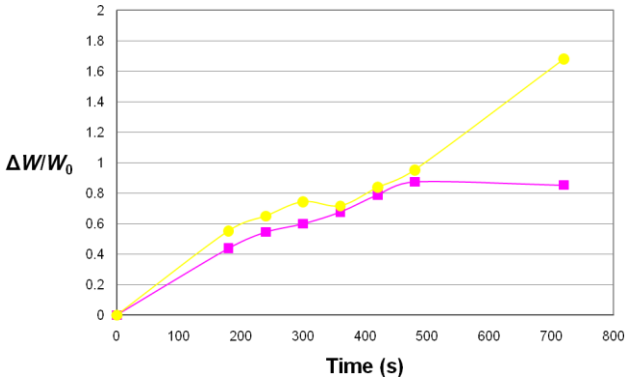

Fig. 3. Weight gain during cooking and overcooking (■) Tortellini, (●) Cappelletti

Table 1. Average microbiological values of cappelletti with ham after RF treatment and during storage for 45 days at 4 °C

Ham (cappelletti)	T0	T15	T30	T45
TVC CFU/g	4.6×10^3	1.1×10^3	4.8×10^4	1.6×10^4
EC CFU/g	<10	<10	<10	<10
TC CFU/g	<10	<10	<10	<10
STA CFU/g	<10	<10	<10	<10
YM CFU/g	<10	<10	<10	<10
EB CFU/g	<10	<10	<10	<10
LAB CFU/g	<100	<100	<100	<100

Table 2. Average microbiological values of tortellini with meat after RF treatment and during storage for 45 days at 4 °C

Meat (tortellini)	T0	T15	T30	T45
TVC CFU/g	5.7×10^3	7.3×10^3	1.7×10^4	3.3×10^3
EC CFU/g	<10	<10	<10	<10
TC CFU/g	<10	<10	<10	<10
STA CFU/g	<10	<10	<10	<10
YM CFU/g	<10	<10	<10	<10
EB CFU/g	<10	<10	<10	<10
LAB CFU/g	<100	<100	<100	<100

Table 3. Average chemical and physical values of cappelletti with ham after RF treatment and during storage for 45 days at 4 °C

Time (days)	Aw	RH%	pH	b*	Chroma	Hue angle
T0	0.94 ± 0.2	26.9 ± 0.2	6.03 ± 0.2	23.9 ± 0.6	28.9	91.9
T15	0.95 ± 0.4	26± 0.6	6.28 ± 0.4	28 ± 0.4	28.0	92.3
T30	0.925 ± 0.2	25.7±0.1	6.13 ± 0,3	27.9 ± 0.4	28.0	92.6
T45	0.94 ± 0.6	25.9± 0.3	6.02 ± 0.2	27.7 ± 0.4	27.9	93.3

Table 4. Average chemical and physical values of tortellini with meat after RF treatment and during storage for 45 days at 4 °C

Time (days)	Aw	RH%	pH	b*	Chroma	Hue angle
T0	0.91 ± 0.2	23.2 ±0.3	6.12 ± 0.2	24.9 ± 0.2	25.01	93.21
T15	0.9 ± 0.5	24.3 ± 0.7	6.15 ± 0.4	22.6 ± 0.5	22.58	92.41
T30	0.89 ± 0.1	25.67 ± 0.5	5.88 ± 0.4	30.50 ± 0.4	30.53	92.31
T45	0.92 ± 0.5	23.43 ± 0.3	6.3 ± 0.2	30.5 ± 0.3	30.47	92.21

CONCLUSIONS

This work shows new possibilities to produce microbiologically safe and high quality fresh filled pasta through a continuous RF industrial plant. By this new process it is possible to pasteurize and pre-dryi fresh filled pasta in very short time and to preserve chemical and physical characteristics for 45 days under refrigeration conditions. The RF treatment, furthermore, guarantees cooking properties and sensorial qualities, in particularly it improves the yellow colour of pasta, and it increases the cooking firmness and lower stickiness. These results strongly encourage further additional efforts to achieve the standardization of the RF processing to produce fresh filled pasta.

ACNOWLEDGEMENT

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INFLUENCE OF NATURAL ZEOLITE CLINOPTILOLITE ON IMMUNOLOGICAL PARAMETERS IN WEANED PIGLETS

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ABSTRACT

The aim of the investigation was to determine influence of the natural zeolite on some immunological parameters, and its performance in combination with live recombinant F4ac$^+$non-ETEC strain 2407 and F18ac+ non ETEC live strain 2143.
The investigation was conducted in two separated models. In the first one group of piglets fed with feed mixture without supplementation was the control group and the other group was fed by feed mixture with 0.5% Vetamin®(Panaceo, Austria) preparation addition, during the whole experimental period. Zeolite is prepared by mechanical micro ionization with high energy, enhances the potency of this material. In the second experimental model sixty piglets were divided into three groups: the control (C) group, the group with the experimental vaccine against *E. coli* (E1) and the group with vaccine and dietary inclusion of zeolite (E2).
There were no statistically differences in body weights between the control and the experimental groups in two trials. Significantly (P<0,05) higher number of leukocytes was found in the E2 group in relation to the control group at 14th day of the experiment. Although, portion of neutrophils and lymphocytes did not statistically differed. But it is noticeably higher share of lymphocytes 35th day of the trial in the E2 group. Significant difference in total protein level is noticeable both between the control and the vaccinated group (P<0,05) and the control and the vaccinated zeolite group (P<0,01) at 35th day of the experiment. Zeolite addition in combination with experimental *E. coli* vaccine stimulated specific immune response.
***Keywords**: weaned piglets, zeolite, Vetamin® , immunohematological parameters*

INTRODUCTION

Natural zeolite clinoptilolite is alumosilicate of volcanic origin, consisting of three-dimensional frameworks of SiO_4^{4-} and AlO_4^{5-} tetrahedra linked through the shared oxygen atoms. After ingestion, powdered clinoptilolite is inert and do not react chemically with food or body fluids. It is porous materials, characterized by the ability to lose and gain water reversibly, to adsorb molecules of appropriate cross-sectional diameter and to exchange their constituent cations without change of their structure. Its dietary inclusion on farm animals' diet is established for last two decades. It is well-known as effective micotoxin and heavy metals adsorbent [15, 20], as well as potent immunomodulator in swine [14, 6]. The effect of dietary addition of clinoptilolite on

growth performances, metabolical parameters and animals' health, depends on different concentration of clinoptilolite [17], particle size [7], as well as chemical modification and stability at different pH values [18]. Positive effect has been established by adding organic zeolites in feed of newborn piglets [14], dairy cows [2], sows and gilts [6, 10]. Weaning includes complex physiological, social, environmental and nutritive stress factors which have an effect upon adaptation and development of digestive and immune system. Following weaning, feed intake is reduced which subsequently leads to malnutrition and undergrowth of the piglets, and the reduction of the intestinal immunity. Colonization of small intestines by enterotoxic strains of the *E. coli* causes colibacilosis and subsequent diarrhea in days after weaning. This condition causes high economic loses as a result of additional veterinary costs, undergrowth, death caused by illness and prolonged time required to archive final body weight [3]. Clinoptilolite and mordenite are capable to adsorb and partially inactivate the thermo-labile *E. coli* enterotoxin in vitro, by disrupting them to attach on the intestinal cell-membrane receptors [13]. Aikoh et al [1], claim that alumosilicates act as the non-specific immunostimulators, similarly to superantigens, by activating large fractions of T-limphocyte population. Šperanda et al [17], found that immunohematological response is being enhanced by addition of tribo-mechanically activated zeolites. Therefore, the aim of the investigation was to determine influence of the natural zeolite on some immunological parameters, and its performance in combination with live recombinant F4ac$^+$non-ETEC strain 2407 and F18ac+ non ETEC live strain 2143.

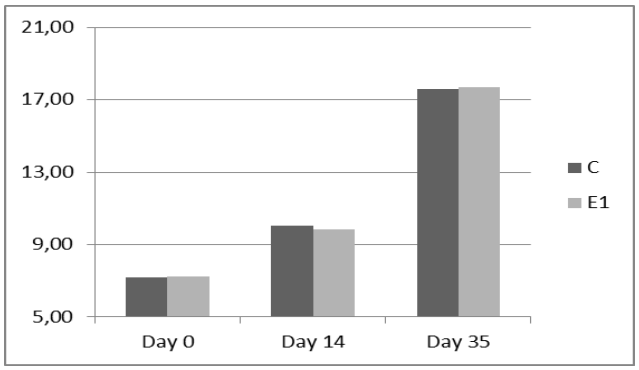
MATERIAL AND METHODS

Animals used in this study were maintained in facilities approved by the Croatian Association for Accreditation of Laboratory Animal Care, and in accordance with current regulation and standards issued by the Croatian Ministry of Agriculture.
The investigation was conducted in two separated models. In the first one we used forty six commercial crossbred piglets (Swedish LandraceX Large White X Pietrein), progeny of seven litters and three boars, weaned at 28th day and attended until 63rd day of life. Piglets were divided into two groups of twenty three piglets. Piglets were housed in isolation pens at 21±2 $^{\circ}$C, received a commercial weaner diet without antibiotics and had an unlimited access to water. Piglets from the both groups were fed on fodder mixture for weaned piglets containing 22% crude protein and 13.84 MJ ME/kg until 21st day after weaning and on fodder mixture for growing pigs with 19% crude protein and 13.74 MJ ME/kg until 35th day of the trial. One group was the control group and the other was the experimental group (E) in which feed mixture was added 0.5% Vetamin$^{®}$(Panaceo, Austria) preparation, during the whole experimental period. In the second experimental model sixty piglets were divided into three groups: the control (C) group, the group with the experimental vaccine against *E. coli* (E1) and the group with vaccine and dietary inclusion of zeolite (E2). Bivalent attenuated nontoxic vaccine, which was removed gene for enterotoxin producing, prepared from F4ac+ non ETEC (enterotoxic *E. coli*) live strain 2407 and F18ac+ non ETEC live strain 2143 (Croatian Veterinary Institute, Zagreb) was used. Vaccine was applied *per os* on the weaning day, dose level 10 x 10^{10} CFU in 60 ml TSB. Body weight in both trials was controlled 14th and 35th day of the trial. In the same time blood samples were taken from the *v. Cavae cranialis* using a

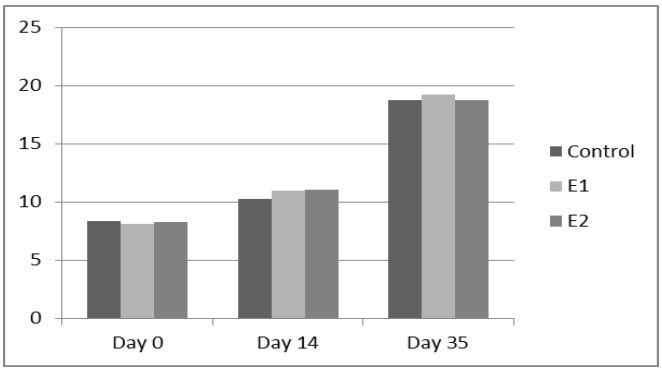
Venoject(R) vacutainer in order to determine haematological and some biochemical values. The number of the leukocytes was established using the Serono Backer 9120 automatic counter. Blood smears were prepared and stained according to Pappenheim and investigated under a microscope in order to determine the differential blood count. The relative ratio of individual cells of the leukocytes is given in percentages in relation to their total number. For the total protein and globulin concentration the automatic analyser Olympus 400 was used. The values obtained from the investigated indicators were processed by the general linear model procedure of the STATISTICA (StatSoft, Inc. 2007). The differences between the control and trial groups were statistically tested using repeated measurement model with the Fischer's *post hoc* test.

RESULTS AND DISCUSSION

There were no statistically differences in body weights between the control and the experimental groups (Graphs 1 and 2) in two trials. Some researchers have proved that dietary inclusion of zeolites improves average daily gain and feed conversion [10,11, 17] as well as Leung et al. [7] claiming that there were some potentials in using high quality clinoptilolite in the ration of grower hogs. Papaioannou et al [11] found better feed efficiency that was manifested by lower feed conversion ratio in the experimental group what is in relation with our results (data not shown). But our trial lasted shorter and that is possible the reason of such results.



Graph 1. Body weight of weaned piglets fed with dietary zeolite addition



Graph 2. Body weight of weaned piglets fed with dietary zeolite addition and vaccined piglets

Results of haematological investigation are summarized in Tables 1 and 2. Dietary inclusion of zeolite had no significant effect neither on the immunohematological nor biochemical parameters. Nevertheless, average of the total leucocytes number was higher in the E1 group, but without statistically differences (P>0.05). Martin-Kleiner et al [8] found higher number of leucocytes and lymphocytes in mice fed with clinoptilolite addition, just like Šperanda et al [17] who reported also higher number of leucocytes in weaned piglets.

Table 1. Immunohematological and some biochemical parameters in weaned piglets fed with zeolite addition

	day	Leuko, $X10^9$ L^{-1}	Neutrophils, %	Lympho, %	Total prot, $g.L^{-1}$	Globulins, $g.L^{-1}$
C	0	21,54±5,21	51,43±13,72	54,86±4,02	58,71±3,59	25,35±3,77
	14	24,07±5,13	43,57±14,79	58,29±2,98	55,86±5,61	29,53±4,31
	35	25,21±7,94	48,86±11,16	58,14±7,86	56,57±6,02	29,42±3,27
E_1	0	23,27±5,69	45,14±19,35	51±18,98	67,71±17,33	34,38±16,99
	14	27,41±8,63	46,57±11,04	51,29±11,79	51,71±5,31	28,1±3,86
	35	29,3±5,45	45±14,97	52,67±14,33	58,57±4,65	31,99±4,66

According to Table 2 significantly (P<0,05) higher number of leukocytes was found in the E2 group in relation to the control group at 14^{th} day of the experiment. Although, portion of neutrophils and lymphocytes did not statistically differed. But it is noticeably higher share of lymphocytes 35^{th} day of the trial in the E2 group. Significant difference in total protein level is noticeable both between the control and the vaccinated group (P<0,05) and the control and the vaccinated zeolite group (P<0,01) at 35^{th} day of the experiment. Concentration of globulins was significantly (P<0,05) higher at 35^{th} day in the E2 group in relation to the C group. Evidence in literature supports a possible

immunostimulatory [12, 4] effect of clinoptilolite when administered orally in humans or mice. Šperanda et al [15] reported that zeolite addition influenced hematological parameters with the meaning of leukocytosis, significantly higher level of neutrophils and band neutrophils, while lymphocytes and monocytes levels did not change. Mohri et al [9] found also higher total protein concentration in calves after clinoptilolite addition, but significantly higher albumin concentration. The mechanisms by which clinoptilolite may enhance the immune response especially after vaccination are not clearly understood. Taking into account that the immune response is positively correlated with the energy balance of the animal [19], a possible explanation is that clinoptilolite may improve the energy status. Stojić et al [14] found higher IgG concentration in serum of clinoptilolite supplemented calves. Karatzia [5] have published that the dietary administration of 200 g/day of a natural zeolite clinoptilolite, during the last months of pregnancy, increases the antibody production of dairy cattle vaccinated against *E. coli*. In the light of our results, especially of share of white blood cells and globulin concentration, we can conclude that zeolite addition in combination with experimental *E. coli* vaccine stimulated specific immune response.

Table 2. Immunohematological and some biochemical parameters in weaned piglets vaccine with non-ETEC and with zeolite addition

	day	Leuko, $X10^9$ L^{-1}	Neutrophils, %	Lympho, %	Total prot, $g.L^{-1}$	Globulins, $g.L^{-1}$
C	0	21.6±4.51	44±12.07	56±12.88	54.29±6.95	26.81±7.7
	14	16.7^{a}±4.53	43.43±14.46	52.86±16.08	51.71±5.77	27.54±5.44
	35	23.5±5.48	42.71±12.57	54.57±13.5	58^{A}±4.08	31.25^{a}±5.38
E_1	0	21.13±8.86	43.14±17.16	56.57±17.5	53.29±3.59	25.4±4.84
	14	18.29±2.72	46.71±9.14	51.14±8.95	53.86±5.98	29.87±7.32
	35	21.41±5.45	50.43±7.85	46.71±7.43	58.86^{a}±2.61	34.58±2.39
E_2	0	16.96±4.24	53.57±6.4	45.57±6.43	51±2.38	24.68±2.91
	14	23.2^{b}±3.87	35.86±9.3	62.43±9.47	56.57±3.31	32.04±5.48
	35	21.94±5.78	38.29±15.16	58.57±15.06	65.43^{Bb}±5.13	38.37^{b}±6.94

$^{a, b}$ values between groups differ significantly $P<0.05$,
$^{A, B}$ $P<0.1$

CONCLUSIONS

Although there were no statistically differences in body weights between the control and the experimental groups and no differences in immunohematological parameters after dietary zeolite addition, in combination with experimental *E. coli* vaccine supplementation of natural zeolite stimulated specific immune response.

ACKNOWLEDGEMENTS

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APPLICATION OF RAPID TECHNIQUE FOR SCREENING OF MYCOTOXINS IN CEREALS

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ABSTRACT

Aim of our investigations was fast determination of kernel contamination of various cereals with mycotoxins, following the recommendations of the EU regulations for food safety, as well as of domestic harmonized regulations.
Investigations were performed using the test kits of R-Biopharm on the samples of various cereals: corn, wheat and oat, which have been stored in storehouse during two months (corn samples) and during a three-year period (wheat and oat samples). The results of our investigations have shown that two out of 20 corn samples were contaminated with fumonisin and aflatoxins, and two out of seven oat and wheat samples with DON. Mycotoxin values did not exceed the MRL proposed by the Regulation, regarding the intended use of cereals (food/feed).
Keywords*: corn, wheat, oat, mycotoxins*

INTRODUCTION

Cereals and cereal products have a great importance for the population and farm animals in regard to their nutrition [1]. The quality of the raw materials and food products is evaluated on the basis of physical, chemical and microbiological criterions [2]. Among the numerous factors which influence on it, is the contamination with microorganisms. During the growing period, depending on the ecologycal factors: temperature, precipitation, before culture and other, cereals can be attacked by particular fungus species, often by the *Fuzarium spp.*, *Aspergilus spp*., and *Penicilium.* These fungi can produce mycotoxins as their secondary metabolites. Two mycotoxin types are connected with *Fuzarium spp.* The first one is zearalenone (ZEA) and the second is deoxynivalenol (DON). Other group of mycotoxins consists of structural similar compounds and they are designated as threechotecene mycotoxins [3].Today, among the hundred known different mycotoxins, only 40 the most current have been analysed. These toxins are often found in various cereals (wheat, corn, barley, rye and oat), and in their products, as well as in hay and straw, and they can cause various damages and have effect on the total yield and the quality [4, 5], as well as on the human and animal health [6].

MATERIAL AND METHODS

Investigations were performed on various cereals: 20 samples of corn intended for animal nutrition, five samples of three wheat varieties (Treska, Milenka and Radika) grown in three different production regions (Skopje, Ovche Pole and Kochani), and two samples of oat variety Slavuj, grown by conventional and organic method. Corn was harvested in 2008, and wheat and oat were harvested in 2006. Cereals samples were stored in storehouse during different period before the analyses, as has been pointed out in the abstract. The safety in regard to the contamination with mycotoxins was investigared with quick immunochromatographic tests from R-Biopharm (Fig.1), based on an antigen-antibody-reaction. Qualitative and quantitative analyses were performed for detection of aflatoxins, fumonisins and deoxynivalenol (DON) in corn samples, and of DON in wheat and oat samples.

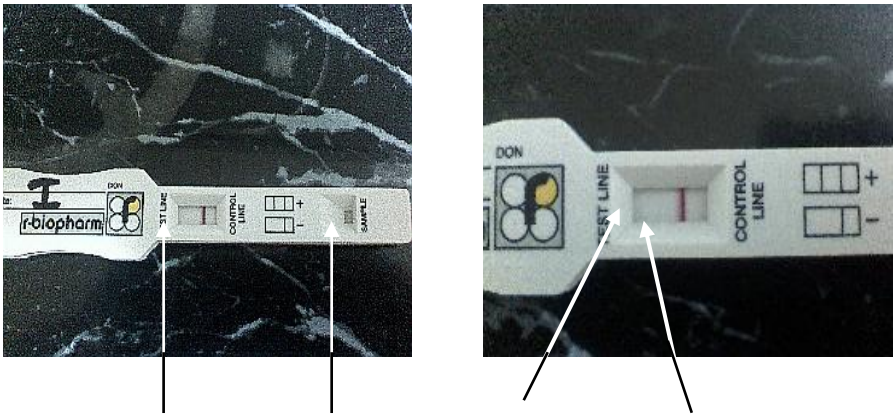

Figure 1. Kit for rapid determination of contamination with mycotoxins

RESULTS AND DISCUSSION

Moisture content and presence of investigated mycotoxins in corn samples are presented in Table 1. Technique used for analyses is assigned for the screening of mycotoxins in cereals. LOD was under 1,7 μg/kg for aflatoxins, under 0,222 mg/kg for fumonisin and under 0,2 mg/kg for DON.

Intensive investigations on the quantitative presence of *Fuzarium spp*. mycotoxins in food and feed based on cereals were performed during the past century and they are continuing now-a-days. Limited levels for cereals and cereal products in Canada, former SSSR and USA for DON were in the range from 500 to 2000 μg/kg for food, up to 4000 μg/kg for feed ingredients. These levels for ZEA in particular countries for some cereals and cereal products were as follows:200 μg/kg for corn in Brazil, 3 μg/kg for all kinds of food in Romania and 1000 μg/kg for cereals in the former SSSR [7]. Data obtained from investigations on the quantitative presence of ZEA in corn, rice, nuts and feed in other

countries in the world and Europe including Egypt, Danmark, Kenya, Taiwan and France, were less than 1000 μg/kg for cereals and food, but higher for feed [7].
According to the recommendation of the European Commission on the presence of different mycotoxins in products intended for animal feeding, the guidance value for DON is in the range from 0,9-12 mg/kg for particular products intended for particular feed, for ZEA is from 0,1-3 mg/kg and for fumonisin B1+B2 from 5-60 mg/kg [8].
The common requirements for food and feed safety according domestic regulations are presented in Table 3.

Table 1. Moisture content and presence of mycotoxins in corn samples

Sample no.	**Moisture content (%)**	**Mycotoxin content**		
		Fumonisins	**Aflatoxins**	**DON**
1	14.58	not detected	not detected	not detected
2	14.69	not detected	not detected	not detected
3	14.08	not detected	4 ppb	not detected
4	13.89	not detected	not detected	not detected
5	13.75	not detected	not detected	not detected
6	13.33	not detected	not detected	not detected
7	14.22	not detected	not detected	not detected
8	14.79	not detected	not detected	not detected
9	11.45	not detected	not detected	not detected
10	10.94	not detected	not detected	not detected
11	13.59	not detected	not detected	not detected
12	14.07	not detected	not detected	not detected
13	16.81	1-2 ppm	not detected	not analysed
14	12.58	not detected	not detected	not analysed
15	11.89	not detected	not detected	not analysed
16	11.75	not detected	not detected	not analysed
17	13.67	not detected	not detected	not analysed
18	13.69	not detected	not detected	not analysed
19	10.18	not detected	not detected	not analysed
20	11.76	not detected	not detected	not analysed

Table 2. Moisture content and presence of DON in cereal samples

Sample no	Material	Moisture content (%)	DON content
1	Oat Slavuj, conventional production	10,53	0.300 ppm
2	Oat Slavuj, organic production	10,47	0.300 ppm
3	Wheat Treska from Kochani region	11,30	0.300 ppm
4	Wheat Treska from Skopje region	10,85	not detected
5	Wheat Milenka from Sv. Nikole region	10,40	not detected
6	Wheat Radika from Ovche Pole region	10,35	not detected
7	Wheat Radika from Kochani region	10,70	0.300 ppm

Intensive investigations on the quantitative presence of *Fuzarium spp*. mycotoxins in food and feed based on cereals were performed during the past century and they are continuing nowdays. Limited levels for cereals and cereal products in Canada, former SSSR and USA for DON were in the range from 500 to 2000 μg/kg for food, up to 4000 μg/kg for feed ingredients. These levels for ZEA in particular countries for some cereals and cereal products were as follows:200 μg/kg for corn in Brazil, 3 μg/kg for all kinds of food in Romania and 1000 μg/kg for cereals in the former SSSR [7]. Data obtained from investigations on the quantitative presence of ZEA in corn, rice, nuts and feed in other countries in the world and Europe including Egypt, Denmark, Kenya, Taywan and France, were less than 1000 μg/kg for cereals and food, but higher for feed [7].
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The common requirements for food and feed safety according domestic regulations are presented in Table 3.

*Table 3. Allowed values of mycotoxins in cereals and feed**

Mycotoxin/Product	**Maximal level**	
Aflatoxin (µg/kg) Processed cereals	B_1	$B_1+B_2+G_1+G_2$
Unprocessed cereals	2.0	4.0
Processed corn	5.0	10.0
Feed (mg/kg)	0.05	
DON (µg/kg) Unprocessed cereals exept durum wheat, oat and corn Unprocessed durum wheat and Unprocessed corn	1250 1750 -	
Fumonizin (µg/kg) Unprocessed corn	4000	

*According to the Regulation of the common requirements for food safety (Macedonian Official Register No.54/2002) and to the Regulation of feed quality (Macedonian Official Register No.15/1989).

According to the Regulation of common requirements for food safety (Official paper of RM No. 54/2002) for the maximal alloweded levels of particular mycotoxins in food which are harmonized with the EU regulation (EC) No. 1881/2006 [9], as well as according to the domestic Regulation of feed quality and that of the European Union, the determined values for the mycotoxins in cereal samples were in the allowed level for the aflatoxins, fumonizin, and for DON.

CONCLUSIONS

From our investigations the following can be concluded:

- Among twenty corn samples analysed, only two were contaminated, one with fumonizin and the other with aflatoxin, and two samples were contaminated with DON, both of oat and of wheat.

- The application of the rapid technique for determination of the contamination of cereal food/feed with mycotoxins enable its rapid, qualitative/quantitative determination.
- It should be continued with the investigations on the contamination of cereals in the food chain in order its real cause and the influence factors for its appearing to be conformed.

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TECHNOLOGICAL PROPERTIES AND PREPARATION OF OLIVE CAKE AS A FEED COMPONENT

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ABSTRACT

Based on its chemical composition olive cake could be a valuable raw material for producing useful organic components, for energy generation, but as animal feed component as well. Olive cake obtained from three – phase decanter process was used in this research. Chemical composition of olive cake was determined, after which was the cake dried using four different air temperatures (40, 60, 80 i 100°C) with the aim to calculate activation energy, that is starting energy for releasing water. To avoid problems with storing fresh and easily spoiled olive cake, and to improve its technological properties (to ease storage, transport and packing) olive cake was pelletized after drying. At last the basic quality parameters were determined for the obtained pellets and the possibility of olive cake utilization in animal feed was evaluated.
***Keywords**: Olive cake, chemical composition, animal feed, pellets*

INTRODUCTION

Olive trees cover the area of about 10 million ha, from which around 10 million tons of olive fruit can be harvested. Nine million is used for olive oil production, and one for the table olives Europe is the greatest olive oil producer and covers around 80% of the whole world production [1]. 98% of olive trees are currently grown within the Mediterranean area where olive products account for about 25% of the farming income Due to its high price, olive oil represents a significant share in the European market, and it is even comparable with the total trade of oil crops [7].

In the recent years olive tree cultivation has significantly grown, therefore in Croatia, around 30 000 tons of olives are produced per year. In the olive oil production process large quantities of waste are generated in a form of olive cake (as a solid residue) and vegetation water (as a liquid residue). From 100 kg of olives, by three-phase processing system, 50 kg of fresh olive cake is obtained [6]. Utilisation of this kind of waste is becoming a larger issue each day. Opening of many little oil mills and olive orchard planting, due to good stimulations and funds from EU, is contributing to that problem. The result is uncontrolled disposal of olive cake in the environment, which has a negative influence not only on the environment, but on the tourism as well; because of the unpleasant odour that derives form this kind of waste.

Olive cake consists of fruit skin, pulp and pit fragments, and the main chemical ingredients are cellulose, proteins, poliphenols and water. Water content varies with the process used for oil production, thus in olive cake obtained by pressing process is lower than in the one obtained by centrifugation. Chemical composition of olive cake depends on the sort, condition and origin of the olives, as well as on the processing used.

Parameter which varies the most is oil content as it most dependable on the sort, cultivation conditions and climate conditions [8].
Regarding its chemical composition, olive cake can present s valuable raw material which could be used for useful organic components production, energy generation but also as a component in animal feed [5]. Since olive cake production is periodical – it is generated only in explicit time of the year, it has to be stored so it would be available for use and so it would maintain its chemical properties. Due to high moisture content, fresh olive cake has very limited storage time, without any chemical changes. Olive cake obtained by centrifugation process deteriorates after four or five days, and the one generating from centrifugation process, after around two weeks. The reason for that is higher moisture content. Olive cake dried to approximately 10% of moisture can be stored for about 45 days, while exhausted and dry olive cake can be stored for more than a year [11].
Storing of olive cake presents a problem due to large space it requires. To avoid the problems with olive cake storing it is necessary to define an optimal process which would simplify and prolong the storage period [9]. That can be achieved by drying and densification (pelleting) of the raw material. Pelleting is a thermoplastic procedure which modifies the particles by pressing them with binder in a cylindrical form, called pellet, and the process is done by a pellet mill. Moisture content of the raw material can be up to 15%, to maintain the pellet durability. Therefore, olive cake needs to be dried before pelleting process. Also it is recommendable to grind the raw material before pelleting, to achieve a fine and even structure, as the particle fineness affects the quality and durability of the pellets. Pellets are compact and therefore easier for handling, with an advantage of having grater density, which decreases transport and storing expenses [12].
The aim of this research was to investigate technological characteristics of olive cake, since knowing these parameters is necessary for possible industrial scale processing of olive cake for the needs of animal feed production. The process of olive cake preparation for storing, by drying and pelleting, has also been investigated, and basic pellet quality parameters have been determined.

MATERIALS AND METHODS

Olive cake from Istria in Croatia, obtained directly from the oil mill, equipped with three-phase decanter, was used in this research. Olive cake was dried from the starting 44,69 % moisture to average 12%, using four different drying air temperatures (40, 60, 80 and 100°C). Drying was conducted using a laboratory drier of 1kW power, in stationary bed, with the air velocity of 1 m/s. During the drying process a loss in sample mass was measured by scale. On the basis of drying procedure analyses, activation energy was calculated, through drying constant (k), determined during drying. Activation energy (Ea) is the energy necessary for water molecules to chemically interact, and it is calculated through given formula [14].

$$\ln k = \frac{-Ea}{R} \cdot \frac{1}{T} + \ln A$$

Where is:

R – Universal gas constant,
T – Drying temperature (K),
A – Exponential factor.

The following analyses, using given methods, were conducted on the olive cake: pH of fresh olive cake (ISO 10390:2005), moisture content (drying at 105°C, 4 hours), oil content (ISO 734-1:2006), ash content (ISO 763:2003), organic matter content (calculation by loss on ignition at 550°C), protein content (Kjeldahl method), fibre content (HRN ISO 6865:2001). Content of calcium, potassium, magnesium and sodium was determined using atomic absorption spectrometry with sample preparation in microwave oven (CEN/TS 15290:2006, CEN/TS 15297:2006), while the contents of nitrogen and carbon were determined simultaneously, by dry incineration using Vario, Macro CHNS Elementar, according to CEN/TS 15104:2005.
Dry olive cake was milled using hammer mill equipped with a 4 mm diameter sieve. On the laboratory sieve–shaker, according to CEN/TS 15149-2:2006, granular structure was determined. After that olive cake was pelletized by a flat die pellet press (Flat die pellet press 14-175, AMANDUS KAHL GmbH & Co. KG, Germany). Diameter of pellet die openings was 6 mm, with die thickness of 20 mm (channel length 18 mm).
Analyses of the basic quality were conducted on the olive cake pellets: moisture and ash (according to above mentioned analyses), particle density (Hydrostatic method), bulk density (CEN/TS 15103:2005), pellet hardness (Pellet hardness tester, AMANDUS KAHL GmbH & Co. KG, Germany), durability (CEN/TS 15210-1:2005) and energetic value (CEN/TS 14918:2005).

RESULTS AND DISSCUSION

Considering that fresh olive cake is a very wet material, before milling, pelleting and storing, it has to be dried to average 12% of moisture. To calculate the activation energy during drying process and to estimate required energy for drying, the drying process was conducted at four different air temperatures. The results of the drying process are presented graphically (Diagram 1 and 2) and they are displaying a curve of moisture loss by drying.

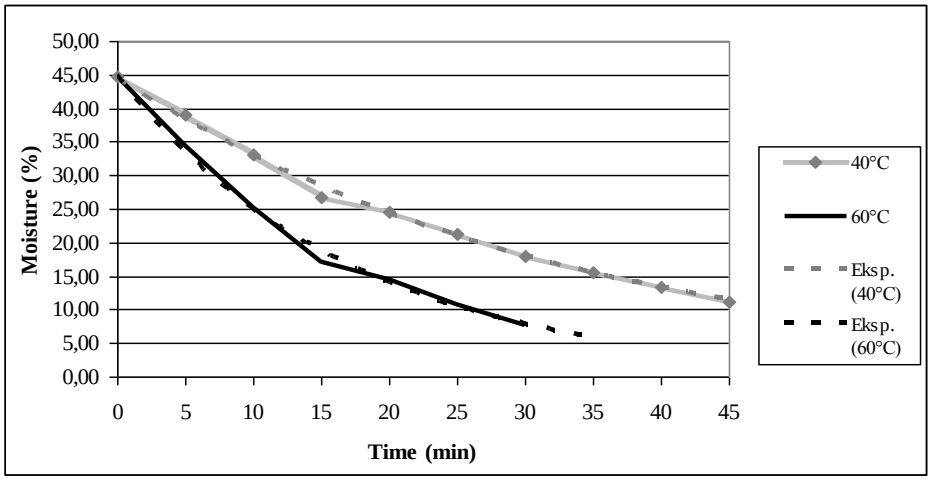

Diagram1. Drying at 40°C and 60°C air temperature

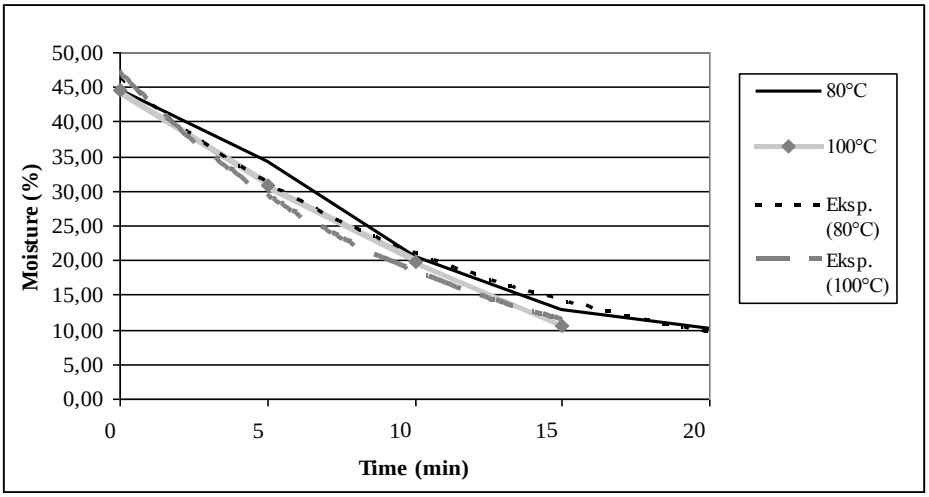

Diagram2. Drying at 60°C and 80°C air temperature

Based on data measured during drying, reaction rate of moisture loss was determined (Table 1) as well as activation energy of 34,35 kJ/mol, which is somewhat higher than the one given in the literature. Namely, Doymaz et all (2004) [3] have determined activation energy value of 26,71 kJ/mol, while the values of activation energy of in example, corn are between 11,01 i 13,80 kJ/mol [14].

Table 1. Coefficient of moisture loss

Drying temperature (°C)	40	60	80	100
k (min^{-1})	0,06685	0,07469	0,07892	0,07984

Dry olive cake has a mealy aggregate structure, the cake clods are heavy to break by hand and sharp parts of pit can be noted. The colour of cake is dark brown, has a characteristic scent on olive oil, but less expressed than in fresh cake. Considering the size of olive cake agglomerates (3 to 6 cm) it is clear that it is necessary to mill it, for the usage in animal feed and pelleting. Based on the granulometric analyses of milled cake, cumulative and selective diagram was made (Diagram 3) from which is possible to note an unsuitable relation between rough and medium sized particles. A cause for that are physical characteristics of olive cake, which contains large proportion of pit parts. Uniformity module of the raw material was 42:49:9, while the medium diameter was 0,718 mm. These results are leading to conclusion that another mill type (roller mill for example) would be more suitable for olive cake milling, in order for pelleting process to be better.

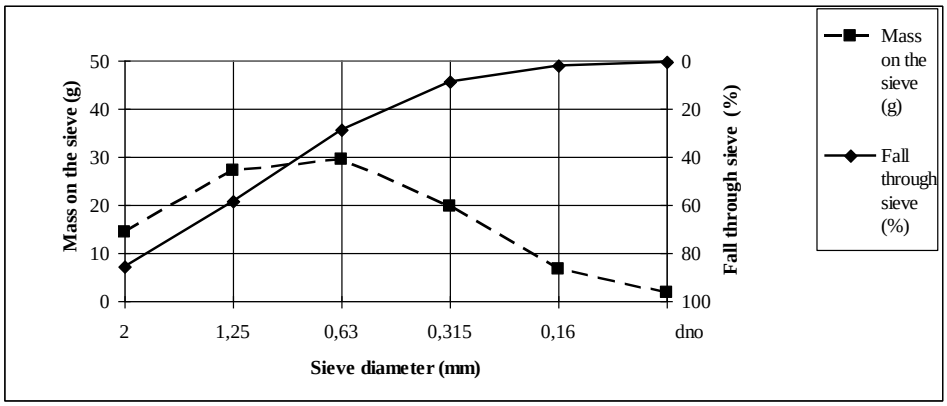

Diagram 3. Cumulative and selective diagram

Based on the chemical composition of olive cake, shown in table 3, it was determined that olive cake, as an animal feed component, is a high fibre feed, considering it has more than 25 % of fibre. As such it can be used in ruminant feeding [13, 2], whose rumen microbiological digestion enables utilization of polysaccharide β band, like cellulose and hemicellulose, who contain most of the energy of this type of feed. It is also determined a favourable oil content of 6,16% which enhances the energetic value of olive cake as animal feed. The cake contained 5,94% of proteins, which is in agreement with the literature, where the values between 3 and 8% are given [10].

Table 2. Chemical compostion of olive cake

Parameter	**Olive cake (% dry weight)**
pH	7,10
Moisture	44,69 (fresh wight)
Ash	1,83
Organic matter	98,17
Oil	6,16
Protein	5,94
Fibre	53,12
N (total)	0,95
C (total)	55,46
Ca	0,33
K	0,30
Mg	0,02
Na	0,74

Most of the analyzed parameters are in the ranges available in the literature [9, 10]. Only the values for metal content (Ca, K, Mg, and Na) are somewhat lower than those given by literature [10].
At the end olive cake, containing approximately 12% of moisture, was pelletized. Pelleting improves microbiological properties of feed as well as digestibility of starch and fibre [4]. It has been determined that olive cake can be easily pelletized with this moisture content, due to oil that it contains. Granular structure has not posed a problem in pelleting process, despite larger content of rough particles. The basic pellet quality parameters are presented in the table 3.

Table 3. Pellet quality analyses

Parameter	**Olive cake (dry weight)**
Moisture	6,65 % (fresh weight)
Ash	1,70 %
Durability	9,80 %
Particle density	1,20 kg/dm^3
Bulk density	0,590 kg/l
Hardness	10,3 KH[a]
Energy value	21 450 kJ/kg

[a] KH-Kahl Hardness-relative units of hardness read off the pellet hardness tester

After natural cooling, pellets had a moisture content of 6,65%. It has been indicated that energy value of the obtained pellets was high, with a low percentage of ash. The values of other quality indicators are within the ranges for animal feed usage, available in the literature.

CONCLUSION

By drying olive cake in this research somewhat higher activation energy was determined (34,35 kJ/mol), than the one given by the literature (26,71 kJ/mol), which was caused by olive sort, as well as the technology for olive oil production. Drying of olive cake is necessary for its pelleting and utilization in animal feed, and based on the calculated activation energy it is possible to estimate the amount of energy needed for this process.
Chemical analyses of the cake showed that based on its characteristics, olive cake can be used in ruminant feeding, but in controlled amounts.
Pelleting of olive cake is done easily without additional substances, thanks to oil content of 6% that it contains, and pellets of satisfactory quality are obtained.

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DEHYDRATED SUGAR BEET MOLASSES AS SUITABLE RAW MATERIAL IN THE PRODUCTION OF ANIMAL FEED

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ABCTRACT

The limiting factor for wider usage of sugar beet molasses as a component of feed is a large viscosity of molasses, especially at lower temperatures. This creates various problems for its industrial applications and requires special devices for transport. Solution for these problems would be dehydration of molasses, in other words, its translation into powder form.
This paper investigated the possibility of using various starchy raw materials for the dehydration of molasses with a view to increase its usage in feeding different categories of animals. At the same time, starchy materials, used in experiment are regular ingredients in animal nutrition – corn grout, wholegrain wheat flour, wheat middlings and corn middlings. The results indicate that the particle size distribution and the amount of starch in the raw materials affect on quality of the molasses mixture. The best appearance, flowability and the lowest moisture content was achieved in the samples of molasses with wholegrain wheat flour.
Keywords: *sugar beet molasses, dehydration, starchy materials*

INTRODUCTION

Sugar beet molasses is a concentrated liquid extract that is a by-product of sugar refining. It has high dry matter content (80%) and contains, on average, about 51% sucrose, 1% raffinose, 0.25% glucose and fructose, 5% protein, 6% betaine, 1.5% nucleosides, purine and pyrimidine bases, organic acids and pectin [20].
In addition to the already mentioned compounds, sugar beet molasses is a good source of many micronutrients (vitamins and minerals), especially potassium, calcium, sodium and magnesium. Very important fact is that all mineral components of molasses are dissolved and that the potassium is in much greater quantities than all other cations with a share of 75% [20].
Molasses, as a feed component, has a pleasant taste and it is a good energy source. In addition to its usage as an energy source, molasses has the following purposes: 1) as appetiser (stimulate appetite), 2) to reduce pulverulent of the meal, 3) as a binder, 4) to stimulate microbial activity in rumen of ruminants, 5) for supplying animals with microelements mikroelementima [8].

Rajčan et al. [17] reported that molasses, in combination with a bulky food, could have a better biological effect in comparison with grains. Recommended amounts of molasses in diets for cattle are 5-10%, while the calves use smaller amounts, concerning the laxative effect of molasses. For pigs and poultry, molasses is usually given through the forage mixture in quantities up to 6%.

Molasses is used for correcting of taste of rough feed ingredients. For this purpose, molasses is dissolved in warm water in the ratio 1:4 and used for spraying over the feed. Feeds spattered with molasses become tastier and less powdered. In that way animals better accept and consume corn straw, straw, malt sprouts and other less palatable feeds [3].

Molasses is a good binder during pelleting of industrial fodder mixtures, usually in quantities up to 5%. It is also grateful material for adding during silage [19].

As sugar beet molasses is a liquid and contains a relatively high percentage of water (about 20%), there are certain difficulties in her handling [10]. Adding of molasses in liquid form leads to the inevitable losses due to a clinging viscous, sticky mass on the walls of package, which has limited the possibility of its practical application, especially in systems with high content of fats and oils [11].

Taking into account all mentioned above, increasing of dry matter content in sugar beet molasses would be very important [7].

For molasses dehydration, different starchy raw materials (carriers), which are, also, the basic components of the animal diet (corn, wheat, corn middlings and wheat middlings) can be used [10, 11, 12].

Corn is the most important chunky feedstuff and, due to the high content of starch, relatively high presence of oil and a small amount of fiber, it is considered as high energy feed [3].

Chemical composition of corn varies depending on the type of hybrids and growing conditions. It contains a high percentage of starch (62%), crude protein (8.1%), crude fat (3.7%), pento, carbohydrates, fiber, etc. Rate of amino acids in corn is also variable. The most abundant amino acid in corn are valine, arginine, isoleucine and lysine [3, 4].

Wheat is primarily used in human nutrition and, in lesser extent, in animal nutrition (20-28%). In recent years, there is a growing tendency for wheat usage in animal feed, due to the high nutritional value of grains and bran and the profitability of the production [3].

According to its nutritive value, it is quite similar to corn, contains more protein (10.5%), less fat (1.5%) and starch (59%) [4].

It can be concluded that wheat is a tasty feed that animals like to eat. Jokić et al. [6] recommended that rate of wheat in poultry diets, age of 1-4 weeks, is max 20% and for the older up to 25%.

Corn middlings is present in the market of animal feed, in lesser extent in comparison with wheat middlings. According to the "Pravilnik o kvalitetu i drugim zahtevima za hranu za životinje" [13] corn middlings is light yellow product obtained by grinding the corn grain, which smells like corn fodder, without bitterness and rancidity. It must contain at least 8% of crude protein and 58% of starch, with maximum of 14% humidity.

Wheat middlings is light brown or reddish product obtained in the process of milling wheat, without bitterness, rancidity and sour taste. Granulation of wheat middlings is such that 95% passes through a square sieve size of 1 mm aperture and the rest through a square sieve with aperture of 2 mm. According to the "Pravilnik o kvalitetu i drugim

zahtevima za hranu za životinje" [13] wheat middlings should contain at least 12% of crude protein and 30% of starch, with maximum of 14.5% of moisture and 8% of crude fiber.

MATERIAL AND METHODS

Sugar beet molasses, used in the experiment, is obtained from sugar factories Pećinci, Serbia.
For dehydration of molasses corn grits, wholegrain wheat flour, corn middlings and wheat middlings were used.
Dehydrated molasses was made by mixing 100g of pure, pre-heated (40 ° C), molasses, and appropriate amounts of these starchy materials (Table 1). After combining these components in the mixer (Forberg, Norway), their mixing lasted an extra 2 minutes.

Table 1. Experimental design

Wholegrain wheat flour (WWF)	**0**	**1**	**2**	**3**	**4**	**5**	**6**
	0g of molasses + 1000g of WWF	100g of molasses + 1000g of WWF	100g of molasses + 500g of WWF	100g of molasses + 330g of WWF	100g of molasses + 250g of WWF	100g of molasses + 200g of WWF	100g of molasses + 160g of WWF
Wheat middlings (WM)	**7**	**8**	**9**	**10**	**11**	**12**	**13**
	0g of molasses + 1000g of WM	100g of molasses + 1000g of WM	100g of molasses + 500g of WM	100g of molasses + 330g of WM	100g of molasses + 250g of WM	100g of molasses + 200g of WM	100g of molasses + 160g of WM
Corn grits (CG)	**14**	**15**	**16**	**17**	**18**	**19**	**20**
	0g of molasses + 1000g of CG	100g of molasses + 1000g of CG	100g of molasses + 500g of CG	100g of molasses + 330g of CG	100g of molasses + 250g of CG	100g of molasses + 200g of CG	100g of molasses + 160g of CG
Corn middlings (CM)	**21**	**22**	**23**	**24**	**25**	**26**	**27**
	100g of molasses + 1000g of CM	100g of molasses + 1000g of CM	100g of molasses + 500g of CM	100g of molasses + 330g of CM	100g of molasses + 250g of CM	100g of molasses + 200g of CM	100g of molasses + 160g of CM

Wholegrain wheat flour was produced in the factory "Fidelinka-milling LLC Subotica, Serbia, corn grout, corn middlings and wheat middlings were purchased at the store of animal feed in Novi Sad. Mixing of the ingredients was carried out in accordance with experimental design presented in Table 1.

Before mixing, the physico-chemical and microbiological properties of raw materials we determined, namely:
- in pure molasses:

a) dry matter content *was determined refractometricaly (refractometer Abbeov, Carl*

Zeis Jenna),
b) content of sucrose, invert sugar, K, Ca, and Mg *was analyzed according to AOAC methods* [9],,

- in the starchy raw materials:
a) particle size distribution [5],
b) the total starch content *was determined by Ewers* [16],
c) water content [9],
d) water activity (aw) *was measured using aw-meter, Testo 650, TESTO-Germany,*
e) flowability *was determined by measuring the angle of repose according to method from handbook* [2],
f) the number of total microorganisms and the number of total yeasts and molds in 1 g of the sample [15].

- in the samples obtained after the mixing of raw materials:
a) water content [9]**,**
b) water activity (aw) *was measured using aw-meter, Testo 650, TESTO-Germany,*
c) flowability *was determined by measuring the angle of repose according to method from handbook* [2],
d) ***the number of total microorganisms and the number of total yeasts and molds in 1 g of the sample*** [15].

RESULTS AND DISCUSSION

1) Analysis of raw materials

a) Sugar beet molasses:

Table 2 shows the physical and chemical properties of sugar beet molasses used in the experiment.

Table 2. Physical and chemical properties of sugar beet molasses

Dry matter, %	80.65
Sucrose, %	40.76
Invert sugar, %	0.47
K, mg/100g	4120
Na, mg/100g	573
Ca, mg/100g	201
Mg, mg/100g	93

Based on the results shown in Table 2, can be concluded that the studied sample of sugar beet molasses has a high dry matter content (80%), which is accordance with published data. Taking into account the high dry matter content, molasses is microbiologically very stable and is not a suitable substrate for growth of microorganisms. Also, due to the high mineral content (K and Na), molasses is nutritionally very rich and very useful if it is

added as a component in feed. Molasses with such characteristics is a high quality supplement in the daily diet of domestic animals.

b) Starchy materials (wholegrain wheat flour, corn grout, corn middlings and wheat middlings):

Figure 1 shows the granulation of certain raw materials.

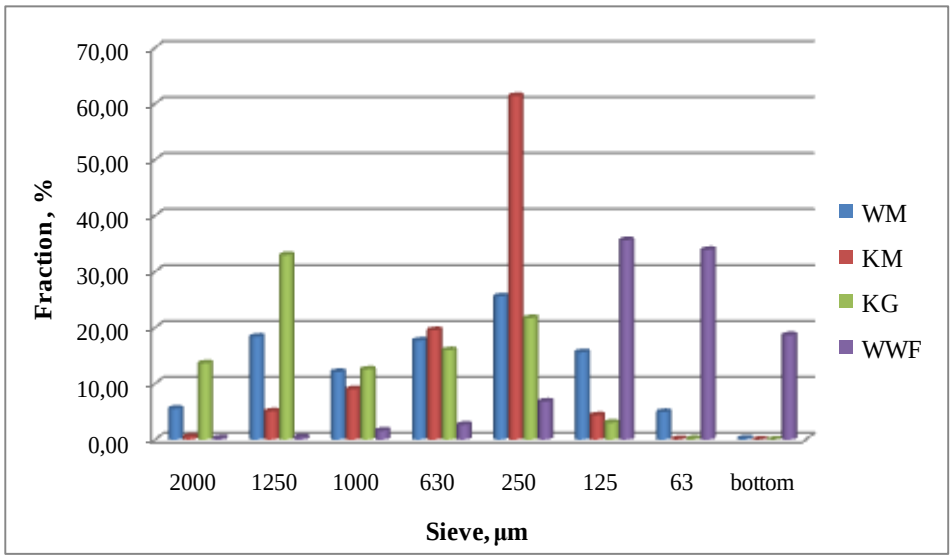

Figure 1. Particle size distribution of row materials

Corn and wholegrain wheat flour have similar particle size distribution - the largest amount of material is size of 250-630 µm. Corn grout has higher rate of coarse fraction while the wholegrain wheat flour has more than 80% of fraction which size is less than 125 micron. Particle size of starchy materials had an influence on appearance, stickiness and flow of the mixture.

Determination of moisture content is the most common chemical analysis of feed. It is important to accurately determine the moisture content in feed for several reasons: 1) the water content in the diet has a significant impact on animal growth and productivity, 2) water content influences on transport costs, 3) the water content affects the sustainability of feed, 4) water is a solvent for feed components, etc. [21].

The content and quality of water in the feed are very important because of their influence on the activity of microorganisms and development of insects. The total water content in the feed does not affect on microbiological stability but essential is amount of water available to microorganisms. The best example is molasses, which contains 20-30% water but inaccessible to microorganisms. The ability of moisture to support growth of microorganisms, especially molds, depends on water activity and its content in feed [1].

Table 3 shows values of starch content, moisture content and water activity values of raw materials. The measured values of moisture content in starchy raw materials are consistent with published data. The measured values of water activity of all the raw materials are in range from 0.566 (0) to 0.683 (7). This range of a_w values allows the growth of certain species of yeast (osmophile) and molds (xerophile). Starch content is the lowest in wheat middlings, which was expected since it contains a significant amount of peel. Starch content in wholegrain wheat flour and its granulation contributed to that, after mixing with molasses, obtained dry products have better flowability compared to other samples.

Table 3. Starch content, moisture content and water activity in starchy materials

	Wholegrain wheat flour (0)	***Wheat middlings (7)***	***Corn grout (14)***	***Corn middlings (21)***
Starch content according to Ewers (%)	68.53	32.82	63.38	49.51
Moisture content (%)	11.51	13.02	12.01	12.46
Water activity (a_w)	0.566	0.683	0.597	0.657

2) Analysis of samples obtained after mixing of raw materials

Table 4 shows the moisture content and a_w value for all samples of starch materials after mixing with molasses (the number of samples is in accordance to Table 1).

Table 4. Moisture content and water activity in the samples obtained by mixing starchy raw materials with molasses

Sample No.	***1***	***2***	***3***	***4***	***5***	***6***
Moisture content (%)	11.88	12.75	13.13	13.62	13.72	13.56
Water activity (a_w)	0.626	0.622	0.645	0.651	0.658	0.656
Sample No.	***8***	***9***	***10***	***11***	***12***	***13***
Moisture content (%)	13.33	13.55	13.60	14.27	14.27	14.37
Water activity (a_w)	0.685	0.688	0.681	0.679	0.656	0.678
Sample No.	***15***	***16***	***17***	***18***	***19***	***20***
Moisture content (%)	13.05	13.23	12.77	12.15	12.34	11.81
Water activity (a_w)	0.689	0.700	0.678	0.683	0.695	0.662
Sample No.	***22***	***23***	***24***	***25***	***26***	***27***
Moisture content (%)	13.11	13.46	14.04	14.11	14.35	14.02
Water activity (a_w)	0.691	0.679	0.664	0.677	0.673	0.662

The moisture content in the samples, obtained after mixing molasses with starchy raw materials, was significantly reduced compared to the moisture content measured in the starting molasses (19.35%).

The values of water activity in mixtures were slightly higher (0.622 to 0.700) compared to the values observed in pure starchy raw materials but enters in range of values that allows the growth of yeasts and molds but not bacteria's. Based on these values, can be assumed that the storage stability of all samples is satisfactory.
Angle of repose is an important physical characteristic that characterizes flowability of powders. Test is commonly used to determine the flow of materials by measuring the angle of repose. Angle of repose is defined as the maximum angle (in degrees) at which the cup material retains its slope. If the values of angle are smaller, flow of the material is better. Lower flows of the material require steeper hoppers and smooth interior walls of the cells and baskets [2]. Determination of flow is necessary to define the shape and size of silos, the ways of their charge and discharge as well as the choice of equipment.
If the value of the angle of repose is less than 35° flow is good, in the range of 36-40° flow is satisfactory, from 41-45 ° acceptable and more than 46 ° is poor [2].
Table 5 shows the values of the angles of repose (°) of starchy raw materials prior to their mixing with molasses and table 6 shows values of the angles of repose of the samples obtained after mixing the basic raw materials.
Comparing the results in Tables 5 and 6, can be noted slightly lower values of angles in samples after mixing compared to the values obtained in pure starchy materials. Since the angle of repose in all treated samples (Tables 5 and 6) are in the range from 15.38 on (16) to 29.5^{o} (21), can be concluded that their flowability is good.
Angle of repose for samples 19 and 20 was not measured because the samples were in the form of sticky mass that couldn't go through the funnel.

Table 5. Angle of repose of starchy raw materials prior mixing with molasses

	Wholegrain wheat flour (0)	***Wheat middlings (7)***	***Corn grout (14)***	***Corn middlings (21)***
Angle of repose (°)	25.64	23.27	22.54	29.25

Table 6. Angle of repose in the samples obtained by mixing raw starch and molasses

Sample No.	***1***	***2***	***3***	***4***	***5***	***6***
Angle of repose (°)	21.55	20.56	20.81	20.30	20.56	21.06
Sample No.	***8***	***9***	***10***	***11***	***12***	***13***
Angle of repose (°)	19.54	20.81	20.81	21.80	22.29	24.47
Sample No.	***15***	***16***	***17***	***18***	***19***	***20***
Angle of repose (°)	16.44	15.38	19.03	24.94	-	-
Sample No.	***22***	***23***	***24***	***25***	***26***	***27***
Angle of repose (°)	20.56	16.44	16.44	16.17	15.91	18.78

Good quality feed, in addition to regular feed composition, must be microbiologically safe. Food microbiology research is wide research area and includes microorganisms used in food production and a large number of pathogenic microorganisms, yeasts and molds that cause various diseases in plants, animals and people. The presence of

pathogenic bacteria in feed may cause disease of animals, reduced productivity, but also animals can be asymptomatic carriers of pathogens to other animals or even humans. Molds contamination can occur feed at all stages of production, processing, storage and use. Grains, as raw material, can be contaminated in the field, during transport and storage [18].
The number of total microorganisms, yeasts and molds were determined by the "Pravilnik o metodama vršenja mikrobioloških analiza i superanaliza životnih namirnica" [15].
Rating of microbiological safety of treated samples was performed according to the "Pravilnik o maksimalnim količinama štetnih materija i sastojaka u stočnoj hrani" [14].

Table 7. Changes in microbiological properties of starchy materials before and after mixing with sugar beet molasses

	Number of total yeasts and molds in 1g		***Number of total microorganisms in 1g***	
Sample No.	***After 48 hours***	***After 15 days***	***After 48 hours***	***After 15 days***
0	1000	1 000	40 000	45 000
6	100	200	60 000	70 000
7	12 000	2 000	1 100 000	1 500 000
13	4 000	2 000	650 000	650 000
14	20 000	8 000	5 000	6 000
20	2 000	2 000	10 000	12 000
21	25 000	8 000	200 000	20 000
27	10 000	8 000	14 000	6 000

The total number of yeasts and molds during storage, in all analyzed samples, was decreased. On the other hand, the total number of microorganisms in most samples was increased during 15 days of storage. Regardless of the previous statements, the results in the Table 7 show that all samples, in terms of microbiological safety, meet the requirements of the "Pravilnik o maksimalnim količinama štetnih materija i sastojaka u stočnoj hrani" [14].

CONCLUSION

Using wholegrain wheat flour, wheat middlings and corn middlings sugar beet molasses can be obtained dehydrated product that contains 38.5% molasses (samples with 160 g of raw material and 100 g molasses). In the case of mixing corn grout and molasses, dehydrated product was obtained when 29% molasses was added (Sample No. 18 - 250 g of raw material and 100 g of molasses). Manipulation of these products in industry application is easier because of their very good flow. This is important for manufacturers of feed, because with the dry mixture into the final product animal can take the required quantity of sugar beet molasses as a valuable component in the diet of all categories of animals.

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PHYSICAL MEASURES FOR STORAGE INSECTS CONTROL

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ABSTRACT

Besides quality and value preservation of stored products, food safety also imposes itself as the highest demand to be met when control measures for insect pests are launched. Although control measures still predominantly rely on conventional insecticides, fumigants and contact insecticides, concerning harmful residues in food and/or storage pests' resistance in the last years, they are oriented towards Integrated Pest Management (IPM) concept, where physical control measures for storage insects have a growing importance. Among all physical measures which may be taken against storage insects, inert dusts and extreme (low and high) temperatures are the most significant.
Inert dust effectiveness against storage insects is directly dependant on their physical and chemical properties (structure, silicon dioxide content, size and presence of particles, pH values, absorptive capacity and geographical origin), but also on their impact on mass density of stored wheat, particle subsidence and binding to wheat surface. It is also dependant on morphological, physiological and ecological properties of every insect species and on environmental conditions and properties of the substrate being protected in the storages.
Optimal temperatures for storage insects are in the range of 25-33 °C, suboptimal 13-25 °C and 33-35 °C, and lethal are below 13 °C and over 35 °C, whereas subject to temperature value and exposition time, the speed of death is measured in minutes or weeks. Practically both, low and high temperatures in storages can be applied either through active ventilation during cold and/or hot months, or by insufflation by specially designed devices and equipment. The drawback of low temperatures can be an increase of humidity of substrate, and of high temperatures (especially > 50 °C) the risk of equipment and stored products damaging.
As physical measures for storage insects control, inert dusts and extreme temperatures are safer than conventional insecticides and have no negative impact on quality of stored products, and it is for this that their use will be increased in the future.
***Key words**: storage insects, inert dusts, extreme temperatures*

INTRODUCTION

The basic task of everyone included in the storing of wheat and other plant products, is preservation of goods quality and value, and the management focus is moved from production to processing and storing of products which will be consumed, and this is where the food safety imposes itself as the highest demand to be met [55]. Protection of stored plant products from insect pests today still heavily relies on application of insecticides, fumigants and contact (residual) insecticides. Yet, after their application harmful residues remain in food and/or over time resistance of insects appears, which leads to the increase of application rate and additional pollution of the environment. For

these reasons, contemporary protection of stored products is in recent years focused on the implementation of the concept of Integrated Pest Management (IPM), where physical measures for stored-product insects control have a growing importance [31, 69, 36].
In general, physical measures for control of insect pests in agriculture can be referred to as passive (covering, enclosing, covering by so – called film, the use of inert dusts and oils), active (mechanical and thermal) and combined (cold storing, use of hot air and flame and immersion in hot water), and in food storages preventive and/or direct. However, they all involve change of environmental conditions (temperature, relative air humidity and substrate humidity) and have completely different mode of action on storage insects compared to conventional insecticides, because they slow down or prevent their development, or produce lethal effects, with simultaneous insignificant or little effect on treated products and environment. Among all physical measures which may be taken on storage insects, inert dusts and extreme (low and high) temperatures are the most significant in practice [30, 61, 18, 69, 13].
The aim of this paper is to underline the role and significance of inert dusts and extreme temperatures in protection of stored plant products from insect pests, especially from the aspect of quality preservation and food safety improvement.

INERT DUSTS

Inert dusts are of natural origin and usually have no impact on the quality of treated products. Although their insecticide action is familiar since ancient civilizations, it was only during the last 20 years that they became the subject of important several studies on their potential for commercial use.
According to characteristics, inert dusts can be classified into four groups: 1) the first group include: clay, sand, ashes created by burning of various plants and volcanic dust, and it has a long tradition of use in Asian and African countries, 2) in this group fall minerals such as dolomite, magnesite, calcium carbonate and various phosphates, but because of their high application rate (> 10 g/kg) they are no longer in use for treatment of stored products, 3) dusts with synthetic silicate content, and 4) dusts containing natural silicates, where diatomaceous earth and natural zeolite belong. It is known that inert dusts have low mammal toxicity (except possible damage to respiratory organs in people who are directly exposed to dust application), act slower than classic insecticides, and the death of storage insects appears as a consequence of: 1) spiracles blocking, 2) water loss for cuticle damage, 3) binding of water from the cuticle, 4) suffocation provoked by small particles inhalation (intake), and 5) binding of lipids from epicuticular layer. Among all inert dusts diatomaceous earth has the highest practical significance and in many countries it is an integral part of program for stored products protection from insect pests [32, 45, 61, 31].

Properties and insecticidal potential of inert dusts

Effectiveness of inert dusts for insect directly depends on their physical and chemical properties (structure, silicon dioxide content (SiO_2), size and presence of particles, pH values, absorptive capacity and geographical origin), and also on their impact on mass density of stored wheat, particles subsidence and binding to wheat surface [61].

It is a common knowledge that dusts with amorphous structure, like diatomaceous earth (DE), have significantly higher efficacy than dusts with crystal structure (sand, minerals). For example, different samples of DE from Serbia applied at rate of 0.75 g/kg after 14 days of exposure in treated wheat caused high mortality (> 95%) of *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst) [39], while the dust Ekokalkon-K on the basis of limestone and sugar molasses, under similar conditions was highly efficient for *S. oryzae*, *Rhyzopertha dominica* (F.) and *T. castaneum*, but with application rate of 10 g/kg of wheat grain [42].
The content of SiO_2 in dusts has a positive correlation with efficacy, which was confirmed by Korunić [46] who examined efficacy of 43 samples of DE from different parts of the world. Researches with samples of DE with Serbian origin also showed that SiO_2 content has a significant impact on the efficacy. For example, it was determined that sample DE-S1 with SiO_2 content of 78.8% has significantly higher efficacy on adults of bean weevil *Acanthoscelides obtectus* (Say) than samples DE S-2, with 63.1% SiO_2 and DE S-3, with 46.5% SiO_2 [38]. Most products on the basis of DE available on the world market contain over 85% of amorphous SiO_2, and many commercial products also contain materials which improve their efficacy. Protect-It contains 10% of silica gel, Insecto 10% of additives, and PyriSec 1.2% of natural pyrethrin [61].
Size and percentage of presence of particles are important parameters not only for the efficacy, but also for the safety of people applying them. Researches showed that dusts with particles size of < 45 µm have significantly higher efficacy than dusts with particles sized 45-150 µm [68], and especially products in which the presence of particles sized 5-15 µm is over 95% [46, 45, 61]. However, besides smaller particles are more efficient for insects, they are potentially more hazardous to people who apply them, and therefore particles smaller than 5 µm, especially those smaller than 1 µm may cause serious health problems in humans, primarily in the respiratory system.
According to data presented by Subramanyam and Roesli [61] and Vayias et al. [68], geographical origin of dusts can also have a significant impact on the efficacy, where the sediments of DE from the sea are more effective than sediments from rivers and the land. Also, it has been noticed that dusts with pH < 7 are usually more efficient than dusts with higher pH reaction, as are dusts with higher sorption capacity of wax and lipids from insect cuticle.

Susceptibility of storage insects to inert dusts

Storage insects show different susceptibility to inert dusts, which depends on morphological, physiological and ecological features of each species. All insect features that affect dust efficacy are related to the mechanism by which insects maintain the optimum water content in the body, because it is found that insects with thinner and gentler wax layer of the cuticle are more susceptible to inert dusts. Insects smaller in size, regarding smaller body surface, such as *Cryptolestes ferrugineus* (Stephens), are significantly more susceptible than e.g. *Tribolium spp*. and *R. dominica*. Also, it is found that the storage insects from various parts of the world express different susceptibility to dusts [25, 32, 45, 61, 67].
Differences in susceptibility between species of storage insects are related to their morphological and physiological features, so among representatives of Coleoptera order,

the most susceptible to diatomaceous earth are species from *Cryptolestes* genus, somewhat less susceptible are species from *Sitophilus* and *Oryzaephilus* genus, while *R. dominica* and species from *Tribolium* genus are the least susceptible [46, 3, 4, 9]. Kljajić et al. [37] in researches with natural zeolite also confirmed the differences in susceptibility between storage weevils and beetles, concerning that the application rate of 0.25 g/kg after 14 days of exposure 100% mortality of *S. oryzae*, 20% of *R. dominica* and 79% of *T. castaneum* was registered.
It was also found that in different development stadiums and stages exists the difference in susceptibility to dusts. Younger larvae of storage insects which develop outside grain are much more susceptible than older, and larvae regardless the stadiums are much more susceptible than adults. Also, researches with DE showed that, for example younger adult stadiums of *T. castaneum* are more susceptible than older stadiums [66, 23, 61].

Environmental conditions and other factors that affect insecticide potential of inert dusts

It is known that storage insects are adapted to living in conditions of low relative air humidity and low water content in substrate, and it is for this that a minimal water loss from insect body is hard to recover. The insect death is found to onset when they loose around 60% of water or 30% of body weight which, concerning the abrasion of cuticle wax layer can be provoked by inert dusts application [25, 30, 61].
The efficacy of dusts significantly decreases with the increase of relative air or substrate humidity, which can be justified by high sorption of inert dusts and binding of water from air or substrate. Moreover, in conditions of increased humidity, storage insects recover water losses provoked by dust action faster, and also for this reason the efficacy can fail [61]. Fields and Korunić [29], after application of Dryacide and Insecto at rate 600 ppm five days after exposition of *T castaneum* recorded mortality in wheat with 12% humidity was 68% and 63%, and in wheat with 14% humidity was 36% and 18%. Arthur [3] founded that efficacy of Protect-It for *Oryzaephilus surinamensis* (L.) adults is the lowest at relative air humidity of 75%, compared to 40% and 57%, and Sathers et al. [57] that efficacy of the Dryacide seven days after *Sitophilus zeamais* (L.) exposure in maize treated with 0.1 g/kg is 75% when air humidity is 50%, and 55% when air humidity is 60%.

Table 1. Efficacy of inert dusts (diatomaceous earth-DE and zeolite) against storage insects under different application conditions

Inert dust (g/kg, g/m^2)	Substrate/surface (temperature and RH)	Test insect (L– larvae, A– adults)	Efficacy (%)	Source
DE	Wheat			Fields and
0.3 g/kg	20°C and 60-70%	*C. ferrugineus* – A	100	Korunić
	30°C		100	[29]
0.4 g/kg	20°C and 60-70%	*S. oryzae* – A	99	
	30°C		97	
0.6 g/kg	20°C and 60-70%	*T. castaneum* – A	92	
	30°C		78	
DE	Wheat			Athanassiou et al.
0.75 g/kg	26°C and 65%	*S. oryzae* – A	100	[9]
		R. dominica – A	99	
		T. confusum – A	39	
Zeolite	Wheat			Kljajić et al.
0.75 g/kg	24±1ºC and 45±5%.	*S. oryzae* – A	100	[37]
		R.. dominica – A	74	
		T. castaneum – A	100	
DE	Wheat			Kljajić et al..
0.75 g/kg	24±1°C and 60±5%	*S. oryzae* – A	96	[38, 39, 40]
		T. castaneum – A	94	
1.0 g/kg	Beans			
	24±1°C and 55±5%	*A. obtectus* – A	48	
1.0 g/kg	Maize			
	24±1°C and 60±5%	*P. interpunctella* – L	100	
DE	Barley, wheat			Athanassiou
1.0 g/kg	26°C and 55 %	*E. küehniella* – L	70	[8]
DE	Wheat			Vayias and
1.5 g/kg	26°C and 55%	*T. confusum* – L	98	Athanassiou [66]
DE	Wood			Collins and Cook
10 g/m^2	15°C and 80%	*S. granarius* – A	82	[22]
		E. küehniella – L	100	

Efficacy of inert dusts under higher temperatures significantly increases, because insects lose water more quickly [25, 61, 11]. For example, Dowdy [24] after 24 hours recovery of *T. castaneum* adults exposed to wheat treated by Insecto under 34ºC at intervals of 15 and 30 minutes, found that efficacy is around 9%, while in the same exposure intervals under 50°C, it is 27% and 62%.

Besides stated, other factors can greatly affect insecticidal potential and effectiveness of inert dusts application, such as, for example, kind of substrate, because it is well known that maize and beans contain more lipids than wheat, barley and rice, and have a higher absorption and dust inactivation potential. Therefore, this fact, as higher percentage of admixtures and impurities in grain mass also, should be kept in mind when making

decisions on application rate [61, 57, 8, 9]. Kljajić et al. [40] examined insecticidal potential and effectiveness of DE samples from Serbia against *Plodia interpunctella* (Hübner) larvae in treated maize with damaged and whole kernels under laboratory conditions. It was found that efficacy of DE S-1 and DE S-2 applied at rate 1 g/kg after 7 days of exposure, on damaged kernels is 60% and 41%, and in whole kernels 98% and 90%, respectively.
Concerning that inert dusts act slower on storage insects than conventional insecticides, it can be concluded that exposure interval is one more very important factor that affects effectiveness of inert dusts. Summarizing all results published to the day present, it can be concluded that most dusts realize high efficacy after 14 or 21 days of exposure [46, 61, 28, 37], whereupon prevention of mass progeny of insects in treated substrate, as indirect action, is pointed out as more important than direct action against their parents.

Practical use of inert dusts: advantages and disadvantages

Advantages of inert dusts application over conventional insecticides are multiple, and the most important is that they are natural materials with very low mammal toxicity, and leave no harmful residues in food. The negative impact is reflected in the reduction of hectolitre grain mass in storages, yet in high risk of possible damage to mills and other facilities in storage houses, which is a consequence of dust abrasiveness when applied in larger quantities. Recently, this problem is mitigated by substitution of older formulations with application rate over 5 g/kg, with new formulations, highly effective even at rate of 0.5-1.5 g/kg [47, 48, 61].
Researches of dust application in interaction with high temperature of 50 °C, as in combination with other natural products e.g. essential oils, entomopathogenic fungi or with new insecticides of natural origin such as spinosad and abamectin, are being conducted with an aim to reduce application rate and to increase efficacy [24, 10, 21].
Taking all information into account, for now, the biggest opportunity for significant practical use of inert dusts is in protection of stored products obtained in organic production.

EXTREME TEMPERATURES

Use of extreme temperatures (low and high) as physical measure against storage insects is becoming more and more used in practice, and it can be considered as preventive and/or direct protection measure of stored products [18, 31, 51].
It is a common knowledge that environmental conditions affect all living beings, especially poikilothermic organisms, where insects are classified, for whom temperature plays a significant role in overall physiological and biochemical processes [71, 56, 20]. For optimal functioning of all functions and smooth development, storage insects require temperature range 25-33 °C. Temperature ranges 13-25 °C and 33-35 °C are suboptimal for storage insects. Under these temperatures specific physio-metabolic processes activate in insects, due to metabolic activation, the organism fights surrounding temperature changes and tries to keep its organism at optimal functioning level as long as possible. Likewise, under suboptimal temperatures, reproduction is stopped, and development period is significantly prolonged. For storage insects temperatures below

13 °C and above 35 °C are lethal, and depending on temperature value and exposition time, speed of death is measured in minutes or weeks [34, 27].
By summarizing the results of various studies, it has been concluded that susceptibility of storage insects to low and high temperatures ranges, and that to a large extent it depends on the species and stage of insect development, substrate characteristics, the type and length of exposure. Results of effects of direct action of different temperatures on stored-product insects, applied individually or in combination with other protection measures and methods, show great advantages over conventional insecticides and distinct possibility of their commercial use [27, 30, 18].

Effects of low temperatures on storage insects

Temperatures below 0 °C lead to freezing of body liquid and hemolymph in insects and formation of ice crystals, especially in intercellular space. Insect mortality onsets due to dehydration, by turning water into ice crystals, or to mechanical cell damage by the crystals formed [27, 18].
Researches showed that storage insects, which for optimal development require temperature of 30 °C, such as *T. castaneum* and *T. confusum*, are more susceptible to low temperatures than insects which develop at temperatures below 20 °C, such as *Sitophilus granarius* (L.) and *Trogoderma granarium* (Everts). Development stages of insects express different susceptibility to low temperatures; the egg stage is the most susceptible, while susceptibility differences between larvae and adults depend on the place of development, in or outside the grain [52, 30].
The speed of action of low temperatures is affected by several factors: humidity, substrate, acclimatization in insects, and above all the value of a given temperature. Researching the effect of temperature range from –6 to 10 °C on survival of previously acclimatized insects *S. granarius, O. surinamensis* and *C. ferrugineus*, Armitage et al. [1] founded that at relative air humidity of 70%, for 99% mortality of *S. granarius* adults, at – 6 °C it takes 35 days, at 6 °C 294 days, at 10 °C 470 days. The shortest time for absolute insect mortality at – 6 °C was observed for *O. surinamensis*, 9 days, and the longest for *C. ferrugineus*, 81 days. However, Stojanović [58] founded that resistance of *S. granarius*, *S. oryzae* and *R. dominica* to low temperatures is higher if wheat humidity is increased, while Kljajić et al. [44], by direct exposure of *S. granarius* adults at -25 and -5 °C recorded that 95% mortality takes around 20 days and around 70 minutes, respectively.

Table 2. Effects of extreme temperatures on storage insects

Temperature (°C)	Substrate	Test insect (E–eggs, L–larvae, A–adults)	LT	Source
46	Flour	*T. confusum* – A	213*	Boina and
50			72	Subramanyam
60			15	[16]
50	Flour	*T. castaneum* – E	105	Mahroof and
		T. castaneum – L	432	Subramanyam
		T. castaneum – A	52	[49]
60		*T. castaneum* – E	16	
		T. castaneum – L	42	
		T. castaneum – A	19	
50	No substrate	*P. interpunctella* – L	34	Mahroof and
		P. interpunctella – A	24	Subramanyam [50]
50	Wheat	*R. dominica* – L	410	Beckett and
60			0.6	Morton
				[15]
50	No substrate	*S. granarius* – A	90	Kljajić [35]
5			27**	
-5			23**	
-7,5	No substrate	*S. oryzae* – A	104***	Burks and
-14		*C. ferrugineus* – A	157***	Hagstrom [17]

* LT lethal time interval during which 99% of given test insect population die (minutes)
** LT_{95} lethal time interval during which 95% of given test insect population die (days)
*** LT_{50} lethal time interval during which 50% of given test insect population die (minutes)

Effect of high temperatures on storage insects

At high temperatures, due to more distinct general mobility, insect mortality is caused by water loss (organism desiccation), and evaporation is proportional to the body surface and greatly depends on air humidity. It is founded that at high temperatures in insect "heat shock proteins" (HSP) are formed, which prevent protein aggregation and denaturization, and in that way protect the organism. However, at longer insect exposure, protein denaturization and disbalance of metabolic processes are observed, which for a consequence have accumulation of toxic substances and death [18, 51].
In terms of susceptibility to high temperatures reverse rule from low temperatures applies. Insects that develop at higher temperatures are more resistant that those which develop at lower, and therefore the attention of scientists over the past decade have been directed towards finding an optimal ratio between efficacy, energy expenditure and substrate to be treated [18]. In the examination of effects of high temperatures on *R. dominica*, Evans [26] singled out the temperature of 70 °C, as the most convenient, bearing in mind the aspect of avoiding the potential damage of wheat grain. However, Beckett and Morton [15] founded that for this species the most effective is heating of wheat up to 50 °C, during 22 h. Kljajić et al. [44] by direct exposure of *S. granarius* adults at 45, 50, 55 and 60 °C founded that for 95% mortality it takes 64, 46, 25 and 17 minutes, and that adults from higher population density are more susceptible to 50 °C. By mortality testing of *S. oryzae* and *R. dominica* adults and younger development

stages, at temperatures of 42-53 °C, depending on the moisture content of wheat (9, 12 and 14%), Beckett et al. [14] founded that all development stages survive longer exposure to these temperatures with an increase in grain moisture. Researching the impact of 50 °C on the reproductive characteristics of *T. castaneum* Mahroof et al. [51] pointed out that this is a minimum temperature by means of which it is possible to effectively suppress this species, which was confirmed by Arthur [5] who exposed all development stages of *T. castaneum* and *T. confusum* at 54 and 51 °C, at intervals of 1 and 2 hours, respectively. Kljajić et al. [41] founded that for *S. granarius* adults from the population which is resistant to insecticide pirimiphos methyl 22% more time is needed for paralysis of 99% of individuals exposed at 50 °C than for laboratory weevils.

Interaction between effects of extreme temperatures and contact insecticides

Environmental conditions, especially different temperatures, in interaction with contact insecticides usually cause changes in toxicity expression of chemical substances, which depends on pest insect species, applied temperature and insecticide properties. Increased or reduced susceptibility of insects is the result of slower or faster absorption of insecticides by individuals, referring to their overall activity, which explains the success of the application of contact insecticides [19, 61].
With normally susceptible and resistant populations of *T. castaneum*, *O. surinamensis*, *S. granarius*, *T. confusum* and *R. dominica* usually higher toxicity was registered for organophosphorus insecticides: bromophos, chlorpyrifos-methyl, fenithrotion, iodfenphos, malathion, foxim, pirimiphos-methyl, tetrachlorvinphos and fenitrothion at higher temperatures (25 and 30 °C), and pyrethroids: cypermethrin, bioallethrin, flucythrinate, d-fenothrin, fenvalerate and cyfluthrin at lower temperatures (5, 10 and 20 °C) [63, 70, 12, 62, 59]. Kljajić et al. [43] recorded that chlorpyrifos-methyl and deltamethrin have a faster action on *S. granarius* adults previously exposed at low temperatures -25, -5, but also at high 45, 50 and 55 °C.
Interaction between temperature of 45 and 55°C and insecticide action of cyfluthrin and hydroprene against *T. castaneum* adults showed significantly higher efficacy of the applied insecticides at these temperatures, which may be a substitute for fumigant methyl-bromide [6]. Wijayaratne and Fields [72] found that for larval and adult stage of *T. castaneum* at temperature of 46 °C effectiveness of all applied rates of methoprene is significantly higher, while the same was observed at 0 °C only for the highest application rate.

Practical use of extreme temperatures: advantages and disadvantages

It was long time ago that the possibility of practical use of high and low temperatures in protection of stored products from insect pests, was pointed out. Navarro et al. [53] studied the possibility of wheat storage during 22 months in metal silo, using active ventilation with a dryer. During the first year they managed to lower grain mass temperature from 26.8-32.2 °C to 10.2-13.8 °C, and the next winter to 10.5-14.3 °C, and therefore insect emergence and activity was at minimum, and there was no reduction in the quality of grain, humidity increase, reduced germination and significant grain damage from insects. There is a number of subsequent highlighting of advantages of hot

air insufflations from the outside, during summer months, in horizontal storage facilities and successful wheat, maize and other plant products protection [33, 2, 7, 64, 65].
Although safe in terms of application and with no negative impact on quality of stored products, extreme temperature treatment is, in proportion to the importance, underused. Problems that accompany extreme temperature treatment still restrict their use, and, above all, these are construction solutions of storage facilities and energy cost. Based on numerous studies on possible temperature application in stored products protection against insect pests, it has been concluded that temperature of 50 ºC is highly effective, because treated products and equipment are less damaged, and the most favorable ratio of consumed energy and efficacy is achieved. Today, in the world, hot air at insufflation of 50 ºC is mostly used in flour and spice plants mills and storages, for what it is expected that by improvement of structure solutions of storage facilities and appliances that utilize temperature, the potential of such control of storage insects will be used more. Also the interaction of extreme temperatures and contact insecticides can be a solution for populations resistant to conventional insecticides, and those less susceptible to new insecticides such as abamectin and spinosad [35].

CONCLUSION

Application of physical measures against storage insects, especially inert dusts and extreme temperatures, can significantly improve the safety and quality of stored plant products, especially if one bears in mind that the protection of stored products from insect pests is a dynamic system that requires constant quality improving and harmonic application (integration) of all effective control measures and methods.

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PROTECTIVE EFFECT OF HULLS *Triticum aestivum* ssp. *spelta* AGAINST INSECT INFESTATION DURING STORAGE

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ABSTRACT

Hulled and dehulled spelt wheat samples were infested with rice weevils (*Sitophilus oryzae* L.*)* and lesser grain borer (*Rhyzopertha dominica* F.*)* and stored for up to 60 days at room temperature. In the case of rice weevils, the protective effect of hull was 100% efficient because the insect did not manage to bore into the kernels of dehulled spelt whereas lesser grain borer did.
The rice weevils reproduced on dehulled kernels caused 9.2% kernel damage. The lesser grain borer caused 14.1% kernel damage on the hulled and 5.2% on the dehulled spelt during storage. Significant decrease in the test weight (7.1-14%) and increase in the moisture (2.4-7.5%) and protein (1.8-6.9%) contents of spelt grains was due to insect infestation.
Keywords: *hulled, dehulled, spelt, grain, rice weevil, lesser grain borer*

INTRODUCTION

Triticum aestivum ssp. s*pelta* (spelt) is an old hulled subspecies of wheat that has lately been grown more frequently in the system of organic certified food. In our region it was already known to the ancient Romans. It was grown in Germany, and the Pannonian Plain [8]. The production was resumed in the seventies of the last century for more than one reason. The prerequisites that this wheat has for the organic farming system is the possibility of production without the application of mineral fertilizers and pesticides, but also the existence of the protective hull which protects the grain from the pathogens [1]. Recently, there exist numerous international publications that compare the yield and nutritive values of spelt and bread wheat [7, 15].
The fact is that about 100 species of insects can find food in our storehouses. The primary pests are capable of damaging the whole and sound kernels causing about 90% of all the damages in storehouses. The rice weevil, *Sitophilus oryzae* (L.) and lesser grain borer *Ryzopertha dominica* (F.), are two the most destructive insect pests of stored grains worldwide [2, 4].
Besides quantitative damage, insects demise the stored grain quality, too. Many authors have reported that moisture contents of insect-infested cereal grains increased with increasing infestation levels [11, 12]. Wheat infested with lesser grain borer showed lower test weights and flour yields and increased ash and protein contents [5].
Storing the spelt wheat in spikes has not been the practice in our country up till now [3].

The objective of this study was to evaluate the protective potential of spelt hull and the grain damage due to infestation with the most prevailing storage pests such as rice weevil and lesser grain borer.

MATERIAL AND METHODS

Three spelt cultivars, Ekö 10 (Hungary), Ostro (Austria) and Nirvana (Serbia) were included in the study. The wheat cultivars were planted at location Bačka Topola in 2009. The samples were dehulled by passing the grains between coated rolls and aspiration was used to remove the hulls.
Both species of insects (rice weevil and lesser grain borer) used in this experiment were grown in a thermostat at 27±1ºC and relative air humidity of 40-60%.
The experiments were done for each variety in two storage variations. The first variation comprised 200 kernels, the second 100 spikes. Both variations were carried out in five replications. In each replication, 20 imagoes of each insect species were placed.
Wheat samples were infested with rice weevil and lesser grain borer and stored at room temperature (24±1ºC i 45±5% r.h.). After 60 days, samples of wheat were evaluated for insect population by passing it over a sieve to separate insects and dust from the grain. Controls were free from insects in all sampling periods.
Kernel weight was determined by Perten SK CS 4100 (Kernel Hardness Tester, Perten Instruments, Reno, Nevada, USA), and test weight of hulled and dehulled spelt was determined by a Schopper scale.
Samples were analyzed by standard ICC (International Association for Cereal Science and Technology) methods for: moisture content of cereals [9] and crude protein [10].
Statistical analysis was performed by analysis of variance (Statistica 9.1,) and Fisher's least significance difference (LSD) was used to compare means. Significance difference was determined at the level of 5%.

RESULTS

Rice weevils cannot feed and reproduce on any variety of hulled spelt wheat when stored in spikes (Table 2). In the same variation, lesser grain borers fed and reproduced although the average number of offspring was small (14.4 imagoes). For all three studied spelt varieties (Ostro, Eko 10 and Nirvana), the number of offspring was small and ranged from 13 to 15 imagoes. Kordan et al. [6] came to similar conclusions and reported that lesser grain borers developed worse on the spelt wheat in spikes.

Table 2. Infestation (n) and kernel damage (%) by rice weevil and lesser grain borer *after 60 days of storage.*

Treatment	Cultivars	Rice weevil infestation (n)	Kernel damage by rice weevil (%)	Lesser grain borer infestation (n)	Kernel damage by lesser grain borer *(%)*
Hulled spelt grain	Nirvana	0 ± 0,00 a	0 ± 0,00 a	14,6 ± 1,61a	5,4 ± 1,41b
	Ekö 10	0 ± 0,00 a	0 ± 0,00 a	14,4 ± 1,93a	4,2 ± 0,98 a
	Ostro	0 ± 0,00 a	0 ± 0,00 a	13,6 ± 1,54a	5,9 ± 1,61 b
Dehulled spelt grain	Nirvana	134,8±17,61 b	9,3 ± 0,91 b	36,0 ± 5,54b	13,4 ± 1,35 c
	Ekö 10	156,8±23,61 b	8,9 ± 1,32 b	49,4± 4,23c	12,9 ± 0,45 c
	Ostro	133,0±19,23 b	9,6 ± 1,45 b	49,8 ± 5,45c	15,9 ± 1,64 d

Mean values in the same column followed with different letters are significantly different ($p<0.05$).

In the experimental variation in which the development of both insects species on the kernels of various dehulled spelt wheat varieties was monitored, the results showed that the kernels of spelt wheat made the development of both species possible (Table 2). In this variation, rice weevils reproduced but the number of the offspring in the F1 generation (141.6 imagoes on average) was greater than the number of offspring of lesser grain borers but smaller than the obtained number of offspring in common wheat (Stojanović, 1966). The greatest number of offspring of rice weevils was obtained on the Eko 10 variety (157 imagoes) and the smallest on the Nirvana variety (133 imagoes). In the same variation, lesser grain borers developed worse (an average number of offspring 45.0 imagoes). The greatest number of offspring was obtained in the Ostro variety (50 imagoes) and the smallest in the Nirvana variety (36 imagoes).
As a result of endosperm reduction caused by insect feeding in the kernel, there was significant reduction in the test weight after infestation with the tested insects in comparison to the control except in the case of infestation with rice rice weevil on hulled spelt varieties (Table 2).

Table 2. Mean values and standard deviations for test weight of hulled and dehulled Tr. aestivum ssp. spelta after 60 day of insect infestation

Test weight (kg/hl)	**Spelt varieties**					
	Hulled spelt grain			**Dehulled spelt grain**		
	Nirvana	**Ekö 10**	**Ostro**	**Nirvana**	**Ekö 10**	**Ostro**
Controls	58.5±3.8a	56.6±4.6a	54.8±2.8a	75.7 ±3.2a	77.3±5.5a	76.4±3.6a
***Sitophilus oryzae* infestation**	58,5±4,2a	56,2±4,8a	55,4±3.8a	70.6 ± 4.8b	71.3±2.8b	71.4±4.6b
***Rhizopertha dominica* infestation**	52,6±4,9b	50,6±3,2b	50,4±6,7b	65.8 ± 3.7c	66.3±4.5c	65.4±3.3c

Mean values in the same column followed with different letters are significantly different ($p<0.05$).

The test weight of dehulled spelt kernels ranged from 75.7 kg/hl to 77.3 kg/hl. According to Reiter et al., [13] the hull weight accounts for 25-30% of the yield which is not completely consistent with our investigations, since our findings showed somewhat broader range of hull percent. The hull percent in *T. aestivum* ssp. *spelta* ranged from 22.7% (Nirvana) to 28.3% (Ostro).

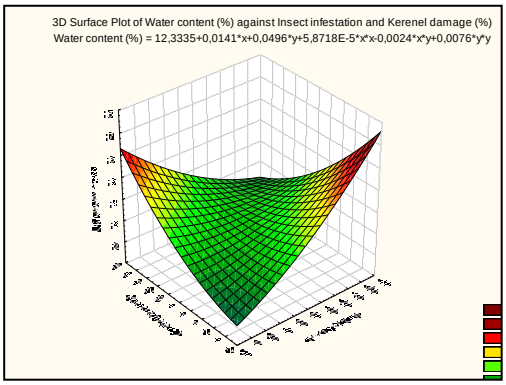

Fig. 1. Effect of insect infestation and kernel damage on the grain moisture content

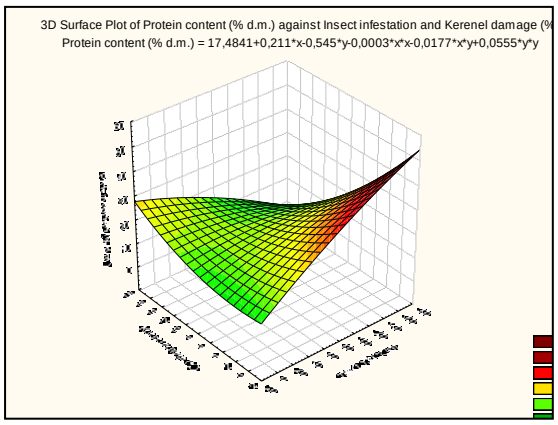

Fig. 2. Effect of insect infestation and kernel damage on the grain protein content

In this experiment, presence of insect infestation in the control samples increased the moisture content from 2,4 to 7,5%, and the protein content from 1,8 to 6,9%, as compared to their initial contents (Fig-s.1 and 2.). Significantly higher moisture content in the infested samples those that in the control samples is owing to the metabolism of insects as suggested by Sánches-Mariñes et al. [14]. The protein content in the grains was positively correlated to the level of insect infestation. This might be as a result of

endosperm reduction caused by insect feeding which reduced the starch content in the kernel and consequently increased the relative proportion of proteins in it.

CONCLUSIONS

Rice weevils do not feed and reproduce when the kernels are hulled. The hull protects kernels from the attack of rice weevils but not from lesser grain borers. Lesser grain borers normally develop both on kernels and spikes of spelt wheat. The results show that the impossibility of rice weevil nutrition resulted from the physical impossibility of nutrition and not from the repellent spike effect.
The number of offspring of lesser grain borers is significantly smaller than the number of offspring of rice weevils on kernels.
Physico-chemical grain quality parameters declined with increased damaged caused by the insects. The test weight was negatively correlated to the insect infestation level whereas the moisture content was positively correlated to the insect infestation level.

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AMINO ACID PURITY DETERMINATION BY MEASUREMENT OF OPTICAL ROTATION

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ABSTRACT

Because of the important metabolic functions, frequent deficit in animal food and impossibility of synthesis in the organisms of domestic animals, special attention is given to supplementing synthetically produced essential amino acids into animal feed. It is necessary to check the purity level before amino acids are added into premixes or mixes.

The objective of this paper was to find out if polarimetric detection can be applied in determining the active substances of amino acids. The results from this experiment were compared to the results obtained by applying the method of total nitrogen, the method of total combustion (according to Dumas) and the standard method. It may be concluded that absolute difference between the mentioned methods, or individual samples, ranged from 0.51 to 0.92% for methionine; from 0.5 to 1.02% for lysine; from 0.03 to 0.96 for threonine and from 0.33 to 0.76 for tryptophan. Since the deviation of the tested method from the standard method was 1%, it can be applied as nonstandard method for checking purity level.

Keywords*: amino acids, optical rotation, animal food*

INTRODUCTION

Animal nutrition presents a coordinated sequence of physical, chemical and physiological activities during which food ingredients are convert into animal body. Its role is to maintain life, help animal production, provide animal health and animal reproduction [6]. In contemporary animal nutrition high nutritive values are demanded, as well as balanced proportion of proteins, fats, amino acids, mineral matters (micro and macro elements) and vitamins. The knowledge on nutritive value of various ingredients is necessary for their proper selection and mix supplementation. Beside total proteins, amino acids have very important role in animal nutrition, and their ratio has to be balanced so that animals can use them to synthesis their own proteins. Even in the case of the smallest deficit of some essential amino acids, animal body can not synthesize tensamine protein. As a consequence, the production of meat, milk and eggs is not adequate, and reproductive disorders may occur. Therefore, there must be an optimal balance of essential amino acids in animal diet. Having in mind that nowadays feed of animal origin are eliminated from animal nutrition and plant protein diet is used for optimal balancing of amino acids, so synthetically produced amino acids have to be supplemented into feed.

Amino acids, which animal body cannot synthesize, are classified as essential amino acids. The ones that can be synthesized in animal organism are non essential amino acids. Amino acids are the basic structural units of proteins. They originate from organic

acids when one H-atom is replaced with an amino group (-NH_2) in their molecule. By introducing amino group into organic acid molecule it becomes optically active compound because C-atom, to which -NH2 group binds, becomes asymmetric. All natural amino acids are α amino acids, and may be right-handed (R) or left-handed (L), depending on whether the plane of polarization is rotated to the right or to the left [3].
Amino acids most frequently used for mix production are lysine, methionine, threonine and tryptophan. The quality and consistent level of active ingredients is of high importance for mix production. The purity of amino acids is checked by potentiometric titration or by determining total nitrogen. The most frequently used methods for determining nitrogen are according to Khjeldahl and according to Dumas. However, expensive equipment is necessary for these methods. Since the property of all amino acids is to rotate plane polarized light, their solutions also show optical activity. The concentration of optically active substance in a solution is proportional to the angle of the polarized light plane and can therefore be polimetrically determined.
The objective of this paper was to determine reliability of polimetric detection of active substances in amino acid examination. The results on amino acid purity were compared to the results obtained by determining total nitrogen quantity, using the method of total combustion (according to Dumas), as a standard method [8].

MATERIAL AND METHODS

The samples of methionine (6), lysine (6), triptophane (6) and threonine (4), were analyzed. The total amino acides originated from Europe, Asia and Southern America, and sampling was carried out by officials, i.e. the Republic and Border Veterinary Inspection.
The solution for measuring optical rotation were prepared by diluting 5.00 g of the examined amino acid in a 100 ml 1 mol/L HCl. If the solution was cloudy and colored, it was filtrated through a paper of medium density and was discolored with activated charcoal [2]. Optical rotation was calculated compared to the dry substance. The temperature correction was carried for each measurement if temperature deviation was more than t=20±2^{o}C. With the temperature increase of 1^{o}C, optical rotation was reduced for 0.3 % [7]. The measures were done by Carl- Zeiss polarimeter, with reading accuracy ± 0.05^o
The range of measured rotation depends on rotary power of optically active substance, as well as on the following variables: the thickness of the layer of optically active substance, temperature, wavelength of the polarized light and concentration of optically active substance. Due to the effect of these variables, certain standards or the conditions of measuring activities have been determined [4]. The measurement is usually carried out under the temperature t=20^{o}C, with light wavelength that responds to yellow sodium D line (589.3 nm) for a layer 1 dm thick.
Procedure: The polarimeter was set to zero degrees with a cuvette filed with a solution (1 M HCl). The angle of rotation for the working solution was measured, in standard conditions. Five measurements were carried out and the mean value was determined. The specific optical rotation was calculated using the following formula [5], where rotation to the right is marked with (+), and rotation to the left with (-):

$$[\alpha] = \frac{1000 \times \alpha_o}{l \times c};$$

From here on, the content of the unknown substance, is measured according to the following formula

$$c = \frac{1000 \times \alpha_o}{l \times [\alpha]},$$

where [α] – specific optical rotation standard;
α_o – specific rotation depending on temperature;
l - cuvette length (1dm);
c – concentration [g/1000 ml].

Measurement of total nitrogen, using the method of total combustion (according to Dumas), as a standard method [9] was performed on the instrument "Elementar, rapid N cube", Germany.

RESULTS AND DISCUSSION

Table 1. The purity of methionine $C_5H_{11}NO_2S$; M_r=149.2 [1] *determined the* combustion method according to Dumas) *and the method of optical rotation*

No.	Purity determined by Dumas method [%]	Purity determined by polarimetric method				
		$[\alpha]_{standard}$	α_o	c [g/1000ml]	purity [%]	|Δ|
1	**100.09**	**+** 22.0°	+ 1.10°	50.00	**100.00**	**0.09**
2	**94.80**	+ 23.0°	+ 1.10	47.83	**95.65**	**0.85**
3	**92.30**	+ 24.0°	+ 1.10°	45.83	**91.67**	**0.63**
4	**96.75**	+ 24.0°	+ 1.15°	47.92	**95.83**	**0.92**
5	**99.27**	+ 23,0°	+ 1.15°	50.00	**100.00**	**0.73**
6	**96.15**	**+** 23.0°	**+** 1.10°	47.83	**95.65**	**0.50**

[α] – specific optical rotation standard; α_o – specific rotation depending on temperature; c – concentration; |Δ| - absolut value of difference between Purity determined by polarimetric method and Dumas method

The results of the purity of methionine determined by polarimetric method, compared to the purity determined by measuring the content of total sodium, the method of total combustion according to Dumas, as well as by a standard method are displayed in Table 1. For each measurement specific optic rotation standard of methionine [α] was measured, as well as the angle of rotation for the samples α_o at the given temperature. From these data unknown concentration *c* [g/1000 ml] of the examined amino acid was calculated. In the last column the absolute value of the difference in the results obtained from measuring methionine purity, according to Dumas and using polarimetric method is displayed. From the given results it can be concluded that the absolute values of the difference for the results obtained by applying the above mentioned methods ranged

from 0.09 to 0.92%. for each sample. The mean value for all individual deviations – absolute difference between the applied methods for determining the purity of methionine was 0.62±0.30%.

Table 2. The purity of lysine-hydrochloride $C_6H_{15}ClN_2O_2$; M_r=182,7 [1] *determined the* combustion method according to Dumas) *and the method of optical rotation*

No.	Purity determined by Dumas method [%]	Purity determined by polarimetric method				
		$[\alpha]_{standard}$	α_o	c [g/1000ml]	Purity [%]	$\|\Delta\|$
1	**91.70**	+ 22.0°	+ 1.00°	45.45	**90.91**	**0.79**
2	**95,75**	+ 21.0°	+ 1.00°	47.62	**95.24**	**0.51**
3	**96.46**	+ 22.0°	+ 1.05°	47.73	**95.45**	**1.01**
4	**98.98**	+ 20.0°	+ 1.00	50.00	**100.00**	**1.02**
5	**99.30**	+ 21.0°	+ 1.05	50.00	**100.00**	**0.70**
6	**96.42**	+ 22.0°	+ 1.05	47.73	**95.45**	**0.97**

[α] – specific optical rotation standard; α_o – specific rotation depending on temperature; c – concentration; |Δ| - absolut value of difference between Purity determined by polarimetric method and Dumas method

In Table 2 are displayed the results of the purity of lysine-hydrochloride determined by polarimetric method, compared to the purity determined by measuring the content of total sodium, the method of total combustion according to Dumas, as well as by a standard method. For each measurement specific optic rotation standard of lysine-hydrochloride [α] was measured, as well as the angle of rotation for the samples α_o at the given temperature. From these data unknown concentration *c* [g/1000 ml] of the examined amino acid was calculated. In the last column the absolute value of the difference results of lysine-hydrochloride purity measured Dumas method and polarimetric method is displayed. From the given results it can be concluded that the absolute values of the difference for the results obtained by applying the above mentioned methods ranged from 0.51 to 1.02% for each sample. The mean value for all individual deviations – absolute difference between the applied methods for determining the purity of lysine -hydrochloride was 0.83±0.20%.

Table 3. The purity of treonine $C_4H_9NO_3$; M_r=119.1 [1] *determined the* combustion method according to Dumas) *and the method of optical rotation*

No.	Purity determined by Dumas method [%]	Purity determined by polarimetric method				
		$[\alpha]_{standard}$	α_o	c [g/1000ml]	Purity [%]	$\lvert\Delta\rvert$
1	**100.8**	-28.5^0	-1.45^0	50.88	**101.76**	**0.96**
2	**100.4**	-28.0^0	-1.40^0	50.00	**100.00**	**0.40**
3	**98.89**	-27.5^0	-1.35^0	49.09	**98.18**	**0.71**
4	**100.92**	-27.5^0	-1.40^0	50.91	**101.82**	**0.90**
5	**99.20**	-28.5^0	-1.40^0	49.12	**98.25**	**0.95**
6	**99.97**	-29.0^0	-1.45^0	50.00	**100.00**	**0.03**

[α] – specific optical rotation standard; α_o – specific rotation depending on temperature; c – concentration; |Δ| - absolut value of difference between purity determined by polarimetric method and Dumas method

In Table 3 are displayed the results of the purity of threonine determined by polarimetric method, compared to the purity determined by measuring the content of total sodium, the method of total combustion according to Dumas, as well as by a standard method. For each measurement specific optic rotation standard of threonine [α] was measured, as well as the angle of rotation for the samples α_o at the given temperature. From these data unknown concentration *c* [g/1000 ml] of the examined amino acid was calculated. In the last column the absolute value of the difference results of threonine purity measured Dumas method and polarimetric method is displayed. From the given results it can be concluded that the absolute values of the difference for the results obtained by applying the above mentioned methods ranged from 0.03 to 0.96 for each sample. The mean value for all individual deviations – absolute difference between the applied methods for determining the purity of threonine was 0.66±0.37%.

Table 4. The purity of tryptophan *$C_{11}H_{12}N_2O_2$; M_r=204.2* [1] *determined the* combustion method according to Dumas) *and the method of optical rotation*

No.	Purity determined by Dumas method [%]	Purity determined by polarimetric method				
		$[\alpha]_{standard}$	α_o	c [g/1000ml]	Purity [%]	$\lvert\Delta\rvert$
1.	**99.67**	$+2.0^o$	$+0.10^o$	50.00	**100.00**	**0.33**
2.	**99.24**	$+2.0^o$	$+0.10^o$	50.00	**100.00**	**0.76**
3.	**99.60**	$+2.0^o$	$+0.10^o$	50.00	**100.00**	**0.40**
4.	**99.32**	$+2.0^o$	$+0.10^o$	50.00	**100.00**	**0.68**

[α] – specific optical rotation standard; α_o – specific rotation depending on temperature; c – concentration; |Δ| - absolut value of difference between purity determined by polarimetric method and Dumas method.

In Table 4 are displayed the results of the purity of tryptophan determined by polarimetric method. compared to the purity determined by measuring the content of total sodium. the method of total combustion according to Dumas. as well as by a standard method. For each measurement specific optic rotation standard of tryptophan [α] was measured. as well as the angle of rotation for the samples α_o at the given temperature. From these data unknown concentration *c* [g/1000 ml] of the examined amino acid was calculated. In the last column the absolute value of the difference results of tryptophan purity measured Dumas method and polarimetric method is displayed. From the given results it can be concluded that the absolute values of the difference for the results obtained by applying the above mentioned methods ranged from 0.33 to 0.76% for each sample. The mean value for all individual deviations – absolute difference between the applied methods for determining the purity of purity of tryptophan was 0.54±0.21%.

CONCLUSION

The results obtained from this experiment point out that polarimetric method. as a method for measuring optical rotation. may be used for purity control of amino acids (methionine. lysine. threonine. tryptophan). because the deviations from standard methods were less than 1%. Since this method is specific. accurate and requires a small number of samples. and at the same time is not time consuming. it can be successfully applied as an alternative method for determination of purity (active substance) for the mentioned amino acids.

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IMPACT OF BENTONITE ON THE QUALITY OF PELLETED FODDER

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ABSTRACT

Main goal of this research was the investigation of the quality of pelleted complete feed mixtures without addition of the binding substance - bentonite (I) and with the supplement of the same material (II). Bentonite was included in feed mixture during the mixing of components, in the quantity of 2%. Testing of pellet quality was carried out in laboratory through establishing the obliteration index, the hardness of pellets, and thorough the examination of microbiological and mycotoxicological correctness. Lower obliteration index was detect in the feed mixture II wherein bentonite was included (10.7%), while in the feed mixture I, wherein bentonite was not included, it was 14.1%. Higher hardness of pellets was detected in mixture II and it was Khal J/kg, while in mixture I it was lower - exactly 3,7 Khal J/kg. There was a higher total count of bacteria in the mixture I (39.000 per 1 g of sample), while in the mixture II it was multiple lower (5.000 per 1 g of sample). A higher amount of yeasts and molds was detected in the mixture I (30 g per 1 g of sample) and 8 species were identified, while in the mixture II amount of yeasts and molds was slightly lower (10 g per 1 g of sample) and just three fungal species were determined. The quality of pelleted fodder mixture was higher when it contained bentonite as binding substance. When decision about the suitability of binding material must be done, it is important to have in mind some of its additional effects as well as price, origin and some other circumstances.
***Key words**:* animal feed, quality, pelleting, bentonite

INTRODUCTION

Production of safe food for humans and animals is the most important task for the producers, and this is the reason for increased importance of technological procedures which result in higher quality. One of those procedures is pelleting of feed mixtures. It is additional technological processing of homogenized, finely grounded feed, in order to produce granules, by passing the mixture thorough openings of press. There is a wide range of positive effects of feed mixtures pelleting. Primary, some of those are more important such as decreasing of decomposition, reducing of the number of total microorganisms, increasing of density, decreasing of losses in transport, achieving of possibilities to use some more finely grounded feed, gaining of better traits of feed for various manipulations [4, 1, 12, 5]. Exposure of mixture to influence of steam, pressure

and temperature, leads to chemical transformations of nutritive ingredients and consequently to increased digestibility of starch, hemicelluloses and pentosans [14, 15, 6, 8]. The result of increased temperature (70 - 80 ^{0}C) is degradation of most of anti-nutritive ingredients in feed. As a result of pelleting of feed mixtures, consumption and utilization of food by domestic animals are increased and the consequence is achieving better results in animal production. There is a wide range of binding materials that can be used in order to improve quality of pellets (increasing of hardness, persistence and resistance to obliteration and crumble). The most commonly used binding materials are Ca – lignosulfonate, Na and Ca – bentonite, as well as other organic and inorganic materials [14, 15].

Bentonite is a hydrous aluminum silicate of volcanic origin, consisting mostly of montmorillonite (50-90%). There are different types of bentonites and their names depend on the exchangeable ions, such as sodium bentonite (Na^+), potassium bentonite (K^+), calcium bentonite (Ca^+) and magnesium bentonite (Mg^+). The chemical composition of bentonite can be different due to the place of mining, but mostly bentonite contains SiO_2, (46-58%), Al_2O_3, (12-22%), K_2O, (0.20-0.40%), Na_2O, (0.04-0.08%), MgO, (1.70-3.50%), CaO, (3.30-5.90%), Fe_2O_3, (3.50-4.70%). Burning loss is 12-17%. Bentonite have a high potential to bind liquids (water and oil). The thickness of layers in crystal lattice of bentonite is approximately 1 nm. In the presence of water occurs the separating of those layers, so the water enters in void between them. Separation of layers can reach the distance up to 0.9-2.1 nm. This is the main explanation for the swelling of mineral. In contact with water weight can be increased 1.5 times and volume 1.2 times as well. Cation exchange capacity (CEC) is 80-120 meq / 100 g. Bentonite have very large covering surface (1 g covers 700-800 m^2).

Due to amphoteric properties (receives and releases hydrogen ions) bentonite can be used for maintaining of pH of rumen content in cattle nutrition and readjustment of unfavorable effects of low rumen pH value on content of milk fat [2, 9]. Bentonite binds aflatoxins (B_1, B_2, G_1 and G_2) in food and reduce presence of aflatoksin M_1 residues in milk (up to 60-90%). However, potential of bentonite to adsorb zearalenone and ochratoxin is limited [10]. Inclusion of bentonite in rations of cows effected on reduction of ^{137}Cs and ^{134}Cs contamination of milk by 50-80%. In rumen content, when concentration of NH_3 is high, bentonite can adsorb it, and later releases it when concentration of NH_3 drops down. In this mode of action it is possible to achieve better utilization of ammonia for synthesis of microbial proteins. Due to bentonites potential for water binding, and consequently because of increased volume of ingested food, it runs slower thorough digestive organs which leads to extended impact of digestive enzymes and increasing of nutrient matters digestibility. Bentonite can reduce solubility of copper (Cu) in rumen as well as Cu concentration in liver, and this may be usefully for solving the problem of chronic copper poisoning of animals. Disadvantage of bentonite usage is that in addition to binding of some minerals it may have an affinity to bind some vitamins [7].

The goal of research presented in this manuscript was to determine effects of bentonite, as a binding material, on quality of feed mixture pellets (hardness and obliteration index) as well as their microbiological and mycotoxicological correctness.

MATERIAL AND METHODS

Complete feed mixtures for hen nutrition were produced in Fodder factory „Component“, in town of Ćuprija. The composition of mixtures is presented in *Table 1*.

Table 1. Composition of mixture for hen nutrition (%)

Component	**I**	**II**
Corn grain	45.9	45.9
Soybean meal	12.5	12.5
Limestone	9.8	9.8
Extruded full fat soybean meal	6	6
Sunflower meal	9.30	9.30
Soybean meal, expeller	5	5
Yeast	1.5	1.5
Wheat middlings	6	4
Soybean oil	1.5	1.5
Na bicarbonate	0.1	0.1
Monocalcium phosphate	1	1
Salt	0.2	0.2
Premix	1	1
Methionine	0.1	0.1
Lysine	0.1	0.1
Bentonite	0	2
Total	**100.0**	**100.0**

Applied bentonite is produced by the special technological procedure (isolation of impurities, washing, drying, grinding and milling) in the Institute for Technology of Nuclear and other Mineral Raw Materials in Belgrade. The ingredients of this bentonite were SiO_2 (48,37%), Al_2O_3 (22,39%), K_2O (0,40%), Na_2O (0,07%), MgO (1,81%), CaO (5,86%), Fe_2O_3 (4,73%), and TiO_2 (0,34%). Particle size was below 50 µm.

After the production of feed mixtures, samples were taken for microbiological, mycotoxicological and other analysis. Samples were deposited in nylon bags, 20 cm above the floor. They were stored in ventilated, low-lighted and dry room, during the 20 days (October). the average temperature in storage room was 18^{o}C. Determination of pellets obliteration index was carried out by methods for pellet quality [3, 19].

Microbiological investigations were performed according to *Regulations on maximal quantity of harmful materials and ingredients in fodder* [18]. Total count of bacteria, molds and yeasts as well as identification of pathogenic microorganisms (*E. coli*, coagul. positive *Staphylococcus* spp., *Proteus* spp., *Salmonella* spp., sulphito-reducing *Clostridium* spp.) was done having in mind Official Gazette of SFRJ [16]. Identifications of fungi were performed according to Samson and van Reenen-Hoekstra [12].

Mycotoxicological investigations. The presence of aflatoxin B1 (AFL B1) and zearalenone (ZON) was determined according to standard method [17], while

diacetoxyscirpenol (DAS) and T-2 toxin were analyzed by applying the method of Pepeljnjak and Babić [11].

RESULTS AND DISCUSSION

Due to presence of similar components in feed mixtures the chemical composition in both of them was similar as well (*Table 2*). Somewhat more prominent differences were notable considering the content of Si and Al. The higher contents of those elements in mixture II were consequences of the bentonite presence in mixture (2%).

Table 2. Chemical composition of feed mixtures (%)

Parameter	**I**	**II**
Moisture	9.59	9.37
Crude rrotein	17.78	17.56
Ether extracted fat	5.32	5.20
Crude fiber	4.55	4.35
Ash	10.07	10.72
Si	0.11	1,12
Al	0.025	0.210
Ca	6.0	5.0
P	0.587	0.648
K	0.865	0.895
Na	0.133	0.182
Mg	0.295	0.293

Examination of appearance pointed out that more proper shape and smooth surface of pellets in mixture II while in mixture I pellets were shorter and with damaged edges. In research about the feed mixture for calves in which bentonite was included in amount of 1.5%, some similar results were obtained [15]. In our research data about pellets appearance were supported by results about hardness and obliteration index. Lower obliteration index was determined in feed mixture wherein bentonite was included (II) and it was 10.7%, while in the control feed mixture (I) it was 14.1%. Higher hardness of pellets was determined in mixture II (6 Khal J/kg) while in the mixture I it was lower (3.7 Khal J/kg), as it is presented in *Table 3.*

Table 3. Quality of feed mixture pellets

Parameter	**I**	**II**
Obliteration of pellets (%)	14.1	10.7
Hardness of pellets (Khal J/kg)	3.7	6

Total number of microorganisms, both bacteria and yeasts and molds, in each of the two samples (*Table 4*) was much lower compared to maximal allowed quantity according to *Regulation about the amounts of harmful matters and other ingredients of feeds for*

domestic animals **[**18**].** However, significantly lower total number of bacteria was noted in feed mixtures II, wherein bentonite was included, (5,000.00 bacteria /g) compared to 39,000.00 bacteria /g in feed mixtures I

Table 4. Microbiological quality

Parameter	**I**	**II**
Total number of bacteria/g	39.000	5.000
Total number of yeasts and molds/g	30	10
Identified molds		
Alternaria alternata	+	
Aspergillus candidus	+	
Aspergillus flavus	+	
Aspergilus fumigatus	+	+
Chrysosporium merdarium	+	
Fusarium verticillioides	+	+
Mucor mucedo	+	+
Rhizopus nigricans	+	

Considering the total number of yeasts and molds, determined differences were not statistically significant (*Table 4*) except the difference about the number of identified mold species in samples I and II (8 compared with 3). Mostly there were present saprophyte species belonging to so-called field fungi (*A. alternata* and *F. verticillioides*) or storage fungi (*Aspergillus* spp.).
Pathogenic bacteria (*E. coli*, coagul. positive *Staphylococcus* spp., *Proteus* spp., *Salmonella* spp., sulphito-reducing *Clostridium* spp.) were not identified during the present investigation.
Mycotoxicological examinations did not establishe the presence of aflatoxin B_1, zearalenone, ochratoxin A and type A trichotecenes (T-2 toxin and diacetoxyscirpenol – DAS). The obtained results are not surprising considering that in samples I and II most of the determined fungal species are mainly not producers of mycotoxins.
Data about the microbiological and mycotoxicological correctness of feed mixtures, suggest high quality and hygienic safety of examined mixtures, certainly due to the excellent quality of applied components and the control of critical points of the process in factory where mixtures were produced as well.

CONSLUSION

In feed mixture II, wherein binding material (bentonite) was added, quality of pellets was higher compared to the mixture I, that did not contain binding material. Obliteration

index of pellets was clearly lower in the feed mixture II, while the hardness of pellets was higher in the same sample. Microbiological and mycotoxicological quality of both examined mixtures was very good, but the total number of microorganisms (bacteria, molds and yeasts) as well as the number of determined fungal species were lower in mixture II, wherein bentonite was added. When decision about the suitability of binding material must be done, it is important to have in mind some of its additional effects as well as composition of mixtures, need to use binding substance, price, origin and some other circumstances.

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THE RESPONSIBILITY OF ANIMAL FEED PRODUCERS IN SERBIA IN THE AREA OF ANIMAL PRODUCTION – AT THE ENTRY TO THE WORLD TRADE ORGANIZATION AND EUROPEAN UNION

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ABSTRACT

From the viewpoint of international market, there is a need for a review of agricultural policy that will benefit livestock farming, where nutrition and advancement of animal feed production technologies are essential for its development. On the other hand, each incident that negatively influences public health within EU member countries, exposes the industrial animal feed production to negative publicity due to negligence during the production of animal feed for own use in livestock farming. This usually results is a recognizable method of European lawmakers to impose stronger laws in this industry, which causes concern among producers and in commerce. In addition to imposing production rules and assessment of risks, attention also draws assessment of relationship in the sector of livestock and feedstuff production, linking sanitary and phytosanitary measures of the WTO with European standards, as well as the influence of livestock production on environmental protection. Those practices point to the responsibility and business seriousness of export-orientated animal feed producers, but also to the need for cautiousness of each national legislation during the process of inclusion into the international market.

Keywords: *producers of animal feed, market, international standards*

INTRODUCTION

Agriculture is one of important components of economic development of Serbia. Its position in the state is specific because, in addition to economic, it also has an exceptional health, social and ecological significance. Trade and technologies support the development of agriculture and animal welfare, but they also represent new risks to food safety and public health. The integration of the Republic of Serbia into the World Trade Organisation (WTO) and European Union (EU) is based on the same mandatory legislative activities so that the Ministry of Agriculture, Forestry and Water Management is faced with a highly responsible situation, namely with the so-called "two-way track".

The maximum creativity in utilisation of national trade potential without creating additional obstacles is in line with changes related to the period of accession into the World Trade Organisation. At the same time, the task of harmonisation of regulations with the European legislation has also been set. While joining the WTO, the countries practically give up a certain extent of national sovereignty and they mainly depend on their own economic and export structure and extent of liberalisation in the key sectors

[1]. Essentially, the process of liberalisation of market services also leads to changes in accordance with the EU legislation [2]. Setting up of the defined legal multilateral framework, with rules and measures for managing development and evidences on adequate implementation of veterinary and phyto-sanitary measures aimed at reducing negative impacts on trade, namely, in the sense of this paper, on products of animal origin. We are witnessing many problems at the national level with focused issues related to the system of food control and safety. On the other hand, we should not neglect the economic significance of market development, which is basically linked to harmonisation of laws and bylaws for the purpose of enhancing of agricultural production and possibilities of utilisation of resources from the EU pre-accession funds in the forthcoming period.
New legal solutions enacted by the Government of the Republic of Serbia and competent Ministry should stimulate the legal identity of agriculture that follows up and makes domestic product recognisable at the international agricultural market.

FARMING OF ANIMALS OF SERBIA IN A NEW LEGAL FRAMEWORK

Harmonisation of laws and introduction of international nomenclature and definitions of terms in the field of animal feed affects primarily the effects of farming of animals, which we analyse through the accomplished profit from trade and export of products generated by farming of animals.
Due to those reasons, we introduce the Law on Food Safety into a new (national) legal framework [4]. This piece of legislation as such should represent the initial step for introduction of standardisation procedure in production of food products. Equally important step in the set legal priorities was made with enactment of the Law on Veterinary Medicine [5]. Positive trends in farming of animals, which include production and transport of animals, can be risky for human and animal life. More precisely, numerous changes in the structure of production, farm, managing plant and animal resources, system of controls in the chain from feed to food, conditions for free access to the market with preservation of the environment, as well as the overall impact on rural development, represent a large economic challenge that is imposed by accession of agriculture of Serbia into the European Union [4]. Animal welfare is a social issue, as well as social category, the advantage of which should be taken for economic progress of the society. Observing and setting up of responsibility towards animals helps achieving better economic efficiency and higher profit. Animal breeders represent active stakeholders that have the key role in the process of development and enhancing of competitiveness of farming of animals [7], but the system of observing of animal welfare in the overall economic development of Serbia has to be taken into account. Due to that reason, the Law on Animal Welfare [6] should be seen as novelty that introduces the obligation of adapting human awareness in the direction of observing, taking care of, and responsibility towards animals. In addition to acquiring positive epizootiological status of the Republic and implementation of the procedures for enhancing of farming of animals business linking based on the parent field laws (veterinary medicine and farming of animals) would result with enhancing of health protection and animal welfare aimed at

preventing damages and injuries of animals, as well as at restructuring of farming of animals in Serbia.

FARMING OF ANIMALS OF SERBIA IN FIGURES

Statistically speaking, despite exceptionally favourable natural conditions, the decline in number of heads of production animals has been recorded within the last decade. It accounts for 2% to 3% at an annual level. The decline in meat production has also been registered – from 550,000 tons (1990) to 460,000 tons (2008). The share of farming of animals accounting for 30.5% in the realised value of agricultural production in 2009 is 10.5% lower compared to 2008.

LEGAL PROCEDURES FOR FREE TRADE WITH FOOD OF ANIMAL ORIGIN

The basic principles and requirements have been defined through setting of national legal framework that is equivalent to the European Food Code [10]. After that, in terms of procedure, it means that third countries that want to export products of anima origin to the EU have to submit a special plan to the European Commission. The plan should include the list of guarantees it offers related to the extent of safety of those products [3]. This implies that the exporting country has undertaken all the measures for monitoring of certain substances and their residues in food of animal origin designated to the European market [8]. The principle is that the national plan for control of residues has to contain the results of monitoring from the previous year and the evidence on check ups carried out during the current year. The guarantees have to be at least equivalent to those existing in the Directive, which deals with requirements for monitoring plans for residues while specific levels and frequency of sampling for the analysis of residues depend on the type of products [9]. After the national plan of control of residues is approved by the European Commission, the "third country" remains on the list of countries from which the EU Member States can import food of animal origin [3]. There is a range of prescribed official methods of the EU for sampling necessary for the purpose of official controls aimed at monitoring of residues in food of animal origin [11].

At the national level, this procedure can be presented through provisions of the Law on Veterinary Science [5] and Law on Food Safety [4] which provides the basis for official controls in Serbia. The control of use of veterinary medicinal products in animals that are used in production of food of animal origin is related to those procedures based on regulations related to medicinal products, which are in compliance with the European legislation [12]. Thus, at the national level there is the prohibition of use of beta-antagonists, bitinol-sulphate, dapsone, colchicine, chynoxaline/qinoxaline (carbadox, olaquinox, ciadox), resorcyclic acid lacones, including zeranol, malachite green, leucomalachite green, nitrofuran, nitroimidazoles, nicozamid, chloramphenicol, chloroform, steroids, stilbenes, stilbene derivatives, their salts and esters, oestradiol 17 β and its ester-like derivatives, substances having oestrogenic action... [13]. It is realistic to expect that due to specific characteristics of national legislation the control and supervision procedures in production of animal and food of animal origin in Serbia are not always

transparent systems, neither the new harmonised laws are always easy for understanding and implementation.

RESPONSIBILITIES AND DOUBTS OF MANUFACTURERS OF ANIMAL FEED

National legislation in the field of animal feed is relatively applicable to industrial product of animal feed. If we take into account that the world population and good manufacturers' practice of animal farming, which move in the direction of meat consumption pays more and more attention to the "source" of meat the question of safety of use of animal feed at the national level arises. Namely, the analysis of risks should unify the results starting from monitoring of quality and safety of plants that are introduced in composition of animal feed up to the use of often uncontrolled feed additives.

It is known that the growth of production of field crops, which are used in diet of animals (e.g. soybean), is accompanied by the growth of processing of those products at small holdings, which started preparing "animal feed" themselves by buying extruders. However, the competitiveness in meat production is largely conditioned by possibilities of access to standardised quality animal feed. On the other hand, if we look at facilities for production of animal feed we can see that most of them have introduced the system of control of critical spots, which is in accordance with the law. We can also notice that procedures for cleaning, as well as the system of planning of production/rotation of production lots have been set. Nevertheless, it happens that animal feed with added medicinal products and other animal feed are manufactured on the same production line. The check ups of cross-contamination, homogeneity (in relation to an active substance) and monitoring of records on the return of animal feed from the market are rare due to the absence of adequate regulations that should follow up that field. Another fact is that no special approval is necessary for production of animal feed with added medicinal products, nor for production of animal feed in the registered "animal feed" factory or on the farm, which implies that adequate controls of such production are not in place, in particular in farm facilities. The lack of national legal grounds is followed up in the European legislation in the way that production of premixes with added medicinal products can be carried out only in facilities that are approved for manufacture of feed containing allowed animal fed supplements... [14, 15].

From the aspect of evaluations and analyses of the competent authorities within the national agricultural policy, the companies dealing with production of animal feed, and later with meat processing or sale, strive to set vertical linking in order to reduce the market and price oscillations typical for meat production. The assessment of the competent analysts is that "manufacturers of merchandise", who mainly prepare animal feed themselves and who deal with growing of field crops and farming of animals deserve the priority of agricultural policy, and that it is necessary to enable them further growth [16].

CONCLUDING CONSIDERATIONS

A logical continuation of Serbia's road into the EU can be found in the light of independent and accepted country in the field of cultural identity and values. However, it is necessary to preserve individual advantages, including market ones and not to allow doubts to put the capacity for completion of the integration process into question. Due to those reasons, it is necessary to impose the planned farming of animals with production of meat and meat preparations that can become significant export products in the forthcoming period. Having in mind that legal framework of agriculture of the Republic of Serbia is harmonised with the set requirements, we can only believe that it will be verified by the EU and World Trade Organisation officials in the form of Agreement on implementation of sanitary and phyto-sanitary measures.
We also believe that legal protection and conditions for its enforcement will be ensured in the way that does not favour any group within the trade chain. This is the conditions for elimination of grey market, which basically creates unequal conditions for doing business. Anima feed manufacturers are expected to direct their activities towards adopting of standards and introducing the system for obtaining of standardised quality of products. Manufacturers of animal feed have to be focused on the market through imposing and emphasising of production of safe animal feed of much higher, balanced quality of their products.

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POTENTIAL OF PLANTING *AMARANTHUS SP.* IN AGROECOLOGICAL CLIMATE OF VOJVODINA

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ABSTRACT

Amaranthus sp. is a new plant in our region that can be grown successfully in our agroclimate as a crop, as well as forage culture. Great production of biomass, the presence of nutrient components that are deficient in grain, such as amino acids (lysine), make it compatible plant species that could be an integral part of the diet of people and domestic animals. Content of protein lysine in *Amaranthus sp.* grain in an average of all three genotypes was 8,87 g/kg.
***Keywords**: Amaranthus sp. grain, biomass, chemical composition*

INTRODUCTION

Species of the genus *Amaranthus L.* are characterized by high productivity of biomass, ecological plasticity and exceptional adaptability potential which allows easy introduction and production in agroecological conditions of Vojvodina [2, 3]. Great nutritious value of leaves and seed speaks in favor of large perspectives of this plant for human and animal consumption [14, 17].
Grain *Amaranthus sp.* has similar processing capabilities and applications as well as cereals and that is the reason why it belong to a group of pseudocereals. The chemical composition of grains of this culture is characterized by higher content of protein, minerals (Ca, Fe) and fat than cereals [1, 5, 7, 14].
Addition of seeds of this culture in a certain small percentage in wheat bread can improve nutritional aspect of this basic foodstuff [4].
Chemical composition of leaves is in correlation with the chemical composition of seeds, but any discussion regarding this issue must take into account the extreme plasticity of this plant, that is its adaptability to external environmental factors. *Amaranthus sp.* leaves contain more dry matter than spinach, because of higher mineral content and fiber [15]. A large number of studies speak in favor of a specific and high-quality amino acid composition of leaf protein of *Amaranthus sp.* which is particularly interesting because of the content of deficient amino acid lysine [15, 16].
Purpose of this study was to investigate the potential of growing *Amaranthus sp.* as a forage crop and culture through the yield and chemical composition of the green mass of leaves and seed of this introduced culture in our region.

MATERIAL AND METHODS

In the research for the purpose of testing the chemical composition of grain, three genotypes of reproduced grain *Amaranthus sp.* (2, 16, 31) have been used from the experiment that was grown for the purpose of work (Bodroža-Solarov, 2001) at the site Futog. Chemical composition of the leaves of *Amaranthus sp.* was taken from various literature data.

Chemical analysis of the grains was investigated on the basis of valid regulations for grain cultures in Serbia. Protein content was estimated by Kjeldahl method and the result was obtained by multiplying the percentage content of N with coefficient of 6,25 [12]. The contents of Mg and Fe were determined after dry ashing at 450º C, by atomic absorption spectrophotometry (AAS) using Varian Spectra AA10 [11].

Filter bag technique (ANKOM Technology) was used to determine Neutral Detergent Fiber (NDF) [9] and Acid Detergent Fiber (ADF) [8] on the analyzer Ankom 220. For amino acid analyses in the *Amaranthus sp.* grain samples was used Shimadzu (Shimadzu corp., Kyoto Japan) low pressure gradient HPLC system consisted of solvent delivery module LC-10AT_{VP} , auto injector SIL-10AD_{VP}, column oven CTO-10AC_{VP}, spectrofluorometric detector RF-10A_{XL}, system controller SCL-10A_{VP} and on-line degasser DGU-14A [10].

Statistical analysis was performed by analysis of variance (Statistica 9.1,) and Fisher's least significance difference (LSD). Significance difference was determined at the level of 5%.

RESULTS AND DISCUSSION

Amaranthus sp. is a plant with robust habitus. In the research of Svirkis (2003), genus *Amaranthus L.* is characterized by high yield up to 70 t/ha which was achieved after 115 days after seeding. In our research yield of green mass is lower with a note that the purpose of experiment was not forage mass production. In a period of 37 days achieved weight of plant in average of all three genotypes was 278 g/plant (Table 1). Under cultivation of 100,000 plants/ha applied in the study, projected yield of green mass in the phase before budding was in the range of 27,5 t/ha to 30,2 t/ha, which is still in favor of the excellent productivity of biomass. The projected yield of *Amaranthus sp.* grain based on this microassay and grain yield per plant is 5,16 t/ha, which is approximately the yield of wheat.

Table 1. The influence of genotype on plant height and mass of Amaranthus sp. in phase before budding and weight of seeds [(2)]

***Amaranthus sp.* genotype**	**The length of period from planting until the beginning of budding** (days)	**Weight of plants in phase before budding** (g/plant)	**Grain yield** (g/plant)
2	36 a	302 a	57,3 a
16	37 a	275 b	51,1 b
31	37 a	280 b	46,6 c
Average	37	278	51,6

[a] Different letters in the same column indicate significant difference (5%)
[(2)]Bodroža-Solarov, M. (2001)

Results of the chemical composition of grains of different genotypes of *Amaranthus sp.* show significant difference in protein content, ash, minerals (Table 2).
According to research from Kuhn (1998), the calcium content of *Amaranthus sp.* is four times and iron two times higher than in wheat. In our research content of Ca in the average of all three genotypes is 299 mg/100g grain, and Fe 8 mg/100g grain (Table 2).
The chemical composition of grains of different genotypes of *Amaranthus sp.* points to the significant difference in the content of cellulose fractions. The lowest percentage level of NDF, which indicates the highest dry matter consumption, is determined in genotype 16. Signicantly lower values of ADF, indicating a higher energy value of dry matter, had genotypes 16 and 31 (Table 2).

Table 2. The chemical composition of Amaranthus sp. grain

Part of the plant	**Gen.**	**Protein** (% d.m.)	**Ash** (% d.m.)	**Ca** (mg/100 g)	**Fe** (mg/100 g)	**NDF** (% d.m.)	**ADF** (% d.m.)
Grain	2	16,12 a	2,76 a	268,5 b	8,4 a	9,54 a	7,18 a
	16	16,19 a	2,46 b	323,5 a	7,2 b	7,58 b	5,74 b
	31	17,68 b	2,68 ab	305,3 ab	8,4 a	8,42 ab	6,11 b
	Average	16,69	2,63	299,1	8,0	8,51	6,34

[a] Different letters in the same column indicate significant difference (5%)

Table 3. Chemical composition of Amaranthus sp. leaves

Part of the plant	**Water** (%)[15]	**Protein** (% d.m.)[15]	**Ash** (% d.m.)[15]	**Fat** (% d.m.)[15]	**Carbohydrates after hydrolysis** (% d.m.)[15]	**Ca** (mg/100 g)[14]	**Fe** (mg/100 g)[14]
Leaf	83,83	23,22	18,23	6,99	18,82	267	3,9

[15] Stanimirović, 1983
[14] Saunders and Becker, 1984

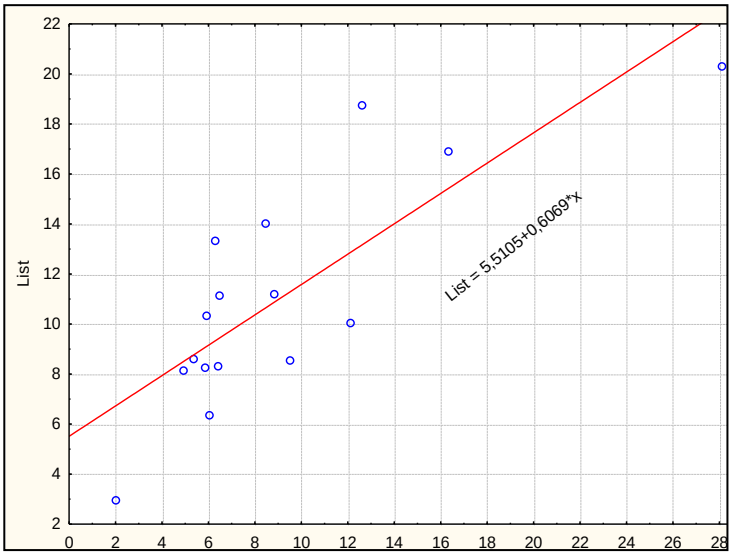
Protein content of *Amaranthus sp*. leaves (Table 3) is approximate to the protein content of alfalfa flour I quality [6]. According to Kuhn (1998), protein content is about 25% higher, and lysine content of *Amaranthus sp.* grain is 2 times higher that in wheat. The lysine content in our research confirms these results and in an average of all three genotypes is 8,87 g/kg (Table 4).

Table 4. Amino acid composition of seed and leaves proteins of Amaranthus sp.

Amino acids	**Amino acid content of *Amaranthus sp*.** (g/kg sample)				
	Grain genotype				**Leaf**[16]
	2	**16**	**31**	**Average**	
Aspartic Acid	12,74	11,89	13,22	12,62	18,70
Threonine	6,16	5,74	5,83	5,91	8,20
Serine	9,71	9,04	9,96	9,57	8,50
Glutamic Acid	28,31	26,35	29,69	28,12	20,30
Proline	4,99	4,60	5,29	4,96	8,10
Glicine	12,50	11,69	12,18	12,12	10,01
Alanine	6,01	5,66	6,21	5,96	10,30
Valine	6,15	5,73	6,33	6,07	6,30
Methionine	2,07	1,82	2,27	2,05	2,90
Isoleucine	6,59	6,17	6,64	6,47	8,30
Leucine	8,60	8,04	8,87	8,50	14,0
Tyrosine	5,43	4,94	5,90	5,42	8,60
Phenylalanine	6,41	5,99	6,61	6,34	13,30
Histidine	6,25	6,14	7,05	6,48	11,13
Lysine	9,14	8,43	9,03	8,87	11,14
Arginine	16,46	15,23	17,49	16,39	16,90

[16] Stojković, 1996

Protein amino acid content of leaves and grain are in high significant correlation that can be expressed through the correlation coefficient of r =0,809 (Graph 1).



Graph 1. Correlation of amino acid content of seeds and leaves of Amaranthus sp.

CONCLUSION

Amaranthus sp. grain with its basic and specific nutritional values deserves attention and has a perspective on the world and in our market of new food products.
On the basis of biological, agronomic properties and biochemical composition of leaf and grain, *Amaranthus sp.* is also a promising forage culture.

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THE INFLUENCE OF VARIOUS FACTORS ON THE DEGREE OF NITROGEN MATTER CHANGES IN LEGUME SILAGES

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ABSTRACT

The influence of wilting, supplementation with maize meal (50 g/kg), inoculant (250 g/5 T green mass) and phosphoric acid (3 ml/kg) on the degree of changes in nitrogenous matters in lucerne and red clover silages was investigated in the paper.
Based on the results of chemical analysis it was found that red clover silages has significantly lower content of ammonia nitrogen while protein nitrogen was much more preserved, and with more intensive lactic acid fermentation in comparison with lucerne silage. Treatment with phosphoric acid significantly reduced proteolysis and fermentation in both plant silages, while inoculant and maize meal addition contributed the higher production of lactic acid. In silages made from wilted material, without additives, the total fermentation was reduced.
Keywords: *lucerne, red clover, wilting, additives, proteolysis, fermentation*

INTRODUCTION

High nutritive value of perennial legumes is most efficiently preserved in the form of silage, with the application of procedures and additives that help to solve the problem of low fermentability of those plant species (7). The great attention is given to the changes in nitrogen matters during the ensiling process (11). Slottner and Bertilsson (2006) record that in living plants there is 75 – 90% of total nitrogen in the form of true proteins, while in silages there is only 30 – 50%. The solubility is in positive correlation with protein degradability (33), which may significantly decrease their utilization (21), or to induce health problems in animals (23). It is therefore recommended that 30-40% of the total protein is in the form of soluble in dairy cow rations (2), while this value is usually above 60% in legume silages. (12). In order to maximally control nitrogen matter degradation during the ensiling process various procedures are used such as wilting, carbohydrate stimulation, inoculation and chemical conservation (5, 16, 28). Also, the legume cultivars are selected on the basis of ruminal degradability (3) and also genetic manipulations are used with the same purpose (14).
There is small amount of data available in our literature about the problem of proteolysis in silages (6, 9, 10, 22, 29). This is the reason while this process is investigated in lucerne and red clover silages, which are the most important legume species in Serbia.

MATERIAL AND METHODS

Experiment was organized as two-factorial trial (3 × 5) with 3 replications, where factor A was legume species and factor B was treatment (additive) type. Lucerne was cut at the end of button phase and red clover at the beginning of flowering phase, the material was collected at second cut and used as fresh of wilted. The inoculant was of the homofermentative type which had following microcapsulated bacteria *Lactobacillus plantarum* (min. $1{,}0\times10^{11}$ CFU), *Lactobacillus acidophilus* (min. 1.0×10^{11} CFU), *Streptococcus faecium* (min. 1.0×10^{11} CFU) and *Pediococcus acidilactici* (min. 1.0×10^{11} CFU). The amount of the used inoculant was according to the producer 250 g/5 T green mass. Maize meal was added in the amount of 50 g/ kg green mass, while phosphoric acid was added as 3 ml/kg green mass.
All silages, after the treatment, were compressed in plastic experimental siloses with the volume of 5 dm^3. After 56 days from the day of closure siloses were opened and samples for chemical analysis were taken. The parameters of chemical composition and silage quality were analyzed in the Laboratory for animal nutrition on the Faculty of Agriculture in Zemun (1). The content of ammonia nitrogen was analyzed with modified Kjeldahl method (8), soluble nitrogen according to Vistahin (8), and protein nitrogen according to Grando (30). Statistical analysis was done by ANOVA process with Statsoft Statistica V.6 with Tukey's Honestly Significant Test used to evaluate significance of differences (32).

RESULTS AND DISCUSSION

Lucerne silages had more crude protein, cellulose, lipids and ash, and significantly less NFE compared to red clover silages, which can be attributed to the botanical differences between those two legume species, and to the different development phase (Table 1).
Red clover silages had less ammonia and soluble in total nitrogen and also higher preservation of true protein nitrogen compared to lucerne silages. Similar results were obtained by McKersie (1985) and Jones et al. (1995-a, b), also comparing the same legume species, with the conclusion that they were not results of differences in dry matter content and pH values. Lower degree of proteolysis in red clover can be explained with the occurrence of soluble enzyme plyphenol-oxidase, which in the presence of oxygen reacts with O-diphenol producing very reactive O-quinine, which with other suitable molecules such as proteins builds polymers (15, 14, 17, 25).

Table 1.Chemical composition of silages, g/kg DM

Treatments	Dry matter, g/kg	Proteins	Lipids	Cellulose	NFE	Ash
Lucerne						
Fresh	217.38a	209.85c	85.68b	246.52ab	335.13a	122.83b
Wilted	364.30bc	201.077	79.34a	258.93bc	334.72a	125.94c
With inoculant	374.30c	193.12a	86.38b	238.04a	363.31b	119.15a
With maize meal	347.87b	201.73b	87.91b	263.24c	321.26a	125.85c
With H_3PO_4	355.88bc	198.99b	78.75a	266.00c	330.75a	125.52c
Red clover						
Fresh	221.60a	183.01b	74.44bc	228.12ns	411.45ab	102.97a
Wilted	332.29b	178.70a	65.93ab	241.51ns	407.58ab	106.28b
With inoculant	359.22c	175.73a	67.85ab	226.88ns	425.19b	104.35ab
With maize meal	338.957c	178.13a	74.65bc	237.69ns	403.60a	105.92b
With H_3PO_4	324.52b	178.40a	61.97a	237.29ns	417.06ab	105.28ab

[a,b,c] Values in the same column for the same plant species with different letter are significantly different ($P<0.05$); ns = non significant

Wilting of the starting material in both lucerne and red clover increased pH values in silages, reduced degree of proteolysis, and reduced total fermentation (Tables 2 and 3). The addition of maize meal and inoculant had stimulating effect on fermentation, which decreased the pH values and reduced degree of proteolysis. The greatest degree of proteolysis and total fermentation reduction was observed with the addition of phosphoric acid. The pH value and dry matter content are the most important factors which determine the proteolysis intensity but cannot completely stop it (4). Similar trend was observed in the works of Hristov and Sandev (1998), Nadeau et al. (2000), Guo et al. (2008) and Grabber (2009).

The amount of ammonia nitrogen in control lucerne silage was by 88% higher that the permitted value for quality silage, which is 100 g/kg total nitrogen (12). This shows the significant activity of proteolytic bacteria which also produce high amounts of butyric acid (5,86 g/kg DM). Control silages in red clover had significantly lower amount of butyric acid (0,24 g/kg DM). The presence of ammonia in silages which had no butyric acid can be explained with the activity of plant enzymes (26). With the use of phosphoric acid as conservant the substantial inhibition in plant enzyme activity occurred, and the production of ammonia nitrogen was significantly reduced. Komprda et al. (1996) also found that chemical conservant (formic acid) obtains best results in terms of preservation of nitrogen matters.

In lucerne control silage the amount of soluble in total nitrogen of 623.41 g/kg was above permitted value for quality silage of 600 g/kg (12), while in red clover control silage it was significantly below this value (457.61 g/kg). With wilting and use of additives there was significant reduction in soluble nitrogen and that is why the other treatments have satisfactory quality parameters (8).

Table 2.The amount of ammonia, soluble and protein nitrogen in total, g/kg N

Treatments	NH_3N	Soluble nitrogen	Protein nitrogen (true protein)
Lucerne			
Fresh	188.49c	623.41d	361.43a
Wilted	142.06b	553.57c	448.71bc
With inoculant	134.49b	533.00b	464.71cd
With maize meal	131.82b	525.45ab	472.94de
With H_3PO_4	90.82a	508.67a	483.11e
Red clover			
Fresh	134.17d	457.61e	546.59a
Wilted	102.36c	388.93d	606.22bc
With inoculant	96.29bc	377.10cd	622.56cd
With maize meal	87.01b	366.46bc	627.20d
With H_3PO_4	48.94a	331.51a	659.96e

[a,b,c,d,e] Values in the same column for the same plant species with different letter are significantly different (P<0.05)

Table 3. Parameters of the biochemical changes in silages, g/kg DM

Treatments	pH	Lactic acid	Acetic acid	Butyric acid
Lucerne				
Fresh	4.86d	52.01a	54.77c	5.86ns
Wilted	5.08e	46.82a	40.58b	0.66ns
With inoculant	4.57c	64.49b	54.62c	0.00ns
With maize meal	4.42b	68.26b	35.84b	0.00ns
With H_3PO_4	4.12a	51.95a	31.48ab	0.00ns
Red clover				
Fresh	4.67d	73.61ab	88.52d	0.24ns
Wilted	4.76e	62.80a	55.20bc	0.00ns
With inoculant	4.49c	80.21bc	49.75ab	0.00ns
With maize meal	4.16b	85.39c	58.25c	0.00ns
With H_3PO_4	3.99a	60.78a	39.47a	0.00ns

[a,b,c,d] Values in the same column for the same plant species with different letter are significantly different (P<0.05); ns = non significant

The low degree of protein nitrogen (true protein) preservation in silage is typical for ensiled feedstuffs (13), while with the use of wilting and additives its content was increased. Protein nitrogen preservation in red clover silages was significantly higher compared to lucerne silages.
The pH values were lower in red clover silages, which is a result of higher production of lactic acid with high degree of dissociation, but also of acetic although it has weaker

dissociation (Table 3). In silages from wilted material there were highest pH values observed as a result of reduced fermentation. The use of carbohydrate additive intensified fermentation with more lactic and acetic acid produced. Butyric acid was observed in higher amounts in silages made from fresh material.

CONCLUSION

Red clover appears to be more suitable material for ensiling than lucerne in the experiment which was done. It can be explained with specific chemical composition of red clover. However, the use of wilting, carbohydrate additive, inoculant and chemical conservant can decrease the protein hydrolysis and increase the quality of obtained silages in both legume species.
The choice of procedure or additive in legume ensiling is having financial effects but also may help in the meeting of the safety standards in feeds and food.

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LABELLING OF LOCAL FOOD: TOWARDS EUROPEAN UNION

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ABSTRACT

In modern times, people expect their food to be safe, healthy and of good quality while respecting ecological and ethical standards. They want to know the origin of the food they eat, and to consume the food based on this information. The basic data regarding quality, quantity and composition of particular food is given via the label. In our country the labelling of packed foods is regulated via Rule Book regarding labelling and marking of packed foods, that was adopted in 2004. Each European Union candidate country must also harmonize its legislature with the legislature of European Union.
Keywords: declaration of food, law regulations, European Union

INTRODUCTION

All citizens of Serbia notice that most food in the market contains a clearly specified label. [8] The content on the label describes the type of food in question. Information on it must be truthful, understandable, visible, accurate and accessible to the average consumer. [7] Additional information on the label which is not prescribed by regulations must not deceive the consumer or assign properties to food that it actually does not have. Depending on the food item the content of the label also varies. [3] However, despite strong sanctions that are prescribed, many labels are not completely clear and accurate. Some have wrongly listed names, expiration dates, names of manufacturers… On the other hand, labels on food in countries within European Union are modernizing themselves with every new scientific discovery and research. Therefore, they must be made aware of all that is scientifically proven and potentially harmful to one's health (allergens). In addition to information contained on ours, „European" labels contain the number of calories, proteins, carbohydrates and fats. Specific mineral materials, such as calcium, sodium or phosphate, and some vitamins (A and D) are also specified, as well as the recommended daily consumption of food as per the body weight of the consumer.

In order to place the food from Serbia into European Union market, high standards of European Union must be met. [2] Foods must not contain potential allergens that are not specified on the label and all ingredients must be specified accurately in order to determine which ingredients negatively affect human health. Shipments must be announced in advance and notified on the border control spots, with all certificates, and foods must originate from approved countries and registered subjects in dealings with food. However, this procedure should be eased by new law regulations in our country.

Legislation must play an important role in introducing order in all areas, as well as primary production, processing and in the food market. European legislation in the area of food tries to include all parts of production process, from production, through to processing, transport, distribution and sales. Legal responsibility for the safety of food is assigned to allsubjects involved in dealings with food. European agency for food safety has an objective of forming a unique network together with similar bodies in member countries.

Agricultural and food production sectors are of great significance to the economy of the European Union. EU is second largest exporter of agricultural products in the world (after USA), the largest in terms of the volume of production of food products and among the largest according to number of consumers. [5] If, in agriculture, the 20th century is signified by an increase in production, then judging from its beginning, 21st century will be the century of increase in safety and responsibility in the area of food production. In the 1970s the rules of nutrition emphasised [2]:

- Increased production in farming of animals
- Free market
- Informing of manufacturers regarding the nutritive value of mixed food

Today the emphasis has been transferred to:

- Protection of health of animals and people
- Protection of the environment

LEGAL ASPECTS NECESSARY FOR CORRECT FUNCTIONING OF EUROPEAN MARKET

In order to ensure the correct functioning of the European market, relevant rules have been established – primarily the harmonization with regulations of the European Union. This means that all member countries have adjusted their regulations, carrying over European law acquisitions (Community Acquis) into their national legislations. In cases that are not regulated by European Union regulations, the principle of "mutual recognition" applies which means that, if the product is produced legally and sold in one of the European Union countries, the rest of the countries must enable its free movement in their markets. The member country has the right to stop selling if there are indications that it may present any danger to general safety, or endanger the health of consumers. [5]

The task of all governments is defining of the strategy regarding food, identification of priorities in solving quality requirements, safety, and satisfying need for food, as well as ensuring availability of food in international market. [5] As the biggest importer and second-biggest exporter of agricultural and food products EU attaches special significance to international laws that regulate the circulation of these products. Therefore, EU is a member of World Trade Organisation since it was founded in 1994. European Union has adopted two different philosophies that through different means enable the free flow of goods. These are:

- New approach
- Sectoral approach (old approach)

The directives of the new approach define important requirements for products which can affect the health and life of people and domestic animals, interests of consumers and protection of the environment. Each directive of the new approach are accompanied by harmonised standards within which other requirements for goods are defined. Harmonisation of legislation is limited to adoption (via directives of the new approach) of important health and safety requirements that goods in the European market must satisfy.

The directives of old, sectoral approach, regulate in detail the basic and other requirements. They are unsuitable due to their prolixity and amount of detail. These directives, among others, relate to food products as well. Member countries have embedded these requirements into their own regulations. In the area of food products, the directive can be horizonatal, and it is always applied, and vertiacal, which relates to specific products and production procedures. European Union enters regulation benchmarks of international organisations into its legislation also with the goal of removing obstacles to free movement of goods, services and capital within its territory and in international market.

The new approach has developed with the goal of preventing obstacles which could arise as a consequence of conflict between various national regulations and is based on the following principles [5]:

- regulatory basic requirements for products – EU directives precisely define basic requirements that the product must fulfill in order for it to be placed on EU market
- Harmonized standards – technical specifications for goods which are the subject of European directives. Assumption of agreement – it is believed that the product fullfills basic requirements if it is manufactured in accordance with European harmonized standards.

FOOD LABELLING IN EUROPEAN UNION

All food placed into the market within European Union is subjected to labelling rules and can be divided into general and special.

General rules are defined in directive 2000/13/EC[1], including its changes and additions. Food labels must contain the following information:

- The name under which the product is sold, including the physical state or the process the food has undergone (e.g. powder, frozen, concentrate, smoked, etc). Application of irradiation must always be indicated;
- List of ingredients. All ingredients must be listed (including additives), with the specific name, in a descending order according to weight (i.e. starting with the

heaviest). It is not necessary to list ingredients in certain foods: fresh food and vegetables, soda water, natural vinegar, cheese, butter, milk and sour cream, etc. In instances where food contains substances that can cause allergies (e.g. gluten), that ingredient must be clearly marked on the label with "contains <name of ingredient>";
- Net weight, in units of volume (for liquids) or weight (in other products);
- Expiry date;
- Special conditions for storage or use;
- Name and address of manufacturer, or packaging company, and importer into European Union;
- Place of origin;
- Instructions for use, if applicable;
- For beverages, the alcohol content if higher than 1.2%;
- Batch number

Special labelling is applicable to certain groups of products:
- Genetically modified food (GMO) or food which contains or is derived from GMO
- Food produced for specific nutritional application, e.g. baby food, diet food, food for weight loss, food for sports people, etc.
- Additives and aromas. Category, name or E-number must be specified.
- Material that comes in contact with food, including packaging and material for packaging, must be marked with "for contact with food" or a knife and fork symbol
- Specific types of foods: cocoa and chocolate products, honey, sugar, fruit juices, jams, natural mineral water, fast-frozen food, etc

INSTEAD OF CONCLUSION

Labelling of food with the goal of ensuring its safety in the diet of people and animals is ensured through collective efforts of all participants in the food chain[2]. In order to avoid potential dangers and ensure production of safe food the entire food chain must be kept under strict control and relevant regulations must be established and respected.

Adoption of the law has been expected in Parliament of Serbia for a long time, and should regulate the production of food in accordance with European standards. One of the adopted laws from area of agriculture is the Law for food safety, which is aligned with with the European directive 178/2002[4]. This law will enable the establishment of Expert advisory board for estimation of food risk, formation of Central registrar of objects for production of food, Directorate for national reference laboratories in the area of food safety, as well as a fast alert system and management of crisis situations. This will enable Serbia to get a modern system where the food is controlled from "farm to fork" and where all entities in dealings with food become responsible for its safety.

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TABLE QUALITY OF LAYER EGGS FROM VARIOUS PRODUCTION SYSTEMS

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ABSTRACT

The quality of table eggs obtained from Hy Line hybrid layers reared in battery system and native population of Naked-neck hens, reared in extensive system, was studied. The external properties were studied (egg mass, shape index, cleanliness and eggshell colour), internal properties (egg-white colour, haugh units, egg yolk colour, egg-white/yolk ratio) properties of egg and egg shell quality (mass, thickness, defomration and breaking force). Trial was carried out on Experimental farm of the Institute and it lasted 28 weeks, i.e. seven 4-week study periods.
Hybrid Hy Line in battery system had eggs of better quality. Eggs had higher average mass, better egg-white quality and thicker and firmer eggshell.
Naked-neck native hen reared in extnesive system with limited range gave eggs with lower shape index (more pointy eggs), significantly lighter colour of egg shell (2,25; 3,37) and significantly more intensive yolk colour (13,03; 11,52).
Keywords: *production system, layer hen, quality of eggs*

INTRODUCTION

Quality of table eggs is a feature of dynamic character and it depends on numerous factors. Quality of eggs is influenced by biological factors, mostly by genotype, sex and age, and some of the major factors of zootechnical nature are housing system, nutrition and health condition of layers. Today, with abundance of eggs on the market, the price is not the only factor influencing the competitiveness of eggs, but also special guranteed quality for which the consumers are willing to pay higher price.
Alternative (non-convetional) housing systems are intorduced into production because of the welfare aspect on one hand, and, on the other, because of the egg quality, i.e. correlation between the quality of life of layer hens and quality of the product is established [5].
Definining of the rearing and egg quality programmes in special, non-conventional housing systems in our country, was the subject of study of only few researchers [2, 4,5, 6, 7 ,9, 10]. Increased demand for new alternative systems arose subsequent to adoption of the Rulebook on animal welfare in Serbia in February 2010, banning housing of layers in battery cages starting from 2012.
In production of eggs in extensive system as one of the alternative systems, domestic native layers are used – Naked-neck hen, domestic populations of breeds Rhode island, New Hampshire, Amrock, Plymouth rock, as well as crosses obtained from crossing of these breeds, i.e. coloured plumage layers, rather than hybrids.
Since there are no data on the quality of eggs produced by naked-neck hens in our country, objective of this study was to determine the differences in quality of eggs

obtained from hens of different genotypes (Hy Line iand Naked-neck hen) reared in different housing systems (battery and extensive).

MATERIAL AND METHODS

Study was carried out using native breed of Naked-neck hen and Hy-Line hybrid, at the age of 24 to 54 weeks, reared in two production systems (battery and extensive) on Experimental farm of the institute for Animal Husbandry, Belgrade-Zemun.
Battery system: layers of hybrid Hy-Line were reared in battery system. There were four hens in each cage, total of 240 hens in a battery. Feeding, watering and collecting of eggs were done manually. Production technology was according to norms of standard technology applied in modern production of table eggs.
Extensive system: 40 Naked-neck hens were reared in a facility where feeders, waterers and nests were placed, starting from the age of hens of 22 to 54 weeks
Hens in both system were fed same mixture containing 17% of protein.
In the period from 24 to 54 weeks of age, every 4 weeks, sample of eggs – 30 eggs (Naked-neck) and 60 Hy-Line eggs, was used to investigate major external (egg mass, colour and cleanliness of eggshell, shape index), internal egg quality properties (yold colour, egg-white height, Haugh units, egg-white/yolk ratio) and eggshell quality properties (mass, thickness, deformation and breaking force). Said properties were investigated according to the method of Pavlovski et al., 1997, and breaking force according to method of Pavlovski and Vitorović (1996), on apparatus „IS-96“ which is modification of the apparatus Wolotkijevich (Institute for Animal Husbandry, Belgrade-Zemun). The data were analyzed by method of variance analysis and Tukey test (Stat.Soft,Inc. STATISTICA, version 6).

RESULTS AND DISCUSSION

In Table 1. the average values of studied properties of external quality for all seven 4-week periods are presented.
Eggs deriving from hybrid Hy Line had statistically greater egg mass (64,54; 53,77). Obtained shape index indicated that eggs from Naked-neck hen were more pointy compared to eggs from Hy-Line hybrid.
In the investigations [6] of eggs obtained from Prelux – R hens reared in extensive system, results showed similar shape index (74,64). Hens Prelux – R were bred in Slovenia and intended for rearing in extensive system and have simialr egg quality traits as native populations, in this case naked-neck hen. Eggs obtained from naked-necj hen reared in extensive system had lighter eggshell colour, similar to results of studies [1, 4, 5, 6, 7]. Based on presented data it can be concluded that housing system and genotype had effect on investigated properties of external egg quality.
In Table 2. the data on investigated properties of internal egg quality are presented. Eggs obtained from Hy Line hybrid hens had statistically significantly greater egg-white height and number of haugh units, which is evidence of significantly better quality of egg-white and more favourable egg-white/yolk ratio, whereas eggs from Naked-neck hens had more intensive yolk colour.

Obtained data was not in accordance to data [1, 2, 3, 4, 5, 6, 7, 9] where better egg quality was established in case of hens reared in extensive system compared to battery aviar system regardless of the studied hybrid, i.e. significant effect of the housing system was expressed. Investigated hen housing systems had significant effect on yolk colour, i.e. hens reared in the extensive system has access to limited range where they consumed green grass mass which had impact on improvement of the yolk colour. For other investigated properties it can be concluded that genotype had not demonstrated significant impact.

Table 1. External egg quality traits

Genotype	No. of eggs	Egg mass, g	Egg shell colour, points	Egg shell cleanliness, points	Shape index
Hy Line	480	64,54±5,42**	3,37±0,60	4,58±0,88**	77,89±2,98**
Naked-neck	240	53,77±6,84	2,25±0,61	4,24±1,31	74,68±3,20

**P≤0.01

Table2. Internal egg quality traits

Genotype	No. of eggs	Yolk colour, Roche	Egg-white height, 0,1mm	Haugh units	Egg-white/yolk ratio
Hy Line	480	11,52±1,57	84,20±15,07**	89,63±8,80**	2,27±0,27**
Naked-neck	240	13,03±0,58**	68,83±16,01	83,39±10,54	1,67±0,25

**P≤0.01

Obtained data on egg shell quality (mass, deformation, thickness and breaking force) are presented in Table 3.

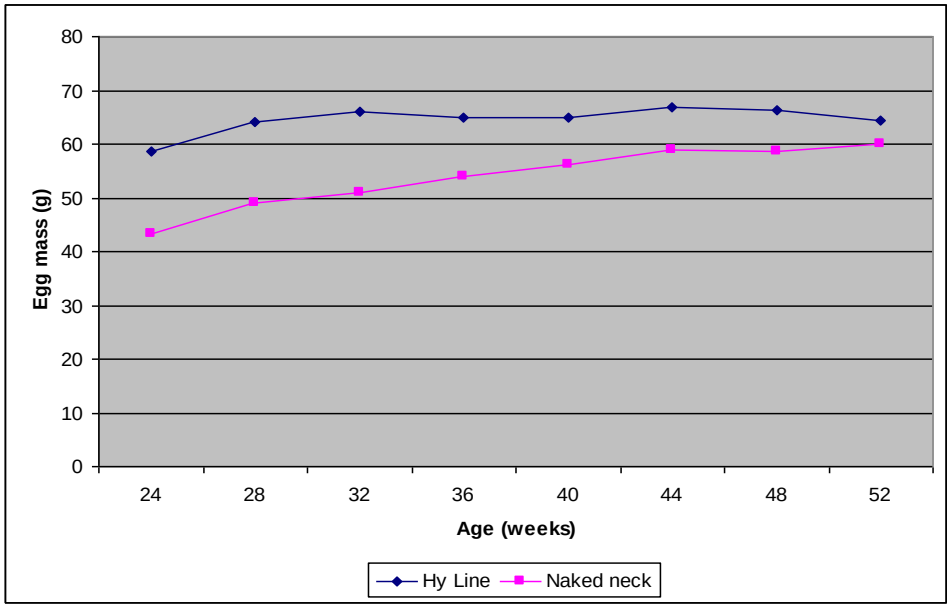
Table 3. Egg shell quality traits

Genotype	No. of eggs	Egg shell mass, g	Egg shell deformation, 0,001mm	Egg shell thickness, 0,01mm	Breaking force, kg
Hy Line	480	8,87±1,18**	21,57±4,06**	34,88±3,00**	17,84±3,45**
Naked-neck	240	6,97±1,12	24,82±4,42	24,82±4,42	15,96±2,87

**P≤0.01

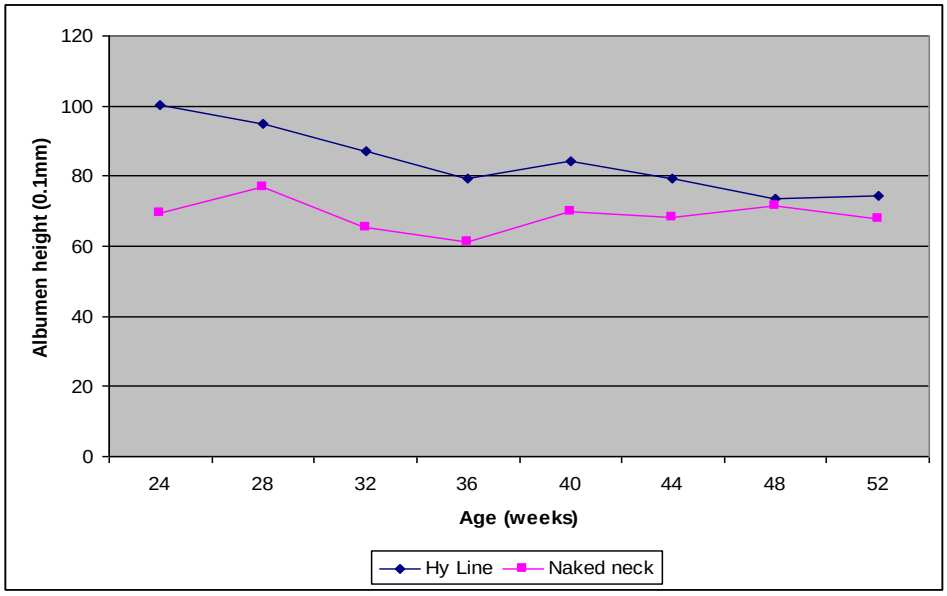
Based on all studied egg shell quality properties it can be concluded that poorer quality of egg shell was determined in eggs obtained from naked-neck hen reared in extensive system. Obtained data are in accordance with researches [4, 5, 7] where hens reared in extensive system had thinner egg shell regardless of the investigated genotype. Reasons for such results can be in the fact that all modern studies in the field of nutrition have significantly influenced the increase of egg shell thickness in case of hens reared in battery system or that areas where rearing of hens is organized in the extensive system

show deficit in lime content in the soil, therefore hens are not able to satisfy the need of their organism for Ca. Contrary to these results are researches [1] where hens reared in the extensive system had eggs of thicker egg shell.
In Graph 1. the average values of the egg mass individually for all study periods of the production duration from 24 to 54 weeks of age of hens are presented. Hens of Hy-Line hybrid realized egg mass of 58,74g, in 24th and in the 28th week of age egg mass was 64,15g and to the end of the research this average egg mass was maintained. Naked-neck hens at the age of 24 weeks realized average egg mass of 43,42g and with the age the egg mass increased and in the 52nd week it was 60,13g. In this study, hen genotype demonstrated different effect on average egg mass. Modern hybrids are selected in the direction to achieve at early age good egg mass and to maintain the persistence of this specific trait as long as possible during one production cycle.



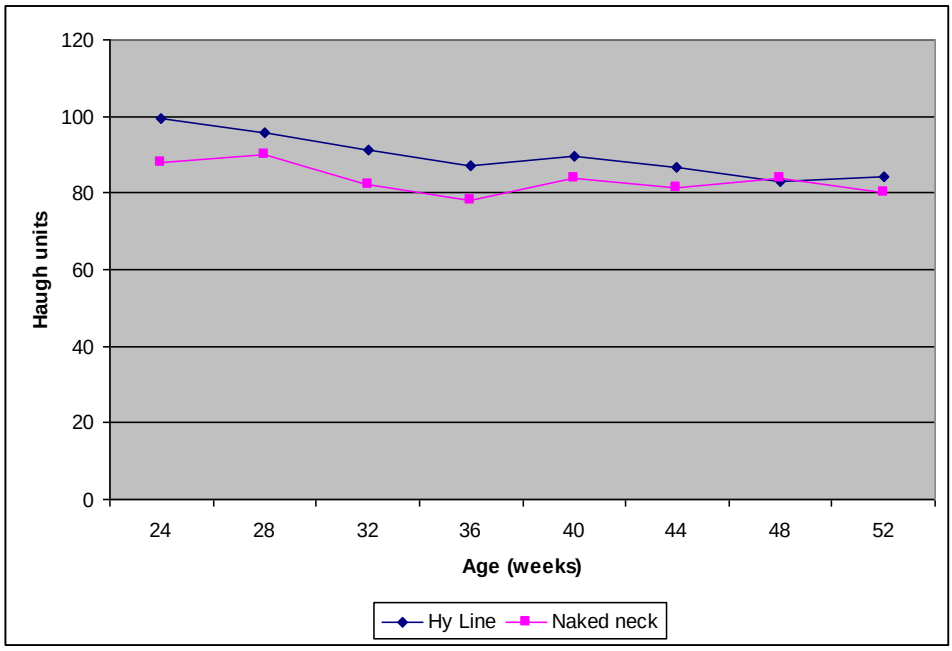
Graph 1. Egg mass, g during 28 weeks of laying

Naked-neck hens demonstrated the feature of the native populations, i.e. with the age of hens also the average egg mass increases.
In Graph 2. data on average values of egg-white height depending on the age of hens, i.e. investigation period are presented. In the first investigation, at the age of hens of 24 weeks, egg-white height was statistically significantly greater in hybrids (100,22; 69,57). With the age of hens, difference in the egg-white quality decreased significantly, in fact the quality of egg-white in Hy-Line hens decreased more rapidly, whereas eggs from naked-neck hens maintained the egg-white height at a similar level throughout the investigation period. In general it can be said that statistically significantly better egg-white quality was established in eggs from hybrid eggs.



Graph 2. Egg-white height in investigated eggs, 0,1 mm

In Graph 3. average values of Haugh units of investigated are presented. Haugh units are calculated using logarithm function based on egg mass and egg-white height, and in this way eggs of lower mass are favored, which is why many researchers have disputed in their studies the Haugh units as objective assessment of the egg quality. Our research confirms this to certain extent. Comparison of egg-white height and number of Haugh units showed significant reduction in differences in quality of egg-white between studied systems and genotypes to the advantage of Naked-neck hens which had lower egg mass and in this way reduced difference in the quality of egg-white and Haugh units.
These studies are contrary to our previous researches [2, 3, 4, 5, 6, 7], which indicated better quality of eggs reared in extensive system of production, regardless of the studied genotype.



Graph 3. Haugh units of investigated eggs

CONCLUSION

Based on obtained data in the research of the system of housing (battery and extensive) and genotype (Hy Line hybrid and Naked-neck native hen) the following can be concluded:

- Hybrid Hy Line reared in battery system gave eggs of better quality. Eggs had greater average mass, better egg-white quality and thicker and firmer egg shell.
- Naked-neck native hen reared in extensive system with limited range gave eggs of lower shape index (more pointy), significantly lighter egg shell colour (2,25; 3,37) and significantly more intensive yolk colour (13,03; 11,52).

In general it can be concluded that in this research the effect of the housing system on egg quality was not observed, and that the quality of eggs was more under the influence of layer hen genotype, i.e. Naked-neck hen laid eggs of poorer quality.

ACKNOWLEDGMENT

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EFFECT OF THERMAL PROCESSING AND TRYPSIN INHIBITOR EFFECT TO PROTEIN DIGESTIBILITY IN SWINE NUTRITION

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ABSTRACT

Usage nonprocessed soybean costs of nutrition will be reduced and aslo the monopoly of processing industry. One of the varieties with lower levels of trypsin inhibitors that are produced in Serbia, it's the soy variety "LANA". It is the result of soybean selection program to reduce the activity of trypsin inhibitor. With the digestibility of proteins using direct and indirect method it is possible to determine the effect of heat treatment and the impact of the presence of Kunitz trypsin inhibitor and thus give an assessment on the possibility of use raw soybean variety "Lana" to nutrition fattening pigs. By using the direct method for determining the digestibility, heat-treated soybean had an apparent digestibility of 83.83% for standard soy and 84.27% in variety "Lana", while the thermal coefficient of raw soybean protein apparent digestibility was 76.90% for the standard variety and 78.43% in variety "Lana".

Key words: *trypsin inhibitor, soybean, digestibility, pigs*

INTRODUCTION

Solubility of proteins is an important physical characteristics. Of the total protein 72-95% is easily soluble in water, which is a necessary condition for their efficiency in feeding and fattening pigs. Digestibility is a major index of quality of the protein and is associated with usability of amino acids. This means that proteins can be used if they are not digestible. The high temperature on digestibility of protein is the impact of the presence of specific biologically active components as well as the chemical form of soy protein. These factors affect the digestibility of the formation of proteins that can not be hydrolysied to amino acids or amino acids obtained by hydrolysis are less usable in the diet. Using raw soybeans comes to depression in both the manufacturing parameters, and cause in digestible of meals. The reason for this is the presence of anti-nutritive factor in soybean grain primarily on trypsin inhibitors. Trypsin inhibitor is a protein fraction of the raw soy beans with anti-nutritive factors which has the property that blocks the activity of trypsin and himotrypsin and thus interferes with and prevents proper digestion in the small intestine Huisman et al. [7], Gabert et al. [4]. Unlike the grain legume crops, which also contain trypsin inhibitors they have not so depressing effect on the digestion of protein because its activity is low. Efforts to increase the use of legumes led to the development of a wide range of media processing, including extrusion. Nutritional effects of extrusion is of special interest because of their increased use in industry.

Extrusion is the thermal process of high production capacity and energy efficiency, with shorter treatment time than other methods of heat treatment. The process consists of operations such as mixing and heating under conditions of high compression and shear stress Fadel et al. [3]. Exposure to high temperatures nutrients in a short time leads to the effective destruction thermolabile anti-nutritive factors and destruction of microorganisms. This technology has numerous advantages, including the possibility of wide application, low cost operations, high productivity, energy efficiency and high quality of the resulting products Brenes et al. [2]. Strong force of friction can also lead to protein denaturation and decomposition of envelope grains, and thus increase the digestibility of nutrients [1]. Kim et al. [11] suggesting that the pigs in fattening and increased use of extruded soybean have greater intestinal digestibility of nutrients, nitrogen and most of the essential amino acids in relation to the fried soybeans, while the digestibility of soybean meal somewhere between these nutrients. Marty et al. [13] observed the apparent intestinal digestibility of soybean protein varies from at least 69.5% of roasted soybeans to a maximum of 75,6% of extruded soybean.
The second is that genetic engineering improves nutritional value of soy beans that is development of varieties with lower levels of Kunitz inhibitor and / or lectins with their omitted heat treatment or lower temperature processing and thus reduce the cost of feeding. New soybean varieties with low levels of anti-nutritive factors were developed the last two decades. Hymowitz [8] discovered soybean seed with low levels of Kunitz [12] trypsin inhibitors. There are claims that the best results are expected when genetically improved varieties subjected to thermal treatment Palacios i sar. [14]. One of the varieties with low levels of TI, which is produced in Serbia, is "LANA." It is the result of the selection program of soybean to reduced trypsin inhibiting activity. Mature grain variety LANA contains a Kunitz trypsin inhibitor but lower concentrations than is the case with standard varieties. KTI is the most important protease inhibitor found in soy beans, and is responsible for approximately half of the trypsin inhibitory activity. The aim of this paper is that, based on protein digestibility determine the use of the thermal efficiency of raw soybean variety "Lana" in pigs, as well as the influence of trypsin inhibitors and thermal treatment on the digestibility of protein.

MATERIAL AND METHODS

The experiment was performed on male pigs, Large White breed, weighing 60 kg, over a period of 25 days while the preparatory period lasted 10 days. The experiment was four experimental groups, with six repetitions. Groups were balanced both phenotypic and genotypic. In the experimental groups mixtures for pigs differed in soybean grain and thermal treatment of the same. In I group were, standard extruded soybean varieties, in Group II extruded soybean seed with low level of trypsin inhibitors variety "Lana" in the third group fallow grain soybean cultivars, in the standard group IV soybean fallow seed with low level of trypsin inhibitors variety "Lana".
The animals were kept in individual metabolic cages, which are adapted to controll feeding and water supply of animals and collection of feces and urine. In this way animals were safely fixed and limited to urinate and defecate in a particular region of cages, and thus it is prevented to mix the contents of urine and feces, and enable the efficient collection of the same, while the same animal didn’t have the possibility of

physical injury. With this type of collection of urine and feces, animals are not subjected to surgical interventions, and physics does not suffer pain, and method is in accordance with the Law on animal welfare. Pigs in the experiment were carried out twice a day, and while feeding the animals consumed food of physiological fullness. The composition of mixtures is shown in *Table 1*

Table 1 Composition of mixtures for pigs nutrition

Nutrients	Group			
	I	II	III	IV
Maze	53.40	53.40	53.40	53.40
Wheat	15.00	15.00	15.00	15.00
Sunflower meal	4.00	4.00	4.00	4.00
Soy with stand. level of TI extruded	24.00			
Soy with lower level of TI extruded		24.00		
Raw soy with stand.level of TI			24.00	
Raw soy with lower level of TI				24.00
Premix	3.00	3.00	3.00	3.00
Lysine	0.10	0.10	0.10	0.10
Chromium oxside Cr_2O_3	0.50	0.50	0.50	0.50
TOTAL	100.00	100.00	100.00	100.00

Standard chemical analysis determined the nutrient content of food and faeces.. Total nitrogen content was determined by Kjeldahl method.

Table 2 The level of protein and chromium in food and excretions of pigs in experiment

Group	I	II	III	IV
Heat tretman	Extruded		Raw	
Type of soy	Standardn soy	„Lana“	Standard soy	„Lana“
Protein in dieti	18.53	16.69	17.94	16.44
Protein u fecesu	19.43	16.55	23.23	19.98
N in urine	0.70	0.93	1.23	1.07
Cr_2O_3 in diet	0.88	0.85	0.86	0.79
Cr_2O_3 in fecesu	4.18	4.66	4.18	4.23

The sample is destroyed with the addition of a catalyst mixture of K_2SO_4 and $CuSO_4$ on 420° C. After distillation of the liberated ammonia are titrated with 4% H_3BO_3 to add the indicator and multiplying by a factor of 6.25 calculates the protein content. Urease activity is determined by the Caskey-Knapp method based on the consumption of 0.1 N NaOH for titration to changes in pH 4.7. Content of trypsin inhibitors is determined by a modified method of Erlanger, which is based on measurement activities anti-trypsin extract soy flour using a synthetic substrate, Na-benzoyl-DL-arginine-paranitroanilide (BAPA).

Table 3 The level of trypsin inhibitors and urease activity in soybean grain used in the experiment

Group	Tretman	Type of soy	T. I. g/mg	Urease activity
I	Extruded	Standard soy	14.53	0.49
II		„Lana“	13.50	0.37
III	Raw	Standard soy	30.51	11.01
IV		„Lana“	17.07	10.27

Digestibility determination was carried out with direct and indirect method. A direct method of determining digestibility means of measuring consumption and the amount of excreted feces during the period. Then, based on chemical analysis of food and feces is calculates the total amount consumed and excreted nitrogen, in order to obtain the results of protein digestibility. As the feces are not only the undigested remains, but the ingredients that come from excreted juices for digestion and digestive tract epithelial cells (minerals, proteins, etc..) digestibility, which was determined as described above is called apparent:

Formula 1. Apparent digestibility, determined with direct methode

$$AD = \frac{Nd - Nf}{Nd} \times 100$$ *Nd- Nutrients in a diet ; Nf – Nutrient in a faeces*

Beside these methods in the experiment were used and indirect methods of determining digestibility using indicators chromium oxide. Prince of digestibility determination of indirect (indicator method) is based on monitoring the relationship between inert and nutrients on the basis of dry matter of food and feces. Inert substance is considered a substance that is not absorbed, which does not affect the digestion of nutrients or the animal health and production, whose concentration in relation to nutrient changes during passage through the digestive tract. To this end meal was added chromium oxide in calculative concentrations of 0.5% in the diet. Calculation of digestibility derived by applying the *Formula 2.*

Formula 2. Apparent digestibility, determined with indirect methode

$$AID[\%] = 100 - (Fd / Ff) \times (Crf / Crd) \times 100$$

AID –apparent digestibility; Fd – nutrient in faeces; Ff –nutrient in diet ; Crd – indicator in faeces; Crf – indicator u diet.

RESULTS AND DISCUSSION

The protein digestibility trial testing where the applied direct method is obtained values shown in *Table 4* indicating that better apparent digestibility of protein was in groups where the pigs consumed extruded soybean seed regardless of whether it comes to the standard variety or cultivar "Lana." Pigs that were fed soybean varieties with the raw soy had worse apparent protein digestibility.

Table 4. Apparent digestibility of protein determined with direct methode

	Repetition	Experimental gruop			
		I	II	III	IV
		Extruded		Raw	
		Standard	„Lana“	Standard	„Lana“
Digestibility %	1.	82.88	85.03	73.22	77.12
	2.	81.71	85.30	73.40	77.34
	3.	85.47	81.54	81.85	80.43
	4.	86.57	82.35	82.02	79.70
	5.	83.18	85.81	75.14	76.50
	6.	83.18	85.61	75.75	79.50
	Average	**83.83**	**84.27**	**76.90**	**78.43**
	Index,%	100.00	100.53	91.73	93.56
	Index,%	99.48	100.00	91.25	93.07

As shown in *Table 4*, heat-treated soy had an apparent digestibility of 83.83% and 84.27%, while the thermal coefficient of raw soybean protein apparent digestibility was 76.90% and 78.43%. When we look achieved an apparent digestibility of protein in determining where the indirect method applied to *Table 5*, it can be concluded that the same trend as in the direct method except that the values obtained by this method and thus lower apparent digestibility coefficient of protein in heat-treated standard cultivars and varieties "Lana" was 77.42% and 81.74%, while the crude variety standard and "Lana" the value was 73.14 and 77.26.

Table 5. Apparent digestibility of protein determined with indirect methode

	Repetition	Experimental group			
		I	II	III	IV
		Extruded		Raw	
		Standard	„Lana“	Standard	„Lana“
Digestibility %	1.	78.16	83.97	71.63	76.65
	2.	70.97	84.00	71.13	76.31
	3.	80.82	76.37	78.02	78.70
	4.	84.16	79.53	77.45	77.91
	5.	74.90	82.21	69.47	74.68
	6.	75.50	84.37	71.12	79.33
	Average	**77.42**	**81.74**	**73.14**	**77.26**
	Index,%	100.00	105.59	94.47	99.80
	Index,%	94.72	100.00	88.48	94.52

The above values are consistent with the results obtained by Holmes et al. [6], when compared with digestibility of heat-treated soybean and raw soybean heat, contrary to the results of which have come Jorgensen et al. *[9]*. For the apparent protein, digestibility of extruded soybeans amounted to 74%, raw soybeans 73%. Marty et al. [13] observed apparent intestinal digestibility of soybean protein varies from at least 69.5% of roasted soybeans to a maximum of 75,6% of extruded soybean. Hancock [5] reported that the total apparent intestinal digestibility of crude protein (69.2% versus 62.4%) was significantly higher in diets based on extruded soy than in diet based on soybean meal.

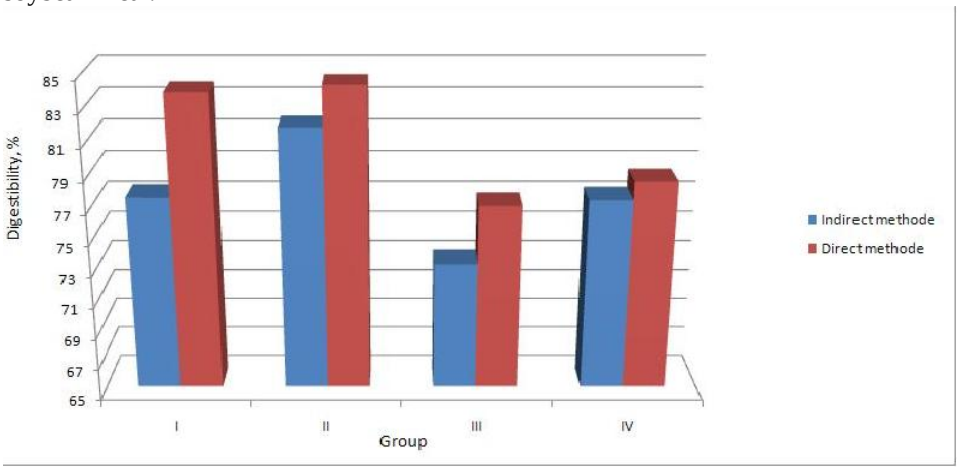

Figure 1 Results of diet digestibility obtained by direct and indirect methode.

If we compare the results obtained by direct and indirect method of calculating digestibility, as shown in *Figure 1*, it can be concluded that the values obtained by indirect method are lower than the direct method, but to follow the same trend. Similar results with Chromium oxide came to the Jorgensen et al. [10] and Sauer [15]. Obtained values are comparable only in the method (direct or indirect) because otherwise it can cause significant errors.

CONCLUSION

Worse digestibility of groups that consumed raw soybean is a consequence of the presence of high levels of anti-nutritive factors in the first place trypsin inhibitors. This is to confirm the results obtained using the direct method and the indirect method (the method of indicators). Both methods in this experiment gave a satisfactory result while the results between these methods can not be comparable.
The use of crude soybean variety "Lana" gave better results digestibility compared to the group with raw soy standard varieties of soybean, but these results were far worse than the group with thermal treatment. Since the variety "Lana" selected in the absence of Kunitz trypsin inhibitor, which is one of the major carriers anti-nutritive effects, besides a grain of soybean contains Bowman-Brik's TI and lectin whose effect can significantly affect the reduced digestibility, especially absorption of nutrients.

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INTOXICATION OF POULTRY CAUSED BY INADEQUATELY HEAT TREATED SOY PRODUCTS AND THE POSSIBILITY OF PREVENTION

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ABSTRACT

The use of soy products in complete diets for poultry has increased abruptly due to aspiration for to exclude feeds of animal origin. Because of higher demands on market for soy products soybean is more represented in sift structure and increased number of packing capacity besides the existing, well established ones. During the regular analysis and the analysis on request made by farmers, the results indicated that the soy products were inadequately heat treated, implying that tripsin inhibitor was not neutralised.
The use of soy products in complete diets in poultry, with content from 28 to 38 % depending on species and category, is very important. In case of inadequate heat treatment of soy products, the disease in poultry develops quickly and is followed by symptoms of: apathy, anorexia, paresis, paralysis, increased intestinal peristaltic with findings of undigested feed in feces, and mortality. By prompt determination of the cause of disease using clinical experience and laboratory examinations, this severe nutritive disorder may be successfully rehabilitated.
Keywords: *poultry, disease, soy products, urease activity.*

INTRODUCTION

Despite the well recognized high nutritional value of soy for a long time, not until the mid twentieth century it was possible to extract and use its nutrients. Several decades later, soy has transformed from almost useless crop to the main source of vegetable oil and proteins. This is particulary important having in mind that soy proteins are cheaper than those in wheet or milk. Also because of the hygiene-sanitary requirements soy is more adequate for the production of ecologically acceptable meat and eggs.
According to recent trends in the development of husbandry, especially a meat industry, the soy and the corn are the primary source of proteins and energy, respectively. The largest consumers of soy products are in the area of pig and poultry industry (38.6 and 29.8% of GDP for meat). The highest growth of meat and egg production is recorded in the USA, China, Brasil and Mexico, the largest producers of soy and corn. The FAO forecasts the world production of poultry meat will reach 95 milion tons till the year 2015.
Soybean contains 35 to 45% of proteins with high conent of irreplaceable aminoacids like lysine, methionine, tryptophan, and also 13 to 20% of vegetable fats. But strong arguments against the use of soy were once stated. The use of soybean was restricted because of its biologically active antinutrients, which decelerate the assimilation of

proteins in organism, lower the efficacy of feed utilization and are toxic to poultry [11]. Crude soy beans contain about 20mg/g of trypsin inhibitor, while the maximum allowed concentration is 4 to 5 mg/g, but also beans have lipid oxidase, hemagglutinin, and several allergens. All mentioned substances are proteins, that are denaturated during the certain temperature regimen, i.e. their activity decreases to the harmless level [3,4].
In order to prevent the outbreak beforehand, our investigations were concentrated to diagnose the disease using tools of clinical and pathological examination, the analysis of production parameters and chemical analysis of different soy products (SP).

MATERIAL AND METHODS

The investigations were carried out during the period from 2009 to 2010 (November-April). We analysed 82 soy products in total: soybean meal, extruded soybean meal and soy cake. The soy products were analysed for the efficacy of heat treatment indirectly, by determination of the urease activity, a method by JUS ISO 5506:2001.
Since the clinical signs of intoxication with inadequately heat treated soy products were present in all categories of poultry, particulary in broiler chickens, the clinical observation and the analysis of production results were conducted on farm facilities. The necropsy was done either on farm or the carcasses were sampled and transported to the Veterinary Institut Novi Sad for the post mortem examination.

RESULTS AND DISCUSSION

During six month period 82 samples of different SP were processed chemically to determine the urease activity, in laboratories of Veterinary institute Novi Sad. The results are presented in Table 1.

Table 1. Results of the chemical examinations of urease activity in soy products

Type of soy product	Soybean meal	Extruded soybean meal	Soybean cake
Maximum allowed urease activity, %	0.4	0.4	0.5
No of analysed samples	30	38	14
No and ration of inadequately treated samples	6 (20.00)	11 (28.95)	2 (14.29)
Variation interval	0.4 – 0.84	0.44 – 2.36	0.68 – 2.00

Totally 82 samples of three different forms of SP were chemically analysed for the urease activity. Overall, the smallest amount of inadequatelly treated SP was present in group of soybean cake (14.29%), while the extruded soybean meal was the most frequently inadequately treated product (28.95%). Comparing to extruded soybean meal, almost twice less inadequately treated soy cake samples were determined, possible because of smaller number of the samples analysed. The urease activity more than maximum allowed was determined in few less percantage (20%) [9].

Table 2. Distribution and variation interval (VI) of soy products according to their urease activity (UA): bellow and up the maximum allowed, optimal or less heat treated

Type of soy product	Soybean meal	Extruded soybean meal	Soybean cake
Maximum allowed urease activity, %	0.4	0.4	0.5
No of analysed samples	30	38	14
No of samples (% of samples overtreated, with UA ≤ 0,05 %)	7 (23.33)	1 (2.61)	0 (0.00)
VI for overtreated samples	0.00 – 0.04	0.04	0.00
No of samples (% of samples optimally treated, with UA from 0.1 to 0.3 %)	10 (33.33)	23 (60.53)	12 (85.71)
VI for optimally treated samples	0.1 – 0.27	0.10 – 0.29	0.13 – 0.27
No of samples (% of samples less treated, with UA from 0.3 to 0.5 %)	7 (23.33)	7 (18.42)	1 (7.14)
VI for less treated samples	0.31 – 0.40	0.31 – 0.48	0.40
No of samples (% of raw samples, with UA >0,5%)	6 (20.00)	7 (18.42)	1 (7.14)
VI for raw samples	0.56 – 1.31	0.56 – 2.36	2.00

The results of distribution of SP based on their urease activity, which was bellow and more than the maximum allowed, optimal or higher but allowed values are presented in Table 2.
Eight out of 82 samples of SP (about 10%) were fortified to be overtreated. Among them, high differences were determined in distribution (23.33 and 2.61% of soybean meal and extruded soybean meal, respectively), while no overtreated soybean cake was assesed.
Also, significant differences were determined in the largest group that comprised of totaly 45 (about 55%) optimally treated SP, with the least represented soybean meal (33.33%), compared to extruded soybean meal (60.53%) and the most represented soybean cake (85.71%).
Totaly 15 SP (round 18%) were determined to be less treated. In this group, similar ratio was present in soybean meal and extruded soybean meal (23.33 and 18.42%, respectively). Only one sample of soybean cake was assesed to be less treated (7%).
Among the SP that were declaired as raw (totaly 14 out of 82, about 17%), similar results were obtained as in the group of less treated SP (Table 2).

Based on the anamnestic data, production results, clinical signs and first findings at necropsy, it was recomended to provide different complete diet until the urease activity analysis in SP was done. At the same time, multivitamine treatment with syntetic amino acids of chickens was prescribed, in order to settle physiological and nutritive requirements.
The examinations of urease activity in SP were done in period (November-April) after the harvest of soy, i.e. during the intensive packing by renowned and also new manufactores with different capacity. The obtained results were defeating (Table 1) indicating that more than 23% of SP were inadequately heat treated. In our

investigations, the determined percantage of proper SP with urease activity in allowed limits was significantly lower than relevant literature data [1, 2,10].

Clinical signs and pathology in broiler chickens

During the investigation period, clinical signs and pathological features indicating intoxication with inadequate heat treated soy products were observed in eight broiler flocks. The flock size varied between 4000 and 15000 chickens.
Farmers mostly complained and asked for inspection in broilers at the age of 3 to 4 weeks. At that age, clear picture of flock ununiformity, low vitality and grouping bellow the heaters became visible, with symptoms of wing and leg paresys and paralysis, and chickens pecking the litter, walls, drinkers and feeders. The litter was moist because of constant yellow to pale red diarrhoea mixed with undigested feed particles.
The control measurements of body weight were bellow the technology, and with negative tendency during the successive intersections. The fattening period was prolonged for 4 to 7 days in average. For the economically justified slaughter, the finishing body weight were hard or impossible to reach.
The carcasses were found in all parts of the floor with their legs extended. The week feathering was observed with significant fluff remains, the skin and visible mucosa were pale - anemic, and thin feces was pasted around the cloaca. Section findings included weekly developed glandular and muscular gizzard, pancreatic tissue either in atrophy or in hypertrophy, small intestines with pronounced catharal enteritis.
Anamnestic data gained from farmers, clinical signs and section findings could not be sufficient to diagnose the disease and we needed the production results and laboratory examination of SP. In comparison to other categories, the most cases of intoxication with inadequate SP that belong to nutriopathies, were registered in broilers. The possible reason for it may lie in the fact that the most percantage of SP is in complete diets for broilers and also because of the highest daily feed consumation in this category.
Interestingly, during the serology testing on regular bases we observed that often antibody response to certain viral vaccines was bellow protective level (data not shown). In several research it was found significantly lower antibody titre in haemagglutination inhibition (HI) test performed from sera of 14 days old chickens vaccinated against Newcastle disease (ND) and fed with diets with 4% less of total proteins. Also, significant differences in HI antibody titre against ND between the experimental and control group were determined in chickens that were fed constantly with complete diet with 2% reduction of the total protein content. Not the content of the amino acids but the total protein content in diet has the primary role in immune response to vaccination [8].
During the fast growth phase in poultry several vaccination procedures are conducted and the syntesis of proteins are particulary intensive. Having in mind the protein nature of antibodies, competent immune tissues and organism overall hold clear requests [7].
In presented cases, secundary bacterial infections were often noted in broiler flocks, because general immunity was supressed. The overall mortality in flocks per production cycle was higher than technology for 2 to 4% in average.
The compilation of data including the production results (average body weight, feed conversion, mortality), the age and the structure of mortality, the chemical analysis and clinical experience, it is possible to rehabilitate numerous diseases [5].

Clinical signs and pathology in commercial layers

Layer production and egg industry are another big consumers of soy products. The same tendency as in broiler production is visible: countries with high production of soy and corn, note the growth of egg rpoduction. One of the main alternative sources of proteins and energy are leguminose and their byproducts that are produced during the packing, including meal and cake. The soy has the essential significans in these technologies.
During our investigation, health disorders and rpoduction fall was noted in two flocks with commercial layers.
The so called «operating atmosphere» is absent in layer flocks that consume inadequately heat treated soy products, including signs of week feed and water consumption, lower reaction on movement of the staff, default of announcement after the eggs are layed. The first sign is gradual reduction of egg mass, followed by the reduction in number of eggs layed. During the section of sacrified layers, we found smaller number of egg follicles and numerous ovarian cysts. Control body weights were bellow the technology. Determined economical losses because of consumption of inadequately heat treated SP could be summerizes in lower number of eggs, smaller total egg mass and higher feed consumption.
In cases when the problem was overlooked or noticed too late, the rehabilitation was not satisfied and the flocks were excluded from further production.

Clinical signs and pathology in parent flocks

The clinical signs, pathology, section findings and the production results in two parent flocks during the lay that were fed with inadequately heat treated SP were similar to those in commercial layers. Besides the reduction of number of fertile eggs, however, approximately 10 to 15 % were unsuitable for the incubation because of their smaller size. Moreover, during the brood, at the firs lamp check round 8 to 12 % of eggs were discarded as unfertilised, and increased embrion mortality was seen at the last incubation phase. The hatching was prolonged with more avital chickens.
It may be usefull to underline that in such circumstances indirect losses are often much higher than the direct ones. Therefore unrealistic estimations of total losses may be provided [6].

CONCLUSIONS

Based on the given results of our investigation, few concluding remarks may be stated:

- The regular and overall monitoring of the safety and the quality of soy products including urease activity is needed, particulary in newly registered feed producers
- For the proper diagnose the following data for certain flock have to be available: case history, clinical and pathological findings, intersections of production results and analysis of urease activity
- It is necessary to replace the faulty SP with the correct one and add multivitamine preparations comprising the essential amino acids.

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PRESENCE OF FUNGI IN POULTRY FEED AND EFFECTS OF CONTAMINANTS ON HEALTH STATUS

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ABSTRACT

Fungi often contaminate cereals already in the field or during storage and, as the basic component in the composition of feed, they are a potential source of various problems in animal production: from fall of performances to death and serious health disorders, not only in animals, but also for people. However, the presence of molds by itself is not the only danger. There is also their ability to synthesize certain secondary products well known as mycotoxins. By developing conditions unfavorable for the growth of molds contamination of cereals by mycotoxins can be also prevented and therefore detrimental effects on health of humans and animals.
This paper presents the results of mycological and mycotoxicological analysis of 747 samples of corn and wheat, and 263 samples of mixtures for feeding of poultry that were received in the NIVS laboratory for control from the territory of the Republic of Serbia during the five-year period.
By mycological analysis of cereals it was found that even 70% of the grains did not meet the requirements regarding the presence of fungi according to Serbian regulations, and the greatest degree of deviation was found in corn naturally dried. The most frequently isolated molds were: *Aspergillus spp., Penicillium spp., Fusarim spp., Mucor spp*. Number of molds in mixtures for poultry was below the maximum permitted limit.
Mycotoxicological testing in most cases determined the presence of mycotoxins, but mainly under the limit allowed by the valid regulations. The most frequently were detected zearalenone (64,6% of samples), then ochratoxin (44.6%), aflatoxin (18.7%) and T-2 toxin (5.4%).
The results of these analysis indicate constant presence of problem of contamination of animal feed by fungi and their metabolites and the need for this issue in practice should not be ignored.
Keywords: *feed, moulds, mycotoxins, poultry*

INTRODUCTION

The question of hygiene of feed for poultry and other animals, from mycological and mycotoxicological standpoint for many years considered to be very important. Mycotoxins are toxic secondary metabolites of fungi, which enter organisms of animals usually over intake of contaminated feed. Through such feed, that contains mycotoxins, in the animal organism appear intoxication, so called mycotoxicoses, that can induce a wide scale of adverse effects [13]. Damages to livestock breeding, which result from mycotoxicoses can be large. They are manifested in the form of direct losses, due to high mortality, or more often they occur as indirect losses due to falling production and reproductive ability of animals. A special problem is that the contaminated feed contains

a variety of mycotoxins in different quantities, which also express the difference in the harmful effects.

So far, several hundred mycotoxins are discovered among which a small number is considered to be detrimental [9], and only 20% to 30% according to the frequency of occurrence and harmful effects have a medical, nutritional, environmental and economic importance. Mould and mycotoxin contamination is a worldwide problem because of the 25% to 35% world cereals are contaminated with fungi [12]. Growth and development of fungi in animal feed causes the change of many properties, reduces the nutritional value of feed, up to 50% reduces content of dry matter [4] and energy value of feed by lowering fat and carbo hydrates content and reduces the metabolic energy [1]. In this way, the costs of feeding are significantly increased. In the period of prevalence of unfavorable external conditions production of fungi secondary metabolites, i.e. mycotoxins, is induced. The most common moulds are from *Aspergillus*, *Penicillium*, *Rhisopus* and *Fusarium* genera, which produce alfatoxins, ochratoxins, zearalenone and trichotecens. According to the primary effect in the organisms, mycotoxins are divided into hepatotoxic, nephrotoxic, neurotoxic and cytotoxic [11]. According to the biological effects they may be carcinogenic, mutagens, teratogens, immuno modulators and inhibitors of protein synthesis.

Aflatoxins are hepatotoxins which are products of the fungi genus *Aspergillus*. They are metabolites of *Aspergillus flavus* and *Aspergillus parasiticus* only [2]. *Aspergillus flavus* is a mould which is very widespread in nature, and can be found in corn, wheat, barley, oat, rice, etc. Fungi produce aflatoxins in the presence of higher moisture, temperature and adequate substratum. Aflatoxicosis is manifested by various patomorphological changes in the liver, kidneys and nervous tissue. The adverse effects are manifested on the production results, especially in the broiler breeding, then in swine production, as well as other types of livestock production.

Ochratoxin is the product of fungi in the field and during storage. Fungi of the genus *Penicillium* and *Aspregillus* produce it [14]. Ochratoxin is slowly apsorbed from the digestive tract. After the resorption most of ochratoxin is present in the kidneys, liver and muscles. According to toxicity ochratoxin is one of the most toxic mycotoxins [13]. Pigs are the most sensitive animals, with the occurrence of nephropathy [3]. Poultry is less sensitive. In laying hans the fall of egg production and the appearance of nephropathy can be noted [5].

Zearalenone is a toxic product of *Fusarium* fungi that contaminate more grain in the field, but the growth of mould and synthesis of toxins also continue in warehouses. After oral ingestion zearalenone resorption is very fast and it is transported to the liver. Pigs are the most sensitive species of animals [6], while ruminants and poultry, especially chickens react less sensitive.

T-2 toxin is also toxic metabolite of fungi from genus *Fusarium*. After ingestion it is very quickly resorbed in front parties of digestive tract. After 3 to 4 hours toxin is in the most organs: liver, kidneys, muscles. It is believed that trichotecenes are significantly more toxic than the other fungi metabolites. The most sensitive animals are pigs and poultry, while the ruminants are the least sensitive because of the effect of their rumen microflora. T-2 toxin has a strong immunosuppressive effect, as well as most of other trichotecens and it damages different tissues and organs [10].

The paper presents results of tests of various samples of grain and feed mixtures for poultry.

MATERIAL AND METHODS

In order to determine the degree of fungi and mycotoxins contamination of cereals and mixtures for poultry, the mycological and mycotoxicological analysis of samples received from different feed mills and poultry farms were done. These data were collected during a five-year period. A total of 747 samples of grains (443 samples of maize and 304 samples of wheat) were analyzed and 263 samples of mixtures for different categories of poultry (68 samples of feed for broilers, 107 samples of feed for hens and 88 samples of feed for breeding chickens).
All samples were prepared for micology tests in accordance to procedure of accredited method in the Institute's laboratory for feed microbiology. Defined amounts of inoculum were transfered into Petry dishes applying pour-plate technique for adding Saburo agar. After 1-14 days of incubation at 28°C microscopic examination of grown fungi colonies was done and afterwards their isolation and determination.
In all samples presence of mycotoxins was detected by ELISA method which was based on antigen-antybody reaction. Into the wells of microtitar plate, according to directions of commercial kit (R-Biopharm®) standards and samples were introduced. To make reaction visible chromogen had to be supplemented, which in contact with enzymes became bly, but after addition of stop reagent it became yellow. At the end adsorbance was measured fotometricly at the ELISA reader and, concidering the standard curve, calculation of mycotoxins content in the sample was done precisely.
The samples were also tested by accredited chemical methods and other parameters of health safety and quality were gained.
All the results were compared and interpreted in accordance with the Rules on the maximum amounts of harmful substances and ingredients in animal feed [8], i.e. Regulation on the quality of animal feed [7].

RESULTS AND DISCUSSION

At the surface of stored grains certain amount of different microorganisms is always present. Microflora of wheat and corn seeds mostly consists of bacteria, primarly geni: *Pseudomonas, Bacillus, Proteus.* But also fungi of geni: *Mucor, Rhisopus, Aspergillus, Penicillium, Alternaria, Fusarium*, and very few actinomycetes could be found.

Table 1. Results of mycological examinations of grains and feed mixtures for poultry

Type of sample	No. of examined samples	No. of samples not in accordance to rules	Determined fungi
Grains	747	523	*Aspergillus spp., Penicillium spp., Fusarim spp., Mucor spp.*
Mixtures	263	0	

By examining all samples various types of bacteria and fungi were isolated. However, attention was primarily focused on mycological examination and it was found that grains in 70% of cases, 523 samples of 747, did not meet the requirements regarding the content of the molds that Rules on the maximum amounts of harmful substances and ingredients in animal feed assigned (the limit of 300 000 molds in gram) [8]. In ready-to-use mixes for poultry mycological examination gave much more favorable picture of hygiene and none of 263 samples did not exceed the limits permitted by legislation (50 000 molds in gram for young animals and 300 000 molds in gram for adult poultry). The most frequently were isolated: *Aspergillus spp., Penicillium spp., Fusarim spp., Mucor spp*. (Table 1).

Table 2. Results of mycotoxicological analyses of poultry feed

Type of mycotoxins	Percentage of contaminated samples [%]	Percentage of samples not in accordance to rules [%]
Zearalenone	64,6	0
Ochratoxin	44,6	1
Aflatoxin	18,7	0
T-2 toxin	5,4	0

Mycotoxicological examination of the samples used in the feeding of poultry in many cases showed the presence of mycotoxins, although mainly not above the limit allowed by regulations. The presence of zearalenone (64,6% of samples), then ochratoxin (44.6%), aflatoxin (18.7%) and T-2 toxin (5.4%) was most frequently detected. However, the content of mycotoxins in feedingstuffs is regulated by the old Rules of the maximum amounts of harmful substances and ingredients in animal feed [8] and the new Regulation on the quality of animal feed [7] only for aflatoxin, so all other specified amounts of mycotoxins can not be interpreted from the legislative standpoint. On the other hand, the poultry is characterized by extreme resistance to certain types of mycotoxins such as zearalenone, and the maximum allowable quantity is very high according to the Rules from 1990 [8] (up to 100mg/kg), while in the new Regulation on the quality of animal feed [7] it is not stated at all.

Therefore, the interpretation of the detected concentrations of mycotoxins in the feedingstuffs and mixtures according to the legislation is often incomplete and inappropriate approach to mycotoxicoses, especially when clinical signs point to them. Because of the possible shortcomings of methods themselves, which are not equally effective in the case of all types of mycotoxins, because of faulty sampling, incomplete homogenization of the sample, inability to test all potential hazards originating from feed and their synergistic effects, also the difference by type, sex and category of animals, environmental conditions and other elements of nutrition, the right diagnosis can be easily missed.

Molds and mycotoxins cause a range of disorders in the body of animals, ranging from biochemical changes, through a functional and morphological damage to various tissues and organs, to the appearance of clinical signs of mycoses and mycotoxicoses, all until possible death.

The improvement of grain storage, processing and storage of finished products (reducing the humidity, ventilation ...) it is possible to reduce the synthesis of mycotoxins, which is one of the most important ways of preventing this problem.

CONCLUSIONS

Results of mycological and mycotoxicological analysis show permanent presence of the problem of contamination of animal feed by fungi and their metabolites. So it is very important always to keep in mind a number of factors that contribute to the complexity of the problem that these natural and unavoidable contaminants carry. First of all, we must not forget that in the case of lower levels of mycotoxins still remains the risk of rigid interpretation of the regulations, as it is well known that the use of feed with low content of mycotoxins in the longer time period shows similar effects as short-term use of feed with higher amount of mycotoxins. The situation is getting more complicated by interaction of mycotoxins present in feed that increase harmful effects of each other. Therefore, the assessment of utilization of feed should be still dependant on the institutions and experts who are specialized for these scientific and professional activities.

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MICROBIOLOGICAL DEGRADATION OF THE T-2 TOXIN AND DIACETOXYSCIRPENOL UNDER LABORATORY CONDITIONS

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ABSTRACT

The activity of 30 different nontoxigenic fungal isolates were observed under laboratory conditions with the aim to decompose fusariotoxins T-2 and diacetoxyscirpenol (DAS), which belong to the group of type A trichothecenes. Isolates of the following test fungi were selected: *Aspergillus niger* (7), *Mucor* spp. (21) and *Syncephalastrum racemosus* (2). The majority of tested fungi originated from the samples of feed and their components that had not been contaminated with fusariotoxins. Selected fungal isolates were cultivated on the modified Vogel's medium supplemented with crude extracts of fusariotoxins. Crude T-2 toxin was produced from a liquid culture of *F. sporotrichioides* (isolate M-1-1), while crude DAS was produced from a liquid culture of *F. semitectum* (isolate SL-B). Fusariotoxins isolated from liquid fungal cultures, evaporated to a dry residue and dissolved in ethanol (1 mg mL^{-1}), were added to the test medium to the final concentration of 0.02 mg mL^{-1}. The presence of fusariotoxin residues were determined after seven and 14 days of fungal cultivation in test medium at 27±1 °C. Residues of T-2 toxin and mycotoxin DAS were not determined in 70% and 53.3% of investigated samples, respectively. The highest number of studied fungal isolates biotransformed both fusariotoxins (36.7%) or just T-2 toxin (33.3%), while a significantly lower number of isolates degraded DAS (16.7%). Isolates of *A. niger* mainly biotransformed T-2 toxin (6/7), while *S. racemosus* biotransformed exclusively T-2 toxin (2/2). Isolates of *Mucor circinelloides* f. *circinelloides* (1/1) and *M. hiemalis* f. *hiemalis* (1/2) degraded only DAS, while a sole isolate of *M. racemosus* f. *racemosus* (1/1) and the majority of unidentified species of the genus *Mucor* (11/17) biodegraded both mycotoxins.
Key words: *T-2 toxin, diacetoxyscirpenol, microbiological degradation, Aspergillus niger, Mucor spp., Syncephalastrum racemosus*

INTRODUCTION

Mycotoxin-contaminated feed pose a health risk to animals and therefore it can cause significant economic losses due to a lower efficiency in animal husbandry. Besides, directly or indirectly (via food chain) contaminated food can also pose a health risk to people. Fusariotoxins (zearalenone, deoxynivalenol, T-2 toxin and fumonisin B_1), aflatoxin B_1 and ochratoxin A are economically most important mycotoxins appearing in food and feed.

Considering a daily increase of data on the mycotoxin presence in both, food and feed mixtures, a need to establish practical and economic procedures for mycotoxin detoxication has arisen. From the economic point of view, it is necessary to reduce mycotoxin contamination of food and to prevent occurrence of a health risk attributed to toxins. In principle, there are several possibilities to avoid harmful effects of mycotoxin-contaminated food: contamination prevention - the development of resistant genotypes, inhibition of fungal development and mycotoxin biosynthesis, decontamination of biosynthesised mycotoxins by the application of physical and chemical methods and hindrance of mycotoxin adsorption in the digestive tract of animals [10]. In the case when mycotoxins are not adsorbed or are poorly adsorbed, the majority of physical and chemical methods of decontamination or the application of adsorbents are not sufficiently efficient, hence it is necessary to apply an alternative approach - enzymatic or microbiological degradation of toxins [11].

The decontamination strategy has to fulfil some fundamental criteria, such as: (a) mycotoxins have to be inactivated or destroyed by the transformation in non-toxic compounds, (b) fungal spores and mycelia have to be destroyed, in a way that new toxins are not formed, (c) food has to maintain its nutritive value and taste, (d) physical properties of a raw material should not be significantly altered and (e) the strategy has to be economically acceptable - costs of decontamination have to be as lower as possible.

The isolation of microorganisms, which are able to degrade mycotoxins and food processing by adequate processes of fermentation are one of the most often applied strategy for biodegradation of mycotoxins. Biotransformation is especially important as it is the only process by which organic compounds are converted into inorganic products in the dark. However, microbiological reaction of degradation encompasses not only mineralization, but also, cometabolism. In the latter case, a microorganism does not grow at the expense of a chemical, hence its degradation is slow and organic compounds similar to the initial substance is a result of this process.

It was determined that concentration of mycotoxins in cultures of certain microorganisms decreases in time, which points out to the existence of functional degradative enzymes [15].

There are some data in literature relating to biotransformation of fusariotoxins of the trichothecenes type, which are one of the most important groups of mycotoxins in agriculture [4]. In the case of trichothecenes, 12,13-epoxide ring is responsible for their toxicity, hence a reductive deepoxidation caused by enzymes and/or living microorganisms has a significant loss of toxicity as a result.

Nakayama et al. [16] isolated 12 strains of bacteria of the genus *Bacillus, Nocardia* like, and unidentified, that were able to use T-2 toxin (type A trichothecene) as the sole source of carbon. The T-2 toxin was transformed into HT-2 toxin, T-2 triol and T-2 tetraol. Acetilisation and deacetilisation of 12, 13-epoxy-9,10-ene are another types of biotransformations [5].

Binder et al. [2] isolated an anaerobic bacterium of the genus *Eubacterium* from the cattle rumen fluid. This bacterium has a property of detoxication of a greater number of types A and B trichothecenes. In the case of the T-2 toxin, de-epoxy HT-2 toxin is a final product of biotransformation of the T-2 toxin over intermediary HT-2 toxin.

Besides bacteria and yeasts [6], there are data showing that T-2 toxin and other trichothecenes can be detoxicated by different fungal species [13, 21].

Accordingly, the objective of the present study was to observe a capability of different nontoxigenic fungal species, in laboratory conditions, to biotransform type A trichothecenes (T-2 toxin and diacetoxyscirpenol), which represent one of the most important group of mycotoxins that biosynthesize *Fusarium* species.

MATERIAL AND METHODS

Microorganisms. Thirty isolates of nontoxigenic fungi belonging to species of *Aspergillus niger* van Tieghem (7), *Mucor* spp. (21) and *Syncephalastrum racemosus* (Cohn) Schroeter (2) were selected for test microorganisms. The majority of tested fungi originated from samples of feed and its components that were not mycotoxin-contaminated. The identification of fungi was done according to Samson and van Reenen-Hoekstra [17]. Fungal cultures were maintained on potato dextrose agar (PDA) at 4-6 ºC.

Production of crude toxins. Crude T-2 toxin was produced from a liquid culture of *F. sporotrichioides* Sherbakoff (isolate M-1-1), while crude diacetoxyscirpenol (DAS) was produced from a liquid culture of *F. semitectum* Berkeley & Ravenel (isolate SL-B). Filtration of liquid fungal cultures was performed after three-day cultivation in a fluid medium SPEK (saccharose 50 g L^{-1}, peptone-1 1 g L^{-1} and yeast extract 1 g L^{-1}; pH 6,2) on a rotary shaker (180 rpm) at room temperature (23-30 ºC). Crude extracts of T-2 toxin and DAS were produced by the use of ethyl acetate. After evaporation of ethyl acetate extracts to the dryness, dry residues of crude fusariotoxins were dissolved in 96% ethanol (1 mg mL^{-1}) and stored until used at 4-6 ºC. Ethanol extracts of fusariotoxins were individually added to the test medium (Vogel's Medium N), immediately prior to its pouring into Petri dishes up to the final concentration of 0.02 mg mL^{-1}.

Cultivation conditions. Test fungi were grown on modified Vogel's medium N (pH 6.3) during 14 days at 27±1 ^{o}C. The medium was supplemented with the extracts of crude T-2 toxin (VAT2 medium) and DAS (VADAS medium) as the sole sources of C atoms. The modification of the minimum Vogel's medium [20] consisted of the exclusion of the solution of biotin and addition of peptone (1 g L^{-1}) and yeast extract (2 g L^{-1}).

Mycotoxicological analyses. Fungal potential for degradation of DAS and T-2 toxin was tested by a simple screening method described by Filtenborg et al. [7], and modified by Bočarov-Stančić et al. [3]. Discs (diameter 6 mm) were cut out of the central part of the fungal colony with a stainless steel borer and were removed by using the sterilised tweezers. The discs with mycelia on the upper side of the discs were directly placed on thin layer chromatography (TLC) plates coated with Kieselgel G (thickenss of 2.5 mm). Then, discs were wetted with 10-20 μL of a chloroform-methanol (2:1, v/v) mixture. Several seconds later discs were carefully removed from the TCL plates with the sterilised tweezers. Discs with VAT2 and VADAS media, with no test fungal culture (control), were simultaneously placed on the same TCL plates. Then, the extraction of controlled discs were done and chromatography plates were developed together with 5 μL of each working standard of tested mycotoxins (T-2 toxins and DAS) in a concentration of 0.01 μg kg^{-1}.

Thin layer chromatography was performed in a saturated tank of toulene-ethyl acetate- formic acid mixture (5:4:1, v/v/v). After the plate development and natural drying in a darkened digester, plates were observed under long wavelength UV rays (366 nm). T-2 toxin and

DAS were visually detected after plates were sprayed with 20% of sulphuric acid in methanol and TCL plates heating at 130 °C for 10 min. All analyses were done in three replicates. Detection limit (LOD) of the applied TLC method amounted to 0.021 μg g^{-1} and was the same for both analyzed trichothecenes of type A.

RESULTS AND DISCUSSION

Under test laboratory conditions, isolates of fungi of the group *A. niger* mainly degraded T-2 toxin (6/7) (*Table 1*). Obtained results are not surprising, because other authors [5] determined that this fungal species degraded T-2 toxin by acetilisation and deacetilisation without attacking the trichothecene skeletnon. *A. niger* isolates are also important from the aspects of biotransformation of other mycotoxins. It was determined that they can degrade ochratoxin A [17], as well as, zearalenone [12].

Table 1. Microbiological degradation of trichothecene A by means of the isolates of fungi of the group A. Niger

Ord. no.	Origin of isolate	Isolate designation	Mycotoxin degradation	
			T-2	DAS
1.	Cob	l 1292/09	yes	no
2.	Cob	l D1/10-1	no	yes
3.	Cob	l D1/10-2	yes	no
4.	Cob	l D1/10-3	yes	no
5.	Cob	l 506/10-2	yes	no
6.	Feed mixture	l 47/10-1	yes	no
7.	Soil	Rb-gr/10	yes	no

Mucor circinelloides f. *circinelloides* van Tieghem (1/1) and *M. hiemalis* f. *hiemalis* (Wehmer) Schipper (1/2) isolates degraded exclusively DAS, while the *M. racemosus* f. *racemosus* Fresenius (1/1) isolate and the majority of unidentified species of the genus *Mucor* (11/17) biotransformed both type A trichothecenes (*Table 2*). Although El-Sharkawy and Abbas [5] state that only the species *M. mucedo* (L.) Fries degrades T-2 toxin, it is obvious that other species of this genus of the phylum Zygomicota can biodegrade this type A trichothecene, as well as DAS, as it is shown by our studies.
In contrast to T-2 toxin, there are little data in literature on the ability of microorganisms to biotransform DAS. Guan et al. [8] studied detoxication of trichothecenes by bacteria from the digestive tract of nine fish species and found out that the association of microorganisms of the species *Ameirus nebulosus* Lesueur (C133) completely transform type B trichothecenes into de-epoxy deoxynivalenol and also biodegrade the majority of other trichothecenes, including DAS, up to diacetil or de-epoxy products.

According to literature data [9], beside stated fusriotoxins, the representatives of the genus *Mucor* can degrade one of the most toxic mycotoxins (aflatoxin B_1), which can be found as a natural contaminant of crops, and thereby of food and feed mixture.
The potential for degradation of type A trichothecenes was tested under laboratory conditions and for the species *S. racemosus*, which also belongs to the phylum of Zygomicota (*Table 3*). This filamentous fungi can usually be isolated from the soil or animal feces in tropical or subtropical regions.

Table 2. Microbiological degradation of type A trichothecenes by the means of isolates of the fungus Mucor spp.

Ord. no.	Origin of isolate	Type of fungi	Isolation designation	Degradation	
				T-2	DAS
1.	Feed mixture	*M. circinelloides* f. *circinelloides*	L00051/10-1	no	yes
2.	Feed mixture	*M. hiemalis* f. *hiemalis*	L00051/10-2	no	no
3.	Sunflower meal	*M. hiemalis f. hiemalis*	l 1216/09	no	yes
4.	Sunflower meal	*M. racemosus* f. *racemosus*	l 1215/09	yes	yes
5.	Additive (Intraco)	*Mucor* sp.	l 1288/09	yes	no
6.	Cob	*Mucor* sp.	l 506/10-3	yes	yes
7.	Cob	*Mucor* sp.	l 506/10-4	yes	yes
8.	Feed mixture	*Mucor* sp.	l 31/10	no	yes
9.	Feed mixture	*Mucor* sp.	l 47/10-1	yes	yes
10.	Feed mixture	*Mucor* sp.	l 48/10-1	yes	yes
11.	Feed mixture	*Mucor* sp.	l 48/10-2	yes	yes
12.	Feed mixture	*Mucor* sp.	l 48/10-3	yes	yes
13.	Feed mixture	*Mucor* sp.	L05809/09	no	yes
14.	Feed mixture	*Mucor* sp.	L00049/10	yes	yes
15.	Feed mixture	*Mucor* sp.	l 685/10	yes	yes
16.	Maize grain	*Mucor* sp.	NS6010/4-1	no	yes
17.	Maize grain	*Mucor* sp.	NS6010/4-2	no	no
18.	Flax seed	*Mucor* sp.	l 1195/09-1	yes	yes
19.	Flax seed	*Mucor* sp.	l 1195/09-2	no	no
20.	Sunflower meal	*Mucor* sp.	l 46/10	yes	yes
21.	Sunflower meal	*Mucor* sp.	l 675/10	yes	no

Table 3. Microbiological degradation of type A trichothecenes by the means of isolates of Syncephalastrum racemosus

Ord. no.	Origin of isolate	Isolate designation	Degradation	
			T-2	DAS
1.	Feed additive (Intraco)	l 1288/09	yes	no
2.	Feed additive (Intraco)	l 1307/09	yes	no

There are not data in available literature on the ability of *S. racemosus* to decontaminate mycotoxins, but it is shown that it is able to biotransform immunosuppressive agent rapamycin that is given to patients after organ transplantations [14]. Therefore, it is especially interesting our finding that this fungus has the ability to degrade T-2 toxin.
In almost all tested cases, results obtained after the 7-day cultivation on media VADAS and VAT2 did not change when the cultivation was prolonged for another seven days. Whether degradation of DAS and T-2 toxin occurs for less than seven days, as stated by Beeton and Bull [1], who found that bacterial associations isolated from water and the soil degraded trichothecene nucleus within 24-28 hours, will be shown by our subsequent studies, which will be devoted more to the dynamics of the process itself.
In contrast to the majority of other authors who investigated the ability of mycotoxins degradation on fluid media [17, 19], we decided on the development of this rapid screening method of fungi growth on agarose media supplemented with mycotoxins, as it is very simple, economic and a great number of isolates can be observed at the same time.

CONCLUSION

Under test laboratory conditions, residues of T-2 toxin were not determined in 70% of cases. At the same time, DAS residues were not detected in 53.3% cases.
Tested isolates of fungi belonging to the group *A. niger* biotransformed one or other type A trichothecenes, while *S. racemosus* biotransformed only T-2 toxin.
In the greatest number of cases (52.4%), isolates of fungi of the genus *Mucor* detoxicated both fusariotoxins.
Obtained results require the continuation of the initiated studies, because biological detoxication of food and feed is an approach that will gain on its importance with the aim to decrease food contamination and prevent occurrence of a health risk related to fusariotoxins and other mycotoxins (aflatoxin B_1, ochratoxin A).

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QUALITY OF INEDIBLE BY-PRODUCTS OF PIGS SLAUGHTERING INTENDED FOR INCLUSION IN PET FOOD

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ABSTRACT

Under industrial conditions of slaughtering of swines, inedible by-products are obtained beside the meat. A number of these by-products have potential as feed ingredients and technical fat.
In this paper the nutritive components of by-products obtained at slaugtering of swines, which could be important for processing into animal feed, were invsetigated. By-products of slaughtering were found to be very valuable ingrediants for production of high protein and energy feeds, ashown by their amino acid and chemical composition.The nitrogen complex in investigated materials consisted mostly of protein. The content of digestible nitrogen in all investigated materials was nearly the same as total nitrogen, showing that all protein from those materials is available for utilization by animal.
Keywords: *by-products, quality, pet food*

INTRODUCTION

Under industrial conditions oj slaughtering of swines, beef cattle and poultry inedible by-products are obtained besides the meat. A number of these by-products have potential to be used as feed ingredients of animal origin and technical fat [12]. This topic attracted attention of many researchers. Solution for the problem on harmless removal of wastes of animal origin has gone through different stages and was always closely linked to diseases of animals and people, as well as to environmental protection [14]. According to *Regulation EU* [10], inedible by-products from healthy pigs may be prepared as feed for pets [7].
For better utilization of raw materials of animal origin, better performance of technological processes and production of the high-quality products, good knowledge of raw material characteristics is necessery [6, 8]. Studying the structure and parameters to be used for calculation the potential amount of animal waste, [13], stated that the processed by-products from slaughtered animal represent nutrient-rich feed suitable for animal.
Nutritional caracteristics as well as other indicators of quality are determined by, first of all, the content and composition of nutrients in initial raw material [4]. Examining the quality of raw materials suitable for the technical processing of feed for animal feeding, *Ristic et al.* [11] determined that the protein feed produced from slaughterhouse by-products is an excellent source of protein of high biological value. According *Gómez-*

Juárez i sar. [2] inedible by-products of slaughtered pigs contained significant amount of essential amino acids and vitamins suitable for feeding poultry. *Park at al.* [9], are found that the collagen of bone and tendon collagen contained 22,2 and 33,0% glycine as opposed to muscle protein that it contained only 5%.
In order to identify the application value of by-products generated at swines slaughtering, the aim of this study was to determine chemical and nutritional characteristics of such materials. furthermore, the goal was to point out the content of nutritive substances that could enable better understanding of the quality of the available raw materials of animal for production of high-quality protein and energy feed and their inclusion in pet food.

MATERIALS AND METHODS

In this study, inedible by-products obtained at slaughtering and processing of pigs, with body weight of about 105 kg, were used. Five samples of blood, intestines (with and without content), mixed meat-fatty wastes and confiscates (lungs) were analyzed. Samples were placed in plastic bags, labeled and put into refrigerator at about 4°C. Four hours after the slaughter, samples were taken to chemical laboratory.
Determination of chemical characteristics of inedible by-products were performed in laboratories of the Institute for food technologies in Novi Sad.
Prior to analisis samples were homogenized in a blender and after that used for chemical composition determination. Basic chemical parameters (water, protein, fat and ash), nitrogen fractions and digestible nitrogen were determined according the *AOAC methods* [5].
For determination of amino acids content an amino-analyzer Biotronic LC 5001 was used. Protein hydrolysis was performed with 6 mol/L HCl for 23 h at 110°C. Cystine and methionine were determined re-oxidization with performic acid [3].

RESULTS AND DISCUSION

Chemical characteristics of by products from pigs slaughter are shown in Table 1.
Results of chemical analysis showed that inedible slaughtering by-products varied considerably between each other. Results showed that inedible by-products of pig slaughtering contained protein as well as a large portion of fat. Crude fat of mixed waste and intestines of all samples over 50% of dry matter, and of konfiskata over 44%. Crude fibre found in intestinal and stomach contens, was originated from the lestover feed. Minerals were present in small quantities.
Results of the chemical composition of pig blood in line with the results of *Ristic et al.*, [11], and were as folows: water 80.4%, protein 18.4%, fat 0.3%, ash 0.8%; *Park et al.*, [9] water 80.82%, protein 18.12%, fat 0.18%, ash 0.85%.
Basic chemical composition of wastes obtained by slaughtering of pigs (Table 1) was different with respect to some of parameters found in the available literature, what can be seen as a consequence of sampling method, depending on the purpose of investigation, as well as on their pretreatment. In case of non-mixed/sole by-product, the partition was done on individual anatomy parts, which were trimmed of other tissues.

This investigation was done in order to obtain raw materials in a status in which they vill be processed further, what generated results more detailed that helped to better understand of their further processing.

Table 1. Proximate analysis of some inedible by-products from swine slaughtering and trimming, %

By product	Watter	Crude protein	Crude fat	Crude fibre	Ash	N-free extract
Blood	79,46	18,90	0,32	-	0,82	0,50
Guts without content	70,38	7,99	13,00	-	0,48	8,15
Varios meat-fat wastes	48,76	11,10	38,00	-	2,04	0,10
Slaughterhouse wastes (lungs, thachea, esophagus	77,36	13,62	5,81	-	1,32	1,89
Stomach content	75,00	4,60	3,80	3,51	2,10	10,99

Inedible by products of slaughtered pigs differed due to their moisture contents. Beside of mixed meat-fatty wastes, all other categories of analyzed by-products contained more than 70% of moisture. This surely may be important for choice of processing procedure of these raw materials, also having in mind the content and nature of individual components that were included in their structure.
By-poducts that contain over 30% of crude protein in dry matter may be concidered as protein raw material. Higher content of fat in raw stomach copntent was the result of primary processing of stomach, and the content of meat-fat tissue.
From these results, it is obvious that the inedible by-products from pig slaughter line are good sources of protein and essential fats. By processing of those wastes, feed of animal origin (blood meal, meat meal and flour from the stomach content of pigs) and technical fat could be obtained.
Fractions of nitrogen and digestible nitrogen in tested samples of blood, gut konfiskata (with and without content), and mixed inedible by-products of pig slaughtering are shown in Table 2.
Nitrogen complex of investigated by products from slaughtering line consisted predominantly of proteins, whereas quantities of ammonia nitrogen indicated that in the period from sampling to analysis, no significant dezamination have taken place.

Table 2. Nitrogen fractions and digestible nitrogen of inedible by products from slaughtering of pigs

	Protein N %	Non-protein N, %	Alpha amino N, mg %	Ammonium N, mg %	Digestible N %
Blood	2,83	0,29	153	107	2,99
Animal wastes (lungs, trachea, esophagus)	1,91	0,17	43	26	1,97
Guts without content	1,21	0,07	18	17	1,21
Guts with content	0,97	0,09	26	21	0,92
Mixed meat - fat offals	1,73	0,05	7	4	1,75

Free amino acid content was also within levels of their normal content when the period between sampling and analyzed of samples is considered. As in production conditions raw material would not be immediately processed, the obtained results for non-protein fractions practically reflect the state of those raw materials at the moment of their generation in a slaughter house.

Digestible nitrogen content of all samples was very close to the total nitrogen content, what indicated that almost total quantity of protein was ready to be metabolized in animal organism after being consumed in the processed form.

Amino acid composition of inedible by-products obtained from slaughtering of pigs (Table 3) showed that those raw materials had valuable potential for processing into animal feed.

The most valuable by-product in this study was, blood, whose proteins contain about 44% of essential and 14% of semi-essential amino acids. Share of essential amino acids in protein of intestine (without contents), confiscates as well as of mixed meat-fatty wastes varied from about 32 to 44%, and of semi-essential from about 10 to 16 %. Non-essential amino acids in those raw materials account for over half of crude protein, while in blood is around 42%.

Looking at individual amino acids, significant by high is lysine content, then threonine, and also phenylalanine content. Methionine is not, according to these results, present in higher quantities, for which is possible explaination could be its degradation during preparing of samples for analyzes. Therefor methionine content is probably lower than the actual in the examined samples. Presence of nonessential amino acids is also important as in case of their defficiency in complete ration, essential amino acids are used. From that point of view, in these raw materials, especially important is presence of glycine in intestine (without contents) [13].

Table 3. Amino acid composition (% of crude proteins)

	Blood	Animal wastes	Guts without content	Guts with content	Mixed offals
Lysine	8,96	7,09	7,69	7,30	7,45
Leucine	11,38	8,68	7,85	8,06	7,53
lsoleucine	2,10	3,67	4,17	4,14	4,95
Methionine	1,23	1,54	1,80	1,43	1,27
Threonine	4,65	3,71	4,15	4,30	3,63
Phenilalanine	7,58	4,00	4,24	4,26	2,86
Tryptophane	1,39	1,02	1,02	0,98	1,30
Histadine	6,35	2,28	1,94	2,00	2,11
Arginine	4,15	6,66	7,49	6,32	6,75
Aspartic acid	9,48	8,61	9,08	8,84	8,31
Serine	5,04	4,42	4,59	4,74	4,32
Glutamic acid	10,78	12,73	14,07	12,96	12,64
Proline	3,79	7,40	6,58	6,42	7,65
Glycine	4,25	12,03	9,40	9,30	10,32
Alanine	6,69	6,56	6,41	6,88	6,62
Valine	8,36	5,64	5,28	4,20	4,81
Cistine	0,58	0,93	0,98	0,97	1,10
Tyrosine	2,93	2,90	3,09	3,16	2,74
Protein content, %	18,90	13,62	7,99	6,60	11,10

There are different methods for processing animal waste wordwide. Each of them has its own characteristics, which are reflected in the quality and efficiency of products. During

processing there is a change in the structure of proteins and reactions of some amino acids with other ingredients in the raw material [8].
Depending on the technological process of processing, the resulting products will, to a greater or lesser extent, have a reduced content of certain amino acids and this is necessary to take into account for balancing of the processing and selection of coating. In this regard particularly is sensitive blood, whose treatment has to be kept under very mild conditions, in order to maintain its high nutritive value [1].
Depending on the type and quality of products wanted, the test results provide the possibility for combining of these materials, respecting the condition of processing which dictates the sensitivity and stability of each individual raw material.

CONCLUSION

Based on the results of characteristics inedible by-products obtained from pig slaughter line the following can be concluded:

1. Inedible by-products from pig slaughter line are an important source of protein and fats and are suitable raw material for technical processing into protein feed and technical fat.
2. The nitrogenous complex materials studied consisted mostly of protein, in different quantities, depending on the structure and typesof material.
3. The digestible nitrogen content of all samples was closed to the content of total nitrogen, indicating that the protein from those materials were available for utilization by animals.
4. Amino acid composition of investigated by-products showed that they have potential for being used in production of protein feed.

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MICROSCOPIC EXAMINATION OF FEED FOR THE PRESENCE OF CONSTITUENTS OF ANIMAL ORIGIN

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ABSTRACT

Monitoring of feed for the presence of ingredients of animal origin is performed in order to prevent transmissible spongiform encephalopathies (TSE). The best known of these diseases is Bovine spongiform encephalopathy (BSE) which, due to the size of the crisis it caused, launched a series of preventive measures including regular control of animal feed all over Europe. In fact, when it was found that the source of infection is feed, i.e. infectious ruminant protein processed in meat and bone meal (MBM), all around the world was introduced legislation which prevents these nutrients to enter a food chain. In order to monitor the implementation of these regulations regular control is carried out by appropriate laboratory methods. Today in the EU use of MBM is completely banned for all farm animals, but in our country since 2001 usage of these nutrients is officially banned just in the diets of ruminants. Such a partial limitation in Serbia still gives the possibility of cross contamination of feed for ruminants with prohibited ingredients, which are used for feeding of other animal species. Therefore, the facilities for the production of animal feed are obligated to separate lines for feed for ruminants, or otherwise, to completely eliminate use of feedstuffs of animal origin (MBM and fish meal). Control of these conditions is carried out by laboratory testing of feed for ruminants, as well as feed for pigs and poultry, for the presence of the MBM using classical microscopy. During year 2009 among 350 samples of mixtures for different animal species which were tested for the presence of constituents of animal origin (MBM and fish meal) it was found 3.81% of positives in feed for cattle and 5.26% of feed for sheep. This result represents a significant improvement compared with the previous years which is a signal of more serious compliance to European standards in the domestic feed industry.
***Keywords**: feed, meat and bone meal, microscopy*

INTRODUCTION

Soon after bovine spongiform encephalopathy (BSE) was first diagnosed in 1986, it was determined that this disease was transmitted through feed, i.e. through infectious ruminant protein processed in meat and bone meal (MBM). Immediately eradication process was started and one of the most important measures was the establishment of legislation which prevents these nutrients to enter a food chain [8].

In the European Union, according to Regulation 999/2001 [7] and Regulation 1234/2003 [8], use of processed animal proteins, including different types of MBM, is prohibited for all farm animals entering the food chain of people, excluding fish meal for nonruminants. Such a measure was taken in strict accordance with the fact that these nutrients represent a potential source of infection not only for animals, but also

indirectly, through food of animal origin, for the people. Due to this strict approach epizootiological situation today is much better in Europe, which shows that the management of the BSE crisis was mostly successful [3].
In Serbia, the control system, compared with the European Union, is more complex, due to the differences in legislation and applied preventive measures. In fact, by the Regulations Amending the Rules of the quality and other requirements for animal feed (OJ FRY 38/2001) [4] since 2001 the use of feedstuffs of animal origin in the diet of ruminants has been officially forbade, but their presence is still allowed in the mixtures for monogastric animals. Identical measures are prescribed by the new Regulation on the quality of animal feed (Official Gazette of RS, no. 4/2010) [5] which is in force since first of May 2010.
On the one hand, these partial restrictions on the use of feedstuffs of animal origin, applied in our country, reduced the negative economic consequences of their complete elimination and kept all the benefits that these nutrients give to animal production and performances, but, on the other hand, the possibility of cross contamination of feed intended for ruminants by prohibited ingredients from feed for monogastric animals is largely open. For this reason, feed manufacturers are obligated by the Veterinary law Art. 110 (Official Gazette 91/05) [10] to separate the special line for the production of feed for ruminants, or otherwise, to eliminate the use of animal feedstuffs completely. Control of these conditions is carried out as part of a special program of the Veterinary Directorate and according to the Order for the measures for preventing, detecting, preventing the spread and eradication of transmissible spongiform encephalopathies (Official Gazette 17/2006) [2] by laboratory testing of feed for ruminants for the presence of MBM using conventional microscopy according to procedure prescribed by Regulation 152/2009 of the European Union Annex VI [6].

MATERIAL AND METHODS

Since 2006 Veterinary inspectors submit samples of feed for ruminants, pigs and poultry from different feed manufacturers to the Institute of Veterinary Medicine of Serbia in Belgrade for examination for the presence of meat, meat and bone, bone and fish meal. During 2009 a total of 350 samples of feed mixtures for different animal species were examined, mostly feed for cattle - 236 samples, 54 samples of mixed feed for pigs and poultry, 31 sample of mixtures for poultry, 19 for sheep, 9 for pigs and one sample of feed for goats.
Tests for the presence of ingredients of animal origin are carried out using conventional microscopy accredited method which is applied in accordance with EU regulations 152/2009 Annex VI [6]. Interpretation and evaluation of results is done according to the Regulations on the quality of animal feed (Official Gazette of RS, 4/2010) [5].

RESULTS AND DISCUSSION

During 2009 total of 350 samples of feed mixtures for different animal species were tested by the classical microscopy method while most of them were of feed for cattle - 236 samples. Among them 3.81% was found positive for the presence of elements of animal origin, what made them unusable as intended, because they did not correspond to

Article 51 of the Rules of the quality of animal feed (Official Gazette of RS, no. 4/2010) [5]. For the same reason, according to Article 54 of the Rules above, 5.26% of samples of feed for sheep were eliminated, while only one sample of feed for goats can not be considered relevant to serious analysis.
Concerning mixtures for pigs and poultry, although according to current Serbian regulations presence of components of animal origin is allowed, based on the results of monitoring significant tendency to avoid all risks of animal feedstuffs usage is observed. Those constituents are found in only 22.22% samples of mixtures for pigs, in 12.96% of mixed samples of feed for pigs and poultry and in only 3.23% samples of poultry feed (Table 1). This extremely reduces the risk of crossing the undesirable substances to feed for ruminants and prevents the possibility of cross contamination in production facilities which are without separated lines for the preparation of feed for ruminants from other animal species.

Table 1. Presence of constituents of animal origin in feed in 2009

	cattle	sheep	pigs	poultry	mixed: pigs+poultry	goats
TOTAL	236	19	9	31	54	1
negative	227	18	7	30	47	0
positive	9	1	2	1	7	1
% of positive	3.81	5.26	22.22	3.23	12.96	100.00

Comparing the results obtained during the four-year period (2006-2009) by regular monitoring of mixtures for different categories of most common species of ruminants, cattle and sheep, a significant improvement in the presence of prohibited components of animal origin could be found (Table 2). Actually, the lowest percentage of positive samples was found in 2009 and established downward trend is expected to continue also during this year.

Table 2. Percentage of positive samples of feed for cattle and sheep from 2006 to 2009

Year	Positive mixtures for cattle [%]	Positive mixtures for sheep [%]
2006	6.12	25.00
2007	6.69	15.79
2008	8.03	13.04
2009	3.81	5.26

Such a favorable situation and the obvious improvement can be interpreted as a consequence of successful harmonization with EU legislation by introducing some new regulations in Serbia: Veterinary Law (Official Gazette 91/05) [10] and the Law on Food Safety (Official Gazette 41/09) [9], as well as a number of other regulations which contribute to better control and progress in the food and feed industry.

CONCLUSIONS

Based on the results of microscopic examination of feed for the presence of constituents of animal origin it could be concluded that in recent years there has been a general raising of production level in our feed industry. In many cases the highest European standards are applied, what makes our manufacturers competitive on foreign markets. On the other hand, laboratory analysts and other professionals close to those profiles, by better future engagement and pragmatic cooperation in this area, can contribute to further improvement in the safe food production.

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THE USE OF CONCENTRATES IN GAME FEEDING WITH THE PURPOSE TO REDUCE DAMAGES

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ABSTRACT

An overview of literature about the use of concentrate feeds and mixtures in game feeding in open hunting grounds, fenced areas and hatcheries. The amount and quality of concentrated feeds used for various game species depend on the availability of natural food and purpose (and intensity) of animal feeding. The highest quantities of concentrates with the appropriate chemical composition are used in wild boar fattening and pheasant chick production. In open hunting grounds mostly cereal grains are used as feeds and in that way the damages on crop, fruit, grape and forest production are reduced
Key words: *game, damages, additional feeding, concentrates*

INTRODUCTION

Supplemental game feeding is one of the most important measures in the management of hunting grounds (11, 20). It is used when natural food is deficient, or to reduce damages that is produced by game on crop, fruit, grape, forest and domestic animal production (6, 7, 8). Supplemental game feeding is organized, depending on animal species, with feeds of plant or animal origin. For all herbivores, omnivores and even some carnivores (bear) supplemental feeding consists of plant materials, in the form of forage or concentrate feedstuffs (9, 12, 24). During winter the most important are concentrated feeds, because they provide high amount of energy and other nutrients (10). In the fenced areas concentrated feeds are used throughout the year in order to produce better trophies. Some game species are very intensively reared in the controlled environment and their meat is sold for a good price to specialized restaurants and markets (19, 21). Artificial reproduction of pheasants (and partridge) is done in hatcheries and their feeding is almost entirely with concentrates (25). In our country but also in the world, concentrates are used mostly for feeding world boars, feathered game, roe and red deer and sometimes bear.

CONCENTRATES IN WILD BOAR FEEDING

In the Europe and Serbia as well the wild boar is increasingly kept in fenced areas, because that is the way to reduce damages that they produce on crops, and more animals can live on per unit of land area, the possibility of crossing with domestic pigs is reduced, and the application of modern methods of selection, controlled reproduction and planed hunting, medication, marking and other methods became easier (2).

In open hunting grounds where wild boars are able to find sufficient amount of natural food the supplemental feeding is required only from December until March, and that depends on the severity of the winter and amount of snow cover, also on the number of piglets. In the situation when food is deficient the body mass of boars is reduced and the composition of some tissues is changed. In preliminary studies with wild boars, it was shown that these animals did not develop ketosis during restricted feeding (33). In order to prevent the formation of ketone bodies, a sufficient supply of glucogenie precursors to the TCA-cycle must occur. It is hypothesized that these precursors became available by mobilization of body protein. As a result, the protein content of body tissue must decrease during undernutrition. It is not known whether such a decrease in protein content of the body tissues is related to the degree of undernutrition. Wolkers et al. (1994) was studied the effect of long term dietary restriction on the composition of liver, kidney, and several muscles in wild boar (*Sus scrofa)* in order to evaluate the tissue protein content as an indicator for the nutritional status. Extreme undernutrition was associated with a relatively large weight reduction in liver and kidney. In liver and kidney, total protein content and the protein/DNA ratio were reduced through a reduction in both cell number and cell size. The lower protein/DNA ratios and the increased DNA concentration in the muscle of food restricted animals suggested only a reduction of cell size (table 1). The response to undernutrition varied considerably between two muscles. It was concluded that the DNA concentrations and the protein/DNA ratio in several tissues provides an additional tool for assessing nutritional status in living or shot wild boar.

Table 1. Mean DNA concentrations (mg/g wet weight) and mean water (H_2O), protein (PR), and triglyceride (TG) fraction (% of wet weight) and standard deviation in 2 muscles of wild boar (Wolkers et al. 1994)

	DNA	**H_2O**	**PR**	**TG**
A control	1.9 ± 0.3	78.1 ± 0.9	16.0 ± 0.6	0.8 ± 0.1
A food restricted	3.4 ± 0.3	79.6 ± 0.7	14.6 ± 0.1	0.5±0.1
	P <0.02	NS	*P <0.05*	*P <0.05*
B control	1.9 ± 0.5	79.3 ± 1.9	15.9 ± 0.6	1.1 ± 0.5
B food restricted	1.9±0.0	80.2 ± 1.2	14.7 ± 0.6	0.8±O.2
	NS	NS	NS	NS

A: *Musculus tensor fasciae latae*; B: M. *psoas minor*, in control and food restricted wild boar. NS = not significant

Keeping boras in fenced areas requires quite intensive feeding, which depends on the nature of the area (amount of natural food and feeds produced as crops), and also on the density of animal (21). In fenced areas where piglets are kept for meat production the supplemental feeding is practiced throughout the year. Wild boar meat is becoming important and is in great demand because of its specific chemical composition. Wild boar meat is reported to contain a lower concentration of fat and cholesterol than the meat from the domestic pig (30.9 g versus 56.6 g fat per 100 g loin meat (4) and 45 mg versus 101 mg cholesterol per 100 g fat (28) in wild boar and domestic pig meat,

respectively). These levels of fat and cholesterol have led to wild boar meat being marketed as a "healthier alternative" to domestic pig meat.
The wild boar belongs to the same species as the domestic pig (*Sus scrofa domesticus*). Historically, wild pigs have consumed diets including high fibre foods such as forages and acorns (26). This high fibre ingestion may have promoted adaptations in the wild boar in terms of efficiency of fibre utilization, in comparison to the domestic pig, which could result in differences in energy digestibility values between the two types of animal, especially for feedstuffs containing a high fibre level. In the experiment reported my Hodgkinson et al. (2008) the differences in apparent digestibility between wild boar (*Sus scrofa* L.) and the domestic pig (*S. scrofa domesticus*, Landrace×Large White) was investigated. Six pure wild boar (*S. scrofa* L.) and six domestic pigs (Landrace×Large White) with liveweights (mean±S.E.M.) of 26±0.6 and 21±1.1 kg, respectively, were fed diets at a daily level of 0.10×metabolic body weight ($W^{0.75}$). The diets included a base diet and three experimental diets containing 700 g basal diet/kg and 300 g maize, oats or alfalfa meal/kg; all animals received all four diets. For ingredients that contain relatively low concentrations of fibre (such as maize and oats), it appears that DE values determined in the domestic pig can be validly applied for diet formulation for wild boars; however, for ingredients with higher fibre levels, the DE values in wild boar appear to be lower than those in the domestic pig (table 2).

Table 2. Coefficient of total tract apparent digestibility (CTTAD) of protein and energy, and digestible protein (g kg^{-1} dry matter) and digestible energy content (MJ kg^{-1} dry matter, mean±S.E.M.) of ingredients determined in the domestic pig and wild boar (n=6)(Hodgkinson et al. 2008)

Ingredient	Component	Apparent digestibility		P	Content		P
		Domestic pig	Wild boar		Domestic pig	Wild boar	
Maize	Protein	0.80±0.084	0.89±0.044	NS	78±8.2	86±4.2	NS
	Energy	0.93±0.039	0.94±0.017	NS	15.45±0.065	15.70±0.276	NS
Oats	Protein	0.74±0.078	0.84±0.074	NS	88±9.2	99±8.8	NS
	Energy	0.81±0.130	0.78±0.102	NS	13.36±2.092	12.80 1.598	NS
Alfalfa	Protein	0.29±0.044	0.25±0.043	NS	58±8.8	51±98.8	NS
	Energy	0.59±0.047	0.47±0.060	**	10.58±0.841	8.48±1.067	**

Probability; NS = Not significant (P>0.05), ** P<0.01.

In the study by van Wieren (2000), wild boar were shown to have a greater capacity to digest neutral detergent fibre than Meishan pigs, which would be expected to result in a greater digestible energy content of diets containing significant concentrations of NDF in the wild boar compared with the domestic pig. If this is the case, then this could affect the concentration of energy-providing ingredients that must be incorporated into diets for wild boar. On the other hand, if the DE content of ingredients in wild boar is the same as that in the domestic pig, this means that the DE values of ingredients determined in the domestic pig could be used to formulate diets for the wild boar.
For supplemental feeding of wild boars maize (the whole ear) is used, also other cereals, or pelleted concentrates. Properly balanced concentrate mixtures are achieving better

results compared to cereals due to the better supply of nutrients. Novaković et al. (1986) in the hunting ground „Sige" fed sows and piglets with complete concentrate mixtures while in hunting ground „Ludaš" sows received cereals (maize and barley) while piglets received maize meal. At control weighing (26-31.07.1986) it was found that piglets in „Siget" hunting ground had almost 4 kg more than those in „Ludaš" hunting ground. Beuković et al. (2003) investigated the influence of concentrate mixtures (with and without fish meal) on productive performances of wild piglets. The authors used mixture with 16% crude protein for nursing sows (1 kg/day) and for their piglets with 18% crude protein (0.3 kg/day). The chemical composition of the mixtures was very similar, while in control group the fish meal was substituted with synthetic lysine and methyonine. Authors observed significant differences between control and experimental groups (Table 3).

Table 3. Average values and variation in body weight of piglets (Beuković et al. 2003)

Group	Sex	Number of piglets	Average value	Standard deviation	Variation coefficient
Mixture with fish meal	Male	16	12.69	3.81	29.99
	Female	15	11.93	4.02	33.67
	Total	31	12.32	3.57	28.97
Mixture without fish meal	Male	38	12.28	2.89	23.52
	Female	29	11.66	3.28	28.10
	Total	67	12.01	3.05	25.43

CONCENTRATES IN RED AND ROE DEER FEEDING

Roe deer are animals usually found in open hunting grounds. The importance of supplemental feeding with concentrates depend on the choice and availability of natural food (10). In years with good production of oak and beech acorns the supplemental feeding is not significant. Also, if there are fields with unharvested maize deer would not respond to feeding spots. The use of concentrates is planned for a period of about 60 days during a year (the days with snow cover), and the needed amount is approximately 300 g/day per animal (22).
Feeding intensity of deer with concentrates depend on the type of hunting ground and amount of natural food available. Ševković et al. (1991) recommend diets for male deer (weight 200 kg), kept in pens, which consist of lucerne hay (1.5 kg), maize silage (4.0 kg) and pelleted concentrate mixture with 30% crude protein (1.5 kg).

CONCENTRATES IN PHEASANT FEEDING

For a large number of hunters pheasants are the favorite game, not only for their meat characterized by low fat content and high essential fatty acids and amino acids content which makes it of a higher quality compared to broilers, duck and geese, but for hunting characteristics, as well (30). Supplemental feeding of pheasants in hunting grounds is done mostly with cereal grains, sometimes from mid summer time, because of the scarcity of natural food. Natural production of pheasants in hunting grounds is

insufficient due to the ever decreasing natural habitat, poor feeding conditions and increasing number of hunters. Supplementary feeding in hunting grounds may to a certain extent affect the number of birds (15). As a result pheasants in captivity are bred under control, similar to broilers, and at a certain age are released in the wild (3). Popović and Stanković (2009) state that the capacity of pheasant farms in Serbia is 900,500 hatched pheasant chicks. According to the authors during the previous four years more than ten million pheasants have been released to the wild.

However, body mass of pheasants in the moment in which they are settling is very important for their survival when feeding conditions on the hunting grounds are poor. Due to the above, great attention is paid to pheasant nutrition in hatcheries. Feeding is at the beginning very intensive and is based mainly on concentrated feeds. Later on, feedstuff such as greens and grains is introduced in order to mimic natural feeding conditions (16).

There are a number of recommendations which differ greatly in the quantity of nutrients. Reference values in the last years are increasing and range from 20 to 40% proteins (27). Đorđević et al. (2010) investigated effects of different levels of dietary protein content (A_1 = 26% up to 4 weeks of age and 20% from 4 up to 6 weeks of age; A_2 = 30% crude proteins up to 4 weeks of age and 24% from 4 up to 6 weeks of age)and population density in growing pheasants (B_1 = 450 and B_2 = 550 birds/group) on production results and mortality. Pheasants fed a mixture containing a higher concentration of proteins had a higher feed conversion rate throughout the entire observed growth period. Mortality was higher during the first growth period compared to the second stage (Table 4). For the duration of the experiment mortality was between 1.27 and 3.00%, on the other hand the observed factors did not significantly influence the mortality rate. Compared to available literature data mortality was at a satisfactory level.

Breeding flocks of pheasants that are used for egg production for incubators are also fed with concentrate mixtures. Chemical composition of the mixtures is important for egg production. Beuković et al. (2001) investigated the influence of three concentrate mixtures (22%, 19% and 16% crude protein) on egg production, egg mass, and chick mass. Authors discovered that decrease in protein level has major influence on egg production (100% : 97.14% : 89.90%).

Table 4. Mortality of pheasants (%)

Proteins	**Density**	**Period**		
		0-15. days	**15-42. days**	**0-42. days**
A_1	B_1	1.45	1.00	2.44
	B_2	0.73	0.55	1.27
A_2	B_1	2.78	0.44	3.00
	B_2	1.55	0.91	2.43
Values for P				
A		0.09ns	0.56ns	0.28ns
B		0.07ns	0.46ns	0.22ns
A×B		0.77ns	0.22ns	0.62ns

ns = not significant

CONCENTRATES IN BEAR FEEDING

According to Naumov (1976) the supplemental feeding of bears on the Prokletije mountains reduced damages by 75%. Considering that bears spend winter in some sort of refuge the feeding needs to be done in other parts of the year (24). According to Dečak et al. (2005) supplemental feeding of bears is done 120 days in a year, in November, February, March and April. Ziegltrum and Nolte (1995) described that bear feeding in the USA is done in the spring and early summer in order to reduce damages on forest trees. Ziegltrum (2004) found that with the founding of feeding spots and supplemental feeding the damages on trees were significantly reduced in some years (Table 5).

Table 5. Average number of damages by black bears on conifer forests with and without additional feeding in the State of Washington (Ziegltrum, 2004)

Year	n	Tratments		Control	
		$\bar{x}$	SE	$\bar{x}$	SE
1999	7	4,9	4,5	26,1	4,5
2000	7	10,3	4,5	21,6	4,5
2001[a]	5	2,8	5,3	14,8	5,3
2002	5	3,4	5,3	16,0	5,3

For supplemental feeding of bears mostly are used cereal grains, beets, fruit, slaughter offal and animal corpses (not died of contagious diseases). In order to reduce tree damages in the USA they use pelleted feeds with 25% sugar (34). For supplemental feeding of adult bear in the period of 120 days the amount of 300 kg cereal grains, 300 of fresh feeds (beet, fruit tec.) and up to 400 kg of slaughter offal (4).

CONCLUSION

Concentrate feeds and mixtures have significant importance in game feeding, especially in places where animal are produced in controlled areas (fenced areas, pens). In the future investigations there is a need to more precisely define optimal chemical composition for various game species, their age, physiological status and amounts of natural food available.

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EFFECT OF OREGANO ESSENTIAL OIL ON ANTIOXIDATIVE SYSTEM OF BROILER'S BLOOD AND LIVER

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ABSTRACT

This report describes an investigation on the effects of oregano essential oil (*Origanum vulgare)* on antioxidative status in hemolysed blood and liver homogenate of broiler chickens (glutathione peroxidase-GSH Px, superoxide dismutase-SOD, concentration of malondialdehyde-MDA and lipid peroxidation-TAOC) . Broilers heavy lines Arbor Acres, both sexes were divided into four groups with 60 individuals. Three levels of origano etheric oil: 0; 150; 200 and 300 mg/kg were incorporated into basal diet of 240 broilers for 42 days. Blood and liver were collected for the subsequent evaluation of antioxidant status. Feeding of diet supplemented with 300mg/kg origano etheric oil significantly decreased the concentration of malondialdehyde (MDA) in plasma in comparison with the control group. The activities of glutathione peroxidase (GSH Px) were significantly higher in blood of chicks fed the diet containing 300 mg/kg of origano etheric oil. Other diets containing 150 i 200 mg/kg of origano etheric oil had no effect on lipid peroxidation and activity of antioxidative protection enzyme in the liver homogenate and blood hemolysate of broilers. The present investigation shows that *Origanum vulgare* exibits a significant antioxidant activity in fatthening chickens and can be used as a source of antioxidant in dietary supplement.
Keywords: *Origanum vulgare, antioxidative system, feed, broilers*

INTRODUCTION

Today, increasing attention is paid to aromatic plants rich in etheric oils and their extracts, because they can act in several directions: antimicrobal, antioxidant, affects on metabolism and can use as potential growth promoters. Essential oils used in the prevention and treatment of bacterial infections [1], and acting antiviral [2] and antimicotic [3].
Oregano (*Origanum vulgare L*.) belongs to the family *Lamiaceae*. In addition to other ingredients, it contains more than 4% essential oil which is mostly composed of two phenols: thymol and carvacrol in variable quantity. In addition to basic biological effects, such as antibacterial and impact of an increase in appetite, essential oil of oregano can be used as an alternative to antibiotic growth promoters in chickens [4], pigs [5] and turkeys [6].
For diagnostics of blood and organ diseases catalytic activity of enzymes in erythrocytes and liver is most comonly monitored. Erythrocytes are directly exposed to molecular

oxygen; their plasma membrane has high levels of polyunsaturated fatty acids and an anionic channel specific for single oxygen. Furthermore, erythrocytes contain a high concentration of hemoglobin that is prone to autooxidation. Erythrocytes have a highly effective system in protecting from free radicals: they contain all the enzymes of antioxidative protection and a high level of glutathion [7].
The liver is an organ with a central metabolic role in the organism, often referred to as „the main laboratory" since it performs the major detoxification tasks. Diverse mechanisms are involved. For this reason the liver is the prime target for the study of the metabolism of xenobiotics and other toxic substances [8]. Lipid peroxidation is a reaction between polyunsaturated fatty acids and oxygen which is initiated by radical intermediates and active oxygen species produced by normal metabolic reactions or during metabolization of chemicals. Antioxidant enzymes counteract excessive formation and deleterious effects of reactive oxygen metabolites. For example, superoxide dismutase (SOD) catalyzes the conversion of superoxide anion radical to H_2O_2, catalase reduces H_2O_2 to water, while glutathine peroxidase (GSH Px) acts in conjuction with other enzymes to reduce H_2O_2 and to terminate lipid peroxidation.
Active components from herbal plants have been explored as possible antioxidants [9], [10]. *Origanum vulgare* is an herbal antioxidant shown to possess strong antibacterial, antioxidative and antiinflammatory activities [11].
The objective of this study was to determine supplemental effects of *Origanum vulgare* on antioxidant system in broiler's blood and liver.

MATERIAL AND METHODS

The experimental protocol was approved by the local Ethics Committee; the principles of animal protection were strictly followed. Experiments under *in vivo* conditions were performed on broilers of the heavy line Arbor Acres, of both sexes. One –day-old broilers, randomly selected, were divided into four groups, each numbering 60 individuals. Bird fed a standard basal diet. All birds had free access to water and feed. Temperature and lighting regimens were in accordance with the recommenditation of the breeder. The initial room temperature 32-33º C was reduced weekly by 1ºC to a final temperature of 28ºC. The control group fed a basal diet; the second, third and fourth groups were fed the some basal diets supplemented with 150, 200 and 300mg/kg of oregano etheric oil, respectively. Oregano etheric oil was extracted from *Origanum vulgare* sp. *hirtum* plants by steam distilation and consists of 81,89% carvacrol, 5,1% χ-terpinen, 3,76% cymen and 2,42% thymol.
Levels of hemoglobin, necessary for the expression of the enzymatic activities in hemolysed blood, were determined using commercial test („Dialab", Vienna, Austria) on a spectrophotometer („MultiscanMCC340, Finland). Protein content was determined by the modified method of Gornall and Bardwall [12].

Praparation of blood hemolysate
Blood was drawn from the hearts of broilers into heparinized test tubes. After centrifugation (10 min. at 3500 rpm and 4ºC) and plasma removal, the pellet was rinsed 3 times in saline. The resulting erythrocyte pellet was suspended in an equal volume of double distilled of water and vortexted. After incubation for 1 hour at room temperature,

the hemolysate was centrifuged for 15 min. at 3500 rpm and the supernatant aliquoted for further analysis.

Preparation of liver homogenate
The exised liver was perfused to eliminate blood and the total mass determined. One gram of the tissue was minced with scissors and homogenized in an ultratorax in 3 volumes of isotonic buffer (0,05 M Tris-HCl, 0,25M sucrose, pH=7,5). The homogenate was filtered through gauze into ice-cold tubes and aliquoted for further analysis.

Determination of enzymatic activity
The SOD (EC 1.15.1.1) activity was determined by the spectrophotometric method based on the inhibition of adrenaline reduction to adrenochrome at pH= 10,2 [7].The GSH Px (EC 1.11.1.9) activity was determined by spectrophotometric measurement of absorbance at 412 nm with cumenhydroperoxide as the substrate [13].

Tissue samples of liver for malondialdehyde (MDA) determination were homogenised with deionised distilled water and 50 ml of butylated hydroxytoluene. The MDA concentrations in homogenates were measured by the fluorimetric method in accordance with Jo and Ahn [14].

Lipid peroxidation was determined with thiobarbituric acid. The oxidation of cellular membrane lipids was measured via reaction of lipid peroxides with thiobarbituric acid [15].

Statistics
Differences between means were evaluated at different levels of significance ($p<0,05$; $p<0,01$; $p<0,001$) using repeated measures analysis of variance [16]. The results are given as means ± SEM.

RESULTS AND DISCUSSION

During the experiments, chickens were regularly observed, autopsies were performed and all findings were carefully recorded. Chickens for fattening did not show any visible clinical changes during the whole experiment.
The results of antioxidant indices in hemolysed blood are shown in Table 1. The MDA activity in blood and plasma was significantly lower ($p<0,05$) only in the group supplemented with 300mg/kg of oregano etheric oil compared to the control group. Birds of group supplemented with 300mg/kg oregano etheric oil had greater ($p<0,05$) lipid peroxidation (TAOC) and SOD than broiler chicken in control group. The activity of GSH Px did not differ among treatments.

Table 1. Concentration of malondialdehyde (MDA) and content of lipid peroxidation (TAOC) and enzymatic activity of glutathione peroxidase (GSH Px) and superoxid dismutase in hemolysed blood of broilers fed diets supplemented with different concentrations of oregano etheric oil(mean±SEM; n=10 in each group)

Parameter	Treatment			
	Control group	150mg/kg et. oil *Origanum vulgare*	200mg/kg et. oil *Origanum vulgare*	300mg/kg et. Oil *Origanum vulgare*
TAOC, U/ml	16,02 ± 0,42[b]	19,21 ± 0,41[b]	19,56 ± 0,41[a]	19,71 ± 0,46[a]
SOD, U/ml	141,04 ± 3,70	140,74 ± 5,00	143,55 ± 4,45[a]	168,44 ± 4,10[b]
GSH Px, U/ml	183,20 ± 4,08[a]	183,90 ± 3,20[a]	184,30 ± 3,30	192,78 ± 4,10[a]
MDA, nmol/ml	7,88 ± 0,06[a]	6,22 ± 0,01	6,23 ± 0,02	5,98 ± 0,01[b]

Significant differences within a row are indicated by the same superscript letter (p<0,05)

Table 2 shows hepatic antioxidant indices of birds. The concentration of MDA was significantly lower (p<0,05) in the group supplemented with 300mg/kg origano etheric oil than control group. MDA concentration in liver were not significantly affected by diets. The GSH Px activity in the liver was significantly higher only in the group of birds fed the diet supplemented with 300 mg/kg of origano etheric oil compared to the control and both experimental groups.

Table 2. Concentration of malondialdehyde (MDA) and content of lipid peroxidation (TAOC) and enzymatic activity of glutathione peroxidase (GSH Px) and superoxid dismutase in liver of broilers fed diets supplemented with different concentrations of oregano etheric oil mean±SEM; n=10 in each group)

Parameter	Treatment			
	Control group	150mg/kg et. oil *Origanum vulgare*	200mg/kg et. oil *Origanum vulgare*	300mg/kg et. oil *Origanum vulgare*
TAOC, U/mg of protein	16,02 ± 0,42[b]	19,21 ± 0,41[b]	19,56 ± 0,41[a]	19,71 ± 0,46[a]
SOD, U/ mg of protein	141,04 ± 3,70	140,74 ± 5,00	143,55 ± 4,45[a]	168,44 ± 4,10[b]
GSHPx, U/ mg of protein	183,20 ± 4,08[a]	183,90 ± 3,20[a]	184,30 ± 3,30	192,78 ± 4,10[a]
MDA, nmol/mg of protein	7,88 ± 0,06[a]	6,22 ± 0,01	6,23 ± 0,02	5,98 ± 0,01[b]

Significant differences within a row are indicated by the same superscript letter (p<0,05)

We found significantly lower lipid peroxidation in blood and plasma of chicks fed the diet supplemented with 300mg/kg of oregano etheric oil. Other diets containing 150 and 200mg/kg of *Origanum vulgare* had no effect on lipid peroxidation.

Additionally, effects on antioxidant enhacement of *Origanum vulgare in vivo* were shown for some antioxidant parameters such as SOD, GSH Px, TAOC and MDA. These measurements were used, because they reflect the effect of *Origanum vulgare* on antioxidant activities. First, MDA can endogenously reflect lipid peroxidation, which is the consequence of diminshed antioxidant protection as levels of ROS (reactive oxygen species) increase. Also, SOD and GSH Px are the main parameters used to assess oxidative status in the enzymatic system. Finally, we assayed the TAOC level, which reflects the nonenzymatic antioxidant defense system. Several enzymatic factors such as SOD and GSH Px can scavenge formed ROS to function as antioxidants. Superoxide can first be degraded into H_2O_2 by SOD and subsequently catalyzed to convert water by a series of enzymes including GSH Px. This could be beneficial for the birds because increased antioxidant activity ensures proper and rapid elimination of ROS. In the present study, *Origanum vulgare* in broiler feed increased SOD levels across serum and liver but GSH Px levels were not altered. Supplementation with oregano etheric oil, therefore, may enhance the ROS scavenging by elevating the SODlevel rather than the GSH Px level. SOD were not significantly affected in chickens fed diet supplemented with *Origanum vulgare*. Lower GSH Px activity is generally accompained with an increase of the MDA concentration. The digestive tract itself is considered to be a major site of free radical production in animals and some of them might be delivered via portal blood system into the liver.

In our study, results shows that oregano etheric oil has an effect on the liver function. Our results indicate that this herb has hepatoprotective effects.

CONCLUSION

The present investigation shows that chosen herb *Origanum vulgare* exibits significant antioxidant activity in broiler chickens. Thus, it appears that this spice exerts antioxidant protection through its ability to activate the antioxidant enzymes. Finding of the present study establish that oregano etheric oil has appreciable immunostimulatory activity.

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OCCURRENCE OF MYCOTOXINS AND GENETICALLY MODIFIED ORGANISMS (GMO) IN FEED AND FOOD CONTAINING CORN AND SOYBEAN

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ABSTRACT

The content of mycotoxins and the presence of genetic modifications (GM) were determined in 50 samples of food and 40 samples of feed. All of the tested samples contained corn kernel and/or soybean. Presence of mycotoxins: aflatoxins (AFs), ochratoxin A (OTA), zearalenone (ZEA), deoxynivalenol (DON) and fumonisins (FUM) was determined using ELISA method. In the examined food samples FUM, ZEA and OTA were found with the frequency of 35.0%, 22.5% and 20.0%, respectively. AFs and DON were not detected in any of the food samples. The content of determined mycotoxins was in accordance with the European Regulations, except for the 2 samples of soybean products that showed higher content than maximum allowed level of OTA. The predominant mycotoxin in feed samples was ZEA with contamination frequency of 52.5%. FUM, OTA, AFs and DON were found in 45.0%, 27.5%, 5.0% and 5.0% of tested feed samples, respectively. Concentrations of mycotoxins in all examined feed samples were in accordance with Serbian and European Regulations.
Quantitative analysis of GM soybean and corn kernel by the use of Real Time PCR System showed that only one food sample (2.0%) contained GM soybean. Its content was below the proposed limit of 0.9%, and therefore labeling was not required. None of the food samples contained GM corn kernel. Among the 40 analyzed samples of feed, GM soybean was found in 18 samples (45.0%), and GM corn in 14 samples (35.0%). Content of the GMO in 15 samples of feed was higher than 0.9%, so labeling of these samples was required.
***Keywords**: mycotoxins, GMO, soybean, corn, food, feed*

INTRODUCTION

Mycotoxins are secondary metabolites produced by filamentous fungi that cause a toxic response (mycotoxicosis) when ingested by humans and animals. Most mycotoxins in feed and food are produced by three genera of fungi: *Aspergillus*, *Penicillium* and *Fusarium.* These toxins could cause contamination of a variety of feed and food consumed by animals and humans, essentially cereals, but also other grains, fruits, forages and manufactured products. Due to their toxic properties and their high stability to heat treatment, the presence of mycotoxins in the food chain is potentially hazardous to the health of both humans and animals [2]. More than 400 mycotoxins are known

today and among the most common as contaminants of feed and food are: aflatoxins (AFs), ochratoxin A (OTA), zearalenone (ZEA), deoxynivalenol (DON) and fumonisins (FUM) [9]. Allowed limits of mycotoxins in food and feed are regulated by corresponding regulations in Serbia [17, 18] as well as in European Union [3, 4, 5]. Noticeably differences exist between regulation from Serbia and European Union (EU) which prescribe maximum allowed limits of mycotoxins in food. The main difference between EU and Serbian regulations for food are: different values for allowed limits, different categories of food, and maximum allowed limits for aflatoxin B_2 and G_2, DON and FUM are not determined in Serbian regulations. Serbian regulation [18] for allowed limits of mycotoxins in feed was adopted in May, 2010. Between the regulations from Serbia and EU difference is noticed in allowed limits, categorization of feed types, and in Serbian Regulation the maximum allowed limits for FUM are not determined. Therefore, in this study results of determined mycotoxins in food were compared with European regulation, and results of determined mycotoxins in feed were compared with new Serbian and EU regulations.

The genetically modified (GM) plants as well as feed/food produced from them, has been present in fields and markets world wide from 1994 [6, 8]. After the first GM crop was developed and commercialized, the amount of land being cultivated for GM crops have increased all over the world [13]. In 2007, twenty-three countries planted commercialized GM crops, out of which seven are members of the European Union [15]. Many countries allowed the presence of genetically modified organisms (GMO) in food, but not allowed their cultivation. For the food that contains more than 0.9% GMO labeling is obligatory in the European Union, Australia, New Zealand, Japan, Norway, and Switzerland. In the USA, as the largest producer of GM plants, labeling of GMO products remains non obligatory. In our country the new law on GMO was adopted in May 2009 [19]. Under that law import of GM seeds and cultivation of GM plants is strictly prohibited in Serbia. The most famous examples of GM plants, which are widely used, are several modifications of corn with incorporated Bt gene from *Bacillus thuringiensis* bacteria, responsible for the synthesis of Bt toxins effective against harmful insects [1], herbicide resistant genetically modified soybean and maize [7] etc.

MATERIAL AND METHODS

The survey was conducted on a total of 90 samples of food and feed from domestic and foreign manufacturers presented on the territory of Vojvodina. All examined samples, contained soybean and/or corn. A total of 50 food samples comprising 15 samples of soybean and its derived products (milk, tofu, paté, soybean flakes) and 35 samples of corn and its derived products (flour, corn flakes, pop corn, grits) were analyzed. A total of 40 representative samples of feed (1-2 kg per sample) included 25 samples of feedstuffs (soybean, soybean meal, soybean grits, soybean cake, corn) and 15 samples of complete feed mixtures.

Content of mycotoxins was determined by the enzyme immunosorbent assay (ELISA) method. The Neogen Veratox® AFs, OTA, ZEA, FUM and DON test kits were used for the analysis. Free mycotoxins in the samples and controls are allowed to compete with enzyme-labelled mycotoxins (conjugates) for the antibody binding sites. After a wash step, substrate is added, which reacts with the bound conjugate to produce blue color.

More blue color means less mycotoxin. The test is read in a microwell reader (Thermolabsystem, Thermo, Finland) to yield optical densities. The optical densities of the controls form the standard curve, and the sample optical densities are plotted against the curve to calculate the exact concentration of mycotoxin. According to the manufacturer's description (Veratox®, Neogen) the detection limits for AFs, OTA, ZEA, FUM and DON are 1, 2, 25, 50 (ppb) and 250 (ppm), respectively.
Quantitative analysis of genetic modifications was performed on 7500 Real Time PCR System (Applied Biosystems). The Certified Reference Materials (CRM) standards consisting of dried soybean powder with 0, 0.1, 0.5, 1.0, 2.0, and 5.0% Roundup Ready soybean (GTS 40-3-2) and MON810 maize produced by the Institute for Research Materials and Measurements (IRMM, Belgium) were used.
For DNA isolation from samples and CRMs DNeasy Mini Plant Kit (Qiagen) was used, according to the manufacturer's manual. Extraction of DNA was performed on 100 mg of sample material, in duplicate. The quality and quantity of the extracted DNA was checked with a UV/VIS spectrophotometer (Evolution 100, Thermo). The A_{260}/A_{280} of extracted DNA ranged from 1.7-2.0. These DNA samples were used as a template for the PCR analysis.
More than 90% of GM plants containing 35S promoter [10], therefore commercial kits for detection of 35S promoter was used for the analysis (GMO Soy 35S TaqMan® Detection Kit and GMO Maize 35S TaqMan® Detection Kit, Applied Biosystems). In addition to primers for the 35S promoter and fluorescent probe, kits for soybean contains primers for lectin and kit for corn contains primer for zein, as reference genes. A positive and a negative control are included in kit.
Each PCR reaction for samples and CRMs was set in the final volume of 25 µl, in triplicates in 96-well optical reaction plates. Temperature program included: initial denaturation during 10 min at 95 °C followed by 40 cycles consisting of denaturation for 15 s at 95 °C, annealing for 1 min at 60 °C and extension for 31 s at 72 °C. Limit of detection (LOD) was 0.1%.
The amount of GMO material (35S promoter) is normalized to the amount of plant material (reference gen for lectin for soy, reference gen for zein for maize) detected in each sample. This produces ΔC_T value which is averaged for replicate samples. These values are compared with a calibration curve produced from the ΔC_T values of the known GMO concentration standards. This enables obtaining the % GMO for each uknown sample.

RESULTS AND DISCUSSION

The presence of mycotoxins and GMO were determined in 50 food samples and 40 samples of feed (Tables 1-3, Graph 1).
Table 1. presents the contamination frequencies and averages of the examined mycotoxins in corn and soy products, in food. None of the samples was contamined with AFs and DON, which was expected since AFs are rarely found in Serbia [11].
In 35 examined samples of corn and corn products FUM, ZEA and OTA were found. The most dominant mycotoxin was FUM with the mean level of 275±234 ppb and contamination frequency of 34.3%. OTA and ZEA were found in 14.3% and 11.4% of

the examined samples, respectively. The concentrations of found mycotoxins in samples of corn and corn products are in accordance with the European regulations [3].
ZEA, OTA and FUM were found in samples of soy and soybean products with contamination frequency of 30.0%, 20.0% and 13.3%, respectively (Table 1). The contents of ZEA and FUM were in accordance with European regulations, but the contents of OTA in 2 samples were higher than maximum level allowed (3 ppb).

Table 1. Contamination frequency (CF), interval (CI) and mean level (CM±SD) of analyzed mycotoxins in food based on soybean and corn

Commodity group (SN)		AF	OTA	ZEA	FUM	DON
Corn and corn products (35)	CF	-[a]	14.3	11.4	34.3	-
	CI	-	2.02-2.94	35.5-53.3	58.2-600	-
	CM	-	2.70±0.39	47.1±7.92	275±234	-
Soy and soybean products (15)	CF	-	20.0	30.0	13.3	-
	CI	-	2.98-4.88	25.1-60.1	56.4-62.4	-
	CM	-	3.86±0.96	34.8±15.3	59.4±4.27	-

CF(%), CI (ppb), CM (ppb), SN (number of samples), SD (standard deviation)
[a] toxin was not detected

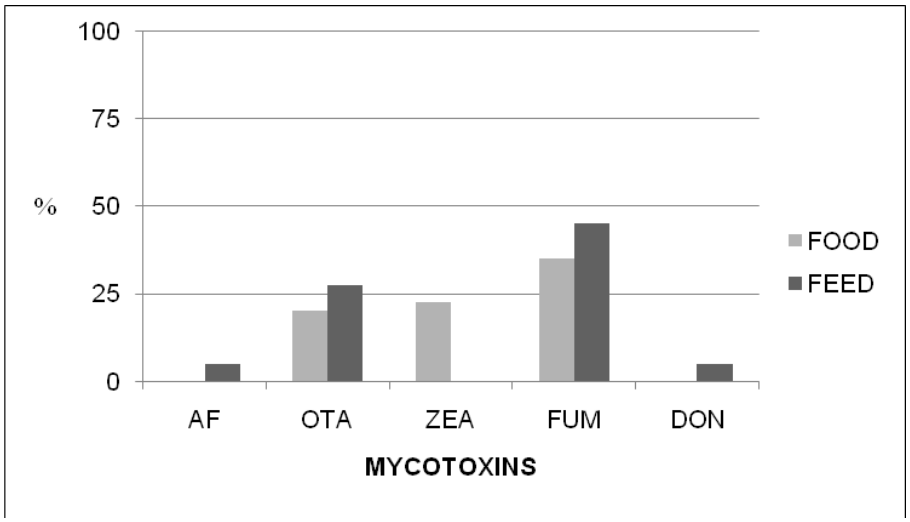
The results of testing contamination with mycotoxins of 40 samples of feed are shown in Table 2. In different types of soybean feed the following mycotoxins were found: ZEA in 11 samples (68.8%), OTA in 8 samples (50.0%), AFs in 2 samples (12.5%), FUM and DON in 1 sample (6.25%).
Four samples (44.4%) and one sample (11.1%) of feed based on corn were found to be contaminated with FUM and DON, respectively. AFs, OTA and ZEA were not found in corn samples. A total of 15 samples of complete feed mixtures were analyzed. FUM was found with the highest incidence (86.6%), followed by ZEA (66.6%) and OTA (20.0%). None of the samples was contaminated with AFs and DON.
Concentrations of mycotoxins in all examined samples of feed were in accordance with Serbian and European Regulations.
The previous investigations of mycotoxicological analyses of soybean and corn showed occurrence of different mycotoxins. Jajić et al. [12] analyzed 38 samples of maize and and 10 samples of soybean. Among the tested samples ZEA, OTA and AFs were found in 86.8%, 78.9% and 73.6% of corn samples, and AFs, ZEA and OTA in 100%, 100%, and 90.0% of soybean samples, respectively. This is not in accordance with the data reported by Mašić et al. [14] who invastigated 53 samples of corn and 43 samples of soybean and found lower incidence of mycotoxins; ZEA were found in 35.8%, OTA in 24.5% and AFs in 16.9% of the analyzed corn samples, and OTA in 6.9%, ZEA in 4.6% and AFs in 2.3% of saoybean samples. Significant differences between the obtained results can be explained by different weather and/or processing conditions.

Table 2. Contamination frequency (CF), interval (CI) and mean level (CM±SD) of analyzed mycotoxins in feed based on soybean and corn

Commodities groups (SN)		AFs	OTA	ZEA	FUM	DON
Soybean (16)	CF	12.5	50.0	68.8	6.25	6.25
	CI	5.20-5.53	2.61-5.12	26.9-74.3	97.4	250
	CM	5.37±0.23	3.72±0.92	54.3±13.6	97.4	250
Corn (9)	CF	-[a]	-	-	44.4	11.1
	CI	-	-	-	350-543	460
	CM	-	-	-	458±84.1	460
Complete feed (15)	CF	-	20.0	66.7	86.7	-
	CI	-	2.66-8.89	27.2-129	232-555	-
	CM	-	5.6±3.13	50.5±29.2	368±105	-

CF(%), CI (ppb), CM (ppb), SN (number of samples), SD (standard deviation)
[a] toxin was not detected

Results of mycotoxins' contaminations of food and feed containing corn and soybean are summarized in Graph 1.
Among the 50 analyzed food samples, FUM, ZEA and OTA were found in 35.0%, 22.5% and 20.0% of samples, respectively. None of the food samples were contaminated with AFs and DON.
All of the investigated mycotoxins were found in feed samples. The predominant mycotoxins was ZEA with contamination frequency of 52.5%. FUM, OTA, AFs and DON were present in 45.0%, 27.5%, 5.0% and 5.0% of samples, respectively.



Graph 1. Contamination frequency of AF, OTA, ZEA, FUM and DON in feed and food containing corn and soybean

Table 3. presents occurrence of GM soybean and corn in food and feed products.
Among 50 the analyzed food samples only one sample (2.0%) contained GM soy. Content of GM soy was below the limit of 0.9%, and therefore did not require labeling.
A total of 40 samples from categories of feed were also analyzed. Among the tested samples that contained only soybean, 7 samples (43.7%) were positive for the presence of GM soybean. Six (37.5%) out of 16 analyzed samples contained GM soy above the limit of 0.9%, of which 5 (31.3%) samples had more than 5%. From samples containing only maize, 3 samples (33.3%) contained GM maize. Among that samples 2 (22.2%) exceeded the limit of 0.9%. From the samples of complete feed, 4 samples (26.7%) contained GM soybean and GM maize below 0.9% while 7 samples (46.7%) contained GM soy and GM maize above 0.9%. Only one sample (6.67%) contained GM soybeans above 5.0%.

Table 3. Results of Real Time PCR analysis of genetically modified soybean and corn food and feed products

Commodity groups(SN)			GM		TSN
			<0.9%	>0.9%	GM >0.9%
Food	Soy and soybean products	(15)	1	0	0
	Corn and corn products	(35)	0	0	
Feed	Soybean	(16)	1	6	15
	Corn	(9)	1	2	
	Complete feed mixture	(15)	4	7	

SN (number of samples), TSN (total number of samples)

Among 40 examined samples from the category of feed, GMO were found in 21 (52.5%) samples, while in 15 (37.5%) samples presence of GMO was above the limit of 0.9%. Possible explanation of these findings is impact of sampling process, as one of the most important factors that determines representative sample composition. Paoletti et al. [16] reported that GM material is not randomly distributed and it shows highly significant deviations from randomness at different hierarchical scales, within and among lots. Also, feed is often a mixture of different agricultural crops from which some or even all may be genetically modified to varying degree. There is no obligation for marking the product in the case when only the RoundupReady soybean is present in the feed sample, although the GM percent is higher than 0.9%. In the case of the presence of GM maize the feed product must be properly labelled.

CONCLUSIONS

Based on the results presented in this paper, it could be concluded that it is necessity to continue the examination of mycotoxins and GMO content in different food and feed products. In the future it will be necessary to extend this study to other plant species present in the human and/or animal diet in order to form a data base of the appearance of mycotoxins and GMO in food and feed originating from the territory of Vojvodina.

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PHYTO ADDITIVES (ALLIUM SATIVUM L.) IN THE DIET OF FATTENING CHICKENS

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ABSTRACT

Garlic (Allium sativum L.) exhibits antimicrobial, antioxidant and antihyperiensic properties in human and animal nutrition according to the presence of bioactive components from which the most important are sulfur compounds, allicin and allin, which make garlic an alternative to antibiotics. It also inhibits the synthesis of key enzymes involved in the synthesis of cholesterol and thus lowers the level of the cholesterol in the tissues of chickens. Based on the above, the goal of this study was to determine the effect of garlic in the diet of fattening chicken production parameters and cholesterol content in white and red meat. The tests were performed in a production environment. At the start of feeding three groups were formed with the 37-day chicken hybrid Ross 308, of the same weight, in five repetitions. In the experimental groups (II and III) 1.5% and 3% of commercial garlic were included. At the end of the experiment, which lasted 42 days, addition of garlic in the diet affected on the increase of body weight in group II (2257.47 g) than it was in the control (2250.26 g), but differences were not statistically significant, while the third group seemed more depressed (2229.39 g) than the control group. Food conversion was better in groups with garlic, while the best was in the second group (1.83%). Cholesterol content was lower in groups with garlic. It was lower in white and red meat compared to control group.
***Keywords**: garlic, chicken, production parameters, cholesterol*

INTRODUCTION

In addition to the nutrients needed for growth and development of chickens, the animal feed is regularly added with pharmacological agents, whether for preventive purposes, to prevent certain diseases (coccidiostatics), or with growth stimulants (antibiotics), particularly in young animals [5, 16].
An alternative to antibiotics as growth stimulants is large and amounts to finding of adequate nonpharmacological agents from the group of prebiotics, probiotics, organic acids, essential and other oils, herbs or parts of plants such as thyme, basil, oregano and other [18, 2].
Useful effect of garlic (Allium sativum L.) on human and animal organism, it is known from ancient times, as it has antimicrobial, antioxidant and antihypertensive properties [11, 19, 14, 13]. Studies have shown that these effects can be attributed to the bioactive components of which are the most important compounds of sulfur, allin, diallylsulphide,

allyldisulphide and allicin [17, 12]. Healing effect on intestinal disorders, abdominal distention, worm and respiratory infections has been established [1].
The investigations focused on growth, conversion and meat quality of different species of animals, show positive effects. Garlic supplement in the amount of 1% in the diet for pigs was established by a better growth rate, conversion and meat quality in relation to the control group. Similar results came in [8], [6] and [3] in their research with broilers, but also there was a realization that lower concentrations, which range from 1-2% show greater effect. Addition of 4,5% of garlic in food for the chickens had no effect on weight gain and feed conversion. Parallel tests with 2% of garlic and 100ppm of copper separated or in combination of both drugs on the production parameters was performed by [20] and found that a group with 2% of garlic achieved the best results. [7] have studied the effect of taste of food with garlic on consumption, specific performances and blood parameters in horses, pigs and sheep.
Garlic also expressed hypocholesterolemic effects in chickens by inhibition of the most important enzymes involved in the synthesis of cholesterol and lipids, tri-hydroxy-tri-methyl glutaril-coenzyme-A-reductase, cholesterol-7α-hydroxylase and fatty acid syntheses.
Cholesterol is alcohol by chemical composition. It belongs to steroids, ring compounds with cyclic core of fenantren to which is linked the ring of ciklopentan. Steroids are often found together with fats and they separate from them after saponification, because they are in the rest that can not be saponificated. It belongs to zoosterole, being a typical product of animal metabolism and occurs in food which has animal origin. It represents the building substance of cell wall, and it is of great importance in the normal functioning of the endocrine glands, primarily the adrenal glands and gonads. Living cells of human and animal body, especially the liver have the ability to synthesize approximately two-thirds of endogenous cholesterol, while the lower part intakes by food. After entering the body, it is related to the proteins dissolved in blood plasma and together with them circulates through the bloodstream. There are two forms of cholesterol-related protein, LDL and HDL cholesterol of low and high density. The difference between them is of great importance, because the level of LDL cholesterol in the plasma determines the risk for atherosclerosis, which is higher if the concentration of these types of particles is larger. HDL particles do not have atherogenic potential, but they represent defenders of blood vessels.
Synthesis of cholesterol is made in the liver, and cholesterol built in low-density lipoproteins (LDL). All carbon atoms of cholesterol come from acetyl-CoA. Varying amounts of cholesterol in the diet is reflected in the production of endogenous cholesterol. The low level in food stimulates its synthesis, otherwise if the exogenous cholesterol increased, endogenous production can not be completely suppressed by the increasing of content of cholesterol in the diet, since it only inhibits the synthesis in the liver, while synthesis in the intestine is inhibited by bile acids. Attempts to reduce the cholesterol levels in peoples plasma by reducing the amount of cholesterol in the diet proved to be successful. Of the factors that reduce blood cholesterol there is usual study of some saturated fatty acids from food, polyunsaturated fatty acids [9, 10, 4].
In addition, the additive is relatively cheap in the market it adds in a small amount of 1-2% and therefore does not increase the cost of production, which is of particular importance for the producers.

According to mentioned, the goal of this study was to examine the influence of different levels of commercial garlic in food of fattening chickens on production parameters and cholesterol content in white and red meat.

MATERIALS AND METHODS

Biological tests were performed in production conditions, on an experimental area named "Pustara" of the Agricultural Faculty in Novi Sad. At the start of feeding three groups were formed with the 37-day chicken hybrid Ross 308, with the same mass. The experiment was conducted in five repetitions on the total of 185 chickens per treatment, and was appointed to the scheme given in Table 1. They used three mixtures for food. Starter was used during the first three weeks of age, then the finisher I and during next 14 days and at the end of the last 7 days they used finisher II, with 23%, 20% and 18% of protein. The experiment lasted 42 days. The composition and quality of the mixtures are shown in Table 2. During the experimental period, chickens were fed and powered by the will, and microclimatic conditions were regularly monitored. Control of body weight and food consumption was performed periodically during the transition to another type of food.

Table 1. The plan of additives supplement

Group	I	II	III
Garlic**,** %	Control	1,5	3

Table 2. Composition of feed for chicks, %

Feeds	Starter	Finisher I	Finisher II
Soy oil	1.80	2.50	3.00
Corn	49.00	55.45	63.00
DL Methionine	0.15	0.15	0.10
Foolfat soya	13.80	12.00	8.00
Soya bean meal	28.00	22.70	18.60
Monocalcium phosphate	1.55	1.50	1.60
Premix	1.00	1.00	1.00
Salt	0.30	0.30	0.30
Limestone	1.40	1.40	1.40
Yeast	3.00	3.00	3.00
Total	100	100	100

The results of research are presented in tables, as well as the average value of treatment and processed by statistical methods, ANOVA. At the end of the experiment, ten chickens from each group were sacrificed and the content of cholesterol was analyzed in white and red meat without the skin.
Total cholesterol was determined by spectrophotometric method using o-phtalaldehidde, according to the method [15].

Table 3. Chemical analysis of feeds

Mixture	Starter	Finisher I	Finisher II
Crude protein,%	22.91	20.57	17.97
Fat,%	6.19	6.76	6.78
Crude fiber,%	4.15	3.88	3.61
Methionine	0.51	0.48	0.39
ME:SP	133	153	178
Metabolic Energy,MJ/kg	12.83	13.206	13.411
Calcium,%	0.90	0.88	0.88
Phosphorus, %	0.76	0.72	0.72

RESULTS AND DISCUSSION

Based on the results we can conclude that the inclusion of commercial garlic in chicken fattening foods in the quantity of 1.5% had positive effect on the intensity of growth (Table 4), while the addition of 3% led to a mild depression in increase.

Table 4. Body weight of chicken, g

Groups	I	II	III
Additive	Control	Garlic, 1,5%	Garlic, 3%
Initial body weight	39.0	39.0	39.0
Body weight at week 3	842,03	840,15	830,24
Body weight at week 5	1809,15	1770,00	1749,83
Body weight at week 6	2250,26	$2257,47^{NS}$	2229,39
SD	299,1918	334,3262	313,9847
DE	21,48072	24,38325	22,83903

NS - Nonsignificant (P>0,05)

In the first fattening period, at the end of the third week, the highest weight achieved the control group (842.03 g), while in experimental groups (II and III) this weight was slightly lower and amounted to 840.15 and 830.24 g, respectively. The reason for this is probably the reduced consumption of food, which is influenced by an intense smell of garlic, which is the reason why there, was needed some time to get used to this kind of chicken feed. Similar findings had Horton et al. (1991a). At the end of the fifth week a growth trend continues. However, at the end of the experiment, which lasted 42 days, adding of garlic influenced on the increase in body weight in group II (2257.47 g) while this weight in control was 2250.26 g, but differences between groups were not statistically significant (Table 4). In the third group, achieved body weight amounted to 2229.39 g.

Use of different levels of additives in this experiment had a different impact on the efficiency of exploitation of food (Table 5). The lowest conversion during the entire experiment of 1.83 kg / kg gain had a second group with 1.5% of garlic in the diet, which is 3.18% less than the control group. The third group also had lower feed conversion (1.85 kg / kg), for 2,12% compared to the control (1.89 kg / kg).

Table 5. Feed conversion

Groups	I	II	III
Additive	Control	Garlic 1,5%	Garlic 3%
1.-3. week	1,55	1,52	1,53
4.-5. week	1,77	1,75	1,76
1.-6. week	1,89	1,83	1,85
Index 1-6, %	100,00	96,82	97,88
SD	0,029693	0,081398	0,055352
DE	0,013279	0,036402	0,024754

Based on the results, it can be concluded that addition of garlic in the diet generally lowers cholesterol levels in the tissues of chickens (Table 6, 7). A significant reduction in the white meat, in the treatment with 3% of garlic is 39.76% compared to the control (Table 6). High lowering of cholesterol is observed in the red meat in the third group with the same amount of garlic. This reduction is 37.16%. It also can determine that the amount of cholesterol in white and red meat in the group with 1.5% of added garlic is lower than the amount in the control group, but there is much weaker effect of phytoadditives compared to the third group.

Table 6. The amount of cholesterol in the white meat of chickens, mg/100g

Groups	I	II	III
Additive	Control	Garlic 1,5%	Garlic 3%
Average	101,76	87,02	61,30
Min	60,87	62,31	36,59
Max	165,32	133,03	105,50
Index,%	100,00	85,51	60,24

Table 7. The amount of cholesterol in red meat (leg and drumstick) of chickens, mg/100g

Groups	I	II	III
Additive	Control	Garlic 1,5%	Garlic 3%
Average	153,24	148,00	96,30
Min	112,54	129,53	51,13
Max	234,12	280,71	198,76
Index,%	100,00	96,58	62,84

CONCLUSION

Based on the presented results we can conclude that the treatment of chickens with 1.5% of garlic has better production results than the control group, but differences between groups were not statistically significant. Body weight was increased and feed conversion decreased by 3.18%. It can also be concluded that the use of commercial garlic in the diet of chickens significantly lowers cholesterol levels in their body. The greatest effect is achieved in white and red meat in the group with the addition of 3% of garlic.

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EFFECTS OF TECHNOLOGICAL TREATMENTS IN PREPARING FORAGES ON PROTEIN FRACTIONS AND THEIR RUMINAL DEGRADABILITY

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ABSTRACT

Technological treatments in preparing forages, which are base for ruminant rations, affect content and ratio of crude protein fractions. Knowing the effects of feeds technological treatments on portions of crude protein (CP) is prerequisite for diet formulation, which will supply sufficient quantity of ruminal degradable protein for microbial fermentation in the rumen. Such diets will also supply an adequate quantity of high-quality rumen undegradable protein and animal requirements in essential amino acids available for intestinal absorption, which cannot be completely supplied from microbial protein. Technological processes for preparing and conserving of forages, plant maturity, wilting time and conditions, and application of chemical and biological additives affect content of fractions and ruminal degradability of CP.
Keywords: *forages, protein, ruminal degradability, fractions*

INTRODUCTION

Ruminal degradability of crude protein (CP) in ration for ruminants is significant factor that affects ruminal fermentation and utilization of consumed amino acids (AA). Fraction of crude protein degradable (RDP) and undegradable (RUP) in rumen, are two components of dietary crude protein with different functions and metabolically pathways in ruminal fermentation and whole digestion tract utilization [18]. Ruminal degradable fraction contains soluble protein, peptides, amino acids and ammonia N, and is used for growth of ruminal microflora and microbial protein synthesis. Ruminally synthesized microbial protein typically supplies most of the AA passing to the small intestine. Ruminally undegraded protein is the second most important source of absorbable AA to the animal. Knowledge of the kinetics of ruminal degradation of feed proteins is fundamental to formulating diets for adequate amounts of RDP for rumen microorganisms and adequate amounts of RUP for the host animal [19].

One of the most complex model that describes fractions of CP in ruminant nutrition is Cornell Net Carbohydrate Protein System (CNCPS), [17]. According to this model, feed crude protein is divided into five fractions (A, B_1, B_2, B_3 and C), which are characterized with different rate of ruminal degradability. Fraction A is instantaneously soluble non-protein N (NPN); B1 fraction is soluble in borate-phosphate buffer and precipitated with trichloroacetic acid (TCA), with rate of ruminal degradation 120-400 %/h; fraction B_3 of

CP is connected with cell walls of plant feeds, calculated as portion of CP insoluble in neutral detergent (NDICP), but soluble in acid detergent, with rate of ruminal degradation 0.06-0.55 %/h; fraction C is considered as completely indigestible portion of CP, insoluble in acid detergent (ADICP); B_2 fraction of CP is calculated as difference between total CP and contents of these fractions, with rate of ruminal degradation 3-16 %/h. According to this model, the contents of RDP and RUP are calculated using equations:

$$RDP = A + B_1 \times [k_d \times B_1/(k_d \times B_1 + k_p)] + B_2 \times [k_d \times B_2/(k_d \times B_2 + k_p)] + B_3 \times [k_d \times B_3/(k_d \times B_3 + k_p)]$$

$$RUP = B_1 \times [k_p/(k_d \times B_1 + k_p)] + B_2 \times [k_p/(k_d \times B_2 + k_p)] + B_3 \times [k_p/(k_d \times B_3 + k_p)] + C$$

k_d – Rate of ruminal degradation of protein fraction,
k_p – Rate of ruminal passage of digesta

Often used model for describing ruminal protein degradation divides feed CP into three fractions (A, B and C), [13]. According to this model, fraction A is nonprotein N (NPN) instantly degraded in rumen, and soluble fraction of true protein with high rate of ruminal degradation; C fraction is completely undegradable portion of CP, and B fraction is potentially degradable protein, with extent of ruminal degradation that depends of rate of passage [14]. According to this model, feed contents of RDP and RUP is calculated:

$$RDP = A + B \times [k_d \times / (k_d + k_p)]$$

$$RUP = B \times [k_p / (k_d + k_p)] + C$$

k_d – Rate of ruminal degradation of protein fraction,
k_p – Rate of ruminal passage of digesta

Technological treatments in preparing forages, which are base for ruminant rations, affect content and ratio of crude protein fractions. Knowing the effects of feeds technological treatments on portions of CP is prerequisite for diet formulation, which will supply sufficient quantity of ruminal degradable protein for microbial fermentation in rumen. Such diets will also supply an adequate quantity of high-quality rumen undegradable protein and animal requirements in essential amino acids available for intestinal absorption, which cannot be completely supplied from microbial protein [21].

EFFECTS OF FORAGES PREPARING TREATMENTS ON PROTEIN FRACTIONS CONTENT

Forages are the base of diets for ruminants supplying a significant portion of total dietary protein. Ruminal degradation and utilization efficiency of forage protein largely affect on supplying of animals with sufficient quantity of AA available for intestinal absorption [8].

Adequate content of ruminal degradable protein (RDP) is necessary for normal ruminal fermentation and optimal utilization of consumed feed. During the technological processes of forages preparing and conservation: wilting, drying, ensiling, great portion of true protein (first of all B_1 fraction, soluble protein) is transformed to NPN (A fraction), [4].

Nonprotein fraction of CP and soluble fraction have a high rate of ruminal degradation, which increases ruminal concentration of NH_3. Rapidly and asynchronous releasing of ammonia relative to available energy (soluble carbohydrates) is a main reason for decreasing utilization of dietary protein, increasing absorption of NH_3 in blood, and increasing of energy deficit of production animals, according to additional energy costs of neutralization of NH_3 (urea synthesis). Because of higher concentration of easily available energy, efficiency of microbial protein synthesis (g of microbial N/kg of fermented organic matter) is higher for corn silage relative to grass silage, or legume silage [20].

In fresh grass, 75-90% of CP is true protein. Intensive proteolytic processes start after cutting, caused by plant proteases, and extent of proteolyses is increased with prolonged wilting time, especially in wet conditions. After ensiling, proteolytic processes are continued, and after 24 h, proportion of true protein in CP is about 60%. Main products of plant proteases activity are AA, which are subsequently degraded to NH_3 by microbial fermentation [2].

Table 1. Nitrogen components of different silages and fresh herbage [5]

N fraction	Italian ryegrass		Red clover		Corn	
	Fresh herbage	Silage	Fresh herbage	Silage	Fresh herbage	Silage
Total N, g/kg DM	20.2	19.6	NR	NR	16.7	13.4
Fractions g/kg of total N						
N-Protein	863	308	760	439	642	210
N-Amino acids	17	369	43	250	241	420
N-Peptides	39	90	44	-	NR	NR
N-Amides	3	-	75	-	NR	NR
NH_3-N	2	121	10	144	9	71
Other NPN	76	112	68	167	108	299

Methods for preparing and conserving of forages, plant maturity, using of additives, affect fractions content and ruminal degradation of CP. Characterization and complete analysis of forage CP availability, has primary importance for feed quality determination

and diet formulation that will enable efficiently utilization of nutrients and maximal animal productivity. Earlier research mainly focused on determination of total protein and NPN content, and there are limited information for forage protein composition.
Earlier cutting and using of red clover for silage (Mean Stage Weight 2.41) relative to later cutting (MSW-2.65) increased portion of A fraction of CP in silage, and decreased content of B_1, B_2 and B_3 fractions [6]. Content of rumen degradable protein is increased above 150 g/kg DM, this value is preferable for red clover silage that is commonly characterized with deficit in RDP. Earlier cutting of alfalfa (MSW 2.61and 3.78) decreased portion of A and B_3 fractions, and increased portion of B_1 and B_2 fractions of CP in silage. Ruminal degradability of CP is significantly increased in this way.

Table 2. Chemical composition (g/kg), protein fractions (g/kg CP) and predicted values for ruminal degradability of protein (g/kg DM) in red clover and alfalfa silages, in early or late cutting [6]

Item	Red clover		Alfalfa	
	Early cut	Late cut	Early cut	Late cut
DM	365	375	368	379
pH	4.26	4.20	4.54	4.61
CP	214	196	241	210
NH_4-N	38	35	53	65
Free AK	266	258	370	357
Peptides	177	155	240	288
Fraction A	481	448	663	710
Fraction B_1	73	80	88	30
Fraction B_2	250	258	162	147
Fraction B_3	142	151	48	58
Fraction C	60	61	39	56
RDP	159	143	209	179
RUP	55	53	32	32

Values for RDP and RUP content are predicted using constants k_d: 150 %/h for B_1, 10 %/h for B_2, 1.5 %/h for B_3, and constant k_p - 5 %/h.

There was trend for increasing content of RDP in total protein based on increased portion of fraction A (NPN), with earlier cutting of red clover, while in alfalfa silage, increasing ruminal degradability of crude protein was based on higher level of B_1 and B_2 fractions (true protein). Determined increasing DM concentration of CP in silages prepared using plants at earlier growing stages, was result of higher level of RDP, while concentration of RUP was almost unchanged.
It was obtained significant increasing of proportion of nonprotein nitrogen (fraction A of CP) with prolonged wilting time (until 35% of DM) in fresh forage of red clover and alfalfa harvested at mid-bloom stage of maturity [15]. Increasing content of NPN is a result of protein hydrolysis during wilting period, affected by plant proteinases.

Table 3. Effect of wilting time of red clover and alfalfa fresh herbage, on content of nonprotein N (NPN, g/kg of total N), [15]

Item	Red clover	Alfalfa
Fresh forage	142	158
Wilted forage[1]		
0	157	181
30	188	197
73	245	286
100	215	302
Silage		
0	385	631
30	364	671
73	414	652
100	383	644

[1]Different wilting time of herbage to targeted dry matter is achieved by different level of shade: 0, 30, 73 and 100%.

There was no significant effect of wilting time on content of NPN in red clover and alfalfa silages.
Ensiling of wilted alfalfa (33% DM) using chemical additives (formic acid or formaldehyde) decreased content of A fraction, and increased proportion of B_1 fraction in CP [9].

Table 4. Protein fractions (g/kg CP) in fresh and wilted herbage, hay and silage of alfalfa [9]

Treat	A	B_1	B_2	B_3	C
Fresh forage	150	570	135	19.5	125
Wilted forage	171	554	145	27.1	103
Hay	287	37.4	411	154	110
Silage	684	14.6	146	32.6	123
Silage-formic acid	507	111	110	140	127
Silage-formaldehyde	572	149	102	34.1	143
Silage, formic acid + formald.	434	97.7	134	216	118

It can be concluded that B_1 fraction makes more than half of CP in fresh and wilted alfalfa. During ensiling, the most of soluble true protein is transformed to A fraction (NPN). Using of preservatives as formic acid and formaldehyde, and especially their combination, significantly reduces this tendency, first of all by rapid acidification of ensiling herbage (effect of formic acid), and by chemical linkage of protein and formaldehyde (partially increases content of fraction C), [16]. Alfalfa hay is characterized with higher level of NPN (fraction A), relative to fresh forage, but also with the highest content of B_2 fraction of CP, which partially passes the rumen (rate of ruminal degradation 50-150 g/kg/h).

Recent research report that up to 10% of soluble N in grass silage passes rumen undegraded, and reaches the duodenum, pointing that methods used for estimating of ruminal degradability of silage CP, overestimating content of degradable N, and underestimating a content of undegradable N that passes rumen [5]. Proportion of soluble N that pass rumen undegraded, first of all consists of shorts peptides' chains, which more rapidly pass rumen, with liquid phase of ruminal content (0.12-0.15 %/h), relative to proteins and longer polypeptides' chains [23]. Portion of silage soluble N that passes rumen, is equivalent to 125 g of protein/day, if intake of grass silage is 10 kg of DM.

Table 5. Content of CP fractions and ruminal degradability, in different silages [12]

Silage	CP g/kg DM	A fraction g/kg CP	B fraction g/kg CP	Rate of ruminal degradation of B fraction %/h	[1]Effective ruminal degradation of CP g/kg
Italian Ryegrass	132.5	749	200	9.5	880
Red clover + grass	163.1	758	191	9.7	880
Orchard grass	179.4	648	293	10.0	840
Maize	106.9	798	147	1.9	840

[1] Effective ruminal degradation is estimated using value of 5 %/h for passage rate

Soluble and rapidly degradable fractions of protein A and B_1 make about 50% of CP in common vetch in stage of flowering and pod filling [1]. Moderate degradable protein fraction B_2, makes 40% of CP. With maturing of common vetch (flowering and pod filling-28% DM in whole plant) proportion of A fraction is increased, and content of B_1 and B_2 in CP decreased. Comparing two phases of pod filling (28 and 38% DM), decreasing of fraction A, and increasing of fraction B_1content, was obtained. Processes of accumulation and distribution of CP, during the period of intensive growth of grain, can explain different effect of maturing on two fractions of soluble protein. Trough out of this period, CP is intensively accumulated in pod in grain, by redistribution from vegetative parts of plant. According to this, the content of true protein is increased, because of decreased proportion of NPN. It was found that these two phases of plant maturity do not affect content of B_2 fraction.

Table 6. Protein fractions of common vetch in different stages of maturity [1]

Item	Flowering	Pod filling 28% DM	Pod filling 38% DM
CP, % DM	21.0	18.8	17.9
Fractions, % CP			
A	19.7	29.6	21.5
B_1	26.2	21.3	27.0
B_2	47.3	39.5	39.3
B_3	2.9	5.3	6.6
C	4.1	4.5	5.6

Polyphenols present in legumes (condensed tannins and o-quinones) have an ability for binding of proteins and precipitation of proteases, thereby decreasing extent of undesirable proteolysis during wilting and ensiling, and increasing content of protein fractions that are rumen undegradable [22]. Presence of 1 g of condensed tannins in hay of birdsfoot trefoil, reduces proportion of soluble protein fraction by 1.9 g, while increases content of rumen undegradable protein fraction by 1.25 g. Presence of o-diphenols in red clover, which are transformed to o-quinones, reduces content of soluble protein fraction, and increases proportion of rumen undegradable protein in hay and silage, providing higher content of this fraction of CP, relative to alfalfa hay and silage by 50%, as also higher proportion of protein available in intestine, by 88%.
Conditioning and conservation methods may interact with polyphenols to alter forage CP solubility and degradability. Shifting from roll conditioning to maceration of alfalfa fresh forage, reduces proportion of soluble in total protein of hay and silage, with insignificant effect on content of rumen undegradable and intestinal available protein. Maceration of birdsfoot trefoil fresh forage in combination with present condensed tannins, reduces proportion of soluble protein in hay and silage, increases content of rumen undegradable protein, and provides higher level of intestinal available protein in CP, by 62% in hay, and by 145% in silage, relative to CP of alfalfa hay and silage. Surprisingly, method of maceration relative to roll conditioning of red clover, reduces content of soluble protein, but also reduces content of rumen undegradable and intestinal available protein [7].

Table 7. Concentration of protein fractions (g/kg CP), in legumes' hay and silage, with different content of polyphenols, and applied method of conditioning [7]

Fraction	Alfalfa	[1]Birdsfoot trefoil LT	Birdsfoot trefoil MT	Birdsfoot trefoil HT	Red clover
Soluble protein					
Hay					
Roll conditioning	440	414	391	376	333
Maceration	323	315	285	249	205
Silage					
Roll conditioning	734	741	707	660	515
Maceration	585	552	524	469	371
Rumen undegradable protein					
Hay					
Roll conditioning	255	291	323	347	365
Maceration	270	336	381	412	304
Silage					
Roll conditioning	199	221	255	302	342
Maceration	243	354	393	456	317

[1]LT-Low tannin, MT-Mid tannin, HT-High tannin

Application of homofermentative lactic acid inoculant based on *Lactobacillus plantarum* and *Enterococcus faecium* (2.5×10^6 CFU/kg ensiling forage) for ensiling wilted millet (25% DM), did not affect on content of nonprotein N, on fraction of soluble protein, as also on proportion of protein fraction insoluble in neutral (NDICP) and acid detergent (ADICP), [10]. Proteolysis is undesirable process during the ensiling, because up to 75% of CP can be transformed to nonprotein N fraction (NPN) by activity of plant proteases, during the first few days of ensiling. One of the predicted positive effect of using of inoculants is reduction of proteolytic processes during early phase of ensiling, by rapidly decreasing of pH in ensiled forage. However, numerous researchings point that positive effect of using inoculants on reduction rate and extent of proteolytic processes in ensiling herbage, is expressed with ensiling legumes (alfalfa, field pea), but is absented with ensiling grains (corn, barley, wheat), [3, 24,11].
Using of inoculants (combination of lactic acid bacteria: *Pediococcus, Lactobacillus*, and *Enterococcus*) at 1.25×10^6 CFU/kg of ensiled forage, for wilted barley ensiling (35% DM), decreased proportion of NH_3-N in total N (37 and 75 g/kg of total N), by reduction of proteolytic processes. Using of this homofermentative inoculant in combination with enzymatic additive (combination of carboxymethylcellulase, xylanase, α-amylase, and β-glucanase) also decreased content of NH_3-N (45 g/kg of total N) in barley silage [25].

CONCLUSION

Technological treatments in preparing forages, which are base for ruminant rations, affect content and ratio of crude protein fractions. First of all, methods for preparing and preserving forages, plant growing stage, and using of additives affect fractions content and rumen degradability of CP. Knowing the effects of feeds technological treatments on portions of CP is prerequisite for diet formulation, which will supply sufficient quantity of ruminal degradable protein for microbial fermentation in rumen, as also an adequate quantity of high-quality rumen undegradable protein and essential amino acids available for intestinal absorption, which cannot be completely supplied from microbial protein

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MEDICINAL PLANTS AS POTENTIAL FUNCTIONAL COMPONENTS IN FOOD AND FEED PRODUCTION

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ABSTRACT

Apart from identification and quantitative determination of phenolic and flavonoid compounds, the objective of this research was to evaluate the antioxidant activity of the ethanolic extracts of parsley fruit (*Petroselini fructus*), buckthorn bark (*Frangulae cortex*), mint leaves (*Mentha piperitae folium*), caraway fruit (*Carvi fructus*) and birch leaves (*Betulae folium*) as well as of the mixture of these medicinal herbs "Vitalplant" (*Frangulae cortex* (35 %), *Menthae piperitae folium* (20%), *Carvi fructus* (20 %), *Petroselini fructus* (25 %)). The results of this experiment show that all the tested plant drugs are a rich source of plant phenolics, and at the same time possess antioxidant activity in all of the tests. Apart from the highest phenolic content, the mint extract was shown to possess the highest antioxidant capacity in all but in the β-carotene-antioxidant (AOA) test. Caraway fruit had the lowest content of total phenolics, as well as the lowest antioxidant capacity of all the tested samples. Commercial mixture "Vitalplant" exhibited a relatively high antioxidant activity in most of the tests, which can be explained by synergistic effects of the components of which it is composed.
***Keywords**: medicinal plants, plant phenolics, flavonoids, antioxidant activity*

INTRODUCTION

Lipid oxidation is one of the major causes of chemical spoilage of foods and feeds, resulting in rancidity and/or deterioration of the nutritional quality, colour, flavour, texture and safety [1].
On the other hand, current researches into free radicals have confirmed that foods rich in antioxidants play an essential role in the prevention of cardiovascular diseases, cancers and neurodegenerative diseases, as well as inflammation and problems caused by process of aging [2].
Spices and herbs have been added to foods since ancient times, not only as flavouring agents, but also as folk medicine and food preservatives. Spices and herbs and their constituents are generally recognized to be safe, either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies [3]. Being natural foodstuffs, spices and herbs appeal to many consumers, who question the safety of synthetic food additives, and as such, these plants could be considered as functional food components and potential animal feed supplements.

Medicinal plants are rich source of secondary biomolecules, many of which exhibit significant pharmacological effects. Birch bark (*Betula pendula* Roth.) is high in betulin and betulinic acid, phytochemicals which have a potential as pharmaceuticals [4]. With the essential oil component apiole, proven kidney stimulant, parsley (*Petroselinum crispum* (Mill.) A.W. Nym. ex Hill) has been known for its diuretic action. Mint (*Mentha x piperita* L.) and caraway (*Carum Carvi* L.) essential oils are frequently used in herbal drugs for treatment of abdominal discomfort and pain, while buckthorn bark (*Rhamnus Frangula* L.) contains anthraquinone glycosides with purgative effect [4].
There is presently an increasing interest both in the industry and in the scientific research of spices and aromatic herbs because of their strong antioxidant properties. These properties are due to many substances, including some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens and minerals [5]. Phenolic substances have been shown to be the most responsible for the antioxidant activity of plant materials [6].
Prevalent phenolics in parsley are apigenin glycosides [7]. In caraway the main phenolics are quercetin and kaempferol glycosides [8]. The main identified compounds in buckthorn bark are anthraquinone glycosides, flavonoids and tannins [9]. In mint, the main individual compounds are eriocitrin and rosmarinic acid, they account for 59 to 67% of total phenolics [10]. Birch is rich in flavonoids (up to 3%) with hyperoside as the main flavonoid compound [4].
Regarding the fact that the above mentioned medicinal plants, nontoxic source of the biomolecules with proven pharmacological action are at the same time a rich source of plant phenolics, the objective of this research was to evaluate the antioxidant activitiy of the ethanolic extracts of parsley fruit (*Petroselini fructus*), buckthorn bark (*Frangulae cortex*), mint leaves (*Mentha piperitae folium*), caraway fruit (*Carvi fructus*) and birch leaves (*Betulae folium*) as well as of the mixture of these medicinal herbs "Vitalplant" (*Frangulae cortex* (35 %), *Menthae piperitae folium* (20%), *Carvi fructus* (20 %), *Petroselini fructus* (25 %)).

MATERIAL AND METHODS

Material

Herbal drugs: parsley fruit (*Petroselini fructus*), buckthorn bark (*Frangulae cortex*), mint leaves (*Mentha piperitae folium*), caraway fruit (*Carvi fructus*) and birch leaves (*Betulae folium)* as well as the herbal mixture "Vitalplant" (*Frangulae cortex* (35 %), *Menthae piperitae folium* (20%), *Carvi fructus* (20 %), *Petroselini fructus* (25 %)) were obtained from the Institute for Medicinal Plants Research "Dr Josif Pančić" from Belgrade. Herbal drugs and the mixture were in the form of powder with granulation of up to 3 mm.

Preparation of plant extracts

Crude plant extracts were obtained by maceration with ethanol/water mixture (80:20, v/v), with the ratio of raw materials to ethanol solution of 1:10, for 24 h at room temperature and subsequently extracted in a ultrasonic bath at room temperature for 10 minutes. Mass of the extracts was measured after filtration through a filter paper

(Whatman, Grade 4 Chr, and UK) and vacuum-evaporation of the solvent at 40°C. The combined extracts were stored at -4°C until further use. Hydrolysis of ethanolic extracts for HPLC determination was performed as described by [11].

Total phenolic and total flavonoid content of plant extracts

Total phenolic content of plant extracts, measured spectrophotometrically and expressed as gallic acid equivalents (GAE) was determined using Folin-Ciocalteau´s reagent [12]. The content of flavonoids in the extracts was measured by the $AlCl_3$ method, which is based on the formation of a flavonoid-aluminium complex [13].

HPLC analysis of plant extracts

The analysis was performed by using a liquid chromatograph (Agilent 1200 series), equipped with a diode array detector (DAD), Chemstation Software (Agilent Technologies), a binary pump, an online vacuum degasser, an auto sampler and a thermostated column compartment, on an Agilent, Eclipse XDB-C18, 1.8 µm, 4.6 x 50 mm column, at a flow-rate of 1 mL/min. Solvent gradient was performed by varying the proportion of solvent A (methanol) to solvent B (1% formic acid in water (v/v)) as follows: initial 10% A; 0-10 min, 10 -25 % A; 10-20 min, 25 - 60 % A; 20-30 min, 60-70 % A. The total running time and post-running time were 45 and 10 min, respectively. The column temperature was held at 30 °C. The injected volume of samples and standards was 5 µl and it was done automatically using auto sampler. The spectra were acquired in the range 210–400 nm and chromatograms plotted at 280, 330 and 350 nm with reference wavelength 550/100 nm. Identification of phenolic compounds in hydrolysates was performed by comparing the retention times and spectra of phenolic compounds of extracts with those of the corresponding external standards.

$DPPH^{\cdot}$ free radical-scavenging activity of plant extracts

Radical-scavenging activity against the stable radical $DPPH^{\cdot}$ (1, 1-diphenyl-2-picryl-hydrazyl) radical was determined spectrophotometrically following the procedure of [14]. The IC_{50} (mg/mL) was defined as the mass concentration of an antioxidant extract which was required to quench 50% of the initial $DPPH^{\cdot}$ under the given experimental conditions; it was obtained by interpolation from linear regression analysis.

Antioxidant activity (AOA) by β-carotene bleaching method of plant extracts

Antioxidant activity of plant extracts, based on coupled oxidation of ß-carotene and linoleic acid was determined according to the method of Moure *et al.* [15]. Degradation rate of the extracts was calculated according to first order kinetics, and the antioxidant activity (AOA) was expressed as % inhibition relative to the control [16]. The IC_{50} (mg extract/mL) was defined as the concentration of an antioxidant extract which was required to inhibit the degradation of ß-carotene by 50% relative to the control under the given experimental conditions; it was obtained by interpolation from linear regression analysis.

Reducing power of plant extracts

The reducing power was determined by measuring the formation of Prussian blue at 700 nm [17]. IC_{50} value (mg extract/mL) is the effective concentration at which the absorbance was 0.500 for reducing power and was obtained by interpolation from linear regression analysis.

Chelating activity on Fe^{2+} ions of plant extracts

Chelating activity of the extracts on Fe^{2+} ions was measured according to the method of Decker & Welch [18]. The IC_{50} value (mg/mL) was defined as the concentration of an antioxidant extract which chelates 50% of present Fe^{2+} ions under the experimental conditions. It was obtained by interpolation from linear regression analysis.

Statistical analysis

All analyses were performed in triplicates, and the mean values with the standard deviations (S.D.) were reported. Analysis of variance and Duncan's multiple range tests were used. Statistical data analysis software system STATISTICA (StatSoft, Inc. (2008). data analysis software system, version 9.0. www.statsoft.com) was used for analysis. *P* values < 0.05 were regarded as significant.

RESULTS AND DISCUSSION

In this experiment ethanol/water mixture was chosen for extraction as a compromise solution between the supposed efficiency and low toxicity. The extraction yields were around 20% (Table 1.). Higher extraction yields for parsley (19.6%) and caraway (24.2%) were reported by Hinneburg *et al.* [19], who applied the hydrodistillation extraction procedure. The amount of total phenolics as well as the composition of the extract is highly dependent on extraction method and the type and polarity of extraction solvent. The total phenolic content as well as the total flavonoids content of each extract was estimated, since phenolics and flavonoids may significantly contribute to its overall antioxidant activity. The amount of total phenolics in the extracts was investigated by the Folin-Ciocalteu method. Significantly higher results were found in the following order: mint > birch and "Vitalplant" > parsley > buckthorn > caraway ($P < 0.05$) (Table 1.). As can be seen from Table 1., total flavonoid content varied from 0.510% (parsley) to 2.05% ("Vitalplant").
Regarding Duncan test results, significant differences are not shown to exist between mint and "Vitalplant" as well as between birch and buckthorn samples in flavonoid content. Both methods, Folin−Ciocalteu method and the method for total flavonoid content determination are recognized as nonspecific and differentially sensitive towards different phenolic and flavonoid compounds and other interfering substances such as sugars and ascorbic acid would also contribute to these specific antioxidant indices. They would not, however, be expected to affect total phenolic content determined by HPLC.

Table 1. Extraction yield; total phenolic content of obtained extracts determined by Folin–Cioacalteu method, expressed as gallic acid equivalents; total flavonoids content of obtained extracts, expressed as rutin equivalents; total phenolic content of obtained extracts determined by HPLC, calculated as the sum of all integrated areas at 280 nm and expressed as gallic acid equivalents

Sample	Extraction yield (%)	Total phenolic content (%)	Total flavonoid content (%)	Total phenolics by HPLC method (%)
Mint leaves	17.2 ± 0.750	18.4 ± 0.001^{e}	1.97 ± 0.037^{b}	61.1 ± 1.25^{e}
Buckthorn bark	22.0 ± 0.890	16.6 ± 0.083^{d}	1.33 ± 0.200^{a}	21.2 ± 0.900^{c}
Birch leaves	26.1 ± 0.820	13.9 ± 0.360^{a}	1.43 ± 0.004^{a}	27.9 ± 1.05^{a}
Caraway fruit	19.5 ± 0.730	2.89 ± 0.020^{b}	1.78 ± 0.070^{d}	14.2 ± 0.950^{b}
Parsley fruit	11.6 ± 0.620	7.13 ± 0.860^{c}	0.510 ± 0.010^{c}	23.9 ± 1.01^{d}
"Vitalplant"	17.3 ± 0.540	13.2 ± 0.950^{a}	2.05 ± 0.060^{b}	29.28 ± 1,03^{a}

Values are means ± SD, n = 3. Values followed by different literals within each column indicate significant differences according to Duncan's test ($P < 0.05$).

Thus, the total phenolic content of the hydrolyzed ethanolic extracts was also calculated on the basis of HPLC determinations, as the sum of all integrated areas at 280 nm, and expressed as gallic acid equivalents (Table 1.). The correlation between the phenolic content obtained spectrophotometrically and HPLC results expressed in gallic acid equivalents was not significant (P<0.05), the HPLC results being higher in all investigated cases. However, both methods revealed mint extract as the richest source and the caraway extract to be the poorest source of plant phenolics among the investigated samples. Large number of papers concerning determination of phenolic content of birch, parsley, mint, caraway, as well as anthraquinone content of buckthorn has been published [4]. The phenolic compounds of birch, caraway and parsley, were investigated by HPLC/DAD method (Table 2.). Obtained results are comparable with the literature data [4].

Four tests were used for testing the efficiency of the extracts: $DPPH^{\cdot}$ radical scavenging activity, reducing activity, antioxidant activity by ß-carotene bleaching method and chelating activity on Fe2+ ions. The results, expressed as IC50 (mg extract/mL) values are shown in Table 3. $DPPH^{\cdot}$ radical scavenging test is widely used to test the scavenging activity of an antioxidant towards the long living free radicals. Regarding the obtained IC_{50} values, the highest $DPPH^{\cdot}$ radical scavenging activity (the lowest IC_{50} value) was obtained for mint extract, while the lowest for parsley. Significant positive correlation between the $DPPH^{\cdot}$ radical scavenging activity and the total flavonoid content has been established (r=0.94; P<0.05), while positive but not significant correlation was found between $DPPH^{\cdot}$ radical scavenging activity and total phenolics.

As in the DPPH· radical scavenging test, the mint extract was shown to possess the highest reducing power. Furthermore, positive correlation between the results of these two tests has been established (r=0.810; $P<0.05$). Referring to the literature data, plant phenolics along with ascorbic acid and reduced glutathione are recognised as the major reducing compounds in plant tissues. This statement is in accordance with our results, where highly positive correlation was established between the reducing power and the total phenolic content of the investigated extracts (r=0.94; $p<0.05$). Also, the antioxidant activity (AOA) was determined by using a β-carotene/linoleic acid model system that undergoes a rapid discoloration in the absence of an antioxidant. In this test (Table 3.), anthraquinone rich, buchthorn bark was shown to possess the highest AOA, while caraway had the lowest activity. Significant correlation between the AOA results and other investigated parameters was not established. Chelating activity test was also used to evaluate the efficiency of investigated extracts. Obtained values indicate that investigated extracts possess chelating activities and may be able to play a protective role against oxidative damage by sequestering Fe^{2+} ions. However, contrary to the activity in AOA test, buckthorn extract possessed the lowest chelating activity.

Table 2. Content of plant phenolics in crude extract after hydrolysis, expressed as mg/g extract.

	Mint leaves	**Buckthorn bark**	**Birch leaves**	**Caraway fruit**	**Parsley fruit**	**"Vitalplant"**
gallic acid	-	0.16 ± 0.02	18.2 ± 0.39	-	11.6 ± 0.45	0.284 ± 0.002
protocatechuic acid	-	0.85 ± 0.01	0.84 ± 0.11	-	0.47 ± 0.02	1.25 ± 0.010
caffeic acid	0.91 ± 0.05	2.26 ± 0.01	0.58 ± 0.03	1.54 ± 0.26	2.26 ± 0.13	1.34 ± 0.012
vanillic acid	-	-	-	-	-	-
chlorogenic acid	1.66 ± 0.29	0.35 ± 0.02	0.29 ± 0.03	1.32 ± 0.03	-	0.885 ± 0.050
syringic acid	-	1.47 ± 0.10	-	-	-	-
ferulic acid	-	0.89 ± 0.02	0.66 ± 0.02	0.52 ± 0.02	-	0.677 ± 0.020
rutin	-	-	-	-	-	-
myricetin	5.37 ± 0.38	0.72 ± 0.01	6.02 ± 0.32	-	-	1.37 ± 0.017
rosmarinic acid	17.5 ± 0.97	-	-	0.20 ± 0.09	-	4.93 ± 0.062
trans-cinnamic acid	-	-	-	-	0.16 ± 0.01	-
quercetin	14.9 ± 0.95	1.68 ± 0.02	7.43 ± 0.35	4.36 ± 0.03	0.82 ± 0.01	4.21± 0.050
naringenin	3.56± 0.22	5.00 ± 0.21	-	-	-	-
luteolin	13.6 ± 0.28	2.32 ± 0.12	-	-	0.56 ± 0.02	4.57 ± 0.062
kaempferol	-	-	0.64 ± 0.03	6.33 ± 0.21	2.99 ± 0.03	1.06 ±0.32
apigenin	3.56 ± 0.26	1.97 ± 0.08	0.67 ± 0.06	-	5.02 ± 0.01	6.49 ± 0.012
aloe-emodin	-	3.48 ± 0.24	-	-	-	2.21± 0.20

Table 3. Antioxidant activity of medicinal plants expressed as IC_{50} (mg extract/mL): DPPH˙ radical scavenging activity, reducing activity, antioxidant activity by β-carotene bleaching method (AOA) and chelating activity on Fe^{2+} ions.

Sample	**DPPH˙ scavenging activity IC_{50} (mg/mL)**	**Reducing power IC_{50} (mg/ml)**	**AOA IC_{50}(mg/mL)**	**Chelating activity IC_{50} (mg/ml)**
Mint leaves	0.172 ± 0.002^{a}	0.677 ± 0.108^{b}	6.71 ± 0.160^{b}	0.435 ± 0.180^{a}
Buckthorn bark	1.18 ± 0.210^{c}	1.76 ± 0.370^{c}	1.38 ± 0.188^{c}	1.39 ± 0.100^{c}
Birch leaves	0.632 ± 0.005^{ab}	1.07 ± 0.028^{a}	5.68 ± 0.270^{a}	0.602 ± 0.117^{a}
Caraway fruit	2.06 ± 0.137^{d}	5.27 ± 0.034^{e}	9.12 ± 0.233^{d}	1.05 ± 0.099^{b}
Parsley fruit	4.65 ± 0.820^{e}	4.68 ± 0.098^{d}	6.32 ± 0.037^{b}	0.583 ± 0.053^{a}
"Vitalplant"	0.893 ± 0.022^{bc}	1.27 ± 0.257^{a}	5.61 ± 0.543^{a}	0.983 ± 0.037^{b}

Values followed by different literals within each column indicate significant differences according to Duncan's test ($P < 0.05$).

CONCLUSIONS

Finally, the results of this experiment show that the tested plant drugs possess antioxidant activity in all of the tests. Commercial mixture "Vitalplant" exhibited a relatively high antioxidant activity in most of the tests, which can be explained by synergistic effects of the components of which it is composed.

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EFFECT OF IRON DEFICIENCY ON HELTH STATUS OF NEONATAL CALVES

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SUMMARY

The aim of this study was to estimate a possible relationship between iron deficiency and health status of neonatal calves during first days of their life. Experiment was done on six Holstein calves that were 5 to 7 days old and expressed disorder in digestive function. Blood samples were taken from sick calves in order to determine important hematological parameters: erythrocyte count, hemoglobin concentration, and hematocrit, total leukocyte count, platelets count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). In blood serum samples total protein, albumin and iron concentrations were determined. Rectal swabs of examined calves were taken for bacteriological, virusological and parasitological examination. Hematocrit value and hemoglobin concentration were lower than physiological (21.16 ± 4.94 % and 5.93 ± 1.36 g/dL, respectively). Iron concentration was lower than physiological in all examined calves (7.35 ± 1.22 μmol/L), while erythrocyte count was within physiological range for that category of calves (5.89 ± 1.24 μmol/L). Based on results for blood picture and calculated hematological indexes it was concluded that all calves suffered from hypochromic microcytes anemia. Total serum protein was within physiological range (57.13 ± 2.59 g/L). Clinical examination showed that all examined calves suffered from diarrhea with high degree of dehydration. Neither of pathogenic bacteria were isolated after 48 hours of anaerobic or aerobic incubation. Intestinal smears were negative for cryptosporidia and coccidian organisms and neither rotavirus nor bovine coronavirus were observed using direct fluorescent antibody tests. Our results indicate on possible influence of nutritional factors like iron deficiency on gastrointestinal disorders in neonatal calves.

Keywords: *neonatal calves, iron deficiency, anemia, diarrhea*

INTRODUCTION

Iron (Fe) is essential for maintaining biological functions in living animals. Some of those functions are: oxygen binding to hemoglobin and myoglobin, energy production in cells (oxidative phosphorilation), cell proliferation (through the control of ribonucleotid reductase enzyme activity). Although iron is 4th element, according to its presence in Earth's crust, deficit of iron is very common in young farm animals as well as in children. Mammals are born with relative small iron depositions that are made during last trimester of intrauterine development [1,13]. Colostrum and milk are not rich in this

microelement. These facts indicate that each disorder that has negative influence on iron depositing in neonatal animals may lead to clinical expression of iron deficit.

Iron deficit in neonatal animals that are on milk feeding is usually expressed as anemia. Anemia caused by iron deficiency is usual in neonatal piglets. Nevertheless, this type of anemia is described in high percent in neonatal calves, too [9,14,27] and may indicate on deficient iron deposition during intrauterine life. Postnatal, iron deficiency in calves is characterized by unregenaritive anemia with typical picture of microcytosis, anisocytosis, poikilocytosis and hypochromic [4,9,17]. Besides, low Fe concentration, additionally to anemia, provoke metabolic and endocrine adaptations, as insulin resistance and decreased IGF-I synthesis by growth hormone [3]. Described clinical manifestations in calves are decreased feed intake, lower body gain and increased predisposition to infections [7]. It is possible that iron deficiency in neonatal animals is associated with homeostasis disturbance in dry period in cows. This assumption is in accordance with the opinion that sickness in calves that leads to its weakness are mainly consequence of health disorders of pregnant mothers [23,27].

Aim of this study was to indicate on possible relationship between health disorders in neonatal calves and iron deficiency in serum of poorly vital calves.

MATERIAL AND METHODS

This study involved six poorly vital Holstein calves five to seven days old that suffered from severe diarrhea. Calves received colostrum and milk from their mothers during first week of their life, and pooled milk after that. Calves were clinically examined, and a type of diarrhea was scaled according to system recommended by McGuiric [15]. Blood was taken from v. jugularis for hematological and biochemical analyses.

Blood picture was done on blood analyzer (Abacus Junior, France). Total protein, albumin and iron concentration in blood serum was done on biochemical analyzer (Secomam, France).

Rectal swabs of examined calves were taken for bacteriological, virusological and parasitological examination. Rectal swabs were inoculated on Tarozzi broth and Zeissler agar plate and incubated anaerobically for 48 hours at 37ºC, or plated onto 5% blood agar, McConkey agar and O157:H7 ID agar (BioMerieux) and incubated aerobically for 24 hours at 37ºC. For the isolation of *Salmonella spp*., Rappaport Vassiliadis (RV) method was used. This consisted of a pre-enrichment step in BPW and incubation at 37ºC; the enrichment step in RV broth and incubation at 43ºC for 48 hours streaking in Endo agar, Brilliant Green agar and XLD agar and incubated aerobically for 24 hours at 37ºC. Bacteria cultures recovered under anaerobic and aerobic incubation conditions were identified on the basis of colonial and cellular morphology, cultural and biochemical characteristics [20] and commercial tests (BBL Crystal, Becton Dickinson; ETEC F5 antiserum, Denka Seiken).

Additionally, rectal swabs were tested for rotavirus and coronavirus by direct fluorescent antibody techniques of intestinal smears, and also intestinal smears were tested for cryptosporidia and coccidian organisms.

The results are expressed as mean (M), standard deviation (SD), standard error (SE) and CV for each group of calves.

RESULTS

Clinical examination shows that all calves were afebrile, with disfunctions in respiration and tachycardia. Mucosa was bloodless and there were no hair in the perineal region and region around basis of tail. All calves had type 3 of diarrhea and severe dehydration. Therapy with wide spectrum antibiotics did not healed diarrhea in calves that were same age and on same farm but died immediately before we started our observation.

Hematological analyses showed that red blood picture of calves was changed compared with physiological values, while white blood picture and platelets number were unchanged (Table 1 and 2). Mean erythrocyte count was within physiological range, but near lowest physiological limit, while hematocrit value and hemoglobin concentration were lower than physiological. But, erythrocyte count was under physiological range in two calves. Besides, mean corpuscular volume was lower (microcytosis), as well as mean corpuscular hemoglobin concentration (hypochromia) than physiological values. Leukocyte and platelets count were within physiological range.

Table 1. Results for hematological parameters of calf's blood are expressed as mean (M) ± standard deviation (SD)

	RBC x 10^{12}	Hb g/dl	Ht %	MCV ft	MCH pg	MCHC g/L
Ref	6-8,5	8,5 – 13,5	26 - 38	37-51	14-19	300-360
X±SD	5,89±1,24	5,93±1,36	21,2±4,9	36,0±4,0	10,0±1,2	280,3±24
CV %	20,73	22,93	23,34	11,11	11,16	8,58

Referent values taken from Baumgartner [2].

RBC – red bllod cells, Hb – hemoglobin, Ht – hematocrit, MCV, MCH, MCHC – hematological indexes, WBC – leukocytes, PLT – platelets

Based on analyses done for biochemical parameters of calves it was observed that iron concentration was lower than physiological in all examined calves, while total serum protein and albumin concentrations were within physiological range.

Table 2. Results for hematological and biochemical parameters of calf's blood

	Fe µmol/l	USP g/l	Albumini g/l	WBC x 10^9	PLT x 10^9
Ref.	15-25	50-70	30 - 40	6-12	200-900
X±SD	7,35±1,22	57,13±2,59	33,00±8,11	7,2±0.5	706±207
CV %	16,59	4,53	24,57	7	30

Referent values taken from Baumgartner [2].

WBC – leukocytes, PLT – platelets, USP – total serum protein

From all tested samples non pathogenic *E. coli* and *Proteus spp*. were isolated. Neither of pathogenic bacteria was isolated after 48 hours of anaerobic or aerobic incubation. Intestinal smears were negative for cryptosporidia and coccidian organisms and neither rotavirus nor bovine coronavirus were observed using direct fluorescent antibody tests (Idexx).

DISCUSSION

Our results showed that poorly vital calves had very low iron concentrations in blood serum (5 to 8 µg/L) during first week of their life. According to data from literature, good calves health is associated with iron concentrations in blood serum higher than 18 µg/L [4], while lower concentrations leads to anemia, immunology dysfunctions and higher sensitivity to infections [7].

Based on red blood picture results it may be concluded that examined calves aged from 5 to 7 days had hypochromic microcytes anemia. Analyzing each result, it was observed that one calf had severe anemia (Ht lower than 13 %), three calves had moderate (Ht lower than 20 %) and two calves had mild anemia (Ht lower than 26 %). Values used for classification of anemia in calves are one that are used in adult cattle [25]. There is no classification for calves, but according to our results of clinical examination it may be supposed that anemia had strong effect on health status of calves. Finding of low iron concentration fulfill picture of anemia in examined calves and, at the same time, indicate the cause of anemia. Very similar findings of red blood picture with anemia and iron deficit as its cause were given by others [14,16]. Since anemia cause tissue hypoxia, clinical signs that were found (bloodless mucosa, tachycardia and tahypnoa) are manifestation of compensatory mechanisms that acts during severe anemia.

Analysis of total serum protein shows that its concentration was 57 g/L. Similar results were presented by others [5]. Total protein concentration in blood serum during first week of life is used as indicator of adequate colostrum supply [22,26]. It is believed that total protein concentration that is higher than 55 g/L presents a transfer of passive

immunity that is equivalent to 15 g/L IgG in blood serum [21]. Nevertheless, results for total protein and albumin concentrations represent a state of dehydration and that is why we may suppose that real values are a little lower than those we get with our analyses.

Severe diarrhea that did not react to antibiotic therapy, afebrile calves, negative bacteriological, virusological and parasitological findings as well as normal leukocyte count in blood suggested that diarrhea is not infective origin. One of the reason for diarrhea in examined calves, that may not be excluded, is dysfunction of esophageal sulcus which leads to accumulation of colostrum in reticulum and rumen, its fermentation and provocation of ruminal acidosis and osmotic diarrhea [6]. On the other hand, we may suppose that diarrhea in examined calves is caused by iron deficit. There are data in literature that suggest that iron deficit leads to digestive dysfunctions [4]. Besides, it was found that low hematocrit and haemonoglobine values are in strong association with diarrhea and respiratory diseases in calves [12,18]. Cause of gut dysfunction in conditions of iron deficit may be dual. On the piglet model, it was shown that anemia (caused by iron defect) by itself cause enterocyte dysfunction [8] which is associated with disrupted oxidative phosphorilation and lower producing of ATP [24]. Oxidative phosphiorilation may be disrupted due to the fact that iron deficit leads to citohrome function in respiratory chain of mitochondria. On the other hand, iron is necessary for stem cell proliferation [19]. Stem cells are present in gut crypts and its dysfunction may disturb morphological and functional integrity of gut. Besides, on piglet model for iron deficit, it was shown that reproduction of nonpathological bacteria in gut (coliform, lactobacillus and total aerobe and anaerobe) is increased in these conditions due to increased pH values in gut lumen [11] which is partly due to hypohlorhidria caused by iron deficit [10]. Increased number of those bacteria may cause diarrhea [11].

CONCLUSION

Based on results for hematological and biochemical parameters for examined neonatal calves, as well as bacteriological, virusological and parasitological analyses, it may be concluded that anemia and diarrhea in examined calves are caused by iron defect. Iron deficit is first shown on cells that have strong proliferation potential like red blood cells and enterocytes. Besides, iron deficit leads to lower synthesis of hemoglobin. Based on all presented in this study it is clear that in intensive farm production must be managed adequately, which means that it can not be allowed that nutritional deficit provoke disruption of health status in neonatal animals

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EFFECT OF TEMPERATURE ON RHEOLOGICAL PROPERTIES OF CORN STARCH

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ABSTRACT

The process of manufacturing animal feed involves physical and chemical changes in ingredients, including the gelatinization of starch. In order to investigate the influence of processing temperature on rheological properties of starch, starch pastes and gels were prepared at different temperatures and subjected to flow and dynamic oscillatory measurement. Microphotographs of samples were also taken for the integration of the information coming from rheological measurements. Starch gel prepared at higher temperature was stronger, having greater extent of thixotropy, higher apparent viscosity, modulus of elasticity and yield stress. On contrary, gel prepared at lower temperatures was the weak one. Differences in rheological behaviour of obtained gels were the result of the differences in their colloidal structure.
***Keywords**: corn starch, pasting, rheology, thixotropy, visoelasticity*

INTRODUCTION

During the feed manufacturing process ingredients are subjected to mechanical (rolling, crushing, grinding), thermal (roasting, micronizing) or thermo-mechanical (flaking, popping, extruding) processes into dry or wet (steam rolling, steam crushing, steam flaking, pressure toasting, steam extruding, pelleting) conditions [5]. These processing conditions promote physical and chemical changes in ingredients, including the gelatinization of starch [7].
Starch gelatinization includes the diffusion of water into a granule, hydration and swelling, uptake of heat, loss of crystallinity, and amylase leaching [4]. Leached amylose forms double helices that may aggregate (hydrogen bond) to each other and create semicrystalline regions [9]. As the gelatinized starch cools, the dispersed matrix forms a gel or paste [6].
Starch gel or paste exhibits thixotropic rheological behaviour, meaning that it displays a decrease in viscosity over time at a constant shear rate, which is a consequence of the progressive structure breakdown. Ignorance and improper handling of materials that possess thixotropic properties can lead to numerous problems in their transport through the tube, mixing, homogenization, as well as storage instability [2]. However, determining the extent of thixotropy, it is possible to predict the stability of the system during transport and storage; and by its controlling through the proper choice of equipment, composition and methods of treatment one can affect the production process and product quality.

The objective of the study was to evaluate different levels of starch pasting produced by different processing temperature on rheological behaviour of starch pastes and gels.

Rheological assessment of starch pastes and gels was performed by using fundamental methods, which included flow curve and small deformation (dynamic oscillatory) measurements. In order to study the structural aspects of starch pasting, microphotography recording was used for the integration of the information coming from rheological measurements.

MATERIAL AND METHODS

Corn starch (Ipok, Zrenjanin, Serbia) and deionised water were used to prepare starch pastes and gels.
Sample preparation and determination of characteristic temperatures and viscosities during the pasting process was carried out using Brabender Viscoamylograph (Duisburg, Germany). The suspension of corn starch (9% w/v, calculated on a dry weight basis) was gradually heated from 25 to 95°C at a constant rate of 1.5°C/min. Samples were taken at the pasting temperature (T_{min}), maximum viscosity temperature (T_{max}) and the temperature corresponding to the middle of the curve between T_{min} and T_{max} (T_{sr}); while the temperatures were calculated from the equation:

$$T = 25 + 1.5 \cdot t$$

where *t* is the time elapsed until reaching the characteristic temperature, estimated from the diagram showing the relationship between viscosity and time of heating. The obtained starch pastes were cooled to 20 °C and the resulting gels were further kept in a refrigerator at 4 °C.

Rheological properties of starch pastes and gels were determined by HAAKE RheoStress600 rheometer (Thermo Scientific, Karlsruhe, Germany) at 20±0.1 °C, using the following measuring geometry: PP60 Ti (plate - plate, 60mm diameter, gap between plate 1mm) for oscillatory measurements and Z20 DIN (cylinders, diameter 20 mm, gap between the cylinders 4,2 mm) for recording the flow curves. Measurements were performed 24 hours after the samples preparation in order to diminish the influence of aging on the viscosity and structure of gels. In order to provide comprehensive and detailed characterization of the system different rheological tests were performed.

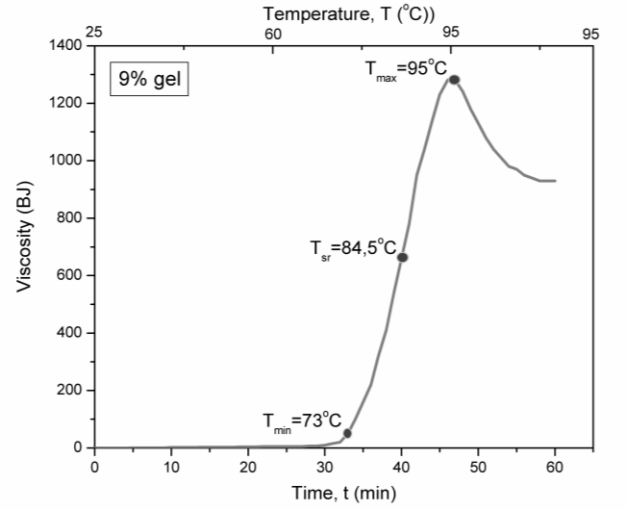
The flow curves were obtained by registering shear stress at shear rate which was increased from 0 to 700 s^{-1} in 1800 s, held constant at 700 s^{-1} until total system destruction, and decreased from 700 to 0 s^{-1} in 1800 s.

Oscillatory stress sweep test was conducted by increasing the stress from 1 to 500 Pa at a constant frequency of 0.1 Hz.

Microscopic structure of the gelatinized starch was investigated using an optical microscope (TP-1001C TOPICA CCD CAMERA, Kruss). The images were processed in the Photoshop image processing program so that the granules magnification was 1000x. All recordings were made 24 hours after the sample preparation.

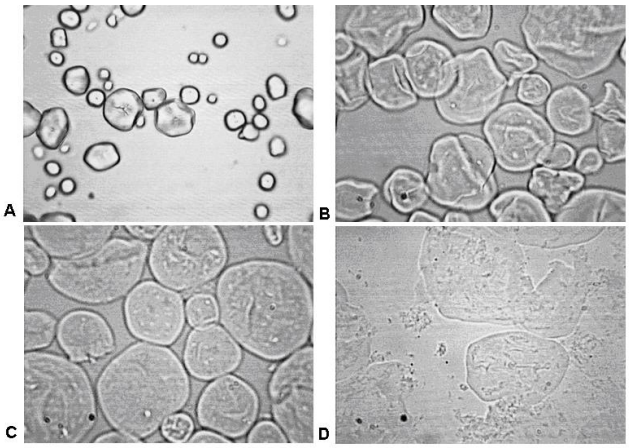
RESULTS AND DISCUSSION

Investigation of rheological properties was carried out on systems obtained by heating the starch suspension until three characteristic temperatures: pasting temperature (T_{min}), maximum viscosity temperature (T_{max}) and the temperature corresponding to the middle of the curve between T_{min} and T_{max} (T_{sr}). The obtained viscoamylogram curves with marked characteristic temperatures are shown in Graph 1.



Graph 1. Viscoamylogram curve of 9% starch suspension

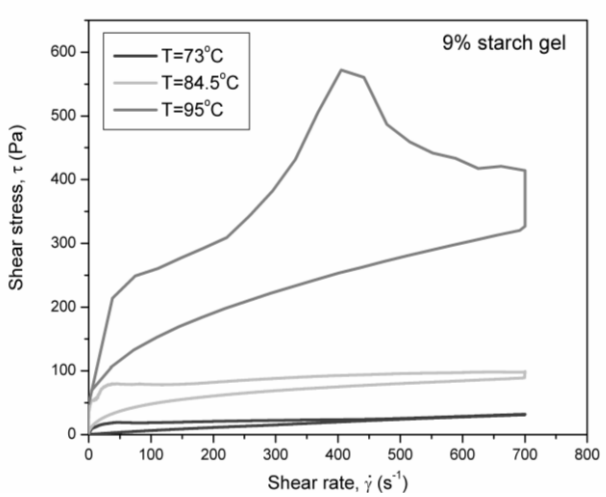
Changes in the appearance of starch granules during the pasting process are shown in Graph 2.



Graph 2. Microscopic structure of starch suspension (A) and 9% starch gel prepared at T_{min} (B), T_{sr} (C) and T_{max} (D)

At room temperature corn starch granules have a distinctive polygonal shape with sharp edges and visible Maltese cross [3]. Picture of the sample at 73 °C (T_{min}), shows that the granules are greatly swollen, but the integrity and shape of granule is well preserved. In the sample recorded at a temperature of 84.5 °C (T_{sr}) there is an increase in the size of starch granules, due to swelling, as well as the change in granule shape which is more rounded. However, the individuality of granule is still preserved. On a recording made at 95 °C (T_{max}) granule structure is completely destroyed, and leached amylose fractions can also be observed.

Detected appearance of starch granules during pasting largely explains differences in the thixotropic behaviour of the system. Flow curves (shear stress versus shear rate) of starch gel prepared under different thermal conditions (T_{min}, T_{sr} and T_{max}) are illustrated in Graph 3.



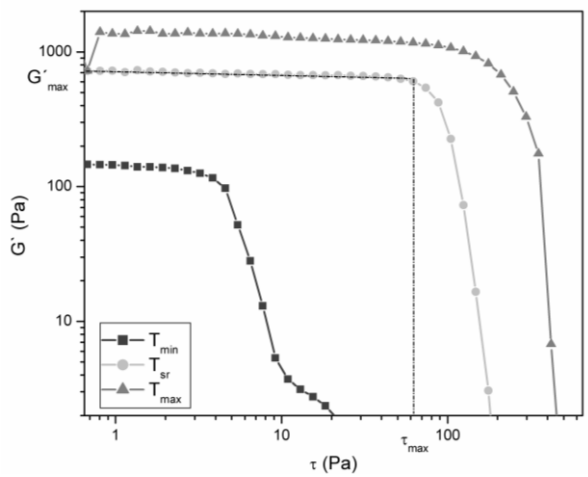
Graph 3. Flow curves of 9% starch gels prepared at different temperatures

The results indicated that gels prepared at higher temperatures, showed the higher value of shear stress and apparent viscosity (the ratio of shear stress to shear rate), as well as the larger thixotropic loop area. Namely, increasing the temperature leads to pronounced granule rupture; and after cooling there are large number of bonds established between the present macromolecules (amylose and amylopectin). As a result, the structuration of gel is much stronger.

In gel heated to T_{max}, at the shear rate of 440 s^{-1}, there is a breakdown of the structure, which is common in macromolecular gel [2]. The cause of this phenomenon is the presence of inhomogeneity in the gel, which is the result of uneven structuration within the gel due to formation of different number of hydrogen bonds between free hydroxyl groups of amylose molecule in the process called retrogradation. Namely, depending on

the orientation of macromolecules, there is different number of available hydroxyl groups in different parts of the gel.
Gels prepared at lower temperatures had lower values of apparent viscosity and lower thixotropic loop areas, indicating that such systems have less tendency to gel and that they contain dispersed, undestroyed starch granules. That structure is close to that observed in polymeric microcrystal-gels, which are obtained by partial degradation of amorphous macromolecular regions, which form a gel with embedded microcrystalline aggregate that due to a greater degree of orderliness could not be destroyed [2].

Since most of macromolecular systems have a certain yield stress value below which no flow occurs, i.e. below which there is no viscous, but only elastic deformation [1], the viscoelastic properties were also investigated (Graph 4).



Graph 4. Oscillatory stress sweep measurements of 9% starch gels prepared at 73, 84.5 and 95 °C

The end of the linear viscoelastic range marked as τ_{max} indicate the maximum stress that the system can resist before failure (yield stress). The results of dynamic oscillatory tests were in accordance to those obtained using flow curve measurements. Namely, gel prepared by heating to T_{max} which was characterized with the highest value of shear stress and largest thixotropic loop area, also showed the highest value of elastic moduli G' (refer to structure rigidity) and τ_{max} (measure of resistance to deformation) [8].

CONCLUSIONS

At different processing temperatures different levels of starch pasting occurred, influencing the rheological behaviour of obtained starch pastes and gels. With the help of microstructural analysis the colloid structure of the obtained gels was revealed. Corn starch gel is a two-phase disperse systems consisting of swollen or destroyed starch granules embedded in the network of macromolecular chains bonded to one another. The results showed that gel prepared at higher temperature, showed the greater extent of

thixotropy (larger thixotropic loop area) as well as the highest shear stress (apparent viscosity), rigidity (modulus of elasticity) and resistance to deformation (yield stress). Namely, that gel was stronger which indicates that it was a system with three-dimensional network structure. On contrary, gel prepared at lower temperature had smaller thixotropic loop area, and lower value of shear stress, modulus of elasticity and yield stress which is typical of weak gels consisting of a suspension of swollen starch granules and macromolecules.

ACKNOWLEDGEMENTS

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AROMA NEKTAR IN NUTRITION OF PIGLETS

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ABSTRACT

The effects of utilization of *Nektar* aroma in nutrition of suckling piglets and weaned pigs were studied. Considering previous positive experience in use of aromas in pig nutrition, object of this research was to evaluate the possibility for use of said aroma in piglet nutrition. Studied aroma was produced according to special technology by company Ireks Aroma in Croatia. Research was carried out on pig farm PD „Halas Jožef" in Ada. Obtained results have shown that introduction of investigated aroma *Nektar* into iso-energy and iso-protein mixtures had positive impact expressed through increase of body mass by 9.19%, improvement of gain by 11.7% in suckling and weaned pigs. Group of suckling piglets fed the mixture based on investigated aroma consumed more pre-starter by 15.66% compared to the control group of piglets fed the diet of same composition without *Nektar* aroma. Investigated aroma showed no differences in gain in weaned pigs, however animals of the trial group fed diet containing *Nektar* aroma have realized better feed conversion ratio by 9.20% compared to the control group of piglets. In general, obtained results showed that use of *Nektar* aroma can be recommended in nutrition of suckling piglets and weaned pigs.
***Key words**: Aroma Nektar, suckling piglets, weaned pigs*

INTRODUCTION

The problem of taste of food dates from the first days of domestication of animals, even though people considered the feed then to be tasteful without much thought. It was natural assumption. Animals grazed food which was naturally tasty to them and the food they preferred, and only in conditions of starving they consumed food which they didn't like.

Since ancient times variety of aromatic and spice herbs are used, as well as plant fruits of characteristic taste, odor and aroma, in order to improve the food intake. Livestock food has its nutritive value and characteristic odor determined by its quality, composition and type of feeds used. Good feed intake is essential for the proper and healthy development of animals where the effects of aroma are indisputable. Animals have extremely well developed sense of taste and smell, so the problems with feed intake mainly occur in younger categories, or with changes of mixtures.

The company Ireks-Aroma is working on models how to fully include i.e. activate natural potentials of animals, especially odor, taste and aroma and in this way attempt to contribute to increase of feed intake. In their programmes they use components free of GMO, as well as ISO and HACCP standards in production of aromas for livestock feed.

Objective of this paper was to continue with the research of potential use of *Nektar* aroma in mixtures for suckling piglets and weaned pigs for the purpose of enhancement of the nutritive value of mixtures used i.e. improvement of production performance of piglets.

MATERIAL AND METHODS

Investigation was carried out on pig farm of the company PD »Halas Jožef«, AD in Ada. In two trials, total of 281 piglets were included, crosses of Large Yorkshire and Swedish Landrace farrowed in 2 x 12 litters.
Trial was formed immediately before farrowing/partus when sows were divided into two feeding/nutrition treatments. Piglets of the first, control, group, were fed farm mixture, and animals of the second, experimental, group, were fed mixtures containing aroma *Nektar* in concentration of 0.04%.
Criteria for evaluation of obtained results were following parameters: number of born piglets, number of weaned piglets, average body mass of piglets at birth and at weaning, average daily gain of piglets during lactation and consumption of pre-starter during creep feeding.
At weaning, piglets were also divided into the groups, ensuring that piglets fed control mixture without studied aroma during lactation period continue with the same nutrition in rearing period, and that suckling piglets from trial group continue to be fed trial mixture containing aroma in the same concentration sin lactation – 0.04%.
During rearing period the following parameters in piglets were observed: average daily gain, average daily food consumption and feed conversion ratio.
Statistical analysis of data on body masses of piglets, gain and consumption of pre-starter was done using conventional methods of statistical processing - variance analysis, and data on differences between average values using t-test.

RESULTS AND DISCUSSION

The possibility of introduction of *Nektar* aroma/flavour into the nutrition of suckling piglets and weaned pigs was investigated.
Obtained results (tab. 1) showed that trial group of animals, fed diet containing supplemental aroma/flavour, realized higher average body mass at weaning, by average 0.61 kg or 9.19% compared to the control group of piglets fed diets without investigated aroma/flavour.

Table 1. Performance of suckling piglets in the experiment

Group	1 control	2 experimental
Suckling piglets		
Aromas Nektar in the diet, %	-	*0.04*
Duration of lactation, days	27.2	27.4
Number of live born piglets/litter	10.92	9.40
Number of equalized piglets/litter*	10.75	9.80
Number of weaned piglets/litter	9.83	9.40
Average body weight of piglets at farrowing, kg	1.45	1.49
In the comparison at 1st group, %	-	+ 2.76
Average body weight of piglets at weaning, kg	6.64	7.25
In the comparison at 1st group, %	-	+ 9.19
Average body weight of litter at weaning, kg	65.3	68.2
In the comparison at 1st group, %	-	+ 4.44
Average daily gain of piglets, g	188	210
In the comparison at 1st group, %	-	+ 11.70
Consumed of prestarter/litter, kg	7.60	8,79
In the comparison at 1st group, %	-	+ 15.66
**) – Equalization of suckling piglets includes the imputation of piglets from litter to litter inside the group after the colostrums consumed.*		

Average daily gain of 210 g in case of piglets fed diet containing aroma/flavour was by 22 g or 11.70% better compared to the value established in the control group.
For realizing of gain the experimental group is consumed for 1.19 kg or 15.66% more prestarter/litter in comparison at the control group.

Table 2. Production performance of piglets in rearing

Group	1 control	2 experimental
Aromas Nektar in the diet, %	-	*0.04*
Weaned pigs		
Body weight of weaned pigs at the beginning of experiment, kg	7.27	7.99
Body weight of weaned pigs at the end of experiment, kg	24.90	25.71
Duration of experiment, days	60	60
Average daily gain, g	294	295
In the comparison at 1st group, %	-	+ 0.34
Average feed intake, kg	0.703	0.641
In the comparison at 1st group, %	-	- 8.95
Feed conversion ratio, kg	2.39	2.17
In the comparison at 1st group, %	-	+ 9.20

In the period of rearing, no difference in gain of piglets was recorded as the result of use of *Nektar* aroma/flavour (tab. 2). Feed intake of piglets fed supplemented diets was by 0.062 kg or 8.95% lower compared to the consumption of piglets in the control group.
Piglets fed diet supplemented with investigated aroma/flavour consumed by 0.22 kg or 9.20% less feed per 1 kg of gain, compared to the control group of animals.
In previous researches of the nutritive value of the aroma/flavour *Vanilla butter cream* used in nutrition of piglets, results were obtained showing the increase of number of piglets by 0.3/litter, their mass at weaning by 0.39 kg/litter, i.e. better realization by 8.12% compared to animals fed mixture of the same composition without supplemented aroma/flavour [8, 15]. Similar studies showed that the piglets fed the diets containing aromas/flavours were heavier by 3.5% [3] and 7.35% [7] compared to the animals fed the diets without supplemented aroma/flavours.
The use of *Vanilla butter cream* aroma/flavour caused improvement of gain by 2.37%, feed conversion ratio by 1.89% and cheaper gain by 12.60% [18].
In case of fatteners, introduction of aroma/flavour influenced the improvement of gain [4, 5] and in case of *Apple* aroma/flavour improvement of gain by 4.78%, feed conversion ratio by 3.75%, level of utilization of crude proteins, slaughter yields and lower price of 1 kg of gain by 2.06% [10, 11, 17] were realized. Use of plant extracts in fattening pigs improves performance [10, 11], and in case of slaughter parameters – use of aroma/flavour in mixtures can increase the content of poly-unsaturated fatty acids in meat [2], but with no effect on meatiness of carcass sides [1].
In fattening chickens, addition of aroma/flavour *Citrus Fennel* improved the gain by 5.72% and feed conversion ratio by 3.1% [13], and in layers introduction of the same aroma/flavour increased the laying capacity by 6.59% with increased feed intake of animals by 6.84% [12].
In general all previous results indicate the positive effect of addition of aroma/flavour in pig and poultry nutiriton [14, 16].

CONCLUSION

The effects of use of *Nektar* aroma/flavour in the nutrition of suckling piglets and weaned pigs were investigated. Obtained results showed that introduction of studied aroma/flavour *Nektar* in iso-energy and iso-peotein mixtures has positive effects demonstrated in the following way:

- Increase of body mass of suckling piglets by 9.19%,
- Improvement of gain by 11.7% in suckling piglets,
- Suckling piglets fed mixture based on studied aroma/flavour consumed more pre-starter by 15.66% compared to the control group of piglets fed the diet of same composition but without *Nektar* aroma/flavour.
- Investigated aroma/flavour showed no differences in regard to gain realized by weaned pigs,
- Animals of the experimental group fed diet containing *Nektar* aroma/flavour realized better feed conversion ratio by 9.20% compared to the control group of piglets.

In general, obtained results showed that use of *Nektar* aroma can be recommended in the nutrition both of suckling piglets and weaned pigs.

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RHEOLOGICAL AND CHEMICAL CHARACTERISTICS OF SUGAR BEET MOLASSES WITH VIEW OF INDUSTRIAL APPLICATION

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ABSTRACT

The fact that sugar beet molasses is a polycomponent system with complex chemical composition makes it very suitable row material for different food technologies. Molasses processing efficiency depends on its rheological characteristics and quality, as the result of the chemical composition of molasses. Range of values of chemical parameters of sugar beet molasses depend on chemical composition of sugar beet, processing conditions, as well as of the amount of remains processing aids. In this study, the quality of sugar beet molasses, from the perspective of its further industrial application was examined. With that aim, the analysis the chemical composition and viscosity of molasses produced in domestic sugar factories were carried out. Results in this paper allowed to determine the relationship of certain ingredients of the molasses and its rheological properties, depending on the processing conditions and possibilities of its application.
***Key words**: sugar beet, molasses, chemical composition, rheological characteristics*

INTRODUCTION

In sugar industry, molasses is considered as the last syrup, produced during multistages crystallization of thick juice, from which is no longer possible to get crystal sugar by usual procedures of crystallization, whose production is economically justified [6].
Molasses, a by-product of the sugar industry, like any product made from biological material, does not have uniform, contstant chemical composition. Range of these values are wide and depend on composition of row material, applied technology for sugar beet processing and used auxiliary materials [10].
It can be taken that the share of the components in molasses is: 50 % sucrose, 20 % water and 30 % nonsugar substances such as nitrogen compounds, minerals, vitamins and macromolecules (starch, cellulose, hemicellulose, lignine, pectin) [9].
Viscosity of the molasses, which is very high at the lower temperatures, is one of the reasons why the significant amount of sucrose, by usual procedure, can not be crystallized at the last stage of crystallization. At the same time, the high viscosity makes difficult to transport molasses, requires special devices for transport, both in the sugar industry and in the other industries that use molasses as row material.

It was found that sugar beet molasses, with 82% of dry matter, at 40ºC, obtained by processing healthy sugar beet, has an average viscosity of 4.4 Pas, with varying in range from 3.7 to 5.0 Pas.
Knowing a rheological behavior of the molasses is very useful from aspect of its application in appropriate technological operations as well as in understanding and control of the transport process [9].
Large number of the processes in biofermentative industry, based on the activities of certain types of microorganisms (yeasts, bacteria and fungi), traditionally use molasses as row material. In recent years, special emphasis is given to the application of molasses as well as other byproducts of sugar beet processing in bioethanol production. Bioethanol represents a modern form of biomass energy, which is substantial replacement for liquide fossil fuels (oil) or, in the mixture with other gases, replace natural gas [1].
Molasses has an important application in agriculture, primarily in the diet of all types and categories of animals. In combination with forages molasses can have a better biological effect than grain. Recommended amounts of molasses in feed mixture are 5-10% for cattle. Less amounts of molasses should be used for calves nutrition. In pigs nutrition, depending on the fattening period, 6-12% of molasses is recommended, while the amount of molasses, recommended for poultry nutrition, is 5-10%. Molasses has a slightly laxstive effect, it improves the taste of other feed components and it is a binder during pelleting of feed.
Also, it should be emphasized the application of molasses in production of biomass of microorganisms, known as "Single Cell Proteins" (SCP), which is rich in proteins and it is used in human nutrition and for feed [8].
The aim of this paper is to determine the quality and rheological characteristics of the molasses produced in domestic sugar factories, from the aspect of its industrial application.

MATERIAL AND METHODS

Research was conducted using samples of molasses from six local sugar factories (A, B, C, D, E and F), taken directly from the production line.
The basic quality parameters of molasses were analyzed according to methods in laboratory manuals for the control of the process of sugar production [7]. Methods are coordinated with the regulations given by the International Commission for Uniform Methods of Sugar Analysis (ICUMSA) [4].
Rheological behavior of the tested molasses is determined in accordance with the IRIS methods [5], i.e. viscosity of the molasses is determined at 50ºC using rotating viscometer (RV Rotovisko 3, Haak, Germany).
For describing of changes in viscosity of the molasses in function of dry matter content, following equations were used [5]:

$$\log \mu = a \cdot n + k$$
$$n = (3.8573 - k) / a$$
$$w_{DS} = 1900 \cdot n / (18 \cdot n + 1)$$

where μ is the viscosity of the molasses at 50ºC, n molar share of dry matter expressed as sucrose, a and k coefficients, 3.8573 constant calculated for the maximal value of viscosity at 50 ºC and w_{DS} refractometric dry matter content.
Statistical analysis was performed using the statistical program *Costat* (Cohort) in order to calculate the basic statistical indicators [3].

RESULTS AND DISCUSSION

In order to better perception of quality of the molasses from six sugar factories, average values of studied parameters and statistical analysis are shown in the Table 1.

Table 1. Parameters of quality of the molasses

Parameters %	Average	Minimum	Maximum	Standard deviation	The coefficient of variation
Dry matter	82.60	78.60	87.00	2.94	3.56
Polarization	49.69	46.70	52.30	1.80	3.62
Reducing substances	0.580	0.279	1.216	0.27	46.55
Mineral matters	12.24	10.43	13.48	0.87	7.11
Potassium	3.02	2.19	3.82	0.45	15.56
Sodium	1.09	0.71	1.48	0.22	20.18
Calcium oxide	0.60	0.34	1.02	0.21	35.00
Magnesium oxide	0.07	0.02	0.17	0.04	57.14
Total nitrogen	1.72	1.34	1.97	0.15	8.72
Alpha-amino nitrogen	0.31	0.19	0.45	0.07	22.58
Betaine	4.01	2.74	6.22	0.91	22.69
pH value	7.58	6.10	9.50	0.82	10.82

Results of the analysis show that the compostion of the tested molasses is typical and usual for the sugar beet processing in local factories. Molasses with this composition is a suitable row material for both the fermentative industry and the feed technology, because, beside fermentable sugars contains mineral matters and nitrogen compounds necessary for the microorganisms activity on the one hand, and improve feed quality on the other hand.
It has been noted that the main indicators of quality molasses, depending on processing conditions, show statistically significant variation and that factories in different ways and with different effects led the process.
The least statistically significant differences, determined for basic quality parameters, and statistical differences between factories are given in Table 2.

Table 2. Significant differences of the basic indicators of the quality of molasses, depending on processing conditions (p = 0.05)

Least significant difference*							
Dry matter ^{0}Bx		Polarization %		pH value		Reducing substances %	
LSD =2.215		LSD =1.699		LSD = 1.116		LSD = 0.382	
Lable of the factory	Ratings	Lable of the factory	Ratings	Lable of the factory	Ratings	Lable of the factory	Ratings
F	a	F	a	F	a	B	a
E	a	E	a	A	a	D	a
A	b	C	b	E	a	C	a
D	b	D	b	C	a	A	a
C	b	A	b	D	a	E	a
B	b	B	b	B	a	F	a

* The differences ranked with the same letter (a, b, c) is not statistically significant

The results show that molasses, from factories E and F, had a statictically significant higher dry matter content and consequently statistically significant higher sucrose content, determined polarimetricaly. The content of reducing substances and pH values, as indicators of possible hydrolysis process in molasses, does not show significant variation depending on processing conditions.

Determination of viscosity of the molasses was performed by recording viscosity curves at 50ºC, as a function of dry matter content, with the dry matter expressed as molar concentration of sucrose.

Table 3 shows the linear regression equations of the molasses viscosity in relation to dry matter content measured at 50ºC, viscosity of molasses from factories and viscosity of molasses with 85ºBx dry matter content, measured at 50ºC.

Linaer regression determination of viscosity allows calculation of viscosity of the molasses for any dry matter content, which contributes to a better process control in order to separate sucrose from molasses, in accordance with present nonsucrose compounds represented with values of the constants a and k.

Viscosity values of molasses from factories are in range of 373 mPas to 4524 mPas (average 1861 mPas) , which indicates that the viscosity is generally low and in function of low dry matter content. When the previously viscosity values are recalculated on dry matter of 85ºBx, as usual value of dry matter content of molasses, viscosity is still below the limit value of 7200 mPas and do not endanger the work of centrifuge.

Viscosity of the molasses is not a limiting factor for its use in fermentative industry because it is a function of dry matter content. For this reason, datas of the viscosity of molasses, excluding the aspect of sugar refining, have a practical significance both, for selection and determination of the maximum capacity of pumps for transport of molasses and for dosage of molasses for feed production.

Table 3. Viscosity of molasses, depending on the dry matter at a temperature of 50°C

Factory	Dry matter °Bx	Equation of linear regression of the viscosity of molasses *	Viscosity of molasses from the factories /mPas/	Viscosity of molasses (85°Bx) /mPas/
A	83.20	logμ= -0.176+15.936·n	1317	3085
B	79.44	logμ= 0.083+14.727·n	373	2953
C	80.12	logμ= 0.040+15.832·n	646	4801
D	80.12	logμ= -0.046+15.001·n	379	2536
E	85.60	logμ= 0.047+15.162·n	4524	3422
F	80.96	logμ= 0.180+13.976·n	3929	2480

*logμ= $k+ a \cdot n$
a,*k* – constants
n – molar share of the dry matter , expresed as sucrose

Table 4. shows the significance of differences in viscosity of molasses, depending on processing conditions

Table 4. Significance of differences in viscosity of molasses, depending on the conditions of processing (p = 0.05)

The least significant differences*	
Viscosity at 50°C, mPas	
LSD = 2250.03	
Lable of the factories	Ratings
F	a
E	a
D	b
A	b
C	b
B	b

* The result differences ranked with the same letter (a, b, c) is not statistically significant

According to the obtained results, it can be concluded that molasses from factories E and F has a significantly higher viscosity than molasses produced in the other four factories, which is consistent with the results shown in Table 2 and confirms the dependence of viscosity of the process conditions i.e. of dry matter content in molasses.

CONCLUSION

Taking into account the fact that molasses is produced in significant quantities (about 4% of sugar beet) and contains on average 50% of sucrose and large number of organic and inorganic compounds, it is very valuable raw material for different technologies.
Confirmed interdependences of chemical composition and viscosity of molasses of processing conditions are very important, both in terms of monitoring of the

technological processing of sugar beet, and in terms of application of the by-products as raw materials for further industrial use (technology, biotechnology and feed).
Deciding factor, when considering the use of molasses as raw material in industry, must be its chemical composition and rheological characteristics, taking into account the economic and energy effects and industrial demands.

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EFFECT OF DIETARY SELENIUM ON BODY WEIGHT AND LIVER MASS IN FATTENING POULTRY

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ABSTRACT

Effect of dietary selenium (Se) on changes of body weight and liver mass in fattening poultry depends on selenium concentration in feed and the period of consumption. The experiment was carred out for 42 days on heavy broiler line Arbor Acres (total number 300) divided in five groups of 60 one-day old chickens of both sexes. The chickens were fed with complete mixtures, with no Se supplemented (control group) or mixes with different selenium concentration (4 working groups): I group (50 μg Se/kg); II experimental group (100 μg Se/kg); III experimental group (150 μg Se/kg) and IV experimental group (250 μg Se/kg).
In first 14 days no statistically important differences were discovered in body gain weight between the experimental group II (1272 g), III (1271 g) and IV (1279 g) comparing to the control (1212 g) and experimental group I (1233 g) of chickens. After six weeks period the highest gain in body weight was noticed in the group IV (250 μg Se/kg) and it was 2451 g. The chickens in the control group (0 μg Se/kg) had the least gain in body mass and at the end of fattening period it was only 2255 g. Dietary Se, when added to feed mix in quantity 50 to 250 μg/kg, does not effect the mass of liver in the experimental animals.
***Keywords**: selenium, body mass, liver, chicken*

INTRODUCTION

In order to provide good animal health and production, besides organic nutrients (proteins, fats, carbohydrates, vitamins) inorganic component, or minerals, are required. Long feeding with deficient meals, insufficiently balanced meals or meals with higher quantity of minerals causes changes in concentration on body tissues. This all results in considerably changed physiological function of organs.
Selenium is an important essential microelement. It may be found in all live systems. Due to its exceptional importance in human and animal nutrition, the interest in selenium has extremely increased. It is mainly found in the kidney, pancreas, endocrine glands, muscles, liver and erythrocytes. Current knowledge points to the importance of selenium: in many tissues and organs micro-quantity of selenium plays very important functional activity; it is a component of antioxidant system in animals and humans [12]. Besides, it has been proved that selenium reduces the effect of toxic elements, as for example arsenic, mercury, cadmium and lead because it binds them into stabile selenide and thus reduces their toxic effect [10].

Selenium is a co-factor in several enzyme systems, of which two enzyme systems have been best examined: glutathionic, where selenium, as a co-factor of glutathion-peroxidase (GSH-Px), has antioxidant effect and takes part in destructs of peroxide radicals that have adverse affect on cell membrane as well as co-factor of iodothyronine 5'-deiodinose that transports thyroxin into triiodothyronine and releases iodinine.
In animal nutrition selenium is provided through herbal and animal raw materials. Low content of selenium in soil and its low biological availability (due to acid soil reaction) results in low content of selenium in plants, what is the main meal for domestic animals. Forage plants and animal feed with low selenium content are not adequate feed for successful animal production [6]. Indirectly, through food chain, selenium comes into human organism, so plant and cattle nutrition with insufficient concentration of selenium is reflected in insufficient level of selenium in human population [14].
Besides quantity, an important parameter is a chemical form of selenium, because not all are useful for organism. Inorganic forms, as Na-selenite and Na-selenate, are most frequently added to food for humans and animals. However, considerable quantity of selenium is secreted from organism. When provided in its natural form (in plants) it is most effective. Selenium-amino acids are retained in body and selenium takes part in antioxidant protection [13].
Having in mind the importance of selenium in animal metabolism and the fact that its deficiency results in creating a risk factor for most of human population, it is necessary to provide an optimal and natural selenium intake in animal organisms. In poultry production selenium is used for prevention from diseases, has a positive impact on immunology system and increases production performances, most of all body mass [4].

MATERIAL AND METHOD

Our examination was carried out *in vivo* condition on hybrid heavy broiler line Arbor Acres. The animals were divided in five groups with 60 one-day old chicken of both sexes (total number 300). They were fed with complete mixtures with no Se (control group), or a mix with different selenium concentration (4 working groups), displayed in Table 1. The feeding and watering of broilers was *ad libitum*. At the beginning of the experiment the chicken were healthy and in good condition. During the experiment (42 days) the chickens were raised on floor.
In order to provide as small as possible addition of selenium in ready made mix by "natural background" the content of selenium in forage mixes (maise, soy flower, sunflower meal) originating from different areas was determined [8,9]. Only the one that contained selenium in traces were used for the mix. In the selenium purified complete mixtures a certain amount of sodium selenite (Na_2SeO_3) was added. The mixing was done by a vertical mixer, type “Nauta”, capacity 50 kg. In this way it was achieved that the mix for feeding the experimental animals contained the planned quantity of selenium, and was the following: < 10 μg Se/kg, 50 ± 10 μg Se/kg, 100 ± 10 μg Se/kg, 150 ± 10 μg Se/kg and 250 ± 10 μg Se/kg.

Table 1. Experimental groups of chickens

0 group – control	Fed with complete mixtures, no selenium added
I experimental group	Fed with complete mixtures, 50 μg kg^{-1} selenium added
II experimental group	Fed with complete mixtures, 100 μg kg^{-1} selenium added
III experimental group	Fed with complete mixtures, 150 μg kg^{-1} selenium added
IV experimental group	Fed with complete mixtures, 250 μg kg^{-1} selenium added

Sacrificing and sampling. Fifteen animals from each experimental group were sacrificed, on days 14, 28 and 42. The chickens were decapitated. Chickens were measured and liver samples were taken. In the autopsy of each chicken, done by an veterinarian, the changes on stomach, small and large intestine and organs (kidneys, liver, spleen, heart, lungs) were noted.
Contemporary statistical programs (SPSS 8.0 for Windows and Sigma Stat 2.0 for Windows) were used for fast data processing. They provided accurate and fast obtaining of statistical parameters (mean values, standard deviation and important statistical difference between the examined parameters depending on the content of selenium and the period of its consumption).

RESULTS AND DISCUSION

Based on data displayed in Table 2. and 2a. it may be seen that after 14 days of feeding no statistically important differences were noticed between the examined groups. The least gain in of average body mass was in the control group (no addition of selenium) and was 418 ± 54 g, and the highest was in the chickens from group IV (455 ± 25 g). At the end of fourth week of fattening, an important statistical difference was noted ($P < 0.01$) between the chickens in group II (fed with 100 μg Se/kg, average body weight 1272 ± 104 g), group III (fed with 150 μg Se/kg, average body weight 1271 ± 128 g) and group IV (fed with 250 g Se/kg, average weight 1279 ± 145 g) comparing to the chickens in the control group (no addition of selenium) and group I (fed with 50 μg Se/kg). Remarkably higher body weight was noted in the group IV (2451 ± 332 g) at the end of the experiment (week 6). The chickens in the control group (0 μg Se/kg) had the worse gain in body weight and at the end of the experiment it was only 2255 ± 306 g. After 42 days the chickens fed with 250 μg Se/kg had average body mass that was statistically considerably higher ($P < 0.01$) comparing to all the experimental groups, what is displayed in Table 2. It is also notable that the average body gain in chickens fed with 250 μg Se/kg was 6 to 9% higher from the body mass in the control group (0 μg Se/kg).

Table 2. Changes in body weight [g] in the experimental animals dependent on the content of selenium in feed and the period of feeding

Selenium concentration, [μg/kg]	Body weight, [g] $X \pm S_d$/ (%)		
	Period of feeding (days)		
	14	28	42
0	418 ± 54 (100%)	1212 ± 129 (100%)	2255 ± 306 (100%)
50	441 ± 28 (106%)	1233 ± 127 (102%)	2287 ± 311 (101%)
100	444 ± 32 (106%)	1272 ± 104 (105%)	2293 ± 250 (102%)
150	454 ± 32 (109%)	1271 ± 128 (105%)	2296 ± 277 (102%)
250	455 ± 25 (109%)	1279 ± 145 (106%)	2451 ± 332 (109%)

Table 2a. Statistically important differences in the body mass in the examined broilers, depending on the content of selenium and the period, obtained by the analyses of variance

t-test	Period of feeding (days)					
	14		28		42	
Risk level Group	P=0,05	P=0,01	P=0,05	P=0,01	P=0,05	P=0,01
G_0-G_{50}	SND	SND	SND	SND	SND	SND
G_0-G_{100}	SND	SND	*	SND	SND	SND
G_0-G_{150}	*	SND	*	**	*	SND
G_0-G_{250}	SND	SND	*	**	*	**
G_{50}-G_{100}	SND	SND	SND	SND	SND	SND
G_{50}-G_{150}	SND	SND	*	**	*	SND
G_{50}-G_{250}	SND	SND	*	**	*	**
G_{100}-G_{150}	SND	SND	SND	SND	*	SND
G_{100}-G_{250}	SND	SND	*	**	*	**
G_{150}-G_{250}	SND	SND	SND	SND	*	SND

SND- statistically no important differences; *- statistically important differences; **- statistically very important differences

It may be concluded that feed deficient in selenium induced lower body mass of chickens. Edens [3] points out that considerably higher gain is noted in the chickens fed with 0.2 mg Se/kg when in form of Na-selenite comparing to the chickens fed without selenium supplementation. Similar data are reported by Matrello and Latshaw [7]; Echevarria et al. [2]; Jokić et al. [5]. Our experiment points that body mass and the rate of survival was considerably improved when selenium was added (at least 250 μg Se/kg feed).

In Table 3. and 3a. are displayed the results of measuring the effect of selenium on liver in fattening poultry. These data point out that in the first four weeks no statistically

important difference was noted between the groups. Only after day 42 statistically important difference was noted ($P < 0.01$) in the liver mass, and this was only in the group IV (53.4±8.1 g) that was fed with 250 μg Se/kg and group I (45.2±7.3 g) that was fed with 50 μg Se/kg. Kantor et al. [1] carried out similar experiments with broilers with 0.3 mg Se/kg selenium supplementation. The results of their trial point out that even after six weeks with selenium supplemented feed, no difference was detected in liver mass. Based on all the aforementioned, it can be concluded that dietary selenium, when added to broiler feed, in quantity of 50–250 μg/kg does not effect the mass gain of liver in the experimental animals.

Table 3. Changes in liver mass [g] in experimental animals depending on selenium concentration and the period of feeding

Selenium concentration , [μg/kg]	Mass of liver, [g] $X \pm S_d$		
	Period of feeding		
	14	28	42
0	28,7 ± 2,3	45,7 ± 3,4	46,9 ± 9,9
50	29,4 ± 1,9	46,3 ± 4,4	45,2 ± 7,3
100	29,8 ± 1,5	44,5 ± 3,9	48,8 ± 8,1
150	29,4 ± 1,2	46,1 ± 2,6	47,1 ± 5,9
250	28,6 ± 2,6	46,3 ± 4,9	53,4 ± 8,1

Tabela 3a. Statistically important differences in the mass of liver between the examined groups of broilers, depending on the content of selenium and the period of consumtion, obtained by the analyses of variance

t-test	Period of feeding (days)					
	14		28		42	
Risk level Group	P=0,05	P=0,01	P=0,05	P=0,01	P=0,05	P=0,01
G_0-G_{50}	SND	SND	SND	SND	SND	SND
G_0-G_{100}	SND	SND	SND	SND	SND	SND
G_0-G_{150}	SND	SND	SND	SND	SND	SND
G_0-G_{250}	SND	SND	SND	SND	SND	SND
G_{50}-G_{100}	SND	SND	SND	SND	SND	SND
G_{50}-G_{150}	SND	SND	SND	SND	SND	SND
G_{50}-G_{250}	SND	SND	SND	SND	*	**
G_{100}-G_{150}	SND	SND	SND	SND	SND	SND
G_{100}-G_{250}	SND	SND	SND	SND	SND	SND
G_{150}-G_{250}	SND	SND	SND	SND	*	SND

SND- statistically no important differences; *- statistically important differences; **- statistically very important differences

CONCLUSION

Based on the results obtained in fattening of chicken fed with mixes with different concentrations of dietary selenium, the following can be concluded:

1. The chickens in the control group (0 μg Se/kg) had the least gain in body mass and in the end it was 2255 g.
2. Statistically important differences were noticed in body mass already after four week of fattening between the groups fed with selenium supplementation 100 μg Se/kg (II group), 150 μg Se/kg (III group) and 250 μg Se/kg (IV group) comparing to the control group (no selenium) and the experimental group (50 μg Se/kg).
3. After 42 days of fattening the chickens in group IV, fed with selenium supplementation 250 μg Se/kg, had the highest body mass (2451 g), with statistically important difference ($P < 0.01$) comparing to all other experimental groups.
4. Dietary selenium, when 50–250 μg/kg supplemented to feed, does not effect on weight of liver in the experimental animal.

The results of our examination point out that the best results in chicken fattening are achieved by adding 250 μg Se/kg mix (0.25 mg Se/kg mix), whant is considerably higher than in the recomendations (minimal 0.15 mg/kg mix) given in the domestic regulations on feed quality [11].

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SYSTEMS AND ECONOMY USE GRAZING IN NUTRITION OF GOATS

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ABSTRACT

Of animals which consume roughage food, goats utilize the greatest number of plant species. However, in intensive production, only few plant species are used in goat feeding, whether on sown surfaces or natural pastures whose plant composition is reduced/simpliefied by proper fertilization. Main objective in grazing of dairy goats is to maximize the amount of fresh pasture in nutrition throughout the year. In this way the principle of goat nutrition using food of maximum nutritious value and minimum expenses is achieved. Food additives are not necessary as grazing supplement, since pasture contains the ideal, natural mixture of energy, proteins and minerals. Additional nutrition using hay, silage, grains, etc., in order to fullfil nutrition requirements, is often necessary in regions where due to climatic changes the grazing cannot last long time (winter and summer), however, on good pasture areas which are managed throughout the year using rotational grazing system, the need for expensive additional feeds can be reduced.
***Keywords**: nutrition, goats, grazing*

INTRODUCTION

Goats are ruminants and they utilize the roughage (grass, hey and silage) very well. That is why roughage is their basic food. Accordingly, the level of production will mostly depend on the quantity and quality of those nutrients [2, 7, 10, 12]. Forage plants that goats like to eat and which are mostly used in their nutrition are: Alfalfa, Red clover, Italian rye grass, Orchard grass, Vetch, Corn, Sorghum and Sudan grass [5, 16]. Under the conditions in our region, various hey, silage, grass, stock kale, turnip, food industry waste and concentrated feed are used in nutrition of goats

Compared to cows and sheep, goats are much better at utilizing food with high level of cellulose [8, 9]. Basic meal in nutrition of goats can in large percent (90%) consists of roughage. Concentrated feed as a supplement meal are given in much lower dosage [13, 15]. However, for a normal or high production the same principles are being applied in nutrition of goats as in nutrition of other ruminants.

Goats are real gourmands, they like regular change in their nutrition so it is recommended for daily meal to consist of various nutrients. Good appetite and high production can be maintained in that way. When a meal doesn't include grass, silage and succulent nutrients (turnips) etc can be used.

Nutrition of goats is similar to the nutrition of sheep except that the choice of nutrients is somewhat different. Basic meal consist of voluminous nutrients (about 95%) while concentrated feed is given as a supplement meal in much lover dosage [6]. However it is

incorrect to consider every type of food which is inadequate for nutrition of cows and sheep as an adequate food for nutrition of goats. It is true that goats will consume nutrients of lower quality, especially in case of malnutrition, but that doesn't mean that those nutrients can ensure the intake of nutriments necessary for normal reproduction. Goats like regular change in their diet so it is recommended for meals to consist of various nutrients and maintain good appetite and high production in that way [4].

Compostition and nutritive value of pastures

During the vegetation period, grass, the green mass, is the best and the richest food nutrient for goats. At the same time, it is the cheapest food and if there is enough of it and if it's of good quality, it can satisfy the requirements of goats completely. That way the adding of concentrated feed is lowered which diminishes the cost of nutrition of goats. Green feed should be the basic nutrient in nutrition of goats for a period of 180 days in a year, at least. However, the yield of green mass from our natural and planted grassland is dangerously low. For green mass production in planted grasslands, various compounds of spear grass and leguminous plants should be used. The most suitable plants for grass compounds are orchard grass, timothy-grass, tall fescue, meadow fescue, ryegrass, hairy brome, smooth meadow-grass, pigweed and leguminous plants such as alfalfa, red clover, birdsfoot trefoil, white clover etc.
Comparison of different categories of grasslands in regard to yield of hay and nutritive substances is presented in the following table:

Table 1. The productivity of different categories of lawn [1]

Category Lawn	Green weight (t/ha)	Hay (t/ha)	CP (kg/ha)	HJ (kg/ha)
Natural nofertizle	2.07	0.65	48.7	263
Natural fertizle	12.9	3.52	367.5	1.508
Sow lawn	38.8	8.73	1.222	4.283

Table 2. Influence of ways of exploiting the lawn on the production of goat [1].

The parameters of livestock production	Pregon grazing		Continuous grazing	
	Natural lawn	Sow lawn	Natural lawn	Sow lawn
Number of grazing days (goat /ha)	3220	6837	2992	5941
Load (ewes, goat/ha)	32.0	38.4	32.2	33.2
Conditional head/ha	2.59	3.2	2.91	2.82
Growth (kg/ha)	318.5	415	355.8	398
Daily gain (g/head)	99.2	61	122.5	63

Grass has many advantages compared to roughage and concentrated feed because it's a very good source of widely available nutriments. It contains a large percent of protein with high biological value, vitamin C and E, carotene, carbohydrates, macro and micro

elements, growth factor etc [11]. These nutriments are directly available to animals unlike with preserved roughage which is transformed by various methods of treatment and loses nutriments in the process. Compared to mowing, pasture amplifies the productivity of grasslands by more than 30% by gaining a richer yield from the start
Pored toga, koze i njihov podmladak na paši su izloženi suncu, što dovodi do sinteze vitamina D u njihovoj koži.
There are different grass types, leguminous plants of different quality, weed, harmful and poisonous plants on our grasslands. The botanical structure of the natural grasslands is less appropriate compared to planted grasslands. Planted grasslands are usually based on only one type of plant (grass or leguminous plant) or a compound of grass and leguminous plants. Grass is rarely harbored as an individual type of plant in grasslands. It is usually harbored as a compound of types of grass and perennial leguminous plants. On the other hand, leguminous plants are primarily harbored as individual crops and some of them are harbored in grass- leguminous compounds (3). When planting grassland, compounds of perennial leguminous plants and types of grass should be given advantage because they provide more dry matter per surface unit, as well as more protein and minerals. Compounds of leguminous plants and types of grass have less variation in quantity of the yield compared to individual crops, and they dry faster when mowed with less mechanical loss during the manipulation.

Table 3. Intake level of green forages used as a sole feed in the basal diet (1)

Forage	State of lactation, days	Intake level* $_{0,75}$ g/kg BW
Italian rye grass	60 – 160	82 (60 – 101)
Orchard grass	60 – 160	65 (59 – 81)
Fescue	60 – 160	68 (62 – 75)
Alfalfa	60 – 160	103 (69 – 157)
Red clover	60 – 160	93 (50 – 118)
Vetch + oats	130 – 150	79 (51 – 150)
Corn (maize)	160 – 200	81 (63 – 99)

*Tolerated refusals: 25-35%; concentrate intake = 0,7 kg DM.

Yield and quality of pasture demonstrate serious fluctuations throughout the year. They greatly depend on the amount and distribution of precipitation and differ significantly deoending on the region, type of soil, fertilizer application and type of plants grown on them. Goats can equally succesfully graze on natural and artificial pastures. Thanks to this fact, goats can survive and give products based on grazing throughout the most of the year, when pasture is the single source of food. Explicit deficit of one or more nutritious substances (proteins, energy, carotin) occur occasionally on natural pastures. Deficit of said substances mainly occurs when plants are completely ripe/mature or insufficiently developed, which is case in excessive grazing of same areas or in time of long lasting drought. The deficit of nutritious substances/nutrients commonly occurs in breeding goats during gestation period or lactation, i.e. production of milk, in the period when the needs are the greatest.

Economic effect of diet by grazing goats

Energy and protein requirements for maintenance, growth and milk production for the different classes and ages of the goat herd should be plotted through the year, and compared against monthly feed supply from pasture, and other sources as needed (15).
Protein is rarely a limiting nutrient factor where pastures are grazed, except when there is a large amount of dry grass. Energy is the most common nutritional deficiency limiting productivity (14).
Energy deficiencies can occur when feed availability is low and when pastures are dry. Feed budgeting based on grazing can be simplified by concentrating on energy supplies and requirements. An example of a simple Feed Budget, based on Energy needs, for a strip grazed herd increasing from 150 to 200 dairy goats (average body weight 60 kg, production milk 2.5 kg 4% mm) na 20 hectares, from late winter throughout early spring, is:

Table 4. Energy requirements for goats depending from season of grazing (18)

	March	April	May
Feed Budget			
Kg dry matter of pasture	6.000	12.000	18.000
MJ for 12 heads in kg	72.000	144.000	216.000
Energy needs			
Maintenance and activity	51.750	60.375	69.000
Milk production	58.500	68.250	78.000
Total energy needs (MJ)	110.250	128.625	147.000
Surplus and deficit in energy	- 38.250	+ 15.375	+ 69.000

Energy deficits can be made up from pasture saved prior to the budget month, and/or supplementary feeds. Energy surpluses can be carried forward to future months, or in the case of spring growth, cut for silage or hay. Early spring pasture may also need supplementation with fibre rich feeds to aid digestibility.

Possibility for utilization of feeds from pastures

For dairy goats to get enough energy from pasture, the feed must be above a certain quality and there has to be enough of it. Pasture availability is measured as kilograms of dry matter per hectare and can be estimated by measuring the height of the pasture, as shown in the following table

Table 5. Pasture availability depending from measuring the height of the pasture (18)

Height of the pasture (cm)	Dry matter of pasture (kg/ha)
1	500
2	800
3	1100
4	1400
5	1700
6	2000
7	2300
8	2600

Pasture height can be measured by special meters developed for the purpose, or by simple measuring sticks. Both techniques require some practice, but once mastered, visual assessments of pasture height can often be confidently made as an alternative from time to time.

Way of utilization of grass surfaces in goat grazing can have significant effect on yield, composition and quality of grass mass. Therefore, use of one grass surface can be organized either in free or planned way. In the system of free grazing, goats can use grass mass on the whole available pasture surface. In that case, they are able to consume mainly young and more tasty plants, whereas the rougher, older plants and weeds are left unconsumed. As they taste the food, animals move around the pasture, soil is trampled and compressed, and poorley used weed species grown undistrubed and release seeds. If the pasture is unorganized, goats will use the pasture surface in very poor way and leave even up to 50% of available green mass. If goats are grazing on sown meadows, and pastures, it is recommended to introduce the system of rotational grazing (Ukoliko se koze napasaju na sejanim livadama, odnosno pašnjacima, preporučuje se da se uvede sistem pregona (partitioning of pasture), so that goats can fully use the grazing surfaces.

Planned grazing can be done in several ways, such as: individual grazing of animals tied to pole, rotational grazing, diet grazing, etc. (10). These systems of grazing can be organized for one or several animal types and categories on the same pasture. Planned grazing system involves restricted stay of goats on pasture, in time and space, and as the result of this better and faster regeneration of plants is ensured, less depletion of high-quality plant species, and higher productivity and longer utilization of sown pastures. Proper fertilization of grasslands can significantly affect the yield of green mass, also changes of the botanical composition and in this way influence better nutritional value of the grass mass. Also, nutritional value of pasture is greatly influenced by the the plant development stage. With the aging of plants the yield of green mass increases, but the quality declines. This means that it is very important to harmonize the time of grazing or cutting of plants, so that goats and their progeny can receive the highest possibly quality of food to satisfy their needs.

Table 6. Requirements of goats and sheep in green forages (19)

Tip pašnjaka / Type of pasture	Species of animal		
	Goat	**Sheep**	**Cow**
	Head[1]		
Good quality of pasture system	6-8	5-6	1
Good grazing system	9-11	6-7	1
	Head/ha.		
Alfalfa	10-12	8-9	1,5
Pasture of alfalfa	12-15	10-11	1,9
[1] Number of animal which intake same quantity food.			

In regard to number of rotations, they are determined based on the duration of the stay of animals on pasture and plant regeneration. According to some recommendations, pasture should be divided into 16 rotations. In the first cycle, 7 to 8 rotations are used, and the remaining ones are cut and the mass obtained is conserved either as hay or silage and used in winter feeding time. In the second and third cycle 10 to 14 rotations are used for grazing, whereas in the last cycle, when yields are the lowest, all 16 are used. Rotational grazing will help increase the productivity of pastures, because there will be rest time between grazing. In these periods, plants will not only develop top plant parts, but also the growth of root is enabled, and plants will be able to utilize more water and nutrients from greater soil volume. This is helping plants grow faster after grazing. Also, resting enables that productive plants and leguminous plants to compete with less prefereble plants and weeds (21).

Goats prefer to graze in the morning and evening, and in the afternoon they usually rest, especially during high summer temperatures. In addition to grazing, green mass can be brought to goat stables (if there are conditions for that), from natural or sown meadows. For instance, goat which produces 1 l of milk daily, needs approx. 7 kg of green mass and 400g of concentrate, whereas for production of 2 l of milk requirements increase to 10–12 kg of green mass and 200 g concentrate daily. In high-yielding goats daily quantity of green mass in diet can be even 15 kg.

In nutrition of animals in first 90 days of lactation and in grazing, it is preferable to add certain quantity of concentrated feeds, as well as mineral substances. Whole cotton seed or some other feed (grain mixture, meals) is excellent supplement for goats, and they are fed quantity which usually doesn't exceed 0,2 kg/animal/day. In case of dry and young goats which are still growing, nutrient requirements are 10-12% of proteinsa and 50-60% TDN (20)

Sometimes it is recommended to produce mineral mixture which contains 20-25% magnesium-oxyde in order to reduce the risk of pašne tetanije, when haigh-yielding dairy animals graze juicy grasses or leguminous plants, especially in period of early lactation.

During grazing in spring-summer season, it is highly probable that goats will satisfy all nutritional needs from any combination of roughage feeds which are at their disposal. Goats should only be given salt with mineral substances, and maybe some phosphorus. However, in the period of late autumn and during winter, quality and quantity of roughage rapidly decrease. Therefore, supplementation of diet with proteins and energy

through concentrated feeds is necessary in order to maintain satisfactory production performance.

CONCLUSION

Goats are ruminants and they utilize the roughage (grass, hey and silage) very well. During the vegetation period, grass, the green mass, is the best and the richest food nutrient for goats. At the same time, it is the cheapest food and if there is enough of it and if it's of good quality, it can satisfy the requirements of goats completely. That way the adding of concentrated feed is lowered which diminishes the cost of nutrition of goats. Green feed should be the basic nutrient in nutrition of goats for a period of 180 days in a year, at least. Koze sa podjednakim uspehom mogu da koriste prirodne i veštačke pašnjake. Zahvaljujući ovoj činjenici, koze mogu da prežive i daju proizvode na paši tokom najvećeg dela godine, kada je ona jedini izvor hrane.

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SPECIFIC NUTRITION OF GOATS IN PRODUCTION CYCLE ON THE BASIS OF BODY CONDITION SCORING

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ABSTRACT

In breeding of goats producers should focus on their body condition. Body condition score is very well developed for dairy cattle, but it equaly relates to to dairy goats (goats are scored from 1 to 5), and basically it is based on assessment/evaluation of the amount of deposited fatty acids.
Body score is visible result of adequately or insufficinetly balanced nutrition of goats in relation to the production level. Goats with body score outside the adequate target ranges (from 0-2 and from 3,5-5) will produce less milk and meat of lower price. Reproductive efficiency ia slo significantly diminished when body scores are outside target ranges. Frequency of occurence of disaeases will also be increase. Adequate body reserves (fatty tissue) increase the milk production, efficiency in reproduction and extand the period of animal exploitation.
***Keywords**: goat, body condition scoring, nutrition*

INTRODUCTION

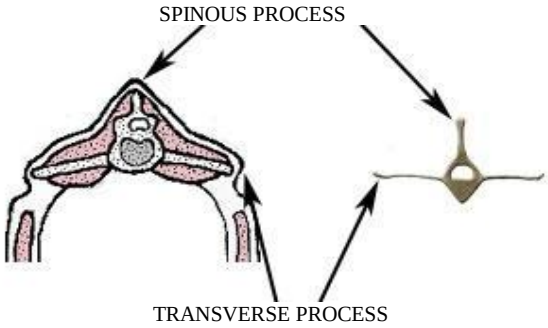
There are numerous instructions for nutrition of goats based on NRC or similar foreign tables of nutritent requirements and diet composition (1,2,3,4,5,8,15,16,17). Combined with regular scoring of body condition of growing goats and goats in lactation, these tables of nutrient requirements should be adjusted in order to ensure adequate providing of nutrients under given conditions with sufficient stimulation for improvement of growth and production, but also enough restricition to prevent overweight and health risks (11).
Goats should not be too thinn or thick, because in such cases they are exposed to risk of various health problems, reduction of milk production, partus problems, etc. Overweight can cause toxichemic pregnancy (6), although overwight in goats is rarely serious problem. Fast changes in body condition or poor condition of goats in early lactation stage can cause serious health problems (7).

PROCEDURES IN SCORING OF BODY CONDITION OF GOATS

Body condition of goats shall be observed in the following phases of production cycle:

1. immediately after drying
2. 15 days before partus
3. 45 days after partus
4. int he middle of lactation period
5. in periods when nutrition is changed

Simple observing of the animal for the purpose of assessment of their body conditionm can lead to misconception. In order to determine the body condition of animals it is necessary to feel gently areas around loin and pelvis, hand pressing down with only few fingers. It is also necessary to determine the quantity of fat covering salients and spinous processes, as well as dents on the loins. Other areas which are observed in goats in body condition socring are blades, rear side of the head, legs and rear bones. Palpation method can be used in all goat breeds, throughout the year and in certain stages of the production cycle, as reliable indicator of body condition of goats, i.e. adequacy of their nutrition. The easiest way of scoring is to select few animals which are in overweight and few underwieght, skinny animals. Then, small groups of animals are brought and compared to animals with extreme body condition compared to prefereable one (19,20).
Application of this method is very important, i.e. body condition scoring in goats in the period before fertilization and partus; in this period, and based on determined score, it is prefereable to divide goats in groups and apply certain level of nutrition for each individual group. Goats with lower body score (1 – 2,5 points) receive grainy food in ratio < 1.2 kg grain: 0,5 kg milk, whereas goats with higher body scores (3,0 – 5,0) are fed diets in the feed:milk ratio of 3:1. This will adjust for the loss in production caused by insufficient nutrition and prevent occurence of thick goat syndrom (13,19).
In scoring of goat body condition the area around the tail root and loin are observed. Main attention is focused to the assessment of the covering of goat body with fat depots. For each animal in the herd, several body condition scores should be evaluated, using average values for the herd in order to correctly evaluate the nutrition program. Average herd body score and nutrition should be considered and not to base the score or change the nutrition because of few animals which are outside preferable score ranges. Each herd has individual animals that are too weak or to fat. If their is a problem with nutrition in the herd, this will affect most of animals in the herd.
In evaluation of body score of goats in a herd, lactation stage or ability to produce milk should not be taken into consideration. Also, once established body condition score will not remain the same forever. Body condition score can indicate presence of a potential problem in nutrition of the herd. Three areas are evaluated in assigning a BCS: the lumbar region, or area containing the loin muscle; the sternum; and the rib cage.
1. Scoring in the lumbar area is based on determining the amount of muscle and fat cover over the vertebrae. Lumbar vertebrae have a vertical protrusion (spinous process) and a horizontal protrusion (transverse process). Both processes are used in determining BCS.



With finger tips, evaluator should feel the vertebrae over thespinous process. Then, evaluator should try to hold spinous process between the thumb and forefinger. Evaluator of body condition should use whole hand to feel the loin muscle and fat depots. Also, evaluator should try to place fingers under cross extensions.
2. The second body area to feel is the fat covering on the sternum (breastbone). Scoring in this area is based upon the size of the fat pad on the sternum that can be pinched.
3. A third area is the rib cage and fat cover over the ribs.

As a doe goes through an entire lactation, her body condition score changes as fat reserves are used for milk production and are restored during late lactation (14). At the start of a new lactation, the doe is in a negative energy balance (12). Her feed intake is not enough to accommodate her high level of milk production (3). To compensate, fat reserves are used until feed intake fulfills milk production requirements. The doe's condition score drops as her fat reserves are used to make milk. Once feed intake matches milk production needs, the body condition score increases as fat reserves are restored.
The following table describes how scores relate to potential problems and the stage of lactation. Scores of a minus (-) and plus (+) are used to further describe body appearance.

Table 1. Body condition score of goats depending on physiological and health conditions of animals

Score	***Condition***
1	Skin and bones. Goat is experiencing a serious health problem and is not eating enough feed.
2 to 2- (low 2)	Severe negative energy balance in doe for early lactation. A problem either exists or may be developing.
2+ (high 2)	High producer in early lactation.
3	Milking doe in good nutrient balance.
3+ to 4-	Late lactation and dry doe in good condition.
4	The doe is over conditioned causing her to be an inefficient milk producer. Suggests a doe with an extremely long lactation if milking and a potential kidding problem if dry.
5	Severely fat: a candidate for fat doe syndrome.

OTHER SYSTEMS OF BODY CONDITION SCORING

There are several systems of goat body condition scoring developed in the world which differ slightly. One of them is system used in North Carolina (USA) applied in scoring of cattle but also adjusted for scoring of body condition of goats. Within this system goats are socred from 1 to 9 In that graduated scale, thin is 1 to 3, moderate is 4 to 6 and fat is 7 to 9. In most situations, goats should be in the range of 4 to 7. Scores of 1 to 3 indicate a problem, and scores of 8 to 9 are almost never seen in goats. The ideal body condition score (BCS) just before the breeding season is between a 5 and a 6 to maximize the number of kids born. (19,20).

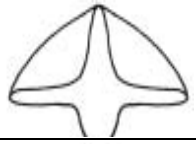
Pregnant does should not have a body condition score of 7 or above toward the end of pregnancy because of the risk of pregnancy toxemia. In addition, a body condition score of 5 to 6 at kidding should not drop off to quickly during lactation. Also they give birth to healthier kids and have better production of milk during lactation period. Based on data for dairy cows, increase of the body condition score by one point within the range of scores from 3 to 5, will lead to increased production of milk by 35 kg per goat during lactation, and to increase of the protein and milk fat percentage by 0,2% during first 5 weeks of lactation period (20).

Body condition score is used to determine whether flushing will be of benefit to breeding does. Flushing means increasing the level of feed offered to breeding does, mostly energy, starting about one month prior to the introduction of the bucks, to increase body weight, ovulation rate and hopefully litter size.

Does in extremely good body condition (BCS = 6-7) will tend not to respond to flushing. On the other hand, does that are in relatively poor condition (BCS = 4 or lower) as a result of summer pastures of poor quality, high worm loads, or late kidding of twins or triplets, will respond favorably to flushing by improving their body condition. Flushing can be accomplished by moving breeding does to a lush nutritious pasture approximately 4 weeks prior to the introduction of the bucks. Another method is feeding 1/2 lb/day of a

high energy supplement. Corn is the grain of choice for flushing. As the goal is to increase the intake and body weight, breeding does should be grouped according to their BCS and fed accordingly to first improve their body condition to 6, and then to maintain it.

Table 2. Illustrate body condition score (North Carolina – USA) (20).

BS	The appearance of the rear body		Description
1		Extremely thin	Extremely thin and weak, near death.
2		Extremely thin	Extremely thin but not weak.
3		Very thin	All ribs visible. Spinous processes prominent and very sharp. No fat cover felt with some muscle wasting.
4		Slightly thin	Most ribs visible. Spinous processes sharp. Individual processes can be easily felt. Slight fat cover can be felt over the eye muscle.
5		Moderate	Spinous processes felt but are smooth. Some fat cover felt over eye muscle.
6		Good	Smooth look with ribs not very visible. Spinous processes smooth and round. Individual processes very smooth, felt with considerable pressure. Significant fat cover felt over eye muscle.
7		Fat	Ribs not visible, spinous process felt under firm pressure. Considerable fat felt over eye muscle.
8		Obese	Animal is very fat with spinous processes difficult to feel. Ribs can not be felt. Animal has blocky obese appearance.
9		Extremely obese	Similar to an eight but more exaggerated. Animal has deep patchy fat over entire body.

- Areas to be monitored:
  - Tail head - Ribs
  - Pins - Hocks
  - Edge of loin - Shoulder
  - Back bone - Longissimus dorsi
- Scale:
  - Thin 1 to 3
  - Moderate 4 to 6
  - Fat 7 to 9
- Recommendation on appropriate assessment of BCS for particular stages of the production cycle:
  - End of pregnancy 5 to 6
  - Start of breeding season 5 to 6
  - Dairy goats in the first part of lactation 3 to 5
  - Animals should never have a body condition score of 1 to 3
  - Kids 5 to 6
  - Bucks (breeding season) – 7
  - Pregnant does should not have a body condition score of 7 or above toward the end of pregnancy because of the risk of pregnancy toxemia.

CONCLUSION

There are as many different ways to feed a goat as there are blades of grass in a pasture. Feeding methods differ according to herd health, weather conditions, feed availability, feed cost, management styles, genetics of the herd and the producer's goal for milk production. All these factors impact the herd's production potential. As you determine your ration, there are many different recommendations that you can use that will improve feed efficiency, reduce incidence of nutritional diseases and promote milk production. Once your ration and feeding management style are established, you must determine whether or not it is meeting the productive and reproductive needs of your goat herd. Body condition scoring is a technique you can use to evaluate if the dietary needs of your herd are being met.

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NORMATIVE OF PROTEIN FOR GOAT IN INTENSIVE PRODUCTION

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ABSTRACT

Protein is the more expensive nutrient in feeding and therefore often limiting maximum productivity. Industry by-products often are less expensive sources besides the traditional major supplies of oilmeals. Protein supplies to the rumen in the form of degradable protein are necessary for optimum growth of rumen bacteria, but they require energy at the same time, without which some proteins will be wasted into ammonia in the rumen. The supply of some rumen protected protein has been effective in increasing milk yield. Excess protein feeding is not only wasting money but is stressing the goat by increasing her blood urea levels, increasing urine excretion and interfering with efficient reproduction. Protein deficiencies will reduce feed intake, rumen function and retard fetal development.

Key words: *nutrition, goats, protein*

INTRODUCTION

Similar to other ruminants, goats can utilize as source of protein not only protein nitrogen from the diet but also non-protein nitrogen (NPN). Within reticulum-rumen microbial activity leads to degradation of large and various quantities of protien from diet. Protein fraction which is decomposed/degraded in the rumen is called degradable protein, whereas the protein fraction which cannot be degraded by bacteria in rumen and which passes through rumen and arives to abomazus and intestines where it is digested and taken in – is called non-degradable protein. Also, it is prefereable not to have certain protein fractions either in deficit or in surplus [6,9,11].

Degradable protein is hydrolyzed in bacterial processes at the beginning, in order to be degraded into amino acids. They can subsequently be used by micro organisms in rumen for synthesis of their proteins, but most of these acids are further degraded to ammonia and organic acids. The ammonia is main source of nitrogen in synthesis of microbial protein and efficiency with which this form of nitrogen is transfered into microbial protein is mainly function of avaliable energy in rumen. Easy digestible sources of carbon hydrate help more efficient conversion of ammonia into microbial protein. When the rate of ammonia production is higher than utilization rate in rumen, ammonia concnetration occurrs which is then aborbed in the blood. In the liver, ammonia turns into urea and is excreted in urin, althoug certain quantity (about 25%) is returned (recycled) into rumen throug saliva.

Nondegradable and microbial proteins generated through bio-synthesis in rumen arrive, carried by the fluid from the rumen, into abomazus and small intestine, where digestion and absorption processes of these proteins in said parts of the digestive tract are slightly different from those in monogastric animals. So the mixture of amino acids which is available for absorption from the small intestine consists of those which are ingredients of the microbial protein and those present in the nondegradable protien.

PROTEIN REQUIREMENTS

Protein is the more expensive nutrient in feeding and therefore often limiting maximum productivity. Industrial by-products are often less expensive sources in addition to traditional plant sources such as oil meals (sunflower and soy bean meal). However, certain feeds, primarily roughages, have higher fibre content and less proteins, as maturity of the plant goes. The cheapest sources of protein in goat nutrition are usually: alfalfa, clovers, well fertilized grasses harvested before blooming or in later stages of maturity. Inflow of protein to rumen in the form of degradable proteins is necessary for optimal growth of rumen bacteria, but at the same time they need easy digestible energy without which certain amount of present proteins will turn into surplus of ammonia in rumen [4,12,13,14,15,16].

Table 1. Daily requirements of protein in dairy and pregnant goats [4]

Physiological state of animals (body weight 55 kg)	RPC[1] g	Crude protein, g	DM kg
Pregnancy:			
1 to 3 months *	43	114	1,5
4 to 5 months	98	164	1,4
Lactation:			
Kg milk with 3% fat			
1	89	152	1,5
2	134	206	1,9
3	179	260	2,3
4	224	314	2,7
5	269	368	3,1

* Plus per kg of produced milk: 45 g RPC-a;
[1] RPC = available protein in intestines (non-degradable protein in rumen + microbial protein)

A minimum of 7 percent crude protein in the diet dry matter is required for normal rumen function, and forage intake will be decreased at lower protein levels.
Excess protein feeding is not only wasting money but is stressing the goat by increasing her blood urea levels, increasing urine excretion and interfering with efficient reproduction. Protein deficiencies will reduce feed intake, rumen function and retard fetal development [11].

According to norms for nutrition of goats, protein requirments issued by NRC, (1981) (Table 1), based on their data, for sustaining of life range from 1,42 – 3,40 g per kg of metabolic body mass of goats (TM ^{0,75}). Protein requirements of goats according to NRC and depending on the physiological situation are presented in Table 1.

Table 1. Protein requirements of goats [18]

Physiological state of animals	Digestible crude protein, g
Maintenance	2,82 g /kg BWG* (W ^{0,75}).
Lactation	51 g/kg 4% masno korigovanog mleka
	6 g po 1% fat content
Maintenance + Pregnancy (4-ti i 5-ti months)	4,79 g/kg BWG* (W ^{0,75}).
Growing	195 g/kg
activity	
Low	2,82 g /kg BWG * (W ^{0,75}) x 1,25
Midle	2,82 g /kg BWG * (W ^{0,75}) x 1,50
High	2,82 g /kg BWG * (W ^{0,75}) x 1,75

* BWG – body weight goats

Sustainable needs increase with the increase of the mobility level of goats. For instance, goat which has to walk further in search for food will have higher sustainable needs than goat on free range for feeding. Environment conditions also have impact on maintenance of body temperature/warmth. When the weather is cold and severe, goats need more feed to sustain body warmth. Additional stress during gestation, lactation and groeth influence further increase of the nutrient requirements. Nutrient requirement of goats depending on the production direction, age, production performance, category of animals and other factors are presented in Tables 2 and 3.

Table 2. Dietary protein, energy and mineral requirements of goats *[20]*

Class of goat	Average feed intake per kg/day[1]	Crude protein, %	% Ca	% P
Growing doeling, 20 kg[a]	1.08	8.8	0.38	0.19
Growing male kid, 30 kg[b]	1.30	9.0	0.33	0.24
Yearling doe, 40 kg[c]	2.07	10.0	0.33	0.23
3 year old doe, 50 kg[d]	2.25	11.7	0.48	0.33
Mature buck, 100 kg[e]	2.38	9.0	0.29	0.20
Dairy doe[f]	3.37	11.6	0.48	0.33

[1] Calculated on basis of the dry matter in the feeds eaten
[a] Growing at the rate of 120 g per day
[b] Growing at the rate of 150 g per day
[c] Yearling female, last trimester of pregnancy and growing
[d] Milking 2 kg per day - enough for twins
[e] Not gaining weight, moderate activity
[f] Nubian, milking 1 gallon per day of 4.0% butterfat

Table 3. Daily nutritional requirement of an adult goat [2]

Item of diet	Daily requirement
Protein	
	Maintenance 20-30 g /50 kg BW
	Lactation 60 – 70 g/kg milk

Information on goat nutrient requirements when they are grazing are rather limited, and most of authors reported on daily requirements in feeds and nutrients which are similar to those recommended for sheep. This is logical, considering the similarity of food digestion in the digestive tract of sheep and goats [11]. However, one of the important factors which casts doubt on these recommendations is that grazing behaviour of goats differs from the behaviour of sheep, as well as that goat graze usually on steeper parts of hilly-mountainous pastures, and they have to walk several kilometers in order to satisfy their daily food requirements. Result of this is increase of the sustainable diet by approx. 20-25%. The other factor is that goats graze food which is not suitable for sheep [9].
In the nutrition of goats it is necessary to pay special attention to optimal satsifying of requirements for high yeild, i.e. production performance [7,8]. For this purpose modern norms are used which take into consideration numerous parameters.
However, the tremendous amount of information, based on additional data, available in the publications [5], NRC published in 2007 new regulations in nutrient requirements of goats which represent a step forward compared to the former regulations from 1981.
Protein value of nutrients is expressed in a new way in this system, in metabolizable protein (MP), protein requirements are also stated in rumen-degradable protein balance (DIP).

Table 4. Nutrient Requirements of goat [17]

Parameter	Body weight, kg	Dry matter intake, kg	Protein Requirements	
			MP,g-d	DIP, g -d
Mature goats, early lactation, milk yield 2.06 – 3.22 kg				
	40	1.97	178	94
	50	2.30	205	110
Mature goats, mid lactation, milk yield 1.47 – 2.30 kg				
	40	1.96	156	93
	50	2.26	178	108
Mature goats, late lactation, milk yield 0.88 – 1.38 kg				
	40	1.69	121	81
	50	1.96	140	94
Mature bucks, prebreeding				
	50	1.36	58	65
	75	1.84	78	88

The protein requirement for lactation suggested in the Nutrient Requirements of Goats [16] was based on a digestible crude protein system for dairy cattle - NRC 1978 [17] due

to a lack of adequate data from studies with lactating goats. This method of calculation resulted in a suggested requirement of 72 grams of total crude protein per kilogram of milk [17].
The AFRC [1] gaves estimates for the metabolisable protein (MP) of dairy and fibre goats in the last 3 months of pregnancy.

Table 5: Metabolisable protein requirements of dairy and fibre goats in the last 3 months of pregnancy. MP requirements (g/d)

Week of pregnancy		Očekivano jaradi		
		Single	Twins	Triplets
9 - 13	D	4.4	7.2	10.0
	F	2.8	4.2	
13 – 17	D	10.1	17.2	23.5
	F	6.1	9.5	
17 and more	D	19.2	32.2	43.2
	F	11.6	17.9	

*D for dairy goats and F for fibre production

In the nutrition of animals in the first 90 days of lactation and on pasture, it is preferable to ass certain amount of concentrated feeds, as well as mineral matter (10). Whole cotton seed or other grains (grain mixtures, meals) is excellent supplement for goats, and the daily quantity does not exceed 0,2 kg/animal/day. In dry and young goats in growth, nutrient requirements range from 10-12% proteins and 50-60% TDN.

Table 6. Protein requirements of goats depending on the quality of roughage and pasture [20].

% Protein in Roughage, Dry Matter Basis	% Protein Needed in Concentrate
15% and over Excellent legume hay or excellent pasture	
➢ High Production (Over 4 qts. per day)	14
➢ Low Production	12
12 to15% Legume - Grass mixed hay or good pasture	
➢ High Production	16
➢ Low Production	14
10 to 12% Good grass hay or fair pasture	
➢ High Production	18
➢ Low Production	16
Below 10% Fair quality grass hay or poor pasture	
➢ High Production	20
➢ Low Production	18

CONCLUSION

Protein is the more expensive nutrient in feeding and therefore often limiting maximum productivity. Industry by-products often are less expensive sources besides the traditional major supplies of oilmeals. Protein supplies to the rumen in the form of degradable protein are necessary for optimum growth of rumen bacteria, but they require energy at the same time, without which some proteins will be wasted into ammonia in the rumen. The supply of some rumen protected protein has been effective in increasing milk yield. Excess protein feeding is not only wasting money but is stressing the goat by increasing her blood urea levels, increasing urine excretion and interfering with efficient reproduction. Protein deficiencies will reduce feed intake, rumen function and retard fetal development.

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MICROBIOLOGICAL SAFETY OF ANIMAL FEED FROM REGION OF VOJVODINA IN 2009

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ABSTRACT

Study results show microbiological safety of 160 samples of animal feed from region Vojvodina in 2009. Out of a total of 160 analyzed feed samples, 105 samples were complete feeding stuffs and 55 samples were animal and plant origin feed. Microbiological safety of analyzed products was evaluated in accordance with the regulations of Serbia [7]. Out of a total of 160 analyzed samples 13 samples (8,12%) did not comply with microbiological criteria specified in the Regulations. Non-compliance with microbiological criteria was due to the increased number of total moulds and yeast in 8 samples and *Ecsherichia coli* in 5 samples. Coagulase-positive *staphylococci*, *Salmonella* species and *Proteus* species were not detected in analyzed samples. Sulfite reducing *Clostridia* were detected in 79 samples (43,37%), but their number was below the limits prescribed by the Regulation, ranging from 10-800 in 1 g.
The aim of this study was to examine the microbiological safety of animal feed from region Vojvodina in 2009.
Keywords*: animal feed, microbiological safety*

INTRODUCTION

Feed hygiene plays an important role in the safety of foods of animal origin [3]. For the effective prevention of the animal and public health, feed safety must be ensured at all stages, including primary production. To this effect, feed safety and wholesomeness are controlled and monitored for years all over the world. Animal feed is exposed to various biological, chemical, physical and other agents. All these factors may adversely affect animal and, indirectly, human health [2], [4], [9].
The main preoccupation of feed industry nowadays is the production of safe and hygienic correct animal feed. It is conditioned by the quality of raw materials, applied technological procedures and stability of the product in the storage conditions [1].
Animal feeds can serve as a carrier for a wide range of microbial contaminants such as bacteria, yeasts, moulds and their toxic metabolites **[**6**].** Feed ingredients and complete feeds contain a wide variety of bacterial species, some of which are pathogenic to humans and animals. Examples of pathogenic species found in feed are *Salmonella*, *E.coli*, *Staphylococcus*, *Streptococcus*, *Pasteurella*, *Pseudomonas*, and *Clostridia*. Other bacteria present such as *Proteus*, *Klebsiella* and *Citrobacte*r may not be recognised as pathogens, but can give rise to sub-clinical effects when presented to the animal in sufficiently high levels in feed.

Feed ingredients can become contaminated at any time during growing, harvesting, processing, storage and delivery to the feed mill. Contamination can occur through direct or indirect contact with the environment, or through cross-contamination with already contaminated ingredients. The primary sources of direct environmental contamination are soil, rodents, wild birds, predators, insects and dust. Indirect contact is through contaminated water, sewage or animal manure used in the fertilisation of crops [5].
As the micro flora found in feed materials comes from a variety of ecological niches, it has to adapt to the conditions in feed and feed components in order to survive and/or grow. The microbial diversity in different feed types is, therefore, dependant on water activity, oxygen tension, pH and nutrient composition of the feed material. Microbial growth is dependant on the moisture content of the feed material. Whereas moulds have adapted to conditions without free water and can actively grow in stored grains, the majority of bacteria must exercise strategies to survive until there is sufficient water to support microbial activity [5].
Though it is not possible to produce microbial-free feed without adversely affecting its nutritional values, microbial feed safety hazards can be minimized through appropriate agricultural and storage practices. Taking into consideration that feed hygiene quality vary among regions and countries because of different environmental and other conditions during animal feed production and processing, objective of this study is to show hygienic wholesomeness and safety of animal feed manufactured in region of Vojvodina.

MATERIALS AND METHODS

In order to evaluate microbiological quality and safety of feedstuffs, 160 samples were analyzed. Out of a total of 160 analyzed feed samples, 105 samples were complete feedingstuffs and 55 samples were animal and plant origin feed (wheat, wheat feed flour, oilseeds, fish meal, meat-bone meal). The analyses were conducted according to current Regulation [7] on the following microbiological parameters:

- ✓ *Salmonella* sp
- ✓ coagulase-positive staphylococci
- ✓ sulfite reducing *Clostridia*
- ✓ *Proteus* sp
- ✓ *Escherichia coli*
- ✓ Total number of bacteria
- ✓ Total number of moulds and yeast

Determining of above mentioned microbiological parameters have been done according to the methods of isolation and determination under the applicable Regulations [8].

RESULTS AND DISCUSSION

The purpose of this paper was to examine the basic hygienic indicators of complete feedstuffs and animal and plant origin feed (total number of bacteria in 1g, total number of moulds and yeast in 1g**,** sulfite reducing *Clostridia* in 1g, and presence of *Salmonella*

sp., coagulase-positive *staphylococci*, *Proteus* sp. and *Escherichia coli* in 50g). Microbiological safety of analyzed products was evaluated in accordance with the regulations of Serbia [7]. These parameters are presented in Tables 1 and 2.

Table 1. Maximum allowed number of saprophitic microorganisms in animal feed[*]

Animal feed	Number of bacteria in 1 g	Number of moulds in1 g
Plant origin feed	100.000.000	300.000
Animal origin feed	50.000.000	10.000
Complete feedingstuffs for young animals	10.000.000	50.000
Complete feedingstuffs for adult animals	10.000.000	300.000

[*] Regulation

Table 2. Maximum alowed number of pathogenic microorganisms in animal feed[*]

Microorganisms	Animal feed	Alowed number
Pathogen microorganisms	Feedstuffs and complete feedingstuffs	0 in 50 g
Salmonella sp.	Feedstuffs and complete feedingstuffs	0 in50 g
Sulfite reducing *Clostridia*	Feedstuffs and complete feedingstuffs	1000 in 1 g

[*] Regulation

The results obtained in this study are shown in Tables 3, 4, 5 and 6 where they presented microbiological characteristics of tested samples.

Table 3. Microbiological quality of feedstuffs of plant origin – wheat and wheat products

Data on samples		Microbiological parameters				Number of valid samples	Number of non-valid samples
Feedstuffs (number of samples)		Number of bacteria in 1 g	Number of moulds and yeasts in1 g	SRC in 1g	Presence of pathogen microorganisms in 50g		
Wheat meal (12)	IC	$1x10^4$ - $2x10^6$	$1x10^2$ - $1x10^4$	SRC/2	-[a]	12	0
Wheat (1)	IC	$4x10^4$	$5x10^2$	SRC	-[a]	1	0
Wheat fracture (3)	IC	$6x10^4$ – $8x10^5$	$17x10^2$ - $25x10^2$	-[a]	-[a]	3	0
Barley (1)	IC	$3x10^4$	$3.2x10^3$	SRC	-[a]	1	0
Corn meal (4)	IC	$2x10^4$ – $4x10^6$	$4x10^3$ – $5x10^4$	SRC/2	-[a]	4	0
Corn grits (1)	IC	$2x10^4$	$9x10^4$	SRC	-[a]	1	0
Milled corn clip (2)	IC	$1x10^5$ - $2x10^5$	$4x10^4$ - $13x10^4$	-[a]	-[a]	2	0
Extruded corn (1)	IC	$1x10^6$	$1.6x10^5$	-[a]	-[a]	1	0
Corn Gluten (1)	IC	$3.7x10^5$	$8.5x10^4$	-[a]	-[a]	1	0
Whole corn plant (1)	IC	$3x10^7$	$16x10^4$	-[a]	-[a]	1	0
Silage corn plant (2)	IC	$1x10^4$- $1x10^5$	$3x10^2$- $13x10^4$	-[a]	-[a]	2	0

Legend: IC-interval of contamination; SRC-Sulfite reducing *Clostridia* / No- number of positive samples, -[a] - was not detected

Table 4. Microbiological quality of feedstuffs of plant origin – oilseeds

Data on samples		Microbiological parameters				Number of valid samples	Number of non-valid samples
Feedstuffs (number of samples)		Number of bacteria in 1 g	Number of moulds and yeasts in1 g	SRC in 1g	Presence of pathogen microorganisms in 50g		
Sunflower meal (6)	IC	$2x10^4$ - $1x10^5$	$5x10^2$ - $4x10^3$	SRC/4	-[a]	6	0
Soybean meal (2)	IC	$5x10^3$ - $15x10^6$	$1x10^3$ – $4x10^3$	SRC	-[a]	2	0
Full fat soy grits (1)	IC	$2.5x10^3$	-[a]	-[a]	-[a]	1	0
Rape seed cake (1)	IC	$2x10^4$	$1x10^4$	SRC	-[a]	1	0

Legend: IC-interval of contamination; SRC/No -Sulfite reducing *Clostridia*/number of positive samples; -[a] was not detected

Table 5. Microbiological quality of feedstuffs

Data on samples		Microbiological parameters				Number of valid samples	Number of non-valid samples
Feedstuffs (number of samples)		Number of bacteria in 1 g	Number of moulds and yeasts in1 g	SRC in 1g	Presence of pathogen microorganisms in 50g		
Pellets noodle (5)	IC	20-250	0-10	-[a]	-[a]	5	0
Brewer's spent grain (1)	IC	$3x10^7$	$5x10^5$	SRC	-[a]	0	1
Yeast (2)	IC	$7x10^2$- $1x10^3$	0-100	SRC/1	-[a]	2	0
Alfalfa seed (1)	IC	$1x10^6$	$3.9x10^3$	-[a]	-[a]	1	0
Fish meal (1)	IC	$4x10^4$	$4x10^2$	SRC	-[a]	1	0
Meat and bone meal(1)	IC	$3x10^3$	100	SRC	-[a]	1	0
Pelleted food for dogs (1)	IC	20	-[a]	-[a]	-[a]	1	0
Concentrate (3)	IC	$2.5x10^5$- $1.5x10^7$	$2x10^4$- $2.5x10^5$	SRC/3	-[a]	3	0
Protein substrates for compound feed(1)	IC	$1.5x10^6$	$1.8x10^4$	-[a]	-[a]	1	0

Legend: IC-interval of contamination; SRC/No- Sulfite reducing *Clostridia* - number of positive samples; -[a] was not detected

As can be seen in Tables 3, 4 and 5, 54 of feed samples examined were of good microbiological quality. One sample did not meet the Code criteria [7] due to increased total mould and yeast in a 1 g sample, which was $5x10^5$. Allowable number of moulds and yeasts according to Regulation [7] is $3x10^5$.
The total number of bacteria ranged from 20 to $5x10^7$ per g of sample, which fits the criteria allowed by the Regulations [7]. From a total of 55 samples analyzed, 22 samples were positive on sulfite-reducing *Clostridia*, whose maximum values were up to 100 in 1 g sample. The results match the specified criteria Ordinance [7]
Salmonella species, coagulasa positive *staphylococci*, *Proteus* species and *Escherichia coli* were not isolated from any sample.

Table 6. Microbiological quality of complete feedindstuffs

Data on samples		Microbiological parameters				Number of valid samples	Number of non-valid samples
Feedstuffs (number of samples)		Number of bacteria in 1 g	Number of moulds and yeasts in1 g	SRC in 1g	Presence of pathogen microorganisms in 50g		
Complete feeding-stuffs for young animals (46)	IC	$3x10^2$-$2x10^6$	0-$8x10^5$	SRC/23	*Escherichia coli*/1	39	7
Complete feeding-stuffs for adult animals (59)	IC	$8x10^2$-$8x10^6$	10-$4x10^5$	SRC/34	*Escherichia coli*/4	54	5

Legend: IC-interval of contamination; SRC/No-Sulfite reducing *Clostridia* - number of positive samples; -[a] - was not detected

Table 6 presents the results of microbiological safety of complete feedingstuffs. We analyzed 105 samples (46 samples of complete feedingstuffs for young animals and 59 samples of complete feedingstuffs for adult animals). In total 12 of the 105 tested samples were microbiological unsafety, because of the presence of *Escherichia coli* and an increased number of mold and yeast.
From a total of 46 analyzed samples of complete feedingstuffs for young animals, 7 samples were contaminated. In one sample has been showed the presence of *Escherichia coli.* Increased total number of moulds and yeasts was detected in 6 samples and ranged in range from $6x10^4$ to $8x10^5$.
From a total of 59 samples analyzed complete feedingstuffs for adult animals, there were 54 samples correctly. The 5 tested samples of complete feedingstuffs for adult animals were microbiological unsafety. The presence of *Escherichia coli* were detected in 4 samples. Increased total number of moulds and yeasts was detected in one tested sample.
The total number of bacteria ranged from $3x10^2$ to $8x10^6$, which fits the criteria allowed by the Regulations [7].
The 57 analyzed samples were positive on sulfite reducing *Clostridia*, whose maximum number were up to 800 in 1 g sample. The results match the specified criteria Ordinance [7].

Salmonella species, coagulasa positive *Staphylococci*, *Proteus* species and *Escherichia coli* were not detected in examined samples.
Results on the microbiological investigation presented in tables 3, 4, 5 and 6 show that all samples meet the Code criteria [7] in terms of total number of bacteria.
Animal feed is a good medium for many microorganisms. Contamination of feed can be in the production, processing, storage, transport and use. Feed may be contaminated by various pathogenic and saprophytic microorganisms. For pathogenic microorganisms feed is the transmitter to the animal organism. That path is conveyed through numerous causes of infectious animal diseases.Therefore, the feed should not contain pathogenic micro-organisms.
The emergence of some toxicoinfections not conditioned only by the presence of certain microorganisms. It is necessary that they are in adequate number to overcome the defensive activity of the organism and produce enough toxin. Therefore, the presence of sulfite reducing *Clostridia* limited to 1000 in 1g. The values in this range are acceptable.
Development and reproduction of microorganisms in animal feed depends on many factors: moisture, temperature, redox potential, pH, type of food, chemical composition, impurities, storage time and degradation products of food. Aqueous nutrient substrate are more favorable than those with lower moisture content. Small ground food is a better medium for microorganisms of granular and coarse fodder.
According to the results shown in Tables 3, 4, 5 and 6 a large number of contaminated samples were in the category of complete feedingstuffs. Complete feedingstuffs consisting of several different components. All components are ground finer than the whole grain of plants from which they originate. Grinding increases the surface area available microorganisms. This can be explained that from 13 unsatisfactory samples; 12 from the category of complete feedingstuffs. Also, samples positive on *Escherichia coli* were from the categories of complete feedingstuffs. *Escherichia coli* is widespread in nature. It is an indicator of fecal contamination and thus points to the lack of hygienic measures. There are pathogenic strains of *Escherichia coli* that can cause a variety of enteritis.
The feed may contain a number of saprophytic microorganisms. They its enzymes break down food nutrients. The food is of poor quality. It is therefore necessary to determine the maximum allowable number of saprophytic microorganisms present. If you are saprophytic organisms present in larger numbers than allowed will cause spoilage of the feed.

CONCLUSIONS

- ✓ The study was conducted on 160 samples of animal feed from region Vojvodina
- ✓ The 105 samples complete feedingstuffs and 55 samples feedstuffs were analyzed
- ✓ From a total of 160 analyzed samples, 13 samples were found to be unsafe on microbiological assay.
- ✓ The reasons of contamination were the presence of *Escherichia coli* in 5 samples of complete feedingstuffs and increased the total number of moulds and yeasts in 8 samples.

- ✓ Sulfite reducing *Clostridia* were isolated in 79 samples and the determined number of this bacteria was within the allowed range.
- ✓ *Salmonella* species, coagulasa positive *Staphylococci*, *Proteus* species and *Escherichia coli* were not detected in examined samples.
- ✓ Out of 13 unsatisfactory samples, 12 were from the category of complete feedingstuffs
- ✓ Food for animals is a good medium for microbial growth.
- ✓ Microbial growth and promote a number of factors such as humidity, temperature, method of manufacture, composition of food, storage.
- ✓ Reduction and the formation of compounds composed of many different components, creates conditions for microbial growth.
- ✓ Size reduction increases the surface area available microorganisms.
- ✓ Thus mixtures that are finely ground have a greater risk to the safety of products
- ✓ In practice, it is not possible to produce food for animals without microorganisms, and not to violate the nutritional properties of food
- ✓ Control of production, storage, distribution and use of contamination can be reduced to a minimum
- ✓ The number of unsatisfactory samples in comparison to the number of analyzed samples indicates the existence of good hygiene and good manufacturing practices in the production of feed

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STABILITY OF MICROTRACER® IN THERMAL FEED PROCESSING

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ABSTRACT

Inert markers are usually used to test completeness of mix, adequacy of batch to batch cleanout of feed manufacturing equipment, etc. One more, but not less important role, is marking feed for recognizing it on market. The aim of this study was to determine stability of diferent type of microtracer markers depending on temperature and moisture content in order to determine initial concentration of tracers. Additionally, using microtracers for tagging feed after processing, such as pelleting, extrusion and expansion was evaluated and its effectiveness in mentioned processes was compared. Two different tracers were added in each mash: F-red and F-blue lake. Conditioning of material caused reduction of number of both tracers' particle, but higher moisture content lead to higher values of F-blue lake/F-red ratio. There has been a similarity between reduction of number of particles in expansion and pelleting processes, which leads to conclusion that moisture content of 16.20% and 17.26% caused very similar mechanism of water effect on tracers' color. Critical moisture content for F-blue lake was about 17.3%, after which its resistance to water became stabile. In all experiments, it was not noticed any dependence between tracers' particle number and temperature of processed material.
***Keywords**: tracers, moisture content, processing, reduction of tracers' particle number*

INTRODUCTION

Inert markers are usually used to test completeness of mix, adequacy of batch to batch cleanout of feed manufacturing equipment, to code the presence (absence) of critical microingredients in feeds and to identify feed additives and feeds containing such additives as propriety. One more, but not less important role, is marking feed for recognizing it on market. External markers, indigestible compounds added to the diets, for example, polyethylene, metallic iron powder or celite, a form of diatomaceous silica, can be used in that purpose [1, 3, 10].

The validity of using inert markers in tagging feed mixtures on market depend on the assumption that it does not interfere with other components from diet, is not absorbed, has the same rate of passage and good stability through all production processes and can be analyzed after taking samples. The location of tracer addition to a mixer can be critical. While most feed manufacturers would first replicate their current production procedures, they often will also test alternate premix addition locations that may yield better mixing [4, 6, 7]. Feed components, together with tracers, are exposed to activity of harsh conditions during processing, such as high temperature and pressure, high moisture content and mechanical forces. Therefore, it is very important to choose

appropriate tracer, which will be damaged in insignificant rate, or not damaged at all. It is also of great importance to add sufficient amount of tracer, considering the possibility of loosing it in production processes [2, 3].
Markers, requiring detailed analytical techniques to quantify, require the services of analytical laboratories and there is often a time lag between submission of samples and receipt of results as well as significant analytical costs. Microtracers® San Francisco, CA has developed a dye impregnated marker particle of iron and steel with magnetic properties as an external marker to test for homogeneity of mixtures and as a tracer for microadditive inclusions, but these markers can be also used for tracing feed and certification of its producers. This compound is considered harmless and rapid analysis with simple equipment can be carried out to provide instantaneous quantitative analysis [5, 8].
The aim of this study was to determine stability of diferent type of microtracer markers depending on temperature and moisture content in order to determine initial concentration tracers. Additionally, using microtracers for tagging feed after processing, such as pelleting, extrusion and expansion was evaluated and its effectiveness in mentioned processes was compared.

MATERIAL AND METHODS

Feed mash for cattle, consisted of the milled corn, soybean grits, sunflower meal and corn-flour as the main components, was processed in three different ways. The mash was mixed and treated with hot steam in the double shaft paddle mixer-conditioner (Muyang Group, China) with capacity of 100 kg per batch, in each trial, in first step, untill reaching targeted moisture content, most suitable for further processes. Moisture contents of the conditioned mash in first, second and third trial were 17.26%, 23.22%, and 16.20%, respectively. After conditioning in first trial, mash was pelleted on flat die pellet press (P = 3 kW) with capacity of 50 kg/h (Amandus Kahl GmbH & Co. KG, Germany) at a temperature of 71 °C, in the second was extruded on single screw extruder/expander with capacity of 100 kg/h, motor (11kW), dosing system (0,37 kW) and hydraulic pump (2,2 kW) at a temperature of 87.7 °C at the end of the barrel, and in the third was expanded on the same equipment as in the second at temperatures of 134.9 °C at the end of the barrel.
Two different tracers were added in each mash in the same amount of 1.25 g as markers for tagging and recognizing the feed: microtracer F-red Colored Iron grit, 25.000 particles per gram) and microtracer F-blue lake (Colored Reduced iron powder 25.000 particles per gram), by producer Micro Tracers, Inc., San Francisco, USA. Each tracer has declared number of particles in it, so it is possibble to count expected number of particles in sample. Twenty five samples (about 60 g) for analyzing were taken successively in all trials; ten before, ten after conditioning, during unloading process, and five after pelleting, extrusion, and expansion. Totally, it was taken seventy five samples.The results were reddened on 100g, due to the possibility of comparing. Previously mixed tracers were separated from analyzed samples by using "***Rotary detector***", which is actually magnetic separator. Separated tracers were placed into a small measuring container, demagnetized, cleaned from other alloy and carefully moved onto a surface of filter paper. Its color was developed in contact with 7% solution of

Na_2CO_3 and after that, the number of particles was counted. Granules formed by pelleting, expansion and extrusion processes were grinded before separation of tracers on laboratory disc mill, GlenMills Inc., Clif-ton, NJ, SAD, model S.500 (capacity of 1kg/min.) in order to liberate iron particles for simplifying further analysis.
Statistical analysis of data was performed with Statistical Analysis System (Statistical, Tulsa, Oklahoma, USA) [9].

RESULTS AND DISCUSSION

Number of particles of both tracers decreased after conditioning in first trial, as it is shown in Table 1 and on Figure 1. F-red tracer's particle number decreased rapidly, due to the fact that its color is soluble in water.

Table 1. Tracers' particle number in separated steps of pelleting process

	Average number of F-blue lake particles per 100g of samples	Average number of F-red particles per 100g of samples	F-blue lake particles/ F-red particles
dry material	74.79	132.33	0.58
conditioned material	61.36	67.54	0.90
pelleted material	34.62	8.25	4.19

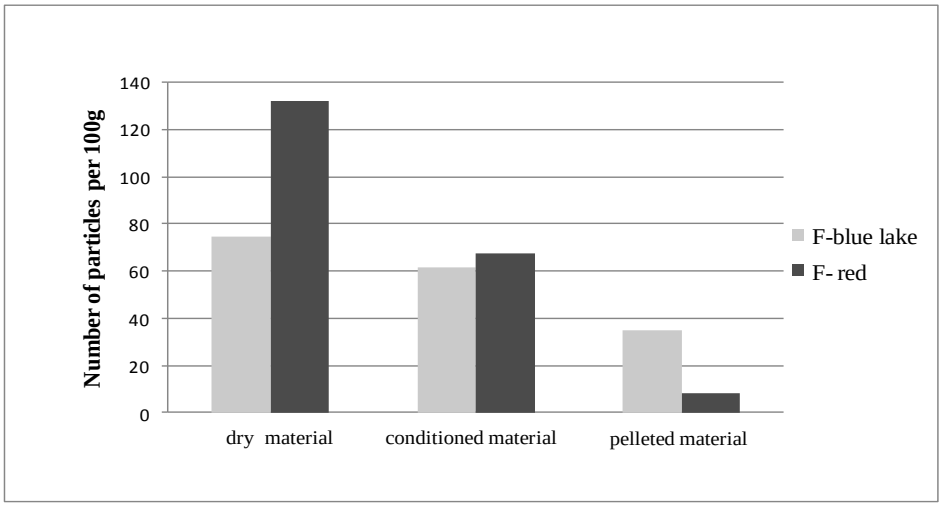

Figure 1. Tracers' particle number in separated steps of pelleting process

Pelleting process continued to reduce number of particles (from 61.36 to 34.62 for F-blue lake and from 67.54 to 8.25 for F-red), but reduction was significantly higher ($p < 0.05$) for F-red tracer, than for F-blue lake tracer, which was shown with ratio F-blue lake/F-red particles number. Milling of pellets did not influence on reduction of F-red tracer particle number, because in that case significant reduction would be present in analysis of F-blue lake tracer. It can be concluded that F-blue lake tracer is more resistant to higher temperature and high moisture content than F-red tracer, especially if moisture influence last for longer period.
Similar results were gained in second trial. Number of particles of both tracers was reduced after conditioning, much more for F-red than for F-blue lake tracer (Table 2 and Figure 2). Number of particles of F-red tracer decreased from 165.56 to 25.81 particles per 100g after conditioning, which indicates that moisture content of 23.22% significantly dissolved color of mentioned tracer.

Table 2. Tracers' particle number in separated steps of extrusion process

	Average number of F-blue lake particles per 100g of samples	Average number of F-red particles per 100g of samples	F-blue lake particles/ F-red particles
dry material	82.37	165.56	0.50
conditioned material	67.19	25.81	2.60
extruded material	65.97	13.29	4.96

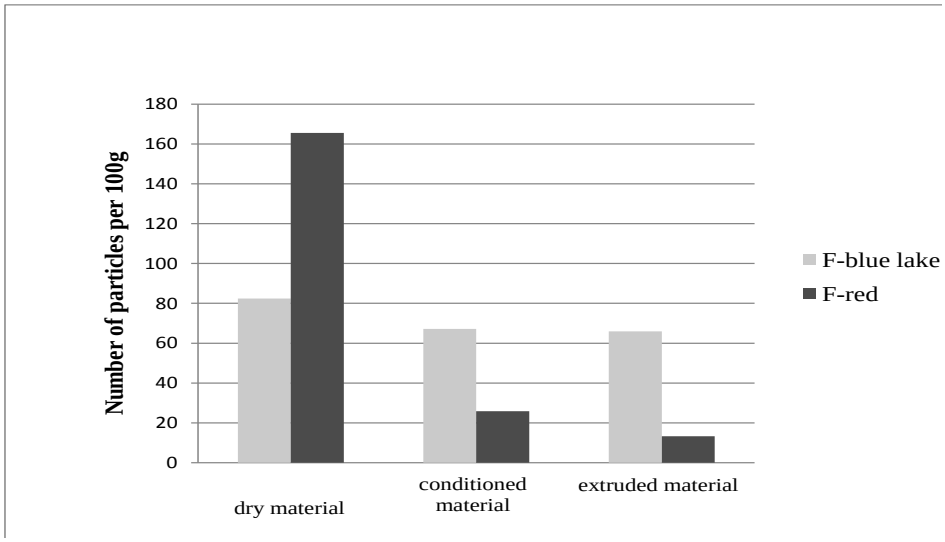

Figure 2. Tracers' particle number in separated steps of extrusion process

Number of F-blue lake tracer of particles was not significantly decreased after extrusion in comparison with conditioned material, and this lead to conclusion that conditions during extrusion did not have strong effect on this tracer.
Higher moisture content after conditioning reduced number of particles for both tracers in third trial, but as it can be seen in Table 3, and on Figure 3.

Table 3. Tracers' particle number in separated steps of expansion process

	Average number of F-blue lake particles per 100g of samples	Average number of F-red particles per 100g of samples	F-blue lake particles/ F-red particles
dry material	69.12	110.29	0.64
conditioned material	64.05	72.51	0.88
expanded material	82.10	26.98	3.04

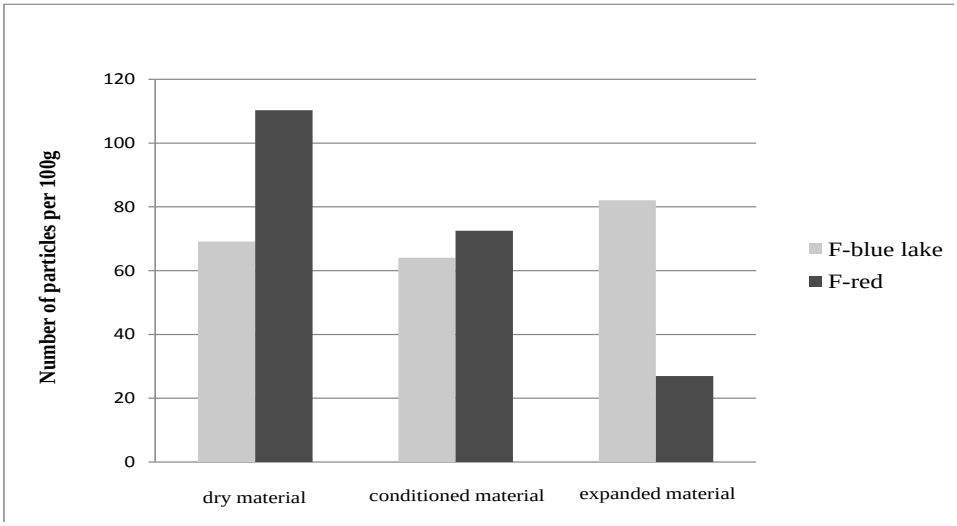

Figure 3. Tracers' particle number in separated steps of expansion process

Figure 4 shows comparison of tracers' particle number ratio during pelleting, extrusion and expansion processes. Conditioning of material caused reduction of tracers' particle number, both F-blue lake and F-red, but higher moisture content lead to higher values of F-blue lake/F-red ratio. The reason for that is stronger resistance of blue lake color to water than it is case for red color. Expanded and pelleted materials were conditioned before processing up to moisture content of 17.26% and 16.20%, respectively. Therefore, ratio between particle number of F-blue lake and F-red tracer was almost equal: 0.90 and 0.88. Extruded material was previously conditioned up to moisture

content of 23.22%, so F-blue lake/F-red ratio was higher and amounted 2.60. Mentioned facts can be graphically represented by angle between horizontal axis and tangent. Greater angle has indicated greater F-blue lake/F-red ratio.

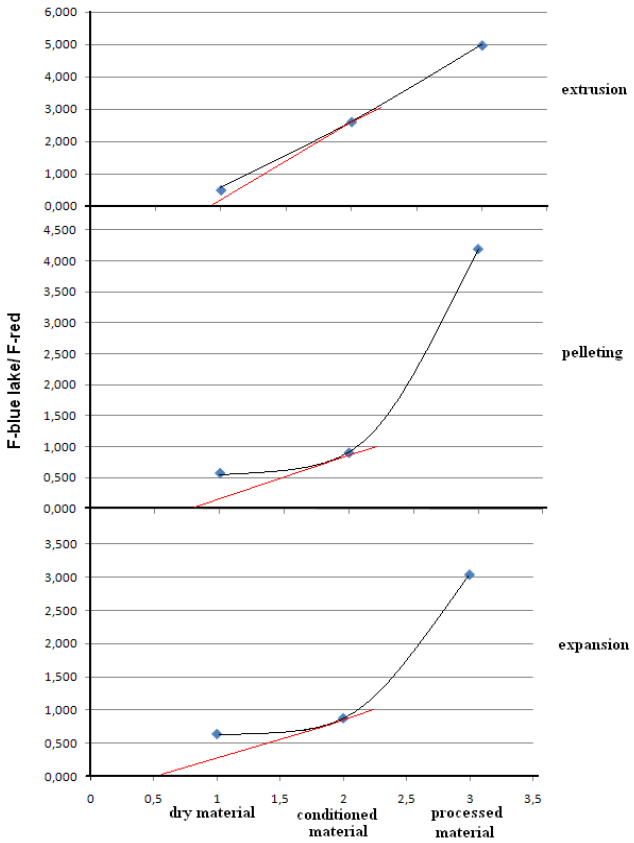

Figure 4. Comparative graph of F-blue lake and F-red particles number ratio in separated steps of used processes

It can be noticed that slope of curve after conditioning have been the highest in pelleting process, although moisture content was lower than in extrusion process. The reason for that is longer period of pause between two steps: conditioning and final processing of material, than it is case for extrusion and expansion, and therefore longer period of contact between tracers and moistened material.
If look the values of particle number reduction for F-blue lake and F-red separately in each process (Table 4 and Figure 5), there has been a similarity between reduction of tracers in expansion and pelleting processes. Lines of graphs that have represented F-blue lake and F-red tracers have had almost the same trend between points of expansion and pelleting, which lead to conclusion that moisture content of 16.20% and 17.26% caused very similar mechanism of water effect on tracers' color.

Table 4. Reduction of tracers' particle number after conditioning for different processes

		Expansion	Pelleting	Extrusion
Moisture content of conditioned material [%]		16.2	17.26	23.22
F-blue lake tracer	Average number of particles before conditioning	68.51	74.79	82.37
	Average number of particles after conditioning	64.05	61.36	67.19
	Reduction of number of particles after conditioning [%]	**6.504**	**17.96**	**18.43**
F-red tracer	Average number of particles before conditioning	110.29	132.33	165.56
	Average number of particles after conditioning	72.50	67.54	25.81
	Reduction of number of particles after conditioning [%]	**34.26**	**48.96**	**84.41**

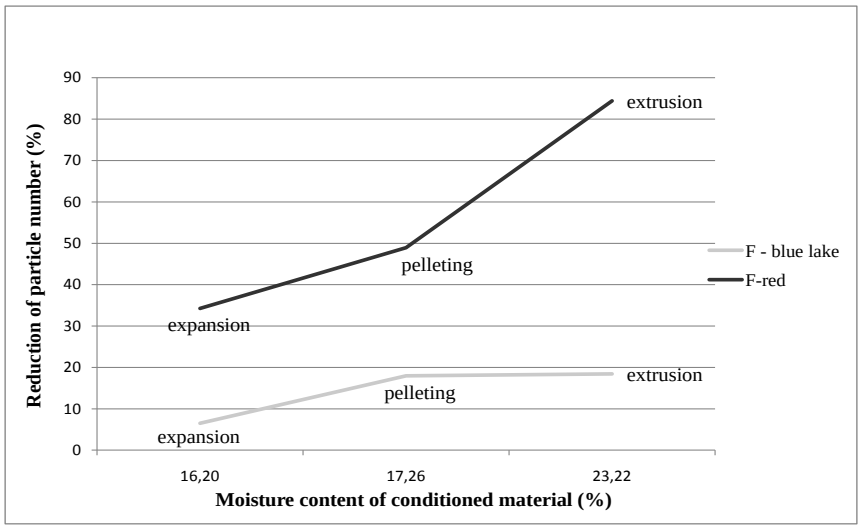

Figure 5. Reduction of tracers' particle number after conditioning for different processes

On the other hand, there was insignificant change in reduction of F-blue lake tracer at moisture content of 22.23% in comparison with content of 17.26% (from 17.96% to 18.43%), while number of F-red tracer particles reduced drastically from 48.96% to 84.41% of initial number of particles before conditioning. Critical moisture content for

F-blue lake was about 17.3%, and after that point F-blue lake particle's number reduction was constant.
In all experiments, it was not noticed any dependence between tracers' particle number and temperature of processed material.

CONCLUSIONS

Conditioning of material caused reduction of number of both tracers' particle. Higher moisture content lead to higher values of F-blue lake/F-red ratio, due to the fact that blue lake color has stronger resistance to water than it is case for red color. Period of contact between moistened material and tracers is also significant parameter. Critical moisture content for F-blue lake was about 17.3%, after that point its reduction was constant. In all experiments, it could not be noticed any dependence between tracers' particle number and temperature of processed material. F-blue lake tracer was more stabile, than F-red tracer and can be used for marking processed feed. It should be added in higher concentration than it is being added in dry material, because of its losses during processing. Authors are suggesting following amounts of F-blue lake tracer: for pelleting and extrusion about 20% more, and for expansion about 7% more than initial concentrations.

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THE INFLUENCE OF ADDING MULTIENZYME PREPARATION ROVABIO™ ON GROWTH AND INCREASE OF CARP (*Cyprinus carpio*)

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ABSTRACT

In this paper we examined the influence of multienzyme preparation Rovabio™ Excel as an additive to the feed for 1^+ year-old carps (*Cyprinus carpio*), on the product performances (growth and increase, food conversion, growth rate, mortality blood parameters).
The experiment was conducted on 336 species divided into two groups (control and experimental) with the density of 28 fish/m^3. The experimental group was fed with pellets with supplementation of Rovabio™ Excel in concentration of 500g /tonne, whereas fish in the control group consumed the same feed, but without addition of the enzyme preparation.
The analysis of the obtained results showed a simulative effect of the used additive on the analysed characteristics of the examined fish. Adding the multienzyme preparation to carp feed had a significant impact on growth and increase ($p<0,01$), feeding coefficinet ($p<0,05$), specific growth rate ($P<0,01$), protein conversion ($P<0,01$) and mortality ($P<0,01$). Statistic significance was not obtained by comparison of hematological blood parameters.
***Key words**: carp, diet, enzym, growth, blood*

INTRODUCTION

Modern concept of intensive breeding and nutrition of fish means using enzyme preparations as necessary ingredients of complete feed with the aim to fulfill maximal production criteria. The use of enzymes provides the development of new nutrition standards which primarily refers to more versatile use of feed. Their use also contributes to reduction of production costs and decrease of production of noxiuos matters (N, P and other minerals) in the environment [4].
The mechanism of enzyme activity in organisms of warm blooded animals and fish is based on their simulative polifactorial effects in the process of digestion, decomposition and metabolism of nutrients. [21]. Positive eubiotic activity of enzymes as additives to

food is seen in the following: improved digestion and absorption; using food and nutritive substances; increased immunity; decreased production losses [8,15,16].
Most authors [7,11,6] think that the most important effect of enzyme activity is breakdown of cell capsule of various herbal raw materials, when nutritive substances are released and anti-nutritive complexes decompose (non-starch polysaccharides, phitat, saponin, tanin, lektin i dr.). According to Graham [12] mixture ksilansa, amilasa i proteinasa added to a meal (broiler), based on corn and soya pellet improved the weight of chicken by 2.5% and food conversion by 3.6%.
Rovabio™ Excel is a multienzyme product gained by formation of fungus *Penicillium funiculosum*, which contains 17 components. The most important are the following: xylanasa (*endo-1,4-ß-xylanasa)*, glukanasa (*endo-1,3(4)-ß-glucanasa*), pectinasa, proteasa (*aspartic protease*) i endo-1,4-ß-mannanasa [3]. Using Rovabia resulted in significant positive experiences in nutrition of monogastric animals, primarily pigs and poultry [3,17].
Having in mind the above mentioned facts about the use of enzyme complexes, the aim of this paper is to point out the potentials of the use of enzymes in the feed for carp nutrition and their influence on aquaculture performances, with a special emphasis on the values of the rate of growth, consumption and food conversion.

MATERIAL AND METHODS

Experimental research was carried out on the fish pond "Ub", Valjevo and it lasted for 42 days. 336 one and a half year old caprs were used in the experiment. Their average weight was 70g ∀ 6g and they were divided into control group (Ko =168 fish) and experimental group (O-I =168 fish).
The dimensions of fish pools were 750x100x80 cm, with the capacity of 6 m^3 and the density of fish was 28 carps/m^3. The control group (Ko) was fed with pelleted carp feed without addition of Rovabia, whereas fish in experimental group (O-I) were fed with pellets with additive in the concentration of 0.05%.
Daily quantity of food was divided into five equal meals depending on the temperature of water and control measurements of fish increase. During blunt, and during to plant young carps were not fed. The food used was sampled and chemically analysed at the beginning of the experiment by standard methods *Wende metod* [25]. The chemical composition of the used feed is shown in table 1.

Table 1. The composition of used feed (%),VSH

No.	Raw materials used ,%	Control group	Experimental group
1.	Corn	**21,35**	**21,30**
2.	Fish flour 64%	**30**	**30**
3.	Soya pellet 44%	**13**	**13**
4.	Soya grits	**12**	**12**
5.	Cattle yeast	**5**	**5**
6.	Wheat flour	**15**	**15**
7.	Sunflower pellet 33%	**2**	**2**
8.	$CaCO_3$	**0,3**	**0,3**
9.	Mono-Ca-phosphate	**0,3**	**0,3**
10.	Ionized salt	**0,05**	**0,05**
11.	Premix	**1**	**1**
12.	Rovabio^{TM} Excel	**-**	**0,05**
13.	TOTAL:	**100**	**100**
Chemical composition of food, VSM (%)			
14.	Water	**8,80**	**8,86**
15.	Ash	**7,95**	**7,93**
16.	Proteins	**35,42**	**35,53**
17.	Cellulose	**3,33**	**3,28**
18.	Dry matter (SM)	**91,20**	**91,14**
19.	Calcium, Ca	**1,578**	**1,582**
20.	Total phosphorous, P	**1,16**	**1,16**
21.	Usvojivi phosphorous, P	**0,82**	**0,82**
22.	ME MJ/kg	**13,10**	**13,10**

Premix composition: Vitamin A (IU/kg) 1.250.000,Vitamin D_3 (IU/kg) 200.000, Vitamin E (IU/kg) 20.000, Biotin (mg/kg) 45, Folic acid (mg/kg) 18, Niacin 35.000, Pantothenic acid 20.000, Vitamin B_6 (mg/kg) 5000, Vitamin B_2 (mg/kg) 8000, Vitamin B_1 (mg/kg) 7000, Vitamin B_6 (mcg/kg) 1500, Fe (mg/kg) 40000, Mn (mg/kg) 10000, Cu (mg/kg) 3500, Zn (mg/kg) 40000, J (mg/kg) 1200, Co (mg/kg) 25, Se (mg/kg) 300.

Physicochemical analysis of water parameters included sampling once a week, and the concentration of dissolved O_2 and water temperature were measured every day. The examined parameters of fish pond water were analysed by standard methods [26].
Hemaethological analysis of basic blood parameters was carried out at the end of the experiment by standard methods of examining blood elements [24]. The following parameters were determined: concentrations of leukocytes (Le), erythtrocytes (Er), hemoglobin (Hb), thrombocytes (Tr) and hematocrit (Hct).
The determination of the following parameters was used as a criterion for evaluation of production results of fish: specific rate of growth (SGR), weight increase (P), food

consumption and conversion (FCR), food protein conversion (PER), mortality and economic justification of the use of Rovabia additive.
SGR, FCR, PER, SFR were calculated according to Hámačkov formulae [14]:

$$\mathbf{SGR} = \frac{\mathrm{LnWt\ \ LnWo}}{\mathrm{t\ x\ 100}}$$

LnWt = natural logarithm of final individual mass; LnWo= natural logarithm of initial individual mass; t = the lenght of experiment (days)

$$\mathbf{SGR} = \frac{\mathrm{F}}{\mathrm{Wt - Wo}}$$

F = food spent; Wt = final fish mass; W_0 = initial fish mass

PER = weight increase / proteins spent
SFR ($\%\cdot dan\cdot ind^{-1}$) = SGR x FCR

Stastical data processing was performed by SPSS program.

RESULTS AND DISCUSSION

The results referring to physical and chemical quality of water are shown in Table 2. The data presented in the table show that the values of basic parameters of water were within the optimal intervals for ciprinidae water [2,1,23,13] and they fulfilled all the necessary criteria for successful fish fattening.

Table 2. Physicochemical analysis of water parameters during the experiment

Parameters of fish pond water	WEEKS						
	I	II	III	IV	V	VI	$\bar{x}$
pH	7,20	7,18	7,30	7,52	7,65	8,00	**7,47**
$KMnO_4$ ($mg\cdot l^{-1}$)	13,60	16,20	22,50	23,40	25,80	31,20	**22,11**
$N\text{-}NH_4$ ($mg\cdot l^{-1}$)	0,28	0,31	0,38	0,42	0,39	0,40	**0,36**
NO_3 ($mg\cdot l^{-1}$)	0,18	0,21	0,26	0,33	0,32	0,31	**0,26**
NO_2 ($mg\cdot l^{-1}$)	0,019	0,028	0,030	0,036	0,042	0,075	**0,038**
$\bar{x}$ O_2 ($mg\cdot l^{-1}$)	6,08	6,35	5,60	5,26	5,15	6,20	**5,77**
$\bar{x}$ temperature C^o	25,30	26,20	27,10	27,70	27,30	26,80	**26,73**

Water temperature varied insignificantly during the research, and the determined values were in accordance with air temperature fluctuations. The maximum temperature was detected during the 4th week of the experiment (27.70 ^{0}C), and the minimum temperature of (25.30 ^{0}C) in the 1st week of the experiment. The pH je was in the interval from neutral to moderately basal (7.18-8.00). Concentrations of other parameters of fish pond water varied within optimal limits for carp waters [1] in the following way: concentrations of dissolved O_2 changed from 5.15 to 6.35 $mg\cdot l^{-1}$, ammonium ion (N-

NH_4) from 0.28 to 0.42 mg·l^{-1}, nitrate from 0.18 to 0.33 mg·l^{-1} and nitrite (NO_2) from 0.019 to 0.075 mg·l^{-1}.
According to the composition shown in the Table 1, it can be concluded that the food used in the experiment contained all the necessary nutritive substances and that the pellets were more or less of the same chemical composition, which is completely appropriate for nutritive needs of young carps [20,22].
Comparative analysis of the results of individual and total ihtiomass (Table 3) shows that both fish groups had approximately equal weight on to plant. However, dynamics of growth and increase of biomass during the further phases of the experiment shows a progressive character of O-I carp group (14.52%) in comparison with Ko fish group. Feord [9] found a significant increase of final body mass of *tilapiae* fed with feeds that contained enzyme additives based on herbal components.

Table 3. Individual and total ihtiomass

Experimental groups	**to plant**			**stocking**		
	n	**x (g·ind^{-1})**	**Σ (g)**	**n**	**x (g·ind^{-1})**	**Σ (g)**
Ko	168	70,4	11827,2	142	101,9	14469,8
O-I	168	70,5	11844,0	150	116,7	17505,1

At the end of the research (Table 4) it was noted that fish fed with feed that contained Rovabia achieved a higher increase of weight than carp from Ko group by 14,7 g·ind^{-1} , i.e. 0,35 g·ind·dan^{-1} or, in absolute values by 31,81 %. Statistical analysis showed highly significant differences in the weight increase between control and experimental group (P>0,01).
Food conversion, as interaction of increase and consumption is one of the most reliable indicators of successfulness of production and acquisition of food in fishery. Analysing this production parameter (table 4) showed that the best food conversion was in O-I carp group (the one with addition of 0.05 % rovabia in food). In comparison with Ko fish group, experimental 0-I group of young carps achieved a better feeding coefficient (FCR) by 0,79 g·g^{-1} or 31,72%. Statistical processing of the results of feeding coefficient showed significant differences between the experimental (O-I) and control (Ko) group (P<0,05).

Table 4. Production indicators at the end of the experiment

Experimental groups	**Increase**		**Food** g·ind^{-1}	**SFR** %·dan^{-1}	**FCR** g·g^{-1}	**SGR** %·dan^{-1}	**PER** %
	g·ind^{-1}	g·in·dan^{-1}					
Ko	31,5	0,75	78,6	1,99	2,49	0,8804	1,13
x ∀ Sd	31,50∀3,4				2,49∀2,17	0,88∀0,32	1,13∀2,41
O-I	46,2	1,10	78,6	1,89	1,70	1,99	1,65
x ∀ Sd	46,20∀3,10				1,70∀1,07	1,99∀0,37	1,65∀2,11
P	p<0.01**	p<0.01**			p<0.05*	p<0.01**	p<0.01**

When it comes to numeric value of protein usage in food (PER), we found that O-I fish group achieved better results for this parameter than Ko group by 0.46 %. PER

significance test also indicated important differences ($p<0.01$) between experimental groups, when it comes to this production parameter. The obtained results are in accordance with the results of other authors who found that addition of multienzyme preparations to fish food has a simulative effect on the increase and protein usage [27,5].
The results shown in the table 4 indicate that young carp fed with addition of enzyme achieved a significantly better rate of growth (SGR) in comparison with control group ($P<0,01$). According to the obtained results we can conclude that the results of this research on the influence of rovabia as food ingredient are in accordance with the data of other authors, who also found that the use of enzyme in fish nutrition results in higher rate of weight in the range 12.8 - 27.9% , on average [19,10,5].
Mortality of examined fish during 42 days of the experiment is shown in the table 6. Fish losses were significant, as 15.47 % of fish died (Ko-group), i.e. 10.17 % (O-I group). According to results of percentage of fish mortality in this experiment, we can conclude that the use of a rovabia as additive had a positive effect on decrease of mortality of fish, which completely justified its technological application in ciprinidae production. The determined differences of mean values of mortality between the groups examined were statistically highly important ($p<0.01$), which is accordance with the research of Zivkovic [17],Wang [28]and Adissea [3].

Table 5. Fish mortality and economic analysis of the use of Rovabia in carp

Indicators	**Ko**			**O-I**		
Mortality	**n – fish**	**%**	**index**	**n – fish**	**%**	**index**
	26	15.47	100	18	10.17	69.23
	x ∀ Sd = 26,00∀3,20 **			x ∀ Sd = 18,00∀2,85 **		
Economic analysis of rentabilty of the use of RovabioTM Excel						
Additive participation %	-			0.05		
Food value %	100			100.66		
Price of increase %	100			99.32		

Concentrations of hematological characteristics of carp blood did not show any statistical significance ($p>0.05$) and they were within the limits of normal physiological values, which is shown in the table 6.

Table 6. Numeric values of hematological parameters of carp blood at the end of the experiment

Indicators	**Ko**	**O-I**
Le (x 10^9/l)	40,56 ± 13,45	40,19 ± 11,58
Er (x 10^{12}/l)	1,73 ± 0,16	1,81 ± 0,12
Hb (g/l)	83,29 ± 6,89	81,66 ± 11,34
Hct (l/l)	0,31 ± 0,06	0,33 ± 0,04
Tr (x 10^9/l)	128,47 ± 16,88	119,14 ± 31,58

Based on the data from the table 5, we can conclude that addition of Rovabia to carp food increased economic value of food by 0.66 %, but on the other hand, the decrease of food consumption also provided the decrease of the total cost feed by 0.10 %. Since the realized value of price of fish meat increase in O-I group was 0.68% lower in comparison with Ko group, we can conclude that the use of nutritive additive rovabia was economically justified.

CONCLUSION

Based on the research on nutrition of young carp with addition of multyenzyme preparation Rovabio™ Excel as food additive (*Cyprinus carpio*) and its impact on growth and survival, the following can be concluded:
The use of additive in concentration of 0.05 % in carp food had a positive effect with statistical significance on all analysed indicators of fish growth rate ($P<0,01$) food conversion ($P<0,05$), protein usage ($P<0,01$) and mortality ($P<0,01$).
The use of rovabia was also economically justified, which again indicates the increase of profitability in aquaculture production.

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MEAT QUALITY OF TWO YEARS-OLD TENCH AND CARP GROWN IN EXTENSIVE CONDITIONS

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ABSTRAKT

The literature does not find data on meat yield of the two-year fish tench and carp, but gives data of consume fish that are age three-years and older. Also, the quality of meat depends on the age and it is necessary to accurately determine this parameter. Apart of meat yield, chemical composition of meat was analyzed in two years tench and carp with emphasis on the presence of fatty acids and the relation of these values in tench and carp was compared. The content of residual materials was analyzed also.
Keywords: *tench, carp, meat yield, unsaturated fatty acids, extensive production*

INTRODUCTION

The placement of freshwater fish produced in our ponds becomes a problem in the past two years. Import lobby "took care" that in the Serbian market can be found a large amount of cheap fish species, especially Pangasius pangasius from Vietnam, which is not present in the U.S. market, Canada and a number of EU countries.
The cost of production is higher than the carp sale prices what raises the question of the survival of our fishing industry in which mostly participate carp. It is therefore necessary to think about the introduction of other freshwater species that would have passed on the EU market, which is primarily related to the tench whose production is practically closed.
Also, it is necessary to think about organic production and extensive methods what was the recommendation at a meeting of Western European and Eastern European countries 1996th in Budapest [2]. It is necessary to take into account the nutritional quality of meat because everybody from fishing industry expect products with low fat and low ratio of unsaturated fatty acids, especially ω-3 and ω-6 fatty acids.
This paper analyzed the meat quality of two years-old tench and carp grown in the extensive system using well water.

MATERIALS AND METHODS

Samples of two-year old tench and carp were taken on experimental pond "Mošorin" in early September 2010. year. Production of these fish was held in extensive systems where the increase of organic production was done by adding burned-out beef and sheep manure. Feeding with additional nutrients was not done. In facility preparations and

during the growing season was used hydrated lime. During the meat yield determination the edible part of each fish was filleting separately. Analysis of meat was carried out from three pooled samples. The chemical composition of fish was determined using standard SRPS ISO methods: protein content (N x 6.25) was determined by Kjeldahl, on a Kjeltec Auto 1030 Analyzer (Tecator, Sweden), the water content was determined by drying at 103 ± 2 ° C to constant mass, total fat determined by fat extraction with petroleum ether, using the Soxhlet apparatus, after acid hydrolysis of the sample, the ash content was determined by measuring the mass of residue after annealing at 550 ± 25 °C.

RESULTS AND DISCUSSION

Results of the morphometric measurements of analyzed tench and carp are given in tables 1 and 2, and dressing percentage in table 3. Results of chemical composition and content of unsaturated fatty acids are presented in tables 4, 5 and 6. The content of residual substances in the meat of two years-old carp and tench is given in table 7.

Table 1. Morphometric measurements of analyzed tench

L (overall length)(cm)	19.5	18	21.2	17	18	17	17.5	17.5	16.7	17.8
l (body length)	12	11.5	13.5	10.5	11.5	11	11.5	11.2	11	11.8
lc (head length)	4.5	4.5	5	4	4.3	4	4.5	4.2	3.6	4
Width	5.5	4.2	5	4.3	4	3.8	3.8	3.8	4	4.1
m (mass)(g)	116	88	129	66	78	71	70	64	67	77

Table 2. Morphometric measurements of analyzed carp

L (overall length) (cm)	30	28	29.5	26	26
l (body length)	19.2	17	18	15.5	15.5
lc (head length)	8	8.5	8.5	8.2	7.5
Width	10.5	10.7	12	11.2	9.5
m (mass)(g)	577	531	647	490	371

Table 3. Dressing percentage of tench and carp meat

	Tench	Carp
The total weight after evisceration (g)	810.72	2308.32
Fillets (g)	496.71	1318. 83
Dressing percentage (%)	60	50.38

Table 4. The chemical composition of tench meat

Tench	Protein content (%)	Water content (%)	The total lipids (%)	Ash content (%)
1.	14.56	82.31	1.06	1.99
2.	14.55	82.15	0.92	2.03
3.	14.48	82.10	1.20	1.89

Table 5. The chemical composition of carp meat

Carp	Protein content (%)	Water content (%)	The total lipids (%)	Ash content (%)
1.	16.15	80.31	2.82	1.02
2.	15.97	80.12	2.47	1.05
3.	16.25	80.14	2.62	1.03

Table 6. Fatty acid composition (% of total fatty acids) - tench and carp

Fatty acid	Tench 1	Tench 2	Tench 3	Carp 1	Carp 2	Carp 3
C14:0	1.22	1.30	1.18	1.16	1.16	1.17
C15:0	0.91	0.95	0.99	0.55	0.53	0.53
C16:0	24.34	24.40	24.18	21.09	20.78	20.89
C16:1	5.45	5.71	5.81	5.00	5.14	5.00
C17:0	1.10	1.12	0.95	0.72	0.70	0.72
C18:0	8.31	8.02	7.94	5.61	5.14	5.61
C18:1cis-9	17.85	18.43	17.64	32.03	32.92	32.03
C18:1cis-11	4.50	4.64	4.07	4.15	4.27	4.15
C18:2n-6	8.98	8.82	8.83	13.34	13.84	13.34
C18:3n-6	0	0	0	0.17	0.21	0.17
C18:3n-3	3.77	4.06	4.11	4.61	4.71	4.61
C20:0	0.46	0.44	0.38	0.28	0.23	0.20
C20:1	0.61	0.66	0.58	1.56	1.51	1.55
C20:2	0.85	0.86	0.82	0.77	0.75	0.77
C20:3n-6	0.82	0.86	0.90	0.74	0.66	0.90
C20:3n-3	1.63	1.49	1.56	0.87	0.91	0.81
C22:1+20:4	6.81	6.43	6.51	2.44	2.82	2.23
C20:5n-3	2.62	2.63	3.15	1.11	1.21	1.06
C22:5n-3	2.13	2.17	2.64	0.97	1.09	0.90
C22:6n-3	7.64	7.01	7.76	2.27	2.71	2.05
SFA	36.34	36.23	35.62	29.43	29.03	28.63
MUFA	28.41	29.44	28.10	43.08	42.69	43.88
PUFA	35.25	34.33	36.28	27.48	28.27	27.48
n-6	17.46	16.97	17.06	17.63	17.74	17.95
n-3	17.79	17.36	19.22	9.85	10.53	9.53
n-3/n-6	1.02	1.02	1.13	0.56	0.59	0.53

Table 7. The content of residual substances in the meat of tench and carp

Testing	Units	Defined value	Tench	Carp
Cadmium	mg/kg	max. 0.100	< 0.005	< 0.005
Lead	mg/kg	max. 0.40	< 0.05	< 0.05
Arsenic	mg/kg	max. 2.00	< 0.01	< 0.01
Mercury	mg/kg	max. 0.500	0.093	0.055
Aldrin and dieldrin	mg/kg	max. 0.020	< 0.001	< 0.001
DDT (DDE + DDD + DDT)	mg/kg	max. 0.100	0.002	0.004
Endrin	mg/kg	max. 0.010	< 0.001	< 0.001
HCB	mg/kg	max. 0.020	< 0.001	< 0.001
HCH (alpha isomer)	mg/kg	max. 0.020	< 0.001	< 0.001
HCH (beta isomer)	mg/kg	max. 0.010	< 0.001	< 0.001
Heptachlor and heptachlorepoxid	mg/kg	max. 0.020	< 0.001	< 0.001
Chlordane (alpha and gamma isomer)	mg/kg	max. 0.010	< 0.001	< 0.001
Lindane	mg/kg	max. 0.010	< 0.001	< 0.001
Polychlorinated biphenyls	mg/kg	max. 3.000	< 0.001	< 0.001

Analyzing morphometric characteristics of fish was found good body form. Dressing percentage of tench is better than the same in carp what can be explained by the smaller mass of the digestive tract of this species [1]. The reason for the lower percentage of protein in the tench and carp than it was descried in the classical literature [6,4] is that it is a two-year old fish meat, while the mentioned literature data relating to older categories that have a lower water content. Higher water content in two-year fish contributes to the higher culinary quality of meat. According to fat content the tench meat is approximately to the big head carp and grass carp meat, but only with more fat than leg meat of green frogs [4], from which it can be concluded that it is very suitable as a dietary food for special people health categories. Two-year old carp have also a low percentage of fat from which it can be concluded that carp do not fall in fatty fish because it has a lower percentage of fat content than trout [7]. Production technology and type of additional feeding is the most responsible for fat percentage [8]. The ratio of unsaturated fatty acids in tench is better than that of carp and trout and in a similar level to the same in marine fish species. [5].
The content of residual substances not only to exceed the limit prescribed by law but is significantly lower than the allowable values and fits into the strict criteria related to the organic production of fish [3].

CONCLUSION

Two years-old fish meat in chemical composition has an advantage over the flesh of fish afther three and more years of farming. Extensive system has the advantage regarding meat quality, but it is necessary to analyze its economic feasibility. Meat quality of tench

has exceptional nutritional value what is the reason for its reintroduction and repopulation. Fish meat with high nutritive value and low residual activity has perspective as an export article to the European Union and other developed countries.

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INFLUENCE OF TECHNOLOGICAL PROCESS OF PRODUCTION ON MICROBIOLOGICAL SAFETY OF CHEESE

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ABSTRACT

This paper examined microbiological safety of 135 samples of cheeses. Of which there were 110 samples of semi-hard cheeses with steamed dough "pasta filata" and 25 samples of albumin cheese. Microbiological safety of analyzed samples was evaluated in accordance with the current regulations (Regulation on microbiological safety of food in trade, Official Gazette of SRJ No. 26/93, 53/95 and 46/02).
The aim of this study was to examine microbiological safety of various types of cheeses and on the basis of the results to detect possible critical control points.
Out of a total of 135 analyzed cheese samples, 37 samples (27,41%) did not comply with microbiological criteria specified in the Regulations. Non-compliance with microbiological criteria was due to the isolation of *Escherichiae coli*. Samples that did not comply with the criteria for microbiological safety were from group of semi-hard cheeses, while all analyzed samples of albumin cheese were correct. In no sample was found the presence of *Salmonella species*, *coagulase positive staphylococcus, Sulphite-reducing Clostridia* and *Proteus species.*
Keywords: *cheeses, microbiological safety, Escherichia coli*

INTRODUCTION

The production of cheese requires raw material – milk, that is of good quality and microbiological safe. The quality of cheeses is under affect of numerous factors such as: the quality of milk, heat treatment, as well as level and type of microbiological safety during production and storage of cheese. In order to get a technological good raw material, it is necessary to heat-process milk, that is to obtain raw materials without pathogenic bacteria and bacterial contaminants, which cause various disadvantages of cheese by reproducing during production.

Milk used for cheese production must be hygienically correct with the parameters [6]:

- The total number of bacteria: $<10^4$ CFU / ml,
- Somatic cells: $<10^5$/ml,
- pathogenic bacteria: absent,
- Antibiotics/inhibitors: absent.

One of the most common problems in the production of cheese is the possibility of production coliform bacteria in some stages of technological process. With their presence a product is marked as defective. The main representative is *E. coli*, which in addition to indicating the lack of food hygiene can cause alimentary infection and intoxication in humans. *E. coli* is the most common cause of the defect cheeses. Heat treatment inhibits large populations of *E. coli*, however, the proceedings after the heat treatment, very often leads to recontamination. Most of the starter micro-organisms leads to a decrease in the number of *E. coli*, and in the early hours of processing of these bacteria, depending on the starter, is decreasing. Furthermore, after the adaptation period, the bacteria may multiply if you do not maintain hygienic and safe conditions [7].
The presence of coliform bacteria in the milk can be minimized with good hygienic conditions. Also rapidly growing lactic acid bacteria used in cheese production cause rapid reduction in the number of coliform bacteria. Enteropathogenic *E. coli* can produce enterotoxins some of which are thermally stable. Many species and strains of *E. coli* 0157 H7 occurs frequently in raw milk and it is very importante because it is fairly tolerant of acidic environment and can grow at refrigerator temperatures [2].
Cheeses such as "paste filata" are very prevalent in the market and here are usually made from cow's milk. The technological process of producing semi-hard cheese with steamed dough such as "pasta filata" is characterized by two phases:

- Production of curd and cheddaring,
- Heat treatment "steaming" of mature curd in hot water (saline solution) and formatting.

The process begins by speeding up the curdling of casein and reproduction of starter culture of microorganisms which leads to the formation of curd. From curd, through the technological process of separation of whey, draining cheese curd, cutting and cheddaring (ripening to pH = 5.2 to 5.3), cutting curd pieces (to separate left whey) and steaming at the temperature 70 to 80°C to which was added 0.5 kg salt/100 liters of water, gets stretched dough, shiny looking, pleasant taste and smell. The most important operation comes after steaming, and that is kneading of dough, followed by shaping and molding of cheese, self-pressing and finally aging and nurturing of cheese 2 to 3 months (15-20°C and relative humidity 75-80%) [3].
Analyzing the specified technology process, from the point of this study special emphasis is placed on the stage which follows the heat treatment of steaming dough at temperature till 80°C. Steamed dough through whole process of further manufacturing, is located at temperatures that contribute to the growth, both targeted and non-targeted microorganisms, since the final processing of steaming dough as well as fostering cheeses during ripening in chambers are done manually in all points. Technological errors and hygiene conditions in these stages has consequences on the microbiological picture of the final product. After the first phase of production of curd whey is obtained. Whey is the most common byproduct in the production of cheese. It contains large portions of nutrients, especially proteins (lactoalbumine and lactoglobuline) which contain the essential amino acids, nutritionally valuable and more digestible [1].

Denaturation of whey proteins, is applied in the production of albumin cheese, which is based on whey warming, and at temperatures of 90°C starts flaky extract 20-25% of protein. In order to make thermal denaturation of proteins the funds for the acidification of whey are added, which at pH 4.4 - 4.6 cause complete denaturation of proteins and the curd is obtained [1]. Albumin cheese whey, is mainly sweet cheese, soft consistency, white to grayish in color with the taste of cooked whey proteins. The technological process is simple and implies a high temperature heat treatment (90-95°C, 3-4min), coagulation and isolation of whey protein, squeezing cheese (curd, 4-6 hours), packaging and distribution [1].
The mentioned technological process of production of semi-hard cheese of steaming dough and albumin cheese allow maximum utilization of milk and whey byproduct. Good microbiological quality of the complete technological process of semi-hard "paste filata " and albumin cheese, you can get with good quality raw material, properly guided technological process of production, adequate hygiene and sanitation in the production process, and above all, good manufacturing and hygienic practices. Set up of control points at all stages of production that will permanently perform microbiological control is of particular importance, and that include control of:

- Raw milk
- Heat-treated milk (pasteurization),
- Control of curd
- Control of fresh whey
- Control of heat-treated whey (curd)
- Control of final products (semi-hard cheese, and albumin).

MATERIAL AND METHODS

In this study we analyzed 135 samples of cheese. There were 110 samples of semi-hard cheeses such as “pasta filata” and 25 samples of albumin cheese. The experiments were conducted according to the current Regulations [5] to following microbiological parameters:

- ✓ *Salmonella spp*
- ✓ *Coagulase positive staphylococcus*
- ✓ *Sulphite reducing Clostridia*
- ✓ *Proteus spp*
- ✓ *Escherichia coli*

In determining these parameters the methods which are used for isolation and determination are under the current Regulations [4].

RESULTS AND DISCUSSION

The obtained results are presented in Tables 1 and 2, which shows the number of analyzed samples, and the number of correct and incorrect samples.

Table 1 shows the results of microbiological safety of analyzed samples semi-hard cheeses.

Table 1. Microbiological safety of semi-hard cheeses

Number of samples	110	*Salmonella spp*. in 25 g	*Coagulase positive staphylococci* in 0,01 g	*Sulphite reducung Clostridia* in 0,01g	*Proteus spp.* in 0,01 g	*Escherichia coli* in 0,001 g
Valid	73	-[a]	-[a]	-[a]	-[a]	-[a]
Non-valid	37	-[a]	-[a]	-[a]	-[a]	+[b]

Legend: -[a] - was not detected
+[b] - detected

According to the Regulation on microbiological safety of food in trade [5] these products are microbiologically correct if there are no pathogenic microorganisms in the sample such as: *Salmonella species* in 25 g of sample, *coagulase-positive staphylococci* in 0.01 g of sample, *sulphite-reducing clostridia* in 0.01 g , *Proteus species* in 0.01 g and *Escherichia coli* in 0.01 g of sample.
Based on the results shown in Table 1, from a total of 110 samples analyzed semi-hard cheese, 73 samples were correct.
Number of unsatisfactory samples were 37. The reason for their microbiological defect was presence of *Escherichia coli* in 0.01 g of sample. Not even from one sample are isolated *Coagulase positive staphylococci, sulphite-reducing clostridia* and *Proteus species.*
Table 2 presents the results of microbiological safety of analyzed samples albumin cheese. According to the Regulation on microbiological safety of food in trade [5] these products are microbiologically correct if there are no pathogenic microorganisms in the sample such as*: Salmonella species* in 25 g of sample, *coagulase-positive staphylococci* in 0.01 g of sample, *sulphite-reducing clostridia* in 0.01 g of sample, *Proteus species* in 0.01 g of sample and *Escherichia coli* in 0.001 g of sample.

Table 2. Microbiological safety of albumin cheese

Number of samples	25	*Salmonella* species in 25 g	Coagulase positive staphylococci in 0,01 g	Sulphite reducung Clostridia in 0,01g	*Proteus* species in 0,01 g	*Escherichia coli* in 0,001 g
Valid	25	-[a]	-[a]	-[a]	-[a]	-[a]
Non-valid	0	-[a]	-[a]	-[a]	-[a]	-[a]

Legend: -[a] - was not detected

Based on the results shown in Table 2, from the total of 25 analyzed samples of semi-hard cheeses, all 25 samples were correct.
The obtained results indicate the possibility of multiple critical points during production. The first could be raw material, or raw milk. All analyzed samples were made by the same manufacturer. It is known that albumin cheese is made from whey, which is

byproduct in cheese production. At the time of the creation of cheese curd, of which is further made steamed dough, the whey is separated, proteins are denaturated and thus gets albumin cheese. Therefore starting material for these two kinds of cheese is the same. Considering that all samples of albumin cheeses were correct, it can be concluded that the raw material was correct. The risks in the production of semi-hard cheeses of steamed dough is a lot of manual labor to obtain the final product which is not present in the production of albumin cheese. During the process of steaming dough, the water in which the process takes place can be a source of contaminants. Then follows the kneading, shaping and molding of cheese, which are the stages of manual labor and can be a source of secondary contamination of cheese. Ripening phase is also one of the possible phase contamination. The process of production of albumin cheese is much shorter and covers after the heat treatment, whey protein curd making, drying and packaging. There are no manual labor, and the possibility of risk is reduced.

CONCLUSIONS

- ✓ The study was conducted on 135 samples of cheese from the same manufacturer
- ✓ A total of 110 samples of semi-hard cheeses such as "pasta filata" were examined
- ✓ 25 samples of albumin cheese were examined
- ✓ Of the total 110 samples of semi-hard cheese tested , 37 samples, which amounts to 33.64% were not correct
- ✓ The reason of contamination was the presence of Escherichia coli
- ✓ From a total of 25 samples of albumin cheese tested, all were correct
- ✓ In the processing of a semi-hard cheese of steamed dough there is a lot of manual labor present, and that gives higher possibility of secondary contamination which leads to failure of the final product
- ✓ In order to better trade placement and improvement of the production of cheeses, a task for manufacturers is to keep the risk at the lowest possible level, which is practically and technically possible to achieve. Starting from the fact that a large number of pathogenic microorganisms, originating from primary production or introduced to the chain during processing, the absolute imperative to increase product safety conditions are fullfilled precondition, which include good manufacturing and hygiene practices.

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CONTRIBUTION MARGIN ANALYSIS IN CHICKEN FATTENING

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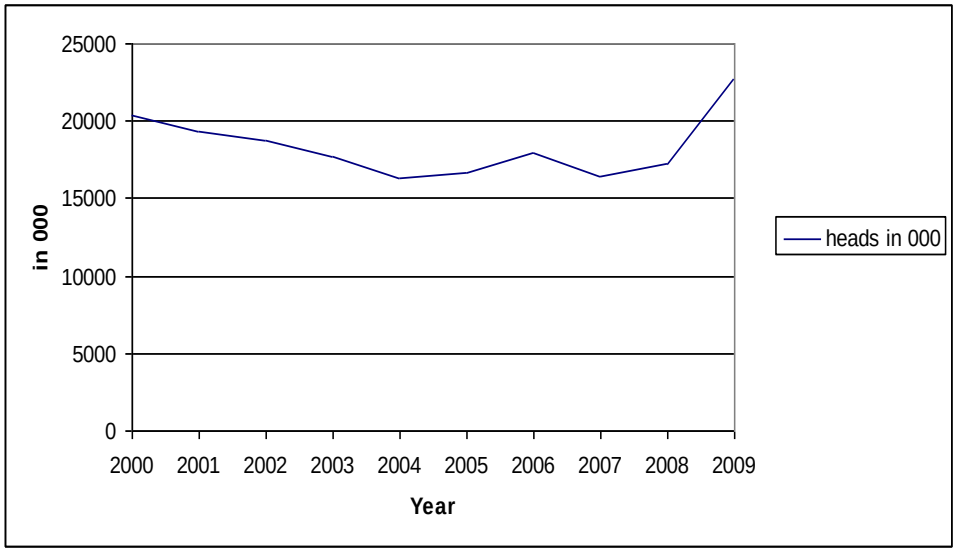
APSTRAKT

Poultry production achieved great improvement in last few decades, as because of poultry meat products quality, as well as because of its lower price in compare to other types of meat. In Republic of Serbia, as like in world too, most important type of poultry meat, by production and consumption volume, is chicken meat.
Main goal of this paper is determination of contribution margin in chicken fattening, that should served as business indicator to market oriented agricultural producers. It was used sensitive analysis for estimation of contribution margin change caused by fattened chicken price, or variable costs change. It was estimated that contribution margin is more sensitive on broilers price fall (contribution margin will be fall on 0 in case that broilers price decrease for 11,64%), in compare to variable costs growth (contribution margin will be equal to 0 in case that variable costs rise for 13,17%).
***Key words**: chicken fattening, costs, production, consumption*

INTRODUCTION

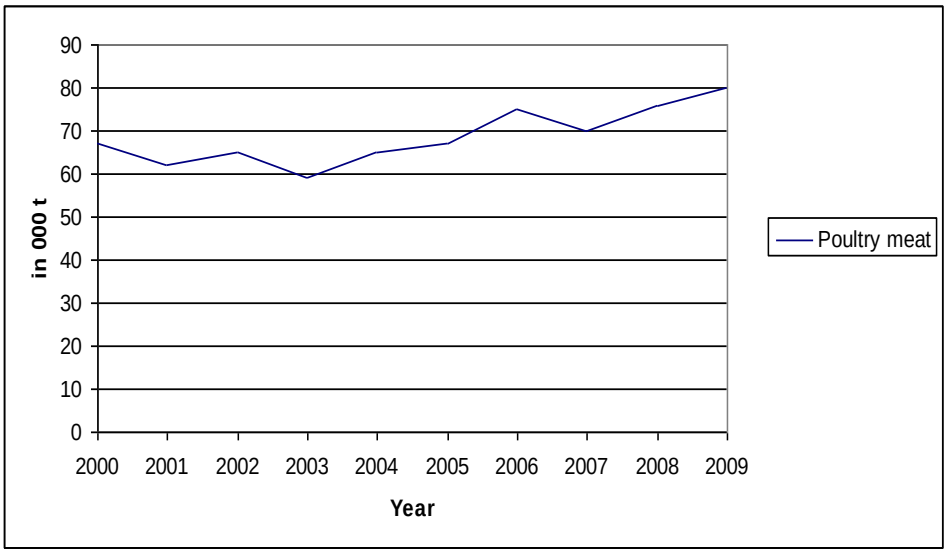
Poultry production is branch of livestock breeding that gains more and more importance within agricultural production in Republic of Serbia. Some of the basic characteristics of poultry production are short production cycle, good food utilization, quick growth of broilers during the fattening process, etc. Under production and consumption volume, chicken meat is the most significant type of poultry meat. Other poultry species are present in far less percentage due to many reasons, such as consumers' habits, technological characteristics of production process, lower demand for secondary products (e.g. feather), etc.
High consumption of poultry meat arises mainly from its quality. Energetic value of chicken meat depends on type of meal and way of its preparation and in average is around 220 kcal/100g. It is great source of vitamins (niacin, B6, B2, B12 and B1), minerals (P, Zn, Se, Fe and Mg) and pantothenic acid, and there are small amounts of folic acid and vitamins A and E present in skin and fat. As meat, chicken meat represents main source of proteins and great substitute for red meat [3].
Chicken fattening used to occur at every husbandry, with meat consumed primarily on farm, and only small amounts (surpluses) being sold on the market. Production modernization caused significant changes in poultry production, so nowadays smaller number of producers that breed more heads and are market orientated, deals with broiler and egg production. To these commercial, usually family run, farms poultry production represents main source of incomes, and in such situation chicken meat cost and market price decline, bringing profitability of chicken meat production for own needs to question. Chicken meat has lower price than other meat types (pork and beef meat). Due to short production cycle and possibility of total control of all production activities, modern poultry production is similar to industrial production.

Total production of chicken meat depends of number of slaughtered heads and their weight during slaughtering. Annual global production is around 60 million of tons, or approximately 10 kg per capita. As per production volume, chicken meat ranks second globally overtaking it from beef meat production, while pork meat still remains intact at first place. Compared to total poultry meat production, chicken meat has dominant share of about 86%. [2]
Poultry production and broiler fattening do not depend directly on natural conditions. However, change in natural conditions still strongly affects crop production and indirectly all branches of live stock production as well. Due to change in natural conditions and decrease of yields in crop production, rise in crop products prices occurs, followed by rise of forage prices, on which profitability of livestock production heavily lies. As poultry production has a short production cycle, often change in fodder prices and fluctuation of output (meat) prices have greater impact on production volume compared to other branches of live stock production. Therefore, due to its sensitivity to change in concentrated stock food price, poultry production along with pork meat production is concerned to be a hazardous business activity.
In order to perceive observed production characteristics on the territory of Republic of Serbia better, total number of poultry (chicken) heads and chicken meat production volume in period 2000-2009 were monitored. The poultry heads in Republic had a decreasing tendency, although in 2009 there was a sudden increase of their number (as shown in Graph 1.). Sudden changes in heads numbers are especially visible in broiler production, due to short production cycle.



Graph 1. Number of poultry heads in period 2000 - 2009 (in 000 heads)
Resource: http://webrzs.stat.gov.rs/axd/index.php

Poultry meat production in the same period has also showed a positive trend (with annual variations) and increased from 67.000 t in 2000 to 80.000 t in 2009. (Graph 2.).



Graph 2. Production of poultry meat in period 2000 - 2009 (in 000 t)
Resource: http://webrzs.stat.gov.rs/axd/index.php

Variations in poultry meat production arose from many reasons, with fodder price fluctuation, chicken meat price, small producers marketing problems, strong market competition, etc, being the most important ones.
Serbia, being a significant chicken meat producer exports it in considerable quantities. Beside export, small quantities of chicken meat are imported, in certain periods when domestic production is not sufficient to meet current market needs for this product. Trends of chicken meat import and export, in period 2000-2009, expressed in quantity and value, are presented in following table.

Table 1. Export and import of poultry meat in period 2000 – 2009 (in RSD, USD and EUR)

Year	Poultry meat							
	Import				Export			
	quantity (in kg)	value (in RSD)	value (in USD)	value (in EUR)	quantity (in kg)	value (in RSD)	value (in USD)	value (in EUR)
2000	2.183.952	47.316.271	1.811.532	2.039.868	279.379	4.683.636	284.901	320.652
2001	1.802.092	135.576.695	2.136.915	2.301.466	66.872	5.228.995	81.568	88.699
2002	1.827.412	104.425.947	1.605.376	1.723.741	2.061	1.975.209	31.388	32.335
2003	732.219	46.334.098	801.068	710.688	6.296	1.703.134	29.283	27.179
2004	568.850	56.794.770	987.911	799.570	426.135	49.789.700	830.124	659.760
2005	196.328	14.276.035	213.030	171.629	1.213.378	156.327.821	2.350.194	1.887.555
2006	47.067	3.104.600	47.610	38.005	1.049.119	141.243.773	2.141.941	1.689.868
2007	196.133	24.178.444	429.108	303.351	2.305.637	31.954.3470	5.495.131	3.984.760
2008	148.264	26.699.555	463.554	323.319	1.650.017	288.047.963	5.259.025	3.543.692
2009	241.755	52.962.148	805.357	564.687	1.691.755	322.900.376	4.790.013	3.434.058

Resource: http://webrzs.stat.gov.rs/axd/index.php

As it could be seen from the table above, imported quantities of poultry meat have a decreasing tendency, while quantities and value of exported meat indicate growth (exception being period 2001 - 2003). These positive trends indicate that in future, in Serbia, further successful development of broiler production could be expected.

MATERIAL AND WORKING METHOD

To determine incomes and expenses in broilers fattening process, data gathered from husbandries (family farms) involved in this production was used. The economic effectiveness of production was determined by use of variable costs coverage calculation method. In other words, contribution margin was determined, as difference between production value and variable costs. In calculations average annual prices of: animal food, performed services and gained products were used.
Contribution margin was calculated for 1.000 heads of broilers in one production cycle (45 days). All values are expressed in RSD and EUR. Variable costs coverage, i.e. contribution margin could be obtained by following formula:

$$PVT = Q - VT$$

Where: $Q = q \; x \; c$

While: PVT - Variable costs coverage; Q - Production value; VT - Variable costs; q - Products quantities; c - Price of product per unit of measure.

In economic analyses, contribution margin is very important indicator, since it can be used for optimal production structure determining (by linear programming), business risk determining, etc.
With results obtained from calculation sensitive analysis was performed. Sensitive analysis was used to track changes in contribution margin in reference to chicken meat price decrease, or increase in production costs caused by change of animal food price.

RESULTS WITH DISSCUSION

As significant number of farms is involved in chickens fattening, results of this research may have great importance for farms involved in this sort of production. Assumed weight of fattened chicken is 2,2 kg with price of 100 RSD per kg, while price of one-day-old chicken is 37 RSD. Other data are presented according to current market prices at the time of calculations. Based on gathered data analytical calculation was created with following results (Table 2.):

Table 2. Variable costs coverage in process of chicken fattening (broiler production)

Description	Unit of measure	Quantity per head	Price per unit of measure	Total RSD/head	Total 1.000 heads (in RSD)	Total 1.000 heads (in EUR)
A. Incomes						
Broiler	kg	2,2	100	220	220.000	2.095,24
Total				**220**	220.000	2.095,24
B. Variable costs						
One day old chicken	pcs	1	37	37	37.000	352,38
Animal food	RSD			132,4	132.400	1.260,95
Veterinary service costs	RSD			5	5.000	47,62
Other costs	RSD			20	20.000	190,48
Total				**194,4**	194.400	1.851,43
C contribution margin (A-B)				**25,6**	25.600	243,81

Resources: Grupa autora - *Analiza učinaka - efekata plasiranih podsticajnih sredstva - povratne informacije (izvještaji) - katalog kalkulacija poljoprivrede*. Institut za ekonomiku poljoprivrede, Beograd 2009.

- Contribution margin is 25.600 RSD per production cycle (one production cycle usually includes 1.000 broilers),
- In variable costs structure, costs of animal food dominate (132.400 RSD), while the lowest share have the costs of veterinary services (5.000 RSD).

Obtained contribution margin in broilers fattening is used for fixed costs coverage, with rest being a profit realized through business activities. In case that the production

optimization is based on contribution margin, production structure ought to have more products with higher contribution margin, since greater potential for fixed costs coverage is thus created. In specific case, profit that could be created in broiler production depends on fixed costs of specific producer. In next table is presented fall of contribution margin caused by fall of broilers price (Table 3.).

Table 3. Contribution margin fall in chicken fattening caused by price decreasing

Broilers price fall (%)	Contribution margin (RSD/head)
0,00	25,60
5,00	14.60
10,00	3.60
15,00	-7.40

Fall of contribution margin in chicken fattening caused by variable costs rise is shown in Table 4.

Table 4. Contribution margin fall in chicken fattening caused by variable costs growth

Growth of variable costs (%)	Contribution margin (RSD/head)
0,00	25,60
5,00	15.88
10,00	6.16
15,00	-3.56

Marginal price of fattened broilers, when contribution margin is equal to 0 is approximately 88,36 RSD. With rising of variable costs in chicken fattening to 220,00 RSD, also comes to equalization of contribution margin with 0. In other words, contribution margin will fall to 0 in case when broilers price decrease for 11,64%, or variable costs increase for 13,17%. So, contribution margin is more sensitive to change of fattened chicken price than on change of variable costs. In any case variable costs covering from achieved incomes enable producers' survival only in short period. In long term perspective for smooth production is necessary to cover all fixed costs and financing costs from contribution margin.

CONCLUSION

It could be said that poultry production is one of the branch of agriculture that is develop intensively, taking in great measure industrial character. It is not only that poultry number increase in 2009 in compares to previous years, but poultry meat production showed increase tendency too. Also, export of poultry meat is far over its import volume.
Because of these reasons in paper is analyzed calculation of chicken fattening based on variable costs, with main goal to determine contribution margin and its sensitivity on relevant parameters change. Sensitive analysis of contribution margin in chicken fattening showed higher sensitivity of this parameter on chicken price fall, than on variable costs growth. Mentioned results direct

producers on maintaining of specified selling price, because its relatively small fall could imperil profitability of examined production.

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THE SIGNIFICANCE OF THE APPPLICATION OF THE *NIRS* METHOD IN ANIMAL FEED FACTORIES WITH THE GOAL OF IMPROVING QUALITY AND COMPETITIVENESS

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ABSTRACT

The production process of animal feed is too fast in relation to the duration of the standard chemical method of analysis of the most important quality parameters of raw materials and finished products. The results of standard methods of chemical analysis are often obtained too late so the process of production cannot be stopped or adjusted at time. Therefore, the application of the NIRS method (Near Infrared Spectroscopy - Spectroscopy in the near infrared area of electromagnetic radiation), as a rapid method, enables that the analysis of the content of important ingredients in feed production is completely in line with the production process.

NIRS represents a spectroscopic method that uses the near-infrared region of the electromagnetic spectrum (700 – 3000 nm) for the scanning of the sample. The obtained result is the spectrum of the analyzed sample in the form of the plot of the transmitted radiation energy (log (1 / T)), or the reflected radiation energy (log (1 / R)), versus wavelength. Since there is no mathematical equation that connects the spectral data with the concentration of the important ingredients (when it comes to quantitative analysis), it is necessary to previously calibrate NIRS devices, using modern mathematical methods and computer software. NIRS is, in fact, an empirical and secondary analytical technique that requires previous calibration by using samples of known composition and properties determined by certain standard (laboratory) methods of analysis. (*Givens and Deaville, 1999*).

This paper also briefly demonstrates the application of NIRS methods in Pre-Mervo, a leading Dutch company for producing mineral premixes. The Pre-Mervo company is one of the pioneers of commercial application of NIRS methods in the processing industry of the Netherlands. Since 1980 Quality Services Laboratory of the company is engaged in the development of calibration curves and improvement of NIRS technology.

***Keywords**: NIRS, animal feed, calibration, PreMervo*

INTRODUCTION

Increased competition within the animal feed industry and narrow profit margins, have increased the need for improved production process efficiency, as well as the reduction of waste. To achieve these objectives, rapid analytical methods need to be used in order to react faster in case of deviation in the product specifications. Consequently, a huge amount of money could be saved and a product with more consistent quality could be offered to the customers. NIRS method is certainly a good solution because it has a great potential for improvement of the monitoring and control of production processes in animal feed factories.

NIRS method has a very wide range of applications so that it is now used for testing a large number of foods of different origin: cereals and cereal-based products, oilseeds, animal feed, various intermediate products of animal origin, as well as for the analysis of the samples with various consistency: liquid, pasty, granular and powdery. A number of techniques of this analytical method have been developed, which use different areas of the spectrum, different methods of recording and collecting the spectrum, as well as various statistical methods for calibration of NIRS devices. (*Osborne and Fearn, 1986; Baeten andi Dardenne, 2002; Murray, 2004; Flinn, 2005*).

Since the 1960s until now NIRS method has developed into a powerful instrument for qualitative and quantitative chemical analysis, as well as for determination of the physical-chemical and other characteristics of various types of samples. The NIRS technique very rapid (usual run time is less than 30 seconds) is non-destructive, and does not usually require sample preparation before the analysis (*Burns*, Ciurczak, *2001*). The major advantage of NIRS method is the possibility of its application without any chemical pretreatment of samples, which reduces the costs the chemical waste (*Barber et al., 1990*). Also, the NIR method shows less variation in repeated analysis of the same sample, than the standard laboratory methods (*Marum and Aastveit, 1990*). Another major advantage of this method is the possibility of an In Line Analysis, when NIR analyzer is interfaced directly to the process stream, enabling continuous process control.

The usual application practice of NIRS methods for determining the concentration of important constituent consists of both obtaining the NIR spectra, by recording the sample containing important constituent, and a traditional chemical analysis on a number of individual samples. The obtained information is then used to develop empirical equations that connect the spectral data with those obtained by chemical analysis, by the application of mathematical and statistical techniques (Chemometrics) the calibration procedure.

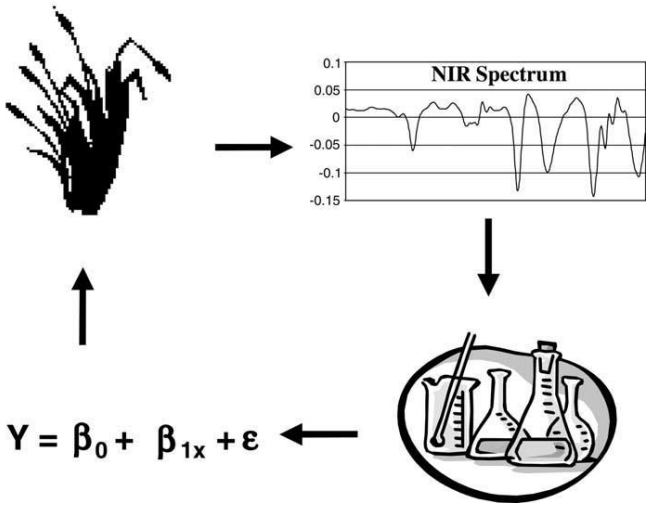


Fig. 1. The simultaneous analysis of a sample by NIRS and chemical methods and comparing the obtained results in the calibration procedure to obtain an empirical equation that connects the spectral data with those obtained by the chemical analysis (Stuth, Jama, Tolleson, 2003)

NIR region of the electromagnetic spectra

All types of spectroscopy are based on the interaction of electromagnetic radiation with a sample to be analyzed. The NIR region of the electromagnetic spectrum lies between lies between the visible and infrared region and is usually defined by a wavelength range 700- 3000 nm. However, most analytical application of this technique is between 1100 to 2500 nm. (*Deaville i Flinn, 2002*).
By exposing the samples to electromagnetic radiation, electromagnetic rays which frequency matches the natural frequency of molecule vibration, causing a change in dipole moment of molecules, may submit its energy to the molecule. Consequently, molecule upgrades from the primary energy state (ύ = 0, ύ - vibration quantum number), to the higher energy state. NIR spectra consists of **overtone** (as a result of the transition between vibration energy states Δύ = ± 2, ± 3,...) and **combination bands** (in the case of the polyatomic molecules as a result of energy changes of two or more vibrations). Hydrogen atom, as the lightest, expresses the strongest vibrations, thus the bands that are observed in the NIR spectral region, are mostly the bonds containing hydrogen: CH, NH, OH, SH bonds. Other important molecular absorption in the NIR region originate from the carbonyl group (C = O), bonds with carbon
(-CC-, C = C), etc.
A NIR spectrum is a result of the absorption of the near infrared radiation by the sample. It represents a dependence of the logarithm of the reciprocal value of the reflectance (or transmittance) of the electromagnetic radiation on the wavelength. The obtained NIR spectrum looks like a smooth, wavy line with some not clearly defined characteristics, as

at the same wavelength occurs the absorption of the radiation by a number of different organic compounds present in the examined material, and the NIR spectrum is actually composed of many overlapping bands which makes it very complex and inhibit the determination of the direct connection between the concentration of important constituents and energy absorption (*Osborne i Fearn, 1986; Murray i Williams, 1987; Wiedemann et al., 1998; Deaville i Flinn, 2002; Cen i He, 2007*).

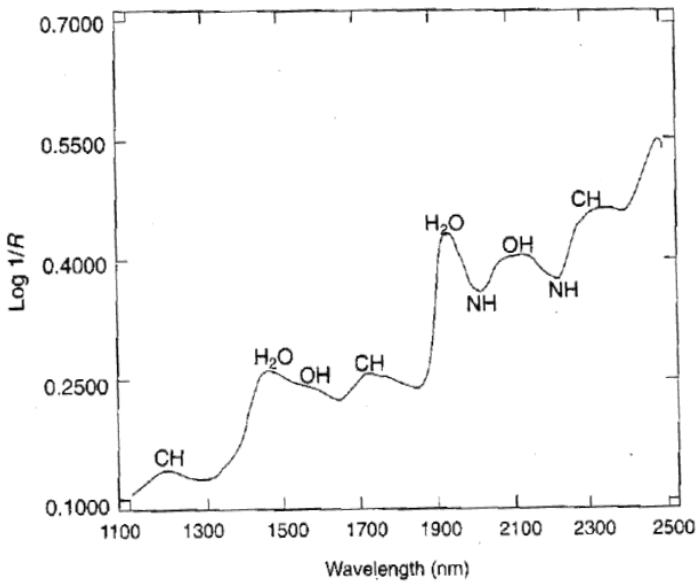


Fig 2. NIR spectrum of the hay sample, demonstrating absorption locations of the main functional groups

The developing procedure of the calibration model

The development of calibration model is a key step for the successful application of the NIRS method. The calibration represents a mathematical process that is necessary for comparing the NIR spectral data with the content of interesting ingredients. It consists of the following stages:

- **The selection of the calibration set of samples, the** robustness and the accuracy of the calibration model is largely conditioned by the variability of the calibration population in terms of the presence of different sample sorts, samples of different degrees of maturity, samples from different growing regions and different production years (*Tsuchikawa, 2007*). Population with a wide range of different types of samples and a wide range of compounds of interest results in a calibration model that allows testing of a wide range of samples, but with reduced accuracy, and populations with little among the

various types of samples and the narrow range of compounds of interest resulting in higher accuracy of model calibration, where a small number of extreme samples is tested with low reliability (Workman, 2008).

— **Outlier** – a sample whose specter is statistically different from the majority of spectra in the population. Outlier samples should not be included in the calibration population. Outlier detection is important in two phases: during the development phase of the calibration model, as in the validation phase of the developed model. In routine analysis, they can be indicators of changes in the production process and should be explored.

- **The collection of spectral and reference data** We need to consider the fact that the work of the NIRS device varies from day to day, and during the day. Readings on the device can change throughout the day with the temperature and time of the lamp and the time when the device is in use. When the temperature of the lamp and the detector are in balance the device reaches maximum stability. Therefore, NIRS devices should not be placed near air conditioning or heating bodies, since the devices may show innacurate measurements on the of the temperature and relative humidity. The air humidity also has an important impact in the application of the NIRS device. Namely, at low values of relative humidity, the tested materials with high moisture content are tested with reduced accuracy.
- **Combining spectral data with the corresponding reference data**,
- **Perform regression models**, The function by which the results of indirect (NIRS) methods are translated into the desired result - the content of the ingredient of interest or a functional property of the surveyed material – is called a *calibration model*. The calibration model is based on the results of measuring by the indirect method (*X variable*) and the results of measurements of the reference method (*Y variable*). Such a model can be used to determine the unknown value (*Ŷ variable*) (Equation 2).

$$X + Y = MODEL \text{ (equation 1)}$$
$$X + MODEL = (\hat{Y} \text{ equation 2)}$$

The development of calibration models is defined either by appropriate standards, or by the manufacturer of the NIRS device within the development of the software aimed at performing calibrations. Leading device manufacturers have developed a so-called. „global“ calibration, which allows for an identical calibration to be used in different locations around the world *(Hruschka, 1987)*.

As the NIR spectrum is characterized by a large number of interrelated variables, a prerequisite of the qualitative application of the NIR spectroscopy is the development of different multivariate calibration models. With the development of **multivariate calibration models,** empiric models were developed for linking the multiple spectral intensities derived from a number of calibration samples with known concentrations of constituents of interest, which can still be used in multivariate prediction analysis of the spectra of the unknown samples for a quick determination of the concentration of the

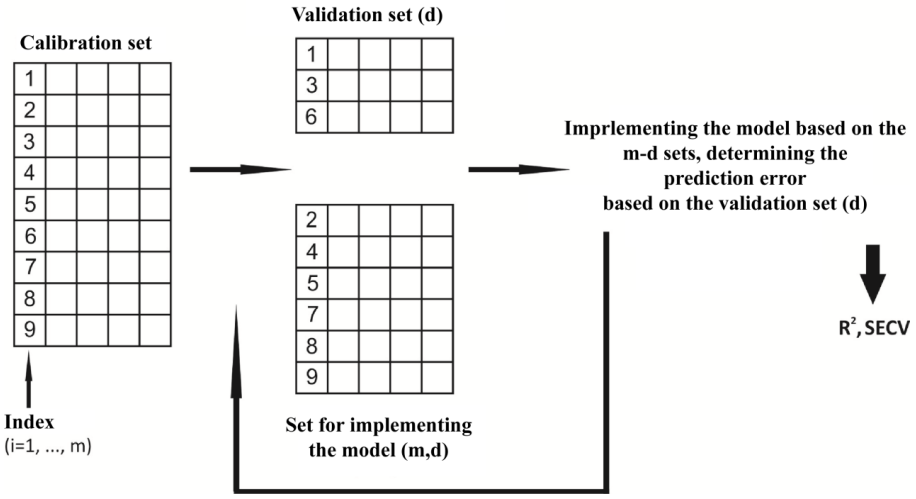
constituents of interest. Multivariate calibration have been historically the main cornerstone of *chemometrics* applied on analytical chemistry (*Brereton, 2000; Dzupin et al., 2004; Fearn, 2005*).

Chemometric quantitative calibration techniques that are mainly used in NIR spectroscopy can be divided into:

-**Multiple Linear Regression**, *MLR* and
-Factoring techniques such as the *Principal Component Analysis, PCA, Principal Component Regression, PCR* and *Partial Least Squares, PLS,* (*Geladi and Kowalski, 1986; Wold et al., 1987; Haaland i Thomas, 1988; Martens i Næs, 1988; Kramer, 1998*).

- **Model validation;** The goal of the validation of the calibration is the estimate of its prediction ability in routine application (*Petersen, 2007; Boysworth i Booksh, 2008*). We differentiate two types of validation procedures of the NIRS calibration model:
-*cross-validation*, and
-external validation i.e. validation with an independent set of .
Both procedures result in a *Prediction Error* i.e. an error which can be expected in the routine application of the model which determines the quality or the effectiveness of the development model (*Esbensen, 2006*), and which is based on the concept of differences between the NIRS results a and the results of the reference laboratory analyses (*Isaksson and Segtnan, 2007; Shenk et al., 2008*).

  Cross validation is a validation technique is based solely on the calibration data. The cross validation procedure consists of successive exclusion of single samples or a group of samples from the calibration sample set, the development of the calibration model with the remaining samples in the calibration group and the and the assessment of the quality of the mode using previously excluded samples or group of samples which serve as a validation set. After returning the previously excluded samples or sample groups into the calibration sample set, we exclude the second single sample or sample group and the procedure is repeated while quality control if simultaneously performed. It often occurs that the application of cross validation which excludes a single sample has a tendency to cause model *overfitting* (which introduces too many wave lengths into the model), resulting in a poorer model quality. On the other hand, cross validation which excludes a sample group shows a lower tendency of overfitting of models and results in a better model quality.



External validation is used to determine the accuracy and repeatability of the developed NIRS calibration model using a set of samples that are not used in the process of model development. Validation set of samples for external validation process can be chosen in two ways: random selection of samples from the calibration set of samples before performing the calibration, or as a separate set of samples. As with the selection of the calibration set of samples special care must be taken on its viability for routine use. Calibration and validation sets of samples must be similar in terms of sampling conditions and sample preparation i.e. they need to be mutually representative and of the same quality. External validation procedure is characterized by a higher prediction error than originally found in the process of cross-validation.

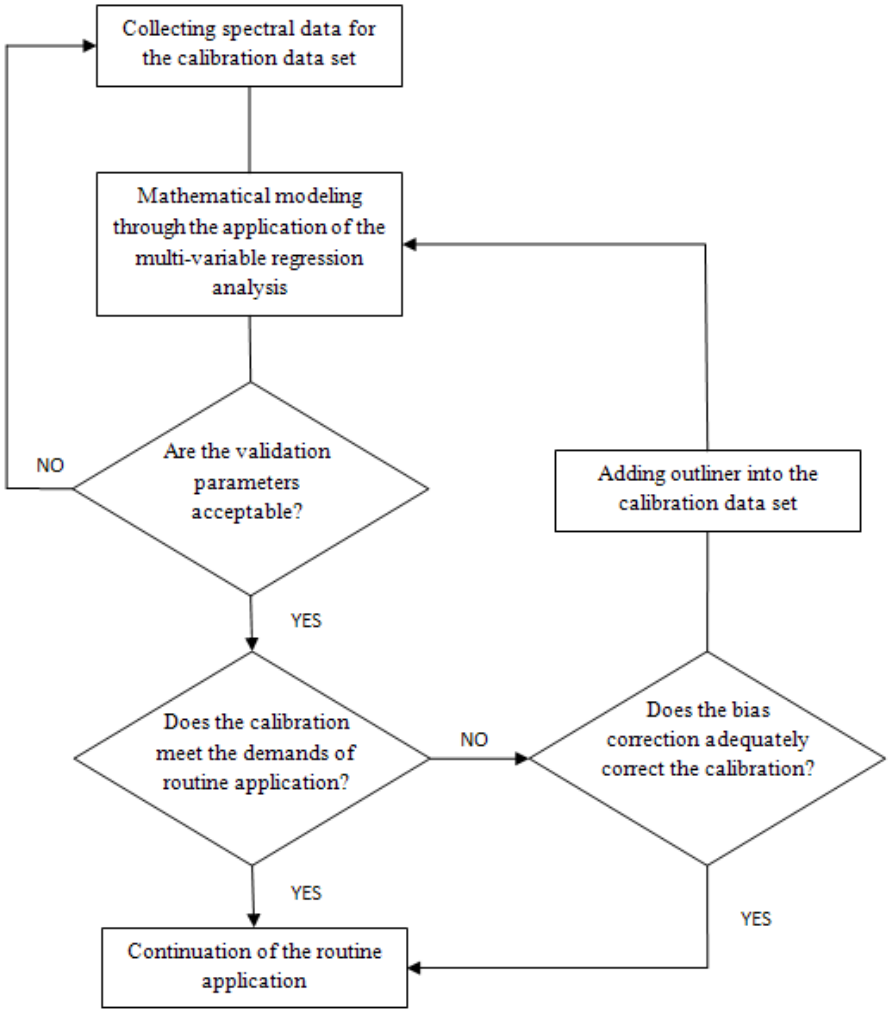


Fig. 3. Scheme of the validation of the calibration model

NIRS calibration model efficiency

Statistical tests used for assessment of eligibility (efficiency) NIRS model include:

- coefficient of determination (R^2),
- coefficient of correlation (r),
- *Standard Error of Calibration, SEC,*
- *Standard Error of Cross Validation, SECV,*
- *Standard Error of Differences, SDD,*
- *Standard Error of Performance, SEP,*
- *Standard Laboratory Error, SEL*
- *Standard Error of Performance, SEP*
- bias (systematic error between the NIRS method and the reference chemical method results)

Application of the NIRS technology in the Pre-Mervo Company

Methods of the analysis

Comparing NIRS and laboratory method of the analysis in terms of the process control is shown in the Table 1.

Table 1. Comparing laboratory method and NIRS

Laboratory	**NIRS**
Reference method	Derived method
Limited amount of samples	High amount of samples
Slow, results are obtained too late	Rapid, results obtained on time
Low frequency	High frequency

NIRS method can provide many results in a short time. There are always differences between the analysis from the laboratory and of the NIR instrument. This also holds true for two laboratories, which analyze the same sample. If it is necessary to make a choice between the two methods for the process control, Pre-Mervo prefers NIRS because it can provide significant information about the raw material shortly after the arrival of the truck with a raw material. When an error occurs in the process, it is very important to react fast. NIRS enables the occurrence of the error in the process to be revealed very quickly, thus the adjustment measures can be taken on time.

Variations in the process

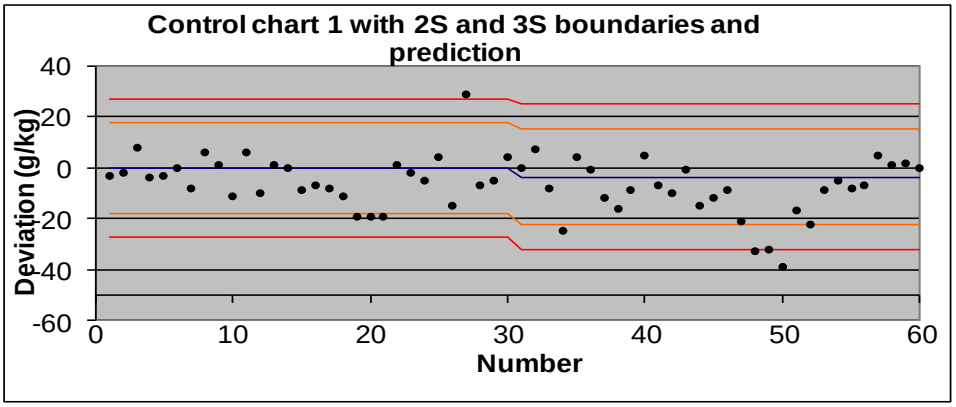
When measuring the important **parameters of the raw material** used in the production process, and calculating the **parameters of finished products**, there are certain expected values of these two items. However, measurement results will always show variations around these values. There are several causes of these variations:

• **Raw material**	• **Finished products**
-Variations in the raw material composition	-Variations in the raw material composition
-Variations in sampling procedure	-Variations in dosing
-Variations in the analysis procedure	-Variations in blending
	-Variations in sampling procedure
	-Variations in the analysis procedure

In order to reduce these variations to a minimum, it is necessary to improve procedures so that all sources of variations are as small as possible, and, above all, to apply the adequate process control.

Control charts

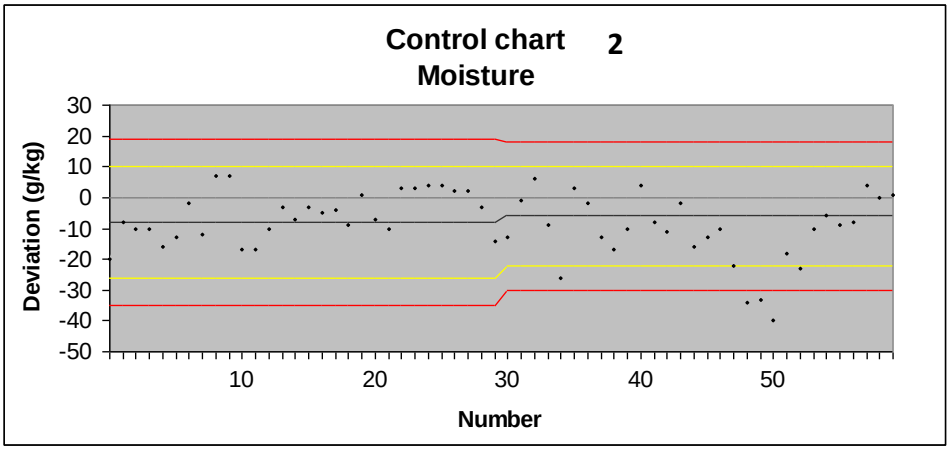
For the monitoring and control of the process, Pre-Mervo uses Control charts. These charts demonstrate the variations as a deviation from the expected, i.e. calculated value. They enable to be on time informed if the result is within the normal range or not. The **control chart No. 1** demonstrates differences between the expected (or calculated value) and the results of the measurements. With more than 30 measurements we can calculate the mean and standard deviation. The results of these calculations are used as boundaries for the next 30 measurements.



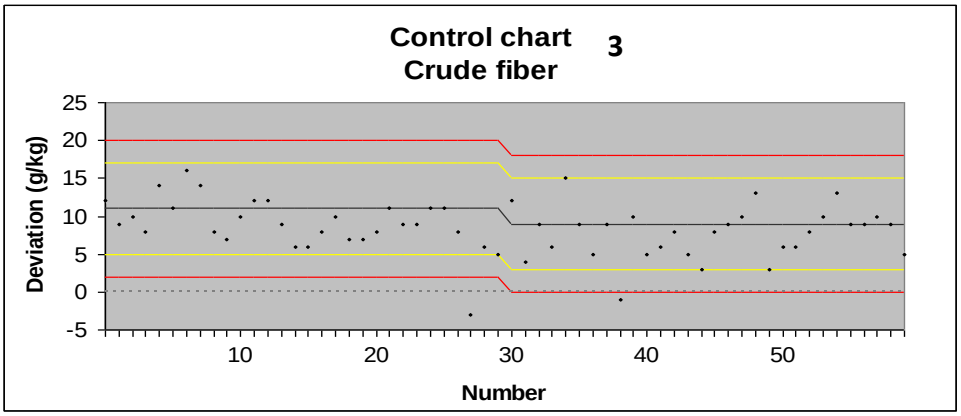
Control chart No. 1 - Demonstrates 2S and 3S boundaries and the deviation from the expected value

Practical results in the animal feed production

The following results are a fairly random selection of charts from one of Pre-Mervo customers. The example piglets feed.



Control chart No. 2 - Demonstrates that after thirty measurements the level of expected mid value little turned up a little, and the tolerance interval becomes narrower. After the measurement No. 40 deviation exceeds the 3s value. The process was not stable. After the measurement No. 50 differences became smaller and the level of deviation became normal.



Control chart No. 3- Demonstrates that the measured contents of crude fiber is higher than the calculated values. The mean value based on the average results from the previous period was above the calculated values. After thirty measurements, the expected value is corrected, according to the average of the last thirty measurements. Two variations of the value exceed -3s, which means that the process was not stable on

these points. Consequently, the employee in charge for the feed formulation has to examine why there is structurally more fiber found than the calculated value is.

CONCLUSION

The introduction of NIRS technology is a good strategy of plant feed if one takes into account their manufacturing process. Implementation of the NIRS technology is a good development strategy for, the animal feed factories, considering the complexity of their production process. NIRS method, as a rapid analytical method, allows timely obtaining of the required results, improvement of the quality control and efficiency, leading to higher profitability and competitiveness.
Advantages of the NIRS technique implementation:

- Fast control of the content of the important components in raw materials and finished products (a large number of measurements in a short period of time)
- Improvement of the product quality
- Costs decrease
- The possibility of installing NIRS device within the production process - continuous process-control
- No chemicals – no chemical waste

Although the implementation of the NIRS technology requires sigbificant start up costs, and relays on chemomtrics, in the long term, and if it is developed properly, it becomes very cost effective.

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THE EFFECT OF A BACTERIAL INOCULANT ON AEROBIC MICROFLORA IN WHOLE CROP MAIZE SILAGE

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ABSTRACT

The quality of silage depends on the competition between different groups of micro-organisms. Lactic acid bacteria, responsible for the silage fermentation, usually dominate the silage microflora. In addition, a number of undesirable micro-organisms that occur at low levels in fresh plant materials may grow during the storage of silage and lead to anaerobic or aerobic spoilage. The aim of this study was to examine the effect of added bacterial inoculant *Bonsilage Mais Flussig* on aerobic microflora during ensiling of whole crop maize. The total number of bacteria in whole crop maize silage was found to be significantly lower with addition of bacterial inoculant *Bonsilage Mais* which, through improved animal health, could indirectly positively influence the somatic cell count in milk. The total number of molds and yeast was significantly lower in the silage inoculated with *Bonsilage Mais Flussig*, thus contributing to lowering the secondary fermentation and related losses of silage nutritive value.
Key words: *whole crop maize, silage, bacterial inoculant, aerobic microflora*

INTRODUCTION

The genus *Lactobacillus* belongs to the large group of lactic acid bacteria (LAB) which are all Gram-positive organisms producing lactic acid by fermentation. Genera of LAB include, among others, *Lactococcus*, *Enterococcus*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Leuconostoc* and *Lactobacillus* **(Kandler and Weiss, 1986).** With over 100 species and subspecies, the genus *Lactobacillus* represents the largest group within the family *Lactobacillaceae*. Members of the genus are rod-shaped, often organized in chains. They are strictly fermentative and aerotolerant, but grow well under anaerobic conditions. There are two groups of species depending on the ability to ferment sugars: homofermentative species, converting sugars mostly into lactic acid, and heterofermentative species, converting sugars into lactic acid, acetic acid, ethanol and CO_2. Because the main catabolite is lactic acid, lactobacilli prefer relatively acidic conditions (pH 5.5-6.5 **(Giraffa et al., 2010).** Lactobacilli are associated with food and feed production because of the preservative action due to acidification, and/or enhancement of flavor, texture and nutrition.

They cause rapid pH decrease in the raw material through the production of lactic acid as the main catabolic product. In addition, the proteolytic activity and production of aroma compounds, bacteriocins and exopolysaccharides are relevant for the quality and

nutritional value of the end product and expand the spectrum of biotechnological applications of this important group of LAB **(Leroy and De Vuyst, 2004).**

Silage is an important feed for livestock, not only in winter in cold and temperate regions, but also during the dry season in the tropics **(Mannetje, 1999).** Whole crop maize (*Zea mays*) is the most popular cereal crop conserved as silage in many parts of the world, and is regarded as an ideal crop for silage making because of its high yields, low buffering capacity and high water soluble carbohydrates (WSC) content **(Nkosi et al., 2009).** The purpose of silage making is to preserve fresh forages while minimizing loss of nutrients and avoiding adverse changes in the chemical composition of the ensiled forages. It is therefore a suitable feed for maintaining the productivity of the livestock. Silage is a product of fermentation which is carried out by LAB under anaerobic conditions. In such an environment, LAB convert WSC of the forages into organic acids, mainly lactic acid. The production of these acids reduces the pH of the ensiled forages and inhibits growth of aerobic spoilage microorganisms **(McDonald et al., 1991).** The fermentation of silage requires several weeks for completion, with a critical time normally taking only a few hours **(Oude Elferink et al., 1999).** Silage fermentation is a complex and sometimes unpredictable process because it involves many species of LAB where interactions among them may occur. It is commonly recommended to add bacterial inoculants, especially those based on lactobacilli, to ensure the fermentation process.

Silage inoculants are selected lactobacilli added to ensiled forage to dominate or outnumber the naturally epiphytic LAB present in the forage. Both homofermentative and heterofermentative lactobacilli have potential advantages as silage inoculants. At the beginning of fermentation, production of lactic acid by homofermentative lactobacilli is preferred to reduce pH faster, which may inhibit growth of undesirable microorganisms and improve fermentation quality **(Cai et al., 1999**). Good aerobic stability is then controlled by the heterofermentative lactobacilli, since the activity of yeast is impaired due to acetic acid produced **(Driehuis et al., 2001; Filya, 2003).** Combining homofermentative and heterofermentative inoculants has become popular and has been used for various forages **(Filya, 2003; Weinberg et al., 1999; Zhang et al., 2009**).

There are many commercial inoculants available on the market. They may vary and be based on several requirements such as their ability to dominate the natural population of microorganisms in the forages and to produce lactic acid rapidly as a result of effective fermentation, which leads to a drastic drop in pH. Ideally, the inoculants should provide 10^6 CFU per g of fresh crop forages **(McDonald et al., 1991).** This concentration may decrease, since their viability declines with increasing temperature. Higher temperature results in even greater losses in viability for some inoculants. Several studies of **Weinberg et al. (1999, 2003, 2004a,b)** showed that some lactobacilli as silage inoculants were able to survive in the rumen fluid. Their survival is a good indication that they might have probiotic effects upon the host animals.

Bonsilage Mais Flussig, a commercial silage bacterial inoculant, has been shown to improve the nutritive value and stability of maize silage **(Nkosi et al., 2009).** This

inoculant has been used in Europe, but has not been evaluated in Serbia. The aim of this study was to determine the effect of *Bonsilage Mais Flussig* on the reduction of aerobic microflora during ensiling of maize.

MATERIALS AND METHODS

Whole crop maize, harvested in season 2009, was chopped by a regular silage chopper at nominal particle length of approximately 5 mm. Fresh material was divided in two parts of 50 kg each. One part was spread in thin layer and sprayed with the solution of commercial bacterial inoculant (*Bonsilage Mais Flussig*, Schaumann, Austria) in concentration according to manufacturer's specification. The other part of the material was used as control. *Bonsilage Mais Flussig* is a combination of hetro- (Lactobacillus buchneri) and homo-fermentative (Lactobacillus plantarum and Lactobacillus pentosaceus) lactic bacteria specially designed for maize silage.

Material was manually compacted into laboratory-scale silos (Figure 1) described by Čolovic *et al*. (2010). The purpose of compaction was to minimize the presence of oxygen and ensure fast initiation of anaerobic conditions. Containers were divided in two groups. Each group consisted of three containers with bacterial inoculant and three control containers without inoculant. The containers were stored at the temperature of 20 ± 3°C. The containers were opened on day 50 and samples for determination of total yeasts and moulds count and total bacteria count were taken.

Total number/count of bacteria (TBC) was determined using the following procedure: 25 g of sample was transferred aseptically into individual stomacher bags, containing 225 ml of sterile Buffered Peptone Water (BPW) solution (0.1%) and homogenized in a stomacher for 60 seconds. For each sample, appropriate serial decimal dilutions were prepared in the BPW solution. From each dilution step, 1.00 ml was transferred to a petri dish. About 15ml of agar tempered at 47°C were poured into the petri dish. Total number of all bacteria (except LAB) was determined aerobically using Plate Count Agar (PCA), after incubation for 3 days at 30 °C. LAB does not grow on this medium under aerobic conditions. Total number/count of yeasts and moulds (TYMC) was determined using Rose Bengal Chloramphenicol agar (RBC) after aerobic incubation at 25 °C for 5 days in the dark.

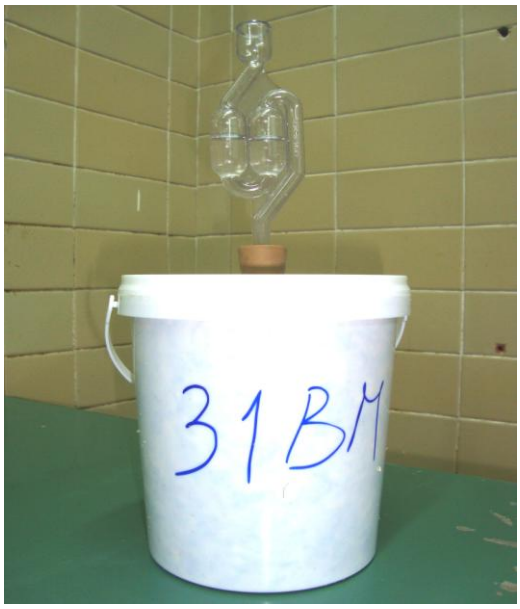

Fig. 1 Mini-silo for silage study

RESULTS AND DISCUSSION

Results for determination of total bacteria number/count (TBC) and total yeasts and moulds number/count (TYMC) in samples with and without inoculants on day 50 are presented in Table 1 and on Figures 2 and 3.

Table 1. Total bacteria count (TBC) and total molds and yeasts count (TYMC) in 1 gram of silage on day 50

	Total bacteria count (TBC)	Total molds and yeasts count (TYMC)
Bonsilage Mais	1.33E+08	7.30E+06
Control	2.43E+08	3.70E+07

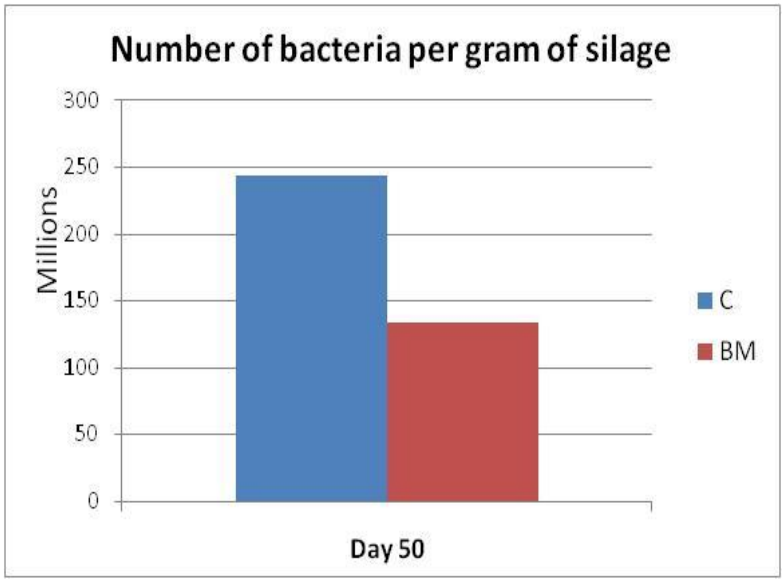

Fig. 2. Total number of bacteria (excluding LAB) in 1 gram of silage with (BM) and without (C) added Bonsilage Mais on day 50

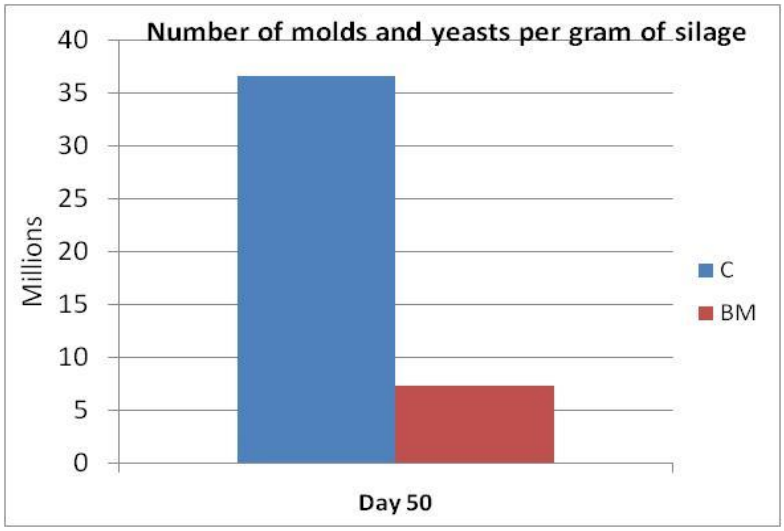

Fig 3. Total number of molds and yeasts in 1 g of silage with (BM) and without (C) added Bonsilage Mais on day 50

From the results presented in Table 1, it can be seen that after 50 days of ensiling, the total bacteria count (TBC) was about 2 times lower then in the samples with bacterial inoculant then in the control samples. With regard to total yeasts and moulds count (TYMC), on day 50 it was is about 5 times lower in inoculated than in the control samples. This was probably due to higher intensity of transformation of simple monosaccharides into the acids in the samples with bacterial inoculant. Therefore, the better effect of conservation has been achieved and growth of microorganisms was suppressed.

CONCLUSION

The total number of bacteria in whole crop maize silage was found to be significantly lower with addition of bacterial inoculant *Bonsilage Mais Flussig* which, through improved animal health, could indirectly positively influence the somatic cell count in milk. The total number of molds and yeast was significantly lower in the silage inoculated with *Bonsilage Mais Flussig*, thus contributing to lowering the secondary fermentation and related losses of silage nutritive value.

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QUALITY OF CARCASS AND BREAST MEAT OF CHICKENS FED WITH EXTRUDED PRODUCTS OF RAPE SEED

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ABSTRACT

In this paper quality of carcass, and nutritive, technological and sensory quality of chicken's breast meat, fed with mixtures where cruched soybean was substituted with extruded products of rapeseed were examined. It was concluded that there were no significant differences in carcass quality and breast meat yield between control and experimental groups (P>0.05). The changes in chicken meal had no influence (P>0.05) on nutritive quality of breast meat (the contents on water, protein, free fat and total ash). It was also found that technological quality of chicken's breast meat averagely corresponds to »normal« meat quality. Sensory quality of fresh chicken's breast meat of control and experimental groups was estimated as »very good«, i.e. optimal.
Keywords: *chicken, meat quality, extruded products of rape seed, meat quality*

INTRODUCTION

Meat quality is a very complex term and the result of action of numerous endogenous and exogenous factors [6)]. Feed as an exogenous factor is considered to have dominant influence, over 30%, on carcass and meat quality [4, 23]. Taking into account the numerous previous knowledge about nutrition and meat quality, it can be concluded that the diet, together with genotype, is the optimal combinatin for obtaining the desired quality of meat [1].

In determination of the quality from crucial importance are two issues, namely: the definition of indicators which express the individual characteristics of quality and quantification of these characteristic properties with respect to overall quality. Quality evaluation is more complete, if it is tested and defined by number of properties [13].

Water, protein, fat, free amino acids and fatty acids are major components of the meat and their qualitative and quantitative ratio determines the quality, that is, the nutritional value of meat [22].

Technological meat quality properties (pH, water holding capacity, colour) are particularly important for industrial production and meat processing. Sensory properties, appearance, colour, texture, juiciness, aroma and taste are the most important characteristics in terms of customers, i.e. consumers of chicken meat.

Standard meals for chicken fattening are based on corm, cruched soybean and fish meal. Domestic production of the basic ingredients of protein feed (soybean and fishmeal) is insufficient, thus they are mostly provided by import.
Containing more that 40% of oil and 18-23% of protein, rape cultivations recently becomes more and more important as sources for biodiesel, and motor oil production in our region, too. Due to low prices and the progress in selection, rape production has increased and the by-products, crushed and meal, are increasingly used as fedd.
The share of these nutrients in a standard diet for feeding animals and poultry depends on the type, age and the purpose and type of food that originates from rape. Nutritive value of rapeseed and its products is mainly determined by the content of protein, fat, crude fiber, and also the content of amino acids lysine, methionine and tryptophan [8].
The negative property of this feed is the increased content on anti-nutritive components (glucosinate, eruca acid, fitates, tanins, sinapine and other insufficiently investigated substances). Thus it is used in limited quantities for chicken feeding. The problem of anti-nutritive substances can be solved by proper selection of rape seed (glyucosinolates < 20 μmol/g), or with heat treatment of feed by expanding, tosting and extruding. Heat treatments of feed reduce glyucosinolates and eruka acid to the lowest possible levels, eliminating in that way the depressive influence of this nutrient in animal feeding [17, 27].
Considering the above mentioned, the aim of this paper was to investigate the carcass quality and quality (nutritive, technological and sensory) of chicken breast meat fed with mixture in which crushed soybean was partially substituted by different portions of rape seed, or with different quantities of extruded crushed rape.

MATERIAL AND METHODS

Ross 308 hybrids were used for the investigations conducted under production conditions. Feed and water supply was *ad libitum* applying floor stocking system. During fattening chickens were fed with standard mixture (K) as control and with experimental mixtures where crushed soybean was substituted by mixture, of extruded rape seed and extruded corn germ in ratio 50:50, in shares of 10% (E1), 15% (E2) and 20% (E3), or substituted by 4% (E4), and 8% (E5) of extruded crushed rape. After 42 days of fattening, broilers were hungered for 12 hours, slaughtered and processed by bloodletting, scalding, plucking and evisceration and chilled.
Eight chickens carcasses "ready to grill" from each group were cut in the basic anatomical parts (National legislation SFRY number 1/81 and 51/88): breasts, whole leg, back, wings and abdominal adipose tissue were measured.
Cutting and deboning of breast was applied in order to determine the breast meat yield and nutritive, technological and sensory quality of meat. Basic chemical composition of meat was estimated by determination of water content [9], protein [12], free fat [10] and total ash [11] content. Technological quality was evaluated by determinations of pH_u, water holding capacity (WHC_u) and $colou_u$. Meat pH value was determined 24 hours *post mortem* (p.m.) using portable pH-meter ULTRA X. WHC_u was determined by compression method and expressed in % of hold water [5). Breast meat colour was determined on the fresh cross section 24 hour p.m. using Minolta Chroma Meter CR-400, and colour characteristics were presented in u *CIEL*a*b** system (lightness *L**,

redness and greenness - *a**, yellowness and blueness - *b** [26]. Sensory analysis (odour and colour) of fresh breast meat was conducted by a group of 6 experienced evaluators of different ages. Sensory evaluation of the was carried out according point system of analytical descriptive test using scale from 1 to 7 (Table A)
All data are presented as mean values. Analysis of variance (Duncan test) was used to test the hypothesis about differences among obtained results. The software package STATISTICA 8.0 [28] was used for analysis.

Table A. Sensory analysis of fresh chicken breast meat

Value	Fresh meat	
	Odour	**Colour**
1	**Extremely bad** (weak, not expressive, strange, too expressive	**Extremely bad** (inappropriate, pale greyish yellow or dark with dotted bleedings)
2	**Extremely bad**	**Extremely bad**
3	**Bad**	**Bad**
4	**Neither good or bad**	**Neither good or bad**
5	**Good**	**Good**
6	**Very good**	**Very good**
7	**Very good** (optimal, very good, pleasant, gentle)	**Very good** (optimal, light red with light nuance of yellowish orange color)

RESULTS AND DISCUSSION

Examination of carcass quality (Table 1) of control and experimental groups shown that maximal weight of cold carcass "ready to grill" was 1514.8 g and it was found for chickens of E5 experimental group, and that the minimum weight of 1397.4 g was in chickens of E1 group. However, the numerical differences found in chilled carcasses weight were not significant (P> 0.05). Biggest breast weight of 524.2 g and maximum weight of whole leg of 470.8 g, the most important parts of the carcass, were found in chickens of E5 (8% extruded rapeseed meal) group.
The differences found between the weight of the breasts and whole leg from control and experimental groups were not significant (P> 0.05). Average values of back and wings mass were the lowest in chicken carcasses from experimental groups E1, and the highest values of these parts were in control group. Weight of abdominal fat (Table 1) was the lowest (12.2 g) in chicken carcasses from E1 experimental group and the highest value (17.2g) was found in the experimental group E4.

Table 1. Mean values of results obtained by cutting of carcasses on main parts chickens of control and experimental groups

Group	Chilled carcass mass^{ns} (g)	Breast mass^{ns} (g)	Mass of whole leg^{ns} (g)	Mass of back portion and tail end^{ns} (g)	Mass of wings^{ns} (g)	Mass of abdominal fat^{ns} (g)
	X±SD	X±SD	X±SD	X±SD	X±SD	X±SD
K	1467.4±125.40	477.0±33.50	448.2±47.76	342.8±35.17	185.0±20.04	14.4±5.59
E1	1397.4±90.45	484.8±56.03	421.4±38.14	306.8±15.17	167.2±11.23	12.2±3.11
E2	1446.2±185.88	493.8±60.56	450.0±62.34	308.2±44.10	179.2±22.83	13.4±4.56
E3	1458.6±151.38	506.0±81.92	444.2±43.10	314.8±18.85	175.4±11.28	14.0±5.70
E4	1429.2±81.69	457.2±34.36	455.4±33.78	321.4±17.60	173.0±13.62	17.2±5.12
E5	1514.8±88.32	524.2±37.63	470.8±39.94	333.4±23.86	171.0±18.15	13.6±2.61

^{ns}P>0.05

The differences between meat yield, breast mass, share of muscle in breast mass and share of breast mass in cold carcass weight (Table 2) among examined groups were numerical, but not significant (P > 0.05). Chicken breast from experimental E3 group had the highest (370.7 g) meat yield, while the largest share of muscle in breast mass was observed in control group (74.23%). The highest (25.48%) share of breast mass in cold carcass weight was found in E1 experimental group and in the other groups this share ranged from 23.13 to 25.41%. The differences found in share of breast mass in cold carcass weight were not significant (P> 0.05).

Table 2. Mean values of mass muscles breast (g), portion of muscles in breast and yields in chilled carcass chickens of control and experimental groups

Group	Muscles breast mass^{ns} (g)	Share of muscle in breast^{ns} (%)	Share of muscle in chilled carcass^{ns} (%)
	X±SD	X±SD	X±SD
K	354.1±17.27	74.23±3.16	24.05±1.85
E1	356.1±47.84	73.45±3.44	25.48±3.00
E2	359.6±56.11	72.82±2.88	24.87±1.22
E3	370.7±48.09	73.26±3.56	25.41±1.47
E4	330.6±26.46	72.31±3.07	23.13±1.56
E5	338.6±54.17	72.13±3.40	23.75±1.27

^{ns}P > 0.05

The study of basic chemical composition of breast meat from control and experimental groups (Table 3) showed that the water content was the lowest in experimental group E3 (74.97%) and the highest (75.34%) in control group K. Lowest protein content (23.08%) was found in E1 experimental group and the highest (23.64%) in E3 group. Further, content of free fat in the breast meat was very low and ranged from 0.22% (E3) to 0.19% (E4).

Differences between the content of water, protein, free fat and total ash from chicken breast in control and experimental groups were not significant (P> 0.05). Obtained results of breast meat basic chemical composition were in agreement with the results of other authors [7, 14, 19, 20, 25] who reported contents of about 23% protein, about 1% fat and 1.5% total ash in chicken meat. Compared with other kinds of meat, poultry meat contains more protein and less fat. Fatty tissues of poultry is composed predominantly of triglycerides with more unsaturated fatty acids, making it easily digestible and does not affect greatly the appearance of sclerotic blood vessels. Its specificity is reflected in the wealth of physiologically important ingredients, in easy digestibility and low energy value [3].

Table 3. Basic chemical composition of chicken breast meat from control and experimental groups

Group	**Content water** ^{ns} **(%)**	**Content Proteins** ^{ns} **(%)**	**Content free fat** ^{ns} **(%)**	**Content total ash** ^{ns} **(%)**
	X±SD	X±SD	X±SD	X±SD
K	75.34±0.09	23.47±0.16	0.32±0.01	1.12±0.02
E1	75.09±1.93	23.08±0.10	0.35±0.07	1.23±0.10
E2	75.18±0.60	23.28±0.24	0.38±0.03	1.16±0.01
E3	74.97±0.16	23.64±0.04	0.22±0.04	1.17±0.01
E4	75.21±0.85	23.17±0.88	0.39±0.06	1.23±0.09
E5	74.99±0.31	23.48±0.21	0.33±0.08	1.21±0.00

$^{ns}P > 0.05$

The study of technological properties of breast meat (Table 4) showed that the average pH_u value in control and experimental groups were equal, about 5.70. Based on pH_u values, as quality parameter and based on quality criteria [2, 24] $5.7 < pH_u < 6.1$ breast meat of control and experimental groups was "normal" - (red-pink, firm, dry) quality.

In the same Table 4 it can be seen that on average the brightest breast muscles were for control group K with lightness (*L**) of 52.28, while the average darkest muscles were for E3 experimental group with lightness (*L**) of 49.05, but these differences were not significant (P > 0.05). Furthermore, the average share of red colour *(a*)* in chicken breast meat ranged from 2.62 in the experimental group E4 to 3.89 in the experimental group E3. From the same table is can be seen that the highest average share of yellow *(b*)* of 3.46 was determined in group K, while the lowest average share of yellow (*b**) of 1.98 was observed in the experimental group E2. Based on the brightness parameter *(L*)* and criteria (15, 21) for "normal" chicken meat quality ($48 < L^* < 52$) meat from control and experimental groups had appropriate lightness of normal meat quality.

Further, is can be seen (Table 4) that the lowest average (78.38%) value for WHC_u was found in the experimental group E5, while the highest average value (87.38%) was detected in E2 groups. The differences found between the average WHC_u of control K and the experimental group E5 were statistically significant (P < 0.05). Statistically

significant (P <0.01) differences were found in average WHC_u in experimental groups E5, E2 and E3.

Table 4. Technological characteristics (pH_u, $colour_u$ (L,a*,b*) and WHC_u (%)) of chicken breast meat of control and experimental groups*

Group	pH_u ns	L* ns	a* ns	b* ns	WHC_u (%)
	X±SD	X±SD	X±SD	X±SD	X±SD
K	5.69 ± 0.12	52.28 ± 3.06	2.95 ± 0.44	3.46 ± 0.63	85.22^{aAB} ± 5.86
E1	5.70 ± 0.07	51.05 ± 1.42	2.84 ± 1.29	2.58 ± 0.80	82.92^{abAB} ± 5.45
E2	5.73 ± 0.07	50.66 ± 2.03	2.67 ± 0.75	1.98 ± 0.15	87.38^{aA} ± 3.59
E3	5.74 ± 0.06	49.05 ± 2.54	3.89 ± 1.44	2.18 ± 1.50	87.24^{aA}± 3.59
E4	5.70± 0.07	50.87± 3.20	2.62± o.42	2.59± 0.65	82.38^{abAB}±4.54
E5	5.73± 0.07	50.40± 2.46	3.52± 0.70	3.43± 0.83	78.38^{bB}±3.08

^{ns}P>0.05; ^{ab} statistically significant differences P<0.05; ^{AB} statistically significant differences P<0.01

Examining the sensory quality of fresh breast meat (Table 5) significant (P < 0.05, P < 0.01) differences were observed in colour between control and experimental groups. Colour of control fresh breast meat was evaluated as "good" or "very good". Odour of fresh breast meat was evaluated with the marks in the range from 5.48 (group E4) to 5.94 (group E3), on average rated as "good" or "very good". Differences in sensory attributes of fresh breast meat among examined groups were numerical, but not significant (P > 0.05).

Table 5. Some parameters of sensory quality of chicken fresh breast meat of control and experimental groups

Group	Colour		Odour ns	
	$\overline{X}$	SD	$\overline{X}$	SD
K	5.69^{aAB}	0.48	5.80	1.02
E1	5.22^{bB}	0.18	5.70	0.42
E2	5.91^{aA}	0.40	5.56	0.74
E3	5.98^{aA}	0.21	5.94	0.37
E4	5.78^{aAB}	0.33	5.48	0.48
E5	5.83^{aAB}	0.30	5.90	0.50

^{ns}P > 0.05; ^{ab} statistically significant differences P < 0.05 ; ^{AB} statistically significant differences P <0.01

CONCLUSION

According to results of this study it can be concluded that the established difference in carcass quality (cold carcass weight, the share of basic parts, and breast meat yield)

between the control and experimental groups of chickens, were numerical but not these not significant (P> 0.05).
Changes in the chicken diet had no effect (P> 0.05) on the nutritional quality of breast meat, i.e. content of water, protein, free fat and total ash. Breast meat of all examined groups had high nutritive value. Also, it was found that according to technological criteria (L^* value and pH_u) for determination of chicken meat quality the control and experimental groups were approximately corresponded to "normal" quality.
Partially substituted chicken standard diet with different products of extruded rapeseed showed no negative effect (P> 0.05) on sensory quality (odour) of fresh breast meat. Differences of breast meat colour obtained between control and experimental groups E1, E2 and E3 were significant (P < 0.05, P < 0.01) Colour of fresh breast meat for experimental groups E2 and E3 was rated as "very good".

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EFFECT OF DIFFERENT THICKNESS OF DIE ON THE STABILITY OF AMINO ACIDS IN PELLETING PIG FEED

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ABSTRACT

In this paper, it was investigated how different thickness of die at pellet press affects stability of amino acids in pelleting process. Diameter of openings of pellet die was 6 mm, and die thickness was 18 mm (1:3) and 48 mm (1:8), respectively. Complete mixture for pigs II (from 15 to 25 kg) was conditioned up to 80°C, and moisture content of about 16 % was achieved. Temperatures of the dies 1:3 and 1:8 were 57,6°C and 64°C, respectively. Amino acids were determined with HPLC using the AccQTag method. It can be concluded that slight decrease of amino acid content can be observed in pelleting process. For die 1:3 total amino acid content decreased by 2,21 % and with die 1:8 (thicker die) it decreased by 3,85 % because of higher temperature in the pelleting process.
Keywords: *amino acids, HPLC, die, pelleting process, conditioning, moisture content*

INTRODUCTION

Conditioning in animal feed production is process of converting mixed mash with use of heat, water, pressure and time, to a physical state which is more suitable for compaction of feed mash. Conditioning increases production capacity and, in the same time, affects physical, nutritional, and hygienic quality of produced feed [5]. Although water has binding properties as well, it is concluded that steam is far superior to water in producing good quality pellets [8]. The application of steam in animal feed manufacturing has long been recognized as a means to facilitate the production of good quality pellets [6]. High steam pressure is used in cases where relatively low amounts of water and higher temperatures are needed. During condensation of the steam, a thin film of water is created around the particles, which, together with the temperature increase, facilitates binding between particles [8]. When steam is used, the temperature of pellets after leaving the die is generally higher in comparison with that of the conditioned meal due to the frictional heat in the die [7]. Finally, pellets are cooled with ambient air. The parameters discussed with respect to the conditioning process are process variables such as steam and water and system parameters such as residence time and pressure [8]. Pelleting is a process of pressing conditioned material trough die with specific dimensions of openings and thickness. A majority of the pellet presses operated in feed manufacturing are of the ring die design. Different designs exist in which usually two or three rollers are used. In most designs, the die revolves around the fixed rollers. A minority is designed as flat-die presses in which the die is static and the horizontal

rollers rotate around a vertical axis while forcing the meal through the die plate. Die sizes may differ in their length-to-diameter ratio, normally expressed as width of the bore times the length of the die-hole [8]. Increasing die-thickness or decreasing bore-width will increase the amount of shear which the feed mash receives. This is limited since a too high amount of shear (thick dies or small bore holes) will block the pellet press [4]. With pelleting, increasing die hole length increases pellet residence time in the die, resulting in improved pellet durability although it may affect lysine reactivity [10]. In general, the effect of ingredient composition upon pellet quality has been attributed to changes that occur in the ingredients when they are subjected to physical compression and shear during the pelleting process [9].

Addition of heat and water leads to changes of components, such as starch and protein, in a way that binding property comes into effect. Livingston [1] presented experimental data showing that thermally induced decrease of protein solubility increased protein efficacy.

As has been shown by Wood [13], partial denaturation during processing may positively affect the hardness and durability of the feed pellets. Denaturation involves the breakdown of the (spatial) three-dimensional structure of the proteins (the secondary, tertiary and quaternary structures), thereby changing the bioactivity of the protein [11]. During the cooling and drying stage, proteins reassociate and so bonds can be established between the different particles. All factors affecting the breaking bonds between molecules of proteins as well as within molecules and their structural arrangements, also affect their physical, chemical and functional properties.

Processing may result in the so-called Maillard-reaction, in which many constituents of raw materials can participate, and which affects many quality attributes. Reactions between reducing sugars and free amino groups from amino acids, lysine in particular, prevail [12]. The Maillard reaction is a general term used to describe a complex series of reactions between reactive carbonyl groups, such as those of reducing sugars, and free amino groups of proteins [2,14]. Lysine is the most important carrier of free amino groups in proteins in the form of ε-amino group, and therefore is the most significant amino acid participant in the Maillard reaction. Beside lysine, arginine, tryptophane, and histidine are also carriers of free amino groups. Low-molecular-weight products of the Maillard reaction have an exceptionally important role in the formation of flavour, aroma, colour and texture in thermally treated foods [14]. Studying the effect of the Maillard reaction products on protein digestion determined that low-molecular-weight compounds developed in the reaction of glucose and lysine inhibited n-amino peptidase [3]. This inhibition resulted in reduced protein absorption in the digestive tract.

In this paper, it was investigated how different thickness of die at pellet press affects stability of amino acids in pelleting process.

MATERIAL AND METHODS

Complete mixture for pigs II (from 15 to 25 kg) was conditioned in double-shaft pedal mixer - steam conditioner, Muyang SLHSJ0.2A, China, up to moisture content of about 16 % until material reached temperature of 80°C. Batch size was 25 kg. Steam was injected in the conditioner under pressure of 2 bars.

Conditioned material was pelleted on flat die pellet press 14-175, AMANDUS KAHL GmbH & Co. KG, Germany (Figure 1).

Fig. 1. Cross-section of flat die pellet press

Diameter of openings of pellet die was 6 mm, and die thickness was 18 mm (1:3) and 48 mm (1:8), respectively. Temperatures of the dies 1:3 and 1:8 were 57,6°C and 64°C, respectively. Pellets were stored for 24 hours under room conditions in order to achieve stabile temperature. Moisture content was determined with moisture analyzer (OHAUS MB 45, Switzerland), in conditioned material. Samples were milled to pass 1 mm sieve. Amino acids were determined with HPLC using the AccQTag method. This method is based on a derivatizing reagent developed specifically for amino acid analysis. Waters AccQ·Fluor Reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate, or AQC) is an N-hydroxy-succinimide-activated heterocyclic carbamate. Separating the derivatives was obtained on reversed-phase HPLC, column Nova Pak C-18 (150×3.9mm, 5µm). The detection was obtained using the Waters 470 Scanning Fluorescence Detector.

RESULTS AND DISCUSSION

Table 1 shows the content of non essential and essential amino acids expressed in g/kg DM, for unprocessed sample and pelleted samples with dies 1:3 and 1:8, which had temperatures 57,6°C and 64°C, respectively. As seen from the presented result, there is a reduction in the level of amino acid content in relation to unprocessed sample for all examined amino acids. For die 1:3 and 1:8 total amino acid content decreased 2,21% and 3,85%, respectively.

Table 1. Amino Acids Content

Amino acids in g/kg DM	unprocessed	Die 1:3 w=15,97%	Die 1:8 w=16%
Non essential amino acids			
Aspartic Acid	14,57	14,25	14,03
Serine	9,35	9,17	9,07
Glicine	11,59	11,47	11,34
Alanine	12,95	12,58	12,62
Essential amino acids			
Threonine	6,56	6,48	6,38
Valine	8,53	8,51	8,23
Methionine	7,42	7,04	7,20
Isoleucine	7,31	7,17	7,05
Leucine	19,06	18,79	18,41
Tyrosine	5,90	5,45	4,96
Phenylalanine	9,07	8,99	8,78
Histidine	6,93	6,80	6,70
Lysine	9,97	9,62	9,56
Arginine	10,56	10,36	10,06
Total content of amino acids	**139,77**	**136,68**	**134,39**

Figure 2 shows % of reduction of non essential amino acid content after thermal treatment (conditioning and pelleting). In comparison to unprocessed sample, for both dies there is a decrease of content of all non essential amino acids. For aspartic acid, serine and glicine, decrease of content is higher when using die 1:8, while for alanine the decrease of content remains the same.

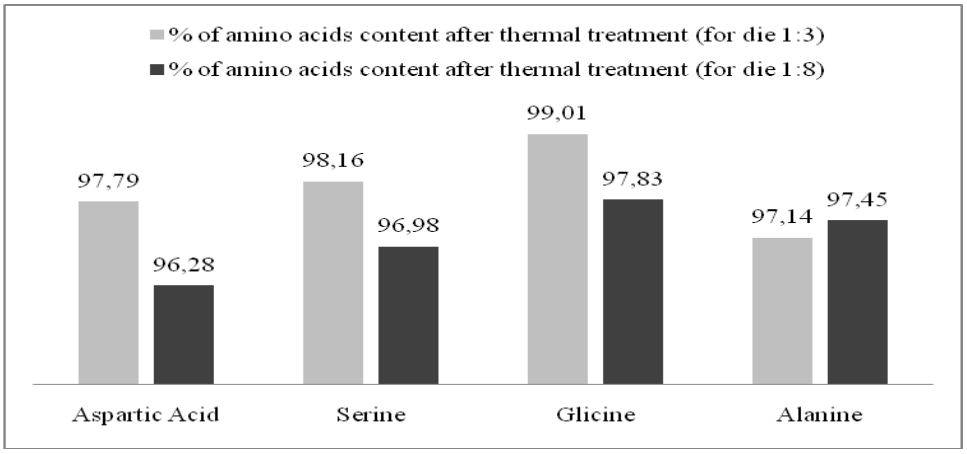

Fig. 2. Decrease of non essential amino acids content after thermal treatment

Figure 3. and 4. show % of reduction of essential amino acid content after thermal treatment. As for non essential amino acids, reduction of content of essential amino acids is higher when using die 1:8, while methionine is the only exception and that result can be explained by error of method. When using die 1:3 and temperature 57,6°C, valine showed to be the most thermostable and tyrosine the most thermolabile. When using die 1:8 and temperature 64°C, glicine showed to be the most thermostable and again tyrosine the most thermolabile. It can be seen that pellet die thickness has negative effect on amino acid stability, because die 1:8 cause higher friction between mash material and thus higher temperature of the die.

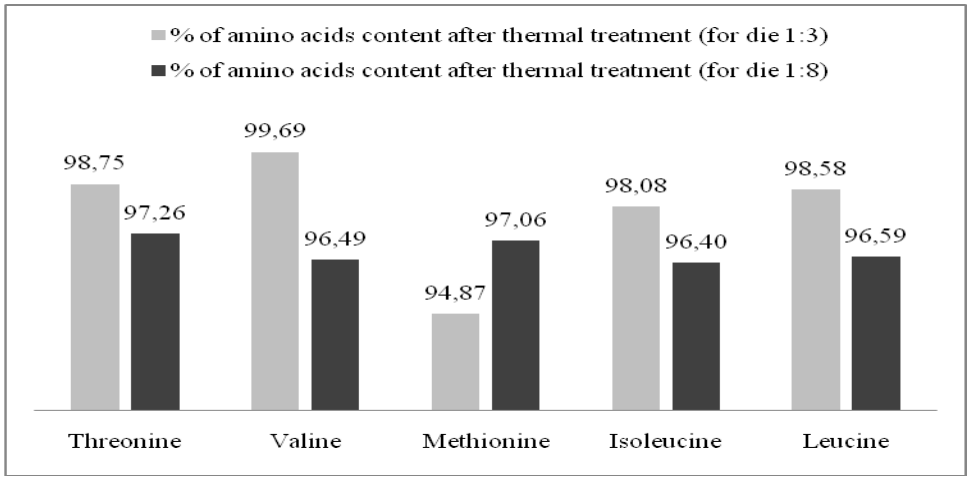

Fig. 3. Decrease of essential amino acids content after thermal treatment (part 1)

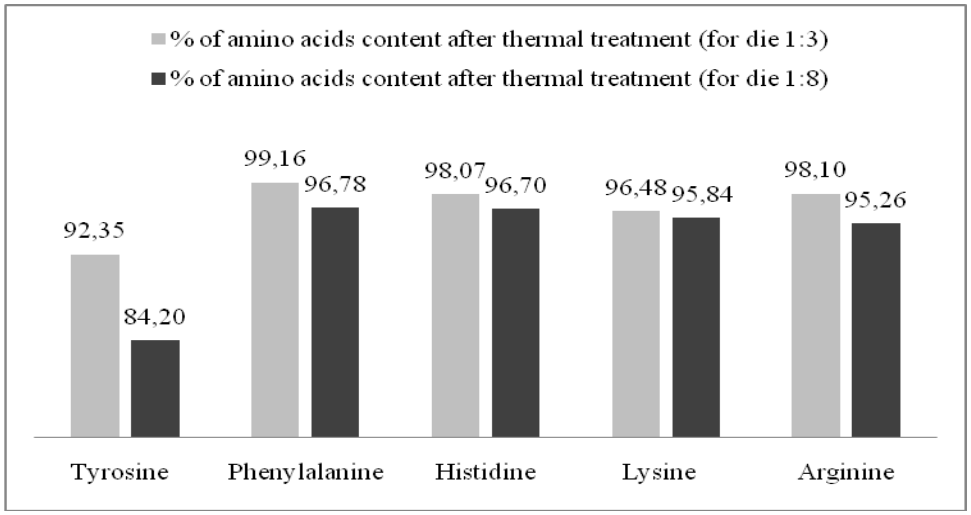

Fig. 4. Decrease of essential amino acids content after thermal treatment (part 2)

CONCLUSION

For die 1:3 total amino acid content decreased by 2,21% and with die 1:8 it decreased by 3,85% because of higher temperature in the pelleting process. As shown in the results and discussion, increased temperature in the process of pelleting pig feed undoubtedly lead to the reduction of amino acids especially essential amino acids. Considering that the essential amino acids human and animal body can not synthesize, in the future more attention should be paid to the choice of die and thermal treatment in the process of pelleting.

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INFLUENCE OF DIFFERENT FEEDING AND HOUSING CONDITION ON METABOLIC PROFILES AND MILK PRODUCTION IN ALPINO GOATS

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ABSTRACT

The aim of the study was to investigate influence of different composition of feeding and different housing conditions on metabolic profiles, and of level of milk production in Alpina goats. All investigated animals were in last of lactation periods, milked twice daily till the last trimester of pregnancy. In clinically healthy pregnant goats (n=14) situated into two farms, at mean 3-4 ages older, between 2-3 stage of lactation. Investigation was provided during month October 2009. The animals (n=7) were kept in indoor (farm A) and feeding with higher concentrate content, per day and (n=7) animals kept indoor-outdoor system (farm B), feeding with lower concentrate content but with longer grazing pasture period. Blood sample for haematochemical parameters: glycaemia level, total protein, albumin, and urea concentration, where collected from *vena jugularis.* For statistical analyses to perform variance analysis we used Statistica and ANOVA computer program. The obtained results we are determined that total protein concentration in the blood serum is statistical higher ($p<0.05$) and urea concentration is statistical higher ($p<0.01$) in the blood serum of goats in farm B. Also, daily production of milk in goats where higher in goats into farm B, but without statistical significance.
***Keywords**: metabolic profiles, feeding, housing, Alpina goats*

INTRODUCTION

Is well known that many factors influenced on production and milk content quality in the dairy cattle, especially feeding and housing conditions but in the dairy goats, about Alpina breed, is smaller different, primary it is genotype, parity and stage of lactation (**Prvanovic et al., 2008).** Metabolic profiles presented with several biochemical parameters in blood and blood serum: glycemia level, total protein, albumin, bilirubin and urea concentration, and all parameters must be according to intake balanced nutrition, but physiological values od metabolic parameters depended on many factors in both species **(Blood, 1994**; **Radojicic et al., 2010).** According to breed, parity, and stage of lactation differences influence on metabolic profiles and level lactation in goats and also is in relationship and accordance of pregnancy and stage of lactation. Also, important factor in pregnant pluriparous is nutrition with important influence on parameters of endocrine-metabolic profiles, especially in goats in the 3trd stage of lactation **(Prvanovic**, **et al., 2008).** Pregnant pluriparous with high individual variation is

compared level of lactation in similar stage of pregnancy that well known that first lactation stage have lower number of somatic cells in milk content in relationship of second lactation **(Ying et al., 2002; Pavlicek et al., 2002).** But, fat and protein content in milk is better in the third lactation stage in relationship to second lactation period **(Antunac et al., 2000).**

MATERIAL AND METHODS

In the clinically healthy Alpina goats (n=14) divided into two groups in farm A (n=7) in feeding is different concentrate content 500 g per day in the farm A, kept into indoor system and farm B (n=7) feeding with 300 g concentrate per day but with longer pasture grazing period, and housing in indoor-outdoor system. Investigation provided during month October 2009**.** The all animals were pregnant and in last of lactation period, between 3-4 ages older, and milked twice dailly till the last trimester of pregnancy. In all investigate animals blood sample were collected from the *vena jugularis.* Glycaemia level are determined in the blood by strips in Presion glucotest. Blood serum was removed by centrifugation imemediatly after collection and stored at -20 ^{o}C until in sera sample determined biochemicaly parameters (total protein, albumin, urea concentration) by biochemical analyser Vet-Screen, using Randox commercial kits. Statistical analysis provided with Statistica compute descriptive program and ANOVA one-way step computer progam to perform variance anlaysis.

Table 1. Composition and away of feeding goats in both farms

Feeding	**Material**		
	Farm A (Per goat)	**Farm B (Per goat)**	
Concentrate	500 g per day	300 g per day	
Grazing pasture *ad libitum*	-	Longer period	
Grain	*ad libitum*	-	
Premix	20 g per day	20 g per day	

The feeding away is different in farm about concentrate content and pasture grazing period shoved in Table 1. Basic differences is in the both farm is in pasture periods and dependent of housing condition. In the farm A animals kept in indoor system without grazing pasture periods and feeding with concentrate (500 g per day) and grain *ad libitum*. The animals in farm B feeding with lower concentrate content (300 g per day) but by longer grazing pasture periods, depending of kept system housing (indoor-outdoor).

RESULTS AND DISCUSSION

After obtained results we are provided next results about milking per day and mean kids per parturition (Table 2). In the goats with 3 ages older, we postulated higher mean number of 3 kids per parturition in the farm B.

Table 2. Milk production per day and mean kids per investigated goat

Animals	**Milking per day (kg)**	**Kids Mean per goats**
Farm A (n=7)	3.4 ±2.41	2.4 -2.6
Farm B (n=7)	3.6 ±1.94	2.4- 2.8

Results in the level of lactation is similar in both farm (3.42±2.41: 3.62±1.94) and higher in the farm B, but without statistical differences. The production of milk in goats depended of many factors especially of feeding, parity and stage of lactation **(Agnihotri et al., 2002).**

Table 3. The mean values of total protein, albumin, urea, and glucose concentration in the blood of Alpina goats into both farms

Alpina goats (n=14)	**Glucose (2.4-3.8) mmol/L**	**Total protein (60-75 g/L)**	**Albumin (37-43 g/L)**	**Urea (4.6-10,2) mmol/L**
Farm A (n=7)	3.62±0.35	75.91±6.77	45.61±8.72	9.91±7.64
Farm B (n=7)	3.52±1.29	80.17± 3.06*	46.64±6.77	11.27± 5.37**

* $p<0.5$
** $p<0.01$

The results in the values of biochemical parameters indicate that investigated parameters is similar x=3.62±0.35:3.52±1.29 mmol/L in glycamia level, and albumin concentration x=45.61±8.72: 46.64±6.77 g/L but of total proteins concentration is x= 80.17±3.06 mmol/L and statistical higher in the goats in farm B ($p<0.5$) and urea (carbamid) concentration is statistical higher ($p<0.01$) in blood serum of goats in farm B (Table 3). The feeding of goats in the farm B is dominant with longer grazing pasture periods and animal kept in the indoor-outdoor system. That increased of total protein and urea concentration indicate that this results directly depended of feeding away, and according with longer pasture grazing period how dominant away of goats feeding **(Radojicic et al., 2010).**

CONCLUSIONS

In the dairy goats of Alpina breed with different feeding and housing conditions we are postulated that no significant differences between glycaemia level. The feeding away with longer grazing pasture periods have influences of increase on milk production, but without important statistical significance. Also, total protein and urea concentration in the blood serum of goats increased with statistical importance and this result directly depended of longer pasture grazing periods.

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PREDICTION POSSIBILITIES OF AMMONIA PRODUCTION, CONSUMPTION AND UTILIZATION OF OXYGEN IN THE CULTIVATION OF RAINBOW TROUT *Oncorhynchus mykiss (Walbaum)*

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ABSTRACT

Considering the fact that intensive aquaculture is inevitably burdened by toxicants the aquatic environment not only fisheries, but also the recipients of water from the pond, the paper examined the theoretical possibility of ammonia and oxygen consumption, as one of the most important parameters of water.
Test were conducted on trout pond for a total of 90 days. It was found that the calculated theoretical values in comparison with labaratory findings obtained differed in average consumption of oxygen by 5,72 %, and 7,66 % for ammonia production.
***Keywords**: troat, oxygen, ammonia, water*

INTRODUCTION

Aquaculture production experienced its greatest expansion in many countries due to which inevitably increases the importance of its impact on environmental conditions and the environment. Knowledge and understanding of physical and chemical properties of water for growing fish is crucial to successfully dealing with aquaculture and a starting point to guarantee positive results in the production.
Impact of intensive cultivation of salmonid ichthyopopulation on the environment depends primarily on the size and quantity of pond fish in it, the technological process of growing, intensity of the biological autopurification, as well as physical and chemical characteristics of water [2]. Inadequate use of high protein fish food and the creation of metabolic excretory products of the fish, lead to an increase in organic and suspended matter in water which can result in eutrophication to not only a pond environment, but also to the immediate vicinity of ponds.
Also, the organic fraction of nutritiens in the water are subject to microbial oxidation, due to which decreases the amount of dissolved oxygen and increases the concentration of dissolved harmful substances, which may call into question the sustainability of fish farming [16]. Non-consumed food, product of metabolism of fish and faeces are subject to intensive biochemical processes which in the water consume dissolved oxygen and produce ammonia, phosphate, carbon and suspended solids. When feeding complete diets in body tissues of fish is mounted about 30% of the total nitrogen ingested food, and the losses amount to up to 12% dry matter of food [20]. The effect of chemical

parameters related to cultured fish is different depending on the age, size, nutritional state and condition factor of fish. Especially important are the amount of dissolved oxygen, water temparature, concentration of nitrogen fractions and pH, which affect the synegistic effect on the toxicity of certain substances and gases dissolved in water.
Nowadays more and more attention is paid to the principles of optimal aquaculture technologies based on ecological principles of breeding and feeding fish, where emissions generated organic matter was little effect on the capacity utilization of waterways and the environment [29].
The aim of this study is a mathematical assessment of oxygen consumption and ammonia in water to create a classic trout ponds on the basis of monitoring the basic parameters of water flows and the amount of feeding, protein composition of food, number and weight of cultivated fish.

MATERIAL AND METHODS

Experimental studies in this paper were carried out during the summer at the trout pond near Cacak, which is located at an altitude of 320 feet, for 90 days. The dimensions of the pool amounted to 10m x 2,1m x 1,2m, the total volume of 25,2 m^3. The entrance of water into the pool during the experiment had an average value of 12 l/sec, or 1.036.800 liters per 24 hours or 41 changes of water for 24 hours.
Mounted material made up of rainbow trout aged 12^+ months unifor body weight (65,2 g) and total body length (17,92cm). Trout were fed with fish food manufactured by "BioMar", that contained 45% raw protein. The amount and number of daily meals were determined according to manufacturer table food, which are adapted to water temparature and fish weight during the test.
Water samples were taken twice a day every day for two control profiles in the water before entering the pool and the release of water from the pool. For each studied parameter was found the average value for a period of seven days. Water parameters were determined using an automatic "MultiDirect" multiparameter phonometer, Lovibond company.
In parallel with examining the characteristics of water, the growth of fish and feed conversion are measured by a seven- day monitoring of health status and mortality, which shows the average values on a monthly basis. Representative control sample of 50 individuals in the pool for the determination the individual body weight and linear dimensions is taken by random sampling method. Individual weight of fish was determined by measuring the decimal technical scale, and total body legth by using ichthyometer.

Investigated production parameters were calculated by the following formula:

Fulton's condition factor [23]: $Fk = \frac{M\,(g)}{L^3\,(cm)}$

Density index [22]: $Ig = \frac{M\,(kg)}{L\,(mm) \times Q\,(m^3)}$

Flow index [22]: $Ip = \frac{M\,(kg)}{L\,(cm) \times P\,(l/sec)}$

M – mass of fish; L – total length of fish; Q – volume pool ; P – water flow

Specific growth rate is determinated by the formula [6].

$$G = \frac{MT - Mt}{T - t} \times 100$$

G = specific growth rate (%); MT = final weight of fish (g); Mt = initial weight of fish (g)
T = number of days at the end of the experiment; t = start of the experiment

Oxygen saturation of water, depending on altitude, temparature and partial pressure was calculated using the following formula [37,3]:

(1) Nv = elevation (m) x 3,28

(2) $PP\,(mm\,Hg) = 10\text{^}\,\frac{2{,}880814 - Nv}{64790{,}7}$

(3) $Bk(L/L\text{-}atm) = 0{,}00099902 \times EXP\,\frac{9{,}7265 - 5268{,}95}{t^0C + 273{,}15} + \frac{1004170}{(t^0C + 273{,}15) \times (t^0C + 273{,}15)}$

(4) $ZO_2\,(mm\,Hg) = 760 * EXP\,\frac{11{,}8571 - 3840{,}7}{t^0C + 273{,}15} - \frac{216961}{(t^0C + 273{,}15) \times (t^0C + 273{,}15)}$

(5) $Pp\,O_2\,(mm\,Hg) = \frac{O_2}{Bk} \times 0{,}5318$

(6) $O_2\,(\%) = \frac{Pp\,O_2}{0{,}20946 \times (PP - ZO_2)}$

Nv- elevation ; PP- partial (barometric) pressure; Bk- Bunsen's coefficient; ZO_2 – O_2 saturation in water, mm Hg; Pp O_2 – partial pressure of O_2 in water; O_2 – concentration of oxygen (mg/l); O_2 (%) – O_2 saturation in the water

The amount of oxygen consumed can be calculated in several ways depending on the parameters we have, where the results are very different. According to some authors, such as Yin-Han Wang [37] consumed oxygen in the pools is calculated using the formula:

$$\text{consumption} = \frac{\text{mg/l } O_2}{\text{kg fish x hour}} \; O_2 \text{ (mg/l)}$$

During these studies we used the most used practical formula for determining the oxygen consumption by Liao [14], which is also used for the formulation of pond carrying capacity:

$PO_2 = K_2 \times T^a \times M^b$

PO_2 - oxygen consumption (Ib/100 Ib weight of fish/day)
K_2 – the ratio of oxygen consumption and water temperature
T – water temperature
T^a – numerical decline in oxygen consumption by reducing the temparature at the same weight of fish
M – weight of fish (g)
M^b - numerical decline in oxygen consumption by the same temparature when increasing the mass of fish

The values of constant coefficient at different water temparatures by Liao [14] as follows:
water temparature is less than or equal to 50 ^{0}F: $K_2 = 1{,}9 \times 10^{-6}$; b = -0,138; a = 3,130
water temparature greater than 50 ^{0}F: $K_2 = 3{,}05 \times 10^{-4}$; b = -0,138; a = 1,855

Using equations with three unknown on the basis of empirical data modification was done with the constants in accordance with the SI system [13], and oxygen consumption PO_2 was expressed g/100g fish weight / day

$0{,}75 = K \times 10^a \times 0{,}01 \times 0{,}4536 \times 10^{3b}$
$1{,}40 = K \times 10^a \times 0{,}0001 \times 0{,}4536 \times 10^{3b}$
$0{,}27 = K \times 7{,}2^a \times 1{,}0 \times 0{,}4536 \times 10^{3b}$

The equation for the water temparature less than or equal to 10 ^{0}C:
$PO_2 = 0{,}05676 \times T^{1{,}2100199} \times M^{-0{,}1355334}$

$1{,}00 = K \times 12{,}77^a \times 0{,}01 \times 0{,}4536 \times 10^{3b}$
$1{,}40 = K \times 12{,}77^a \times 0{,}0001 \times 0{,}4536 \times 10^{3b}$
$0{,}85 = K \times 15{,}55^a \times 1{,}0 \times 0{,}4536 \times 10^{3b}$

The equation for the water temparature greater than 10 ^{0}C:
$PO_2 = 0{,}1310712 \times T^{0{,}8843114} \times M^{-0{,}146128}$

The amount of available oxygen was calculated by the formulai: $R_{O2} = Pv \times (O_2 - 5) / 1000$

However, defined by the mathematical model does not show oxygen consumption depending on the used amount of food. Depending on the authors, the quantities of oxygen consumption per kilogram of used food are different. The numerical values of the coefficient of oxygen consumption ranging from 0,20 [27], 0,22 [34], 0,25 to 0,40 [32] depending on the flow of water.
In this paper, the ratio of oxygen consumption compared to 0,40 pounds of food, because it is taken into account the insufficient number of changes of water for 24 hours:

$$PO_2 = \frac{0{,}25 \text{ x kg food}}{Pv} \text{ x } 1000 \quad (2)$$

Pv – water flow l/24 hours

Concentration of dissolved oxygen in water O_2, mg/l (Ce Ce_{SL}) at normal atmospheric pressure of 760 mm Hg (101,325 kPa ili 1013,25 mbar) and different temparatures were calculated according to Soderberg's modified formula [26,27]. Displayed equations can also be used for different altitude:

$$(1)\ Ce_{SL} = 14{,}161 - 0{,}3943\ t + 0{,}0077147\ t^2 - 0{,}0000646\ t^3$$

Correction factor for altitude is calculated using the formula:

$$(2)\ Ce_k = \frac{760}{760 + (3{,}28 \text{x} Nv)/32{,}8} \quad \text{or} \quad Ce_{k(a)} = \frac{Nv}{760}$$

Equilibrium with atmosphering oxygen pressure at different water temparature and altitude were obtained by the following equation:

$$(3)\ Ce_{SL}^{1} = Ce_{SL} \text{ x } Ce_k \quad \text{or} \quad Ce_{SL}^{1} = Ce_k - Ce_{k(a)}$$

If there is no possibility of using empirical data, predicting the concentration of total ammonia NH_4-N (g) can be determined using the calculated conversion ratio of the total amount of food (NH_4-N, g = Kh x amount of food, kg) or by Colt's formula [7], using the ratio of total ammonia:

NH_4-N (kg/day) = mass of fish (kg) x % food x K NH_4 (0,0289)

In this experiment we used **the formula prediction of ammonia** by Piper [22], based on the quantity of food and water flow, and based on the model presented by other authors, which are numbered I, II and III.

I - $(1)\ Fn = \frac{NH_4 \text{ - N x Pv}}{\text{food (kg/day)}}$

$$(2)\ NH_4\text{-N (g)} = \frac{Fn \text{ x food (kg/day)}}{Pv}$$

Depending on water temparature and pH values, the concentration of nonionic NH_3 compared to the total ammonia concentration of NH_4-N was calculated, according to Emerson [8] and Piper's tabular values[22].

$$(3)\ NH_3\,(mg/l) = \frac{NH_4\text{ - N x \%}NH_3}{100}$$

NH_4-N – total ammonia; Fn – the factor of total ammonia; Pv – water flow l/24 hour

Predicting ammonia can be viewed on a much simplier way, based on total protein content in food and modified conversion factor [7], with theoretical results obtained by Piper's similar method [22]:

$$\textbf{II -}\ NH_4\text{-N (mg/l)} = (1\text{-Fp}) \times 1000 \times \frac{\%\ \text{proteins}}{6{,}25}$$

Fp – conversion factor (0,65 – 0,80)

According to the data of most authors [8,36,3] nonionic concentration of NH_3 can be calculated based on the determined pH values and water temperature :

$$\textbf{III -}\ (1)\ pKa = \frac{0{,}09018 + 2729{,}92}{273{,}2 + t\ ^0C}$$

$$(2)\ K_{NH3} = \frac{1}{10^{(pKa\ -\ pH)}} + 1$$

$$(3)\ NH_3\,(mg/l) = K_{NH3} \times \text{food amount (kg)}$$

RESULTS AND DISCUSSION

The results achieved by weight of the individual linear growth and linear dimensions, and their growth (table1), show the normal dynamics of achieving productive results during the test. This fact confirms the value of total specific growth rate that was 120 % during the test.

Losses of cultivated fish were relatively low. From the total number of fish with which the experiment stared until the end of the experiment, 52 individuals died or 1,57 %. From the results of fish mortality in this study, it can be concluded that the ichthyhygienic measures aplied had a positive effect on reducing mortality of cultivated fish.

Flow index (Ip) and the density index (Ig) are among the most important ichtyosanitary parameters because according to their values, the optium density plantation of fish is estimated. Flow index indicates the amount of fish per unit volume compared to the flow of water, and the index of density is expressed as a ratio of weight of fish per unit area. Practically, this means that in spite of adequate water flow and adequate oxygen concentration, too many fish in relation to the area can cause many adverse situation. The determined values Ip of 1,021 to 1,917 and Ig values of 0,048 to 0,091 are in accordance with standards recommended by the majority of planting fish [22,17,11].

Table 1. The average values of technological parameters during the test

Parameters	days			
	plantation	**30**	**60**	**90**
Total number of fish	3370	3347	3330	3318
Average length (cm)	17,92	19,10	23,20	24,80
Average mass (g)	65,20	81,10	134,50	172,00
Mortality (%)	-	0,687	0,510	0,361
Average weight gain(g)	-	15,90	53,40	37,50
Total gain weight (kg)	-	51,72	176,44	122,81
Total weight (kg/m^3)	-	2,05	7,00	4,87
Average increase in length (cm)	-	1,18	4,10	1,60
Average increase in length per day (cm)	-	0,039	0,136	0,053
Average weight gain per day (g)	-	0,53	1,78	1,25
Condition factor (FK)	0,0113	0,0116	0,0108	0,0112
Total mass of fish (kg)	219,72	271,44	447,88	570,69
Number of fish (m^3)	133,73	132,81	132,14	131,66
Density index (Ig)	0,048	0,056	0,076	0,091
Flow index (Ip)	1,021	1,184	1,608	1,917
Average feed consumption (kg/dan)	2,63	3,53	5,82	7,42
Feeding coefficient (HK)	-	0,068	0,032	0,060
Daily food consumption per fish (g)	0,78	1,05	1,74	2,23
Specific growth rate (G %)	-	54,82	178,00	125,00

Water as a habitat, crucial effects on fish and other hidrology biotic with its physical and chemical characteristics. The largest part of the biological process directly depends on water temparature, which is particularly important when converting ammonia to nitrate with the intensity of transformation increases with increasing temparature. For trout, the optimum temparature at which they maximum grow, take adventage of food and have the greatest immune potential is from 12-16 ^{0}C, but the tolerant values of the temparature are fromo10 do 18 ^{0}C [25].

Temperature of water as one of the major environmental factor,depended primarily on the temparature of the surrounding air. The values shown in table 2, were within the normal temparature ranges from 12,30 ^{0}C to 14,00 ^{0}C. The concentration of hydrogen ions show quite uniform values during the experiment with the lowest value of pH 7,40 to a maximum of pH 7,90.

Table 2. The parameter values of oxygen consumption per days

PARAMETERS	days							
	1-7	**7-14**	**14-21**	**21-28**	**62-69**	**69-76**	**76-83**	**83-90**
water temparature ^{0}C	12,50	12,30	13,20	13,50	12,80	13,10	13,90	14,00
pH	7,70	7,60	7,40	7,55	7,80	7,75	7,80	7,90
consolidated conc. O_2 at the entrance (mg/l)	10,90	10,70	10,20	10,40	10,60	10,30	10,10	10,00
saturated oxygen (O_2 %)	106,24	103,82	100,99	103,66	104,02	101,75	101,57	100,78
concentration O_2 Ce_{SL} (mg/l)	10,31	10,36	10,15	10,08	10,24	10,17	9,98	9,97
concentration O_2 $Ce_{SL}{}^1$ (mg/l)	9,89	9,94	9,74	9,68	9,83	9,76	9,59	9,57
available O_2 (g/day)	6117,12	5961,60	5391,36	5598,72	5806,08	5495,04	5287,68	5184,00
consumption (g/day)	1804,12	1769,25	1927,08	1980,20	2860,50	2941,82	3160,58	3929,13
consumption (mg/l)	1,74	1,71	1,86	1,91	2,76	2,84	3,05	3,79
consumption per fish (g/day)	0,54	0,52	0,57	0,59	0,86	0,88	0,95	1,18
% utilization O_2	29,49	29,67	35,74	35,37	49,26	53,53	59,77	75,79
total consumption O_2 with food (mg/l)	3,10	3,06	3,22	3,27	5,99	6,04	6,23	7,38
expected concentration O_2 at the exit (mg/l)	7,80	7,64	6,98	7,13	4,61	4,26	3,87	2,62
consolidated concentration O_2 at the exit (mg/l)	7,45	7,30	6,50	6,85	4,20	4,00	3,65	2,50

During these investigations the concentration of dissolved oxygen inlet water was quite uneven and primarily depends on water temparature. The lowest limit of dissolved oxygen was 10,00 mg/l in August, and in June, the highest value of 10,90 mg/l was recorded. Determined values of this parameter were in the optimum range. As confirmed by many authors [22,5,17,24,9] indicative value for this significant water parameters are (table 3):

Table 3. The values of the parameters of water for fish

Parameter	Formula	j.m.	Normal values	Negative level
Ammonia	NH_3	mg/l	<0,025	>0,01
Ammonium ion	NH_4^+	mg/l	0 – 2,5	>2,5
Nitrate	NO_2^-	mg/l	0 – 0,5	>0,5
Nitrate	NO_3^-	mg/l	100 - 200	>300
Oxygen	O_2	%	70 - 110	<40 i >250
Carbon dioxide	CO_2	mg/l	10 - 20	›20
pH			6,5 – 7,9	<6,2 i >8,0

Available oxygen for fish is the difference between the amount of dissolved oxygen in the incoming water and physiological minimum concentration, which for the trout is 5 mg/l [35,33]. Exposure of trout to a dissolved concentration of oxygen from 3 to 5 mg/l increase food consumption, interferes with general health and survival. Drop in oxygen levels below 3 mg/l has a lethal effect for the trout [34].

The amount of consumed oxygen during the experiment was directly proportional to the number or weight of fish, then the water temparature, intensity of metabolic processes and quantity of meals. These data in table 3, closer show to the point that oxygen consumption increased with increasing water temparature and fish biomass. However, oxygen dissolves in water easily at lower temparatures. According to Alabaster[1], at higher temparatures the flow of water should be increased or make aeration. Some authors, such as Pillay [21], believe that the growing trout in earthen ponds of $1m^2$ at water temparature of 10 ^{0}C spends about 70 mg of oxygen, and at 20 ^{0}C to 150 mg.

The calculated concentrations of dissolved oxygen at the exit from the pool have shown a little more value to the control profiles compared with the measured values, which is due to biochemical processes the body of fish, and decomposition of organic matter, especially in the second third of the experiment, which is consistent with the assertions of some authors [28,19]. During this experiment the average difference between the expected and theoretically determined oxygen concntration was 5,72 %, on this basis we can conclude that the empirical oxygen consumption was greater than the theoretical and numerical difference ranged from 4,09 % (21-28 days) to 9,76 % (62-69 days).

According to many authors [4,17,30,12], oxygen consumption and ammonia excretion in fish subject to a variety of daily variation and change in value over one hour, depending on the mode of nutrition and overall physical and chemical properties of water. For comparison, 1kg of fishfood to feed the fish bass affects the creation in the wateri: 0,28 kg CO_2; 0,03 kg total ammonia; 0,3 kg of suspended solids, and 0,2 kg of dissolved oxygen is spent [7].

The creation of nitroge in fish pond water is directly depended on water temparature, pH fluctuation, water flow, number of changes of water for 24 hours, the protein content in food, number and weight of fish and quantity of meals. Ammonia is the end product of decomposition of amino acids, and particularly toxic to fish are nitrite NO_2^- and nitrogen fractions of NH_3. In aqueous solution there is a constant balance between nonionic form NH_3 and ammonium ions NH_4^+ depending on other parameters of water, and the special significance is given to pH and temparature. With increasing pH value of water, the

concentration of ammonia increases, too. And, the amount of ammonium ions reduces [31].

Table 4. Ammonia production per days

PARAMETERS		days							
		1-7	**7-14**	**14-21**	**21-28**	**62-69**	**69-76**	**76-83**	**83-90**
consolidated conc. NH_3 at the entrance (mg/l)		<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001
minimum productionNH_4 (mg/l)		0,050	0,050	0,050	0,050	0,080	0,080	0,080	0,080
maximal production NH_4 (mg/l)		0,075	0,075	0,080	0,080	0,185	0,185	0,190	0,190
production NH_4-N (g/day)	**I**	54,88	55,15	64,81	76,26	116,69	130,33	144,84	151,36
	II	52,07	53,46	59,40	69,89	115,23	128,70	140,58	146,92
production NH_4-N (mg/l/day)	**I**	0,0529	0,0532	0,0625	0,0735	0,1125	0,1257	0,1397	0,1459
	II	0,0502	0,0515	0,0572	0,0674	0,1111	0,1241	0,1356	0,1417
production NH_3 (mg/l/day) - **I**		0,00059	0,00047	0,00037	0,00057	0,00164	0,00145	0,00220	0,00289
production NH_3 (mg/l/day) - **II**		0,00055	0,00045	0,00034	0,00053	0,00162	0,00143	0,00214	0,00280
pKa		9,64	9,65	9,62	9,61	9,63	9,62	9,59	9,59
K_{NH3}		0,011	0,008	0,006	0,008	0,140	0,011	0,015	0,019
production NH_3 (mg/l/day) - **III**		0,00059	0,00046	0,00037	0,00060	0,00162	0,00147	0,00218	0,00288
consolidated concentration NH_3 at the exit (mg/l)		0,00060	0,00049	0,00042	0,00061	0,00176	0,00158	0,0024	0,0031

Based on these results, which are presented in Table 4 can be concluded that the established concentration of NH_3 at the exit of the pool had a higher value of the used mathematical models. The determined concentrations of these two water parameters (NH_3 i NH_4) are in agreement with the optimum limit values for growing trout. The average difference between the set and obtained numerical values ranged from 7,66 % for the I Piper [22] method to 11,16 % for the II Colt [7] method. According to the results shown in tables, the biggest coincidence between the theoretical results and methods and determined concentration of NH_3 is noticed.

According to some researches [11,5], excessive concentrations of ammonia cause damage to gills, reduce growth and increase mortality. But, thanks to the developed adaptive organic systems, trout relatively well tolerate higher concentrations of ammonia on limited values. Data on limited values for ammonia are varied and they are from 0,0125 mg/l to 0,025 mg/l.

Aquatic production of pollutants of the aquatic environment is indirect correlation with the amount of daily meals [10,15,27]. Coefficients that create the most important nutritients in water at trouts that are fed per kilogram food pellets as a water temparature of 10 to 15 ^{0}C are: total ammonia (0,289), phosphate (0,0162), suspended solids (0,52), biochemical oxygen consumption (0,60), chemical oxygen consumption (1,89).
Bearing in mind the presented results, there is the further task of determining the precise calculation procedures for obtaining closer values in the comparison with the results of labaratory analysis.

CONCLUSION

On the basis of the test, the following conclusions can be done:

a) oxygen consumption and ammonia excretion directly depend on the temparature and water flow, pH, the amount of food and protein content in it and the weight of fish.
b) the average difference between the expected and theoreticaly determined oxygen concentration is ranged from 4,09 % (21-28 days) to 9,76 % (62-69 days), or an average of 5,72 %.
c) calculated ammonia production differed depending on the applied mathematical models. The highest concordance between the results and methods I, Piper [22] and determined concentration of NH_3 was found.

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TECHNOLOGY FOR ACHIEVING QUALITY PARAMETERS OF CARP FRY FEED

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APSTRACT

Production of fish feed with exactly defined composition and physical properties is the complex technological process with many interrelated and intertwined influences. It is necessary to achieve proper shape and size of granules, specific or apparent density and stability of fish feed in the water, to meet nutritional requirements while adhering to the regulations for environment protection. In this study, chemical composition and physical characteristics of raw materials (grain size, bulk density and angle of repose), as a prerequisite for the formulation and achievement of quality parameters of the finished product, were determined. As a result, three types of carp fry feed with different particle sizes (<0.5 mm, 0.5-1 mm and 1-2 mm) were produced. The obtained products were defined by the physical and nutritional quality parameters. The overview of the impact of certain technological operations and specific requirements to be met in order to achieve the required quality parameters of carp fry feed was presented.
Keywords: *physical properties, carp fry feed, quality, technology*

INTRODUCTION

Nutrition contributes the most to the costs of intensive and semi-intensive production of fish. Therefore, high quality fish feed, provides a high daily gain, good health of animals and products and desirable quality of fish meat for human consumption. The quality of feed includes several aspects such as nutritional, physical, hygienic and sensory quality. Nutritional quality is defined as the ability of feeds to meet nutritional requirements of specific animal species and categories in order to achieve optimal growth. Physical quality is not usually associated with nutritional quality, although there is mutual influence between these two factors, but is defined as the ability to file produced food handling without generating large amounts of dust (losses), to maintain the necessary level of stability in the water and to minimize pollution of water by dissolving ingredients and the remains of non-consumed and / or undigested feed. Hygienic quality includes content of mycotoxins and microorganisms in the area of feeding, and sensory appearance, smell and taste of fish feed [20]. For some fish species is necessary to produce a special form of pellets to stimulate feeding [4, 5]. All these characteristics must be taken into account in the fish feed production. Technological process particularily has decisive influence on quality parameters.
Intensive farming, which always involves a large number of fishes in a small pond or a certain volume of water, in addition to economy in nutrition, has strict requirements

regarding the composition and physical properties of fish feed in order to minimize water pollution remains non-consumed and / or undigested feed [11, 18].
Fish feed must be formulated to provide the optimum combination of nutrients and an adequate energy content for each type and category of fish in accordance with their physical needs and conditions of breeding, taking into account the total costs. Increase of digestibility and availability of feed and reducing the amount of non-consumed feed significantly affect the reduction of water pollution in intensive fish production. It is also important that feed must be balanced in accordance with the maximum production potential of fish. The more accurately feed meet the needs, according to availability and quantity, the less water pollution will be [12.18].
There are a number of strategies to reduce losses of nutrients from fish feed by dissolving in water, which are primarily [22]:

- reduce the time between the giving of feed and consumption, achieved by additives that will attract fish and increase their impact on hunger
- achieve the optimal size of grains in accordance with consumption and losses by dissolving
- coating of granules or a mixture of water soluble components with protective layer that will not adversely affect the digestibility and availability of feed
- different methods of application for water soluble compounds that will "capture" and protect consuming

To meet nutritional requirements and more stringent regulations for the environment protection, it is necessary to achieve proper shape and size of granules, specific or apparent density and stability in the water. All these characteristics must be tailored to the level of growth and size of fish and its natural feeding mode. For fish fry feeding, there is additional research challenge, in order to reduce loss of feed, increase consumption and utilization and it should be provided at the level of very small granules of complete mixture [10, 17, 18]. If granule is too big or too small fish will not consume it. The American Association of Soybean Producers (ASA) in collaboration with the Research Institute of China ZFRI found out the size of particles of feed suitable for the consumption by carp of 2 to 7 cm lenght. The results are shown in Table 1 [5].

Table 1. Total lenght, weight, mouth opening and particle size of feed for 2-7 cm long carp fry [5]

Fish lenght [cm]	Fish weight [g]	Mouth opening [mm]	Particle size of feed [mm]
2	0.1	1.0	0.5
3	0.4	2.7	1.3
4	1.0	3.0	1.5
5	3.0	3.9	1.9
6	4.0	4.2	2.1
7	5.5	4.5	2.2

Sinking or floating of fish feed depends on the specific gravity or density. Floating granules have a specific gravity of 900-1000 g/dm^3 and the sinking 1000-1200 g/dm^3. In practice, measuring the specific gravity is more complicated so it is determining by the bulk density. This value strongly depends on the size of granules, porosity and presence of air and it is also affected by temperature and water hardness. Table 2 shows the requirements for the bulk density according to the behaviour of granules in fresh water at 20 ° C [17].

Table 2. Relation between bulk density and buoyancy of fish feed[17]

Buoyancy	Bulk density [g/dm^3]
Fast sinking	>600
Slow sinking	540- 560
Neutral floating	480-520
Floating	< 440

Powder mixture is not suitable for fish feeding because the nutrients are unnecessarily lost by segregation and dissolution in water. Granulating the mixture by the process of pelleting and/or extrusion is preferable. Therefore, pelleting is not suitable in the production of high quality fish feed because it is not possible to produce floating pellets. Also, the abrasion of pellets and 1-3% of dust that is always present cause a significant loss and negative impact on the water purity[12].
By using conventional processes for the production of fish feed (pelleting and extrusion) it is possible to produce granules of a few millimeters lenght. Granules with size of 2.5 mm can be achieved by single–screw extruder and 1.5 mm lenght by twin-screw with an acceptable capacity and power consumption. Since there are requirements to produce even smaller particles for carp fry feed, produced granules of homogeneous composition must be additionaly processed and classified by size [4, 9.18].
Production of fish feed with exactly defined composition and physical properties is the complex technological process with many interrelated and intertwined influences. A modern equipment with the appropriate measuring and control devices and extensive

knowledge and experience of nutritionists, technologists and operators are necessary. Technology of fish feed production includes the basic technological processes, such as grinding, weighing and dosing, mixing, extrusion, drying, cooling, crushing, sieving, dosing of liquid components and transportation and storage of the raw materials to the packaging and distribution of finished products. However, the entire process of carp fry feed production is complex and specific [10.15, 21]. The aim of this paper is to highlight the most important characteristics of the technological process of production of carp fry feed, starting with the raw materials used, to the process parameters related to the characteristics of components and finished products.

MATERIAL AND METHODS

The raw materials commonly used in the production of fish feed, including some specific, that contribute to a better nutritional quality of the product or improve its physical properties has been examined. Particle size distribution of raw material, was analyzed by the standard ISO method [8], bulk density was determined by the method according to DIN [6], and angle of repose by method according to Appel [1].
Raw materials with nonsuitable particle size distribution (corn, corn gluten, fish meal, soybean cake, soybean meal) were milled by pulveriser before the determination of physical characteristics.
Selected raw materials were those that are used in the formulation of the mixture in the production (corn, corn gluten, fish meal, soybean cake, soybean meal, wheat flour, egg powder, chalk, premix, DL-methionine, and L Lysine - HCl). In addition, soybean oil was used
Chemical composition of components used in the formulation and chemical composition of finished products were determined by standard AOAC methods [14]. Metabolic energy was calculated according to the AEC tables [23]. The homogeneity of the mixture was measured by Microtracers ® method [2].
Based on the results of previous research and literature review [9, 10, 19, 20, 21] processes of milling, mixing, extrusion, crushing, screening, fractions and vacuum coating were adjusted and controlled. As a result, three types of carp fry feed were produced. Chemical composition and the physical parameters of quality (particle size and bulk density) of finished products were determined.
Figure 1 shows the technological scheme of production of carp fry feed, which was applied in the framework of our research.

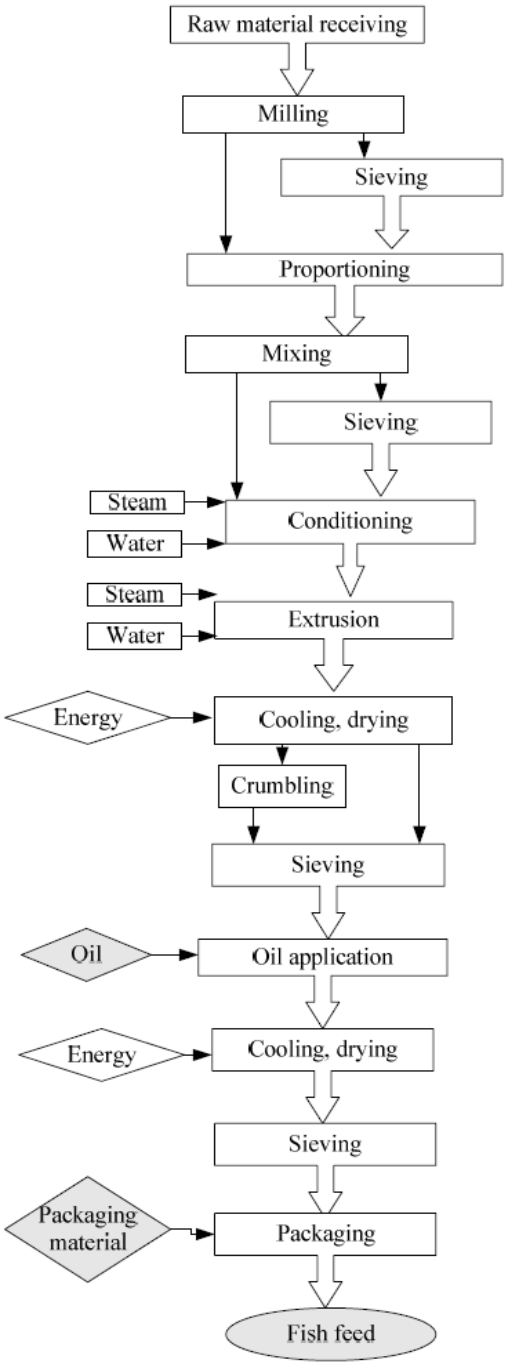

Figure 1. Technological scheme of production of carp fry feed

RESULTS AND DISCUSION

According to the nutritional requirements for carp fry feeding, formulation was made using available components tested: corn, corn gluten, fish meal, soybean cake, soybean meal, wheat flour, egg powder, limestone, premix, DL methionine, L-Lysine HCl and soybean oil. The main chemical composition of the produced mixture is shown in Table 3

Table 3. Chemical composition of carp fry feed

Parameter	Content
Moisture [%]	9.95
Protein [%]	42.78
Ash [%]	8.67
Fat [%]	10.87
Celulose [%]	3.17
Lysin [%]	3.53
Methionin [%]	1.77
Methionin+cystin [%]	2.38
Ca [g/kg]	18.90
P [g/kg]	12.39
Na [g/kg])	2.69
Metabolic energy [MJ]	15.05

The processes of milling, extrusion, fat addition, crushing and fractionating of granules are very specific in the production of carp fry feed. Special attention should be paid to grinding, as a precondition for achieving homogeneity and guaranteed composition at the level of granules. It requires a great number of very fine particles of all components [20]. Grinding of feed for larvae requires particle size of 250-300 μm [4]. It is preferable to grind each component separately to achieve uniform particle size distribution, since the raw materials and particles of different fractions do not behave on the same way during milling. It is very difficult to get enough small particles by hammer mills. Even a good aspiration can not avoid bridging of materials and adequate cleaning of screens with very small openings [20]. Granulometric profile of tested components milled or prepared by prior processes and components which are not milled (wheat flour, egg powder, amino acids, premix and limestone), is shown in Figure 2

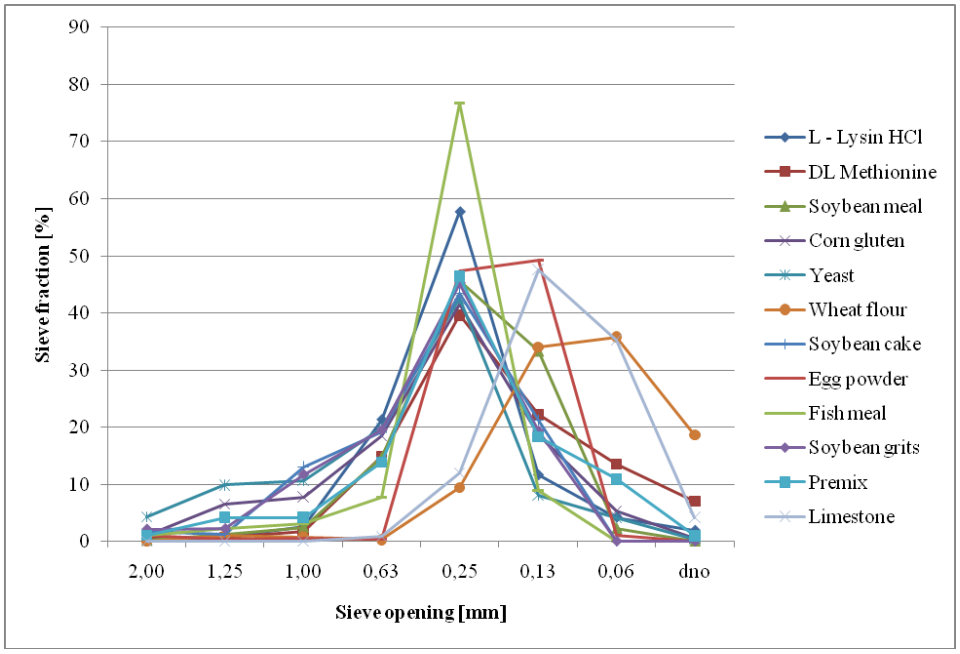

Figure 2. Granulometriic profile of raw materials for carp fry feed

From the results shown in Figure 2 can be concluded that the grinding was achieved by uniform size and size distribution, although they were components of different chemical composition and different physical characteristics. Raw materials grounded by pulveriser (corn, corn gluten, fish meal, soybean cake, soybean meal) 60-79% of particles were ranging from 250 to 630 µm, while the egg powder particles, wheat flour and limestone were significantly smaller. Egg powder had 96.5% of particles smaller than 630 µm, and wheat flour and limestone 88.5% and 82.9% of particles smaller than 250 µm respectively.

Values for the angle of repose of components as an important feature for handling raw materials and mixing in the process, were within the range of 14 to 29.2 o, as shown in Table 4. According to Appel [1], materials with angle of repose below 30° belong to the group a good flow, suggesting that the selection of raw materials reduced the risk of bridging. According to data showed in Table 4, comparing the embankment weight components, it can be seen that most values range from 520-590 [g/dm3], but for premix and methionine, whose bulk density exceeds 750 [g/dm3], chalk to fill a mass of 1043 [g/dm3] and egg powder which was bulk density below 400 [g/dm3]. Although these components have the bulk densities that interferes with the uniformity characteristics of the components, they were selected because of the nutritional characteristics that must be met in the compound.

Table 4. Physical properties of raw materials used for carp fry feed

	Raw materials	**Bulk density** [**g/dm^3**]	**Angle of repose** [°]
1	Corn	540	22.9
2	Corn gluten	559	15.3
3	Soybean meal	564	18.0
4	Soybean cake	575	20.8
5	Soybean grits	524	22.5
6	Fish meal	597	29.2
7	Yeast	558	19.3
8	Wheat flour	586	27.2
9	Egg powder	383	28.4
10	Limestone	1043	40.8
11	Premix	760	19.8
12	L Lysin HCl	575	14.0
13	Dl Methionin	789	21.0

For effectively mixing of accurate and precise dosed, carefully selected components is important to use technological process in producing all kinds of compound feeds. General requirement is that the stirrer, regardless of shape, size, design and installation is expected to produce a homogeneous mixture, and it must be ensured that homogeneous distribution of ingredients by the coefficient of variation up to 10%. [2, 3, 21]. Mixing efficiency can be improved by extending the mixing time, changing the level of charge, repair the physical characteristics of components or selecting acceptable substitute, and by removing faults and defects on the device (bad closure, the curvature of shaft and tools for mixing, etc ...) [21]. Selection of materials and devices available for mixing enabled a homogeneous mixture of ingredients distribution with coefficient of variation of 5.8%, thus fulfilling one of the main prerequisites for achieving the required composition of the finished product.

Process without an alternative in producing high quality fish feed is extrusion, a method of cooking the material by friction. Extrusion is performed according to the principle of suppression of material, which is processed, a strong screw through the cylinder, from which the treated material is extruded in the form of granules. Due to the design parameters of the device, by extrusion is carried out a large number of operations such as hydration, homogenizing, mixing, dispersion, compression, heat treatment, destruction of microorganisms, inactivation of antinutritional factors, protein denaturation, gelatinization, compression, expansion, connectivity of particles, formation, the formation of porous structure and partial dehydration [11, 16]. Extrusion cooking on the principle of "high temperature-short time" (up to 200^0 C, 20-30 s) achieves multiple changes in the treated material, such as higher digestibility and inactivation of undesirable ingredients - trypsin inhibitor, lipoxygenase. Due to such a

short time exposure to high temperatures of the treated material, there is no significant damage to amino acids and vitamins [10]. Controlling of the process parameters of extrusion, which affect the level of expansion, provides a swimming or sinking granules. Proportional to the increase of pressure and temperature in the extruder, the material dissolves and the degree of dissolution depends on the level of expansion at the exit of the extruder. Adding water steam to viscoelasticity material have a decisive influence on the expansion. Higher temperatures and higher humidity reduces the viscosity of the material and facilitate its expansion [15]. The process of extrusion of fish feed density can be influenced by changing pressure and temperature, ie. characteristics of the extruder (screw configuration and pipe extruder, the rotation speed of a screw, engine power, opening the matrix output, ventilation tube extrusion ...) and added the quantity and quality of water and steam [4, 12, 13, 16]. Given that the available production line did not offer the ability to change configuration of extruder a hands-on experience was used to ensure a controlled production in accordance with set objectives. Extrusion pellets were produced by crushing and sieving adjusted to the required size in accordance with the requirements of carp fry. Rotary screens were used for sieving. Fractional separation of the cracked granules was done by size and three products with different particle sizes for different categories of carp fry feed were obtained. Basic physical properties of products obtained (range of particle size and bulk density) are shown in Table 5. Based on the achieved bulk density obtained products could be classified as "floating neutral" by Rokey classification [17].

Table 5. Physical properties of carp fry feed

Type of feed	**Bulk density [g/dm^3]**
Carp fry feed < 0.5 mm	480
Carp fry feed 0.5-1.0 mm	460
Carp fry feed 1-2 mm	445

Size distribution for all three types of feed produced for carp fry obtained by sieving analysis is shown in Figure 3. Results show that the granules obtained by fractionating of cracked products of different particle sizes for different categories of feeding carp fry with a very narrow range of variation of particle size in relation to the most frequently used size. The product with the smallest particles had more than 90% of particles ranging from 0.25 to 0.63 mm. Products were free of dust. On the sieve with the smallest openings no residue after sieving were found.

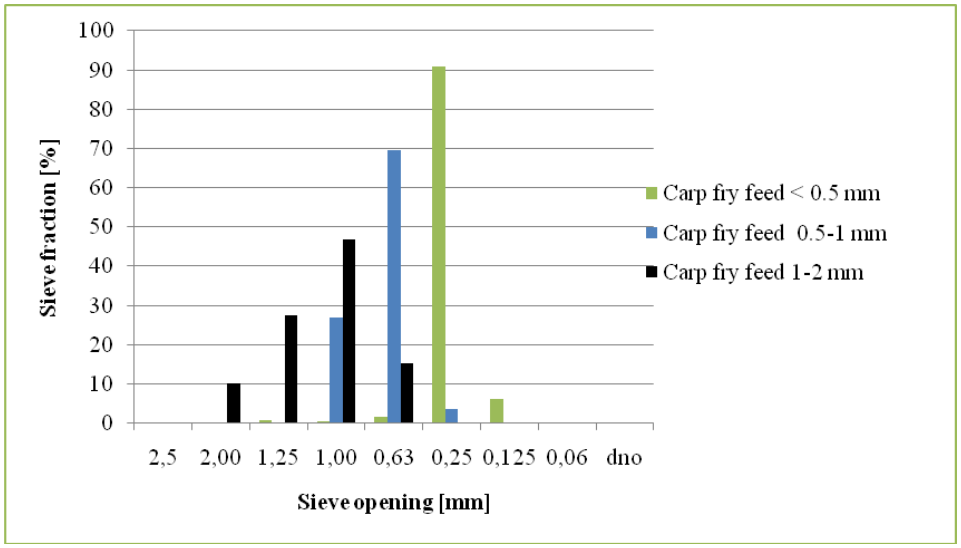

Figure 3. Granulometric profile of carp fry feed

The production of high-energy fish feed, requires a high level of fat in the granules, which is a particular technological problem because of the negative effects of fat on the strength of granules. This problem of adding large amounts of fat was solved by a device for subsequent coating under vacuum, a system that allows the deployment of oil on the entire volume of granules [7, 19, 25]. Usually the oil is added at 40-50^{o} C, which is above the solidification temperature of fat, but due to the low viscosity it facilitates dosing [10].
Extruded products have more pores and therefore, can receive a higher percentage of fat than pellets. The prescribed formulation has necessitated the need for dosing large amounts of fat. There has been the content of 10.87% fat in feed for carp fry adgered by using a dosing device for subsequent addition of fat (vacuum coater). Produced granules were solid, did not have "greasy look" and have maintained stability in the water over 4 hours. The average acceptable stability of fish food in water is about 4 hours, although some products achieve stability for up to 24 hours. Stability can be increased by adding binders [15].

CONCLUSIONS

In order to produce fish feed which will meet nutritional requirements is necessary to achieve proper shape and size of granules, specific or apparent density and stability in water. All these characteristics must be tailored to the level of development and size of fish and its natural way of feeding. Fish feed of exactly defined composition and physical properties was produced by complex technological processes with many interrelated and intertwined influences. For the development of this processes, modern equipment with the appropriate measuring and control devices and extensive knowledge

and experience of nutritionists, technologists and operators is necessary. Based on the research and literature review, controlled parameters of the processes of milling, mixing, extrusion, crushing, screening, fractionating and subsequent addition of fat under vacuum were adjusted and three types of carp fry feed that have met the requirements for quality parameters, were produced .

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THE EFFECTS OF PARTIAL REPLACEMENT OF GRAINS WITH MOLASSES ON RUMINAL MICROBIAL PROTEOSYNTHESIS IN GROWING RAMS

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ABSTRACT

The effect on rumen microbial proteosynthesis of a partial replacement of starch (from grains) with sugars (from molasses) was assessed on 8 Merino growing rams, within a 3 weeks digestibility trial. The diet consisted in low quality Sudangrass hay fed ad libitum to both groups and a compound fed based on grains and cold-extracted sunflower meal, which was restricted to 300 g/d in control group (without molasses) and 180 g/d in experimental group (with molasses), which also received 120 g of a mixture of molasses and sunflower meal (83:17). The microbial proteosynhesis was estimated on the base of purine derivatives determined in urine. The amount of purine derivatives excreted in urine were 4.54±1.58 mmols/d and 4.49±1.75 mmols/d, for the control and experimental group, respectively and the quantities of microbial protein were 15.54±9.75 and 16.08±9.98, respectively. It is concluded that replacement of grains with molasses did not significantly influenced microbial proteosynthesis.
***Keywords**: microbial proteosynthesis, molasses, sheep, purine derivatives*

INTRODUCTION

Molasses represents an important feed resource for ruminants; approximately half of the worldwide production being used in animal feeding. The largest consumption is recorded in the developed countries, due to the technological capacity allowing the incorporation of molasses in the commercial products; on the other hand, the consumption is low in developing countries and has declined in Eastern Europe (because of the changes in the production systems). From the point of view of ruminants, molasses are important as source of readily available carbohydrates, one of the direct effects being the stimulation of microbial proteosynthesis, especially when the digestibility of the basal diet is low.
Among the most cited problems for limited microbial proteosynthesis is the low degree of synchronization of energy and protein supply. However, the results of synchronization trials are often controversial, because of the high variety of feeding situations (Hersom, 2008). One of the proposed approaches, beside supplementation, is to replace starch with

sugars (Chamberlain, 1993, Araba, 2001) in order to match the ruminal availability of nitrogen, when this is provided by degradable sources.
The objective of the study was to assess the effects of partial replacement of grains with molasses when on microbial proteosynthesis in sheep when basal diet is provided by a low quality hay (Sudangrass) and the source of nitrogen is a rapidly degradable protein meal (cold-extracted sunflower meal).

MATERIAL AND METHODS

Eight Merinos rams, 55–60 kg liveweight, randomly assigned to a control and an experimental group, were used for determination of microbial proteosynthesis. The animals were kept in individual digestibility cages and usual procedure for digestibility trial (Burlacu, 1996) was applied. Animals were fed experimental diets for three weeks, in the winter time, of which 2 weeks of adaptation and one week of data recordings. Diets consisted in low quality Sudangrass hay (ad libitum) and compound feed (restricted) and were designed to meet the nutrients requirements of the animals' category and to and to ensure equal supplies of nutrients to both groups. The compound feed consisted in 30% wheat, 30% barley, 15.9% corn, 20% sunflower meal and 4.1% minerals & vitamins specific to animal category. Consumption of diets, recorded daily and individually, is shown in Table 1. Control group was fed 300 g/d compound fed while the experimental group received only 180 g/d compound feed and 120 g/d of a molasses & cold-extracted sunflower meal mixture (83:17). This led to a partial replacement (40%) of grains with molasses. Nutritive value of feeds and dietary supply of nutrients were calculated according to Burlacu, 1996.
Urinary excretion of purine derivatives was determined using the method of Chen et. al, 1992: total daily collection of urine and dosage of allantoin, uric acid, xanthine and hypoxanthine. Concentration of creatinine was also determined. The daily retained volumes were pooled every two days; therefore 3 samples for each animal were taken, leading to 12 observations per group. Of the two days pooled samples, 50 ml were placed in vials and frozen until analysis of purine derivatives.
Allantoin, uric acid, xanthine and hypoxanthine were assessed by HPLC. Purine derivatives standards were prepared with ultrapure water and 1N sodium hydroxide solution was added until pH reached 7.8. Elution flow was 1 ml/min, detection was performed at 218 nm, column temperature was 25°C and samples were priory passed through Teflon filters. Before determination, samples were stabilized with sodium hydroxide and passed through a 0.45 micrometer filter.

Table 1. Consumption of experimental diets

	Control group (grains)	Experimental group (molasses)
Sudangrass hay, g/d	631	624
molasses & sunflower meal, g/d*	-	119
compound feed, g/d**	293	179

** 8:1 (molasses : sunflower meal)*
*** 30% wheat, 30% barley, 15.9% corn, 20% subflower meal, 4.1% minerals&vitamins*

Concentrations of purine derivatives were multiplied with the daily output of urine (recorded daily and individually) and divided to the molar weights, in order to obtain the daily excreted quantities (mmols/d). The sums of excreted quantities were used in the equations proposed by Chen et al., 1992, in order to estimate the microbial synthesis of intestinally digestible protein (IDMP). Differences between groups were assessed with GLM procedure using Minitab software.

RESULTS AND DISCUSSION

The consumed diets let to theoretical supplies of 0.59 and 0.57 feeding units/d, 52.08 g/d and 49.89 g/d intestinally digestible protein allowed by nitrogen (IDPN), 49.21 g/d and 47.97 g/d intestinally digestible protein allowed by energy (IDPE), for the control and experimental group, respectively.
As also reported by other authors (Chen, 1992), high individual variability of purine derivatives output was observed. In average, allantoin accounted for 79.3% of the total purine derivatives, a value which is near the upper limit of the range of values reported in literature. Overall, the excreta of purine derivatives is also in the range of the literature data, toward the lower level. In fact such values are associated with restricted diets

Table 2. Daily output of urine and purine derivatives

Specification	Control group (grains)	Experimental group (molasses)
Urine volume, (ml)	524.6±99.7	556.0±198.3
Total allantoin, mmols/d	4.536±1.585	4.489±1.759
Total uric acid, mmols/d	0.584±0.265	0.680±0.275
Total xanthine, mmols/d	0.054±0.031	0.039±0.024
Total hipoxanthine, mmols/d	0.471±0.174	0.499±0.181
Total purine derivatives, mmols/d	**5.645±2.020**	**5.707±2.193**

Replacement of 40% of the grain quantity ingested by control group (222 g) with molasses did not influence the total daily output of purine derivatives (P=0.91). This led to a similar effect on estimated production of microbial protein, which was increased only by 4.8% comparing to the level of the control group and this difference was not detected as significant (P=0.84). On the other hand, it is known that high intra-group variability of purine derivatives data lead to less powerful statistical tests, therefore only large differences can be detected as being significant.
In general, it is usual that at least half of the protein supply of a diet comes from microbial proteosynthesis. In the current study, where protein value of diets is around 50 g of intestinally digestible protein, we would expect at least 25 g of digestible microbial protein, especially in the experimental group, where a certain stimulation of proteosynthesis was expected (Chamberlain, 1993). However, the values for microbial proteosynthesis were much lower (15-16 g/d) in both groups, suggesting either an underestimation of purine derivatives concentrations in urine or a overestimation of nutritive value of the diet.

Along the determination of allantoin, uric acid, xanthine and hypoxanthine, concentration of creatinine in urine was also assessed.

Table 3. Daily production of rumen microbial protein

Specification	Control group (grains)	Experimental group (molasses)
BW, Kg	58.33±2.98	62.08±0.48
Purine derivatives in urine, mmols/d	5.645±2.02	5.707±2.193
Purine derivatives in microbes, mmols/d	5.275±3.354	5.53±3.431
Microbial N, g/d	3.835±2.439	4.02±2.494
Microbial CP, g/d	23.97±15.24	25.13±15.59
IDMP, g/d	**15.34±9.75**	**16.08±9.98**

IDMP = intestinally digestible microbial protein

Creatinine is largely used in spot sampling method, in cases where total collection of urine is impossible or too difficult. PDC index (ratio of concentrations of total purine derivatives and creatinine, multiplied by metabolic weight) was calculated and similar values were obtained for control and experimental groups: 36.43±5.68 and 33.91±2.86, respectively. However, when calculating total derivatives, using known daily excretion if creatinine reported in literature (Hodgen, 1967), underestimated values were obtained (data not shown). As this method heavily rely on this estimation of daily excretion of creatinine, is is important that such values are obtained from sufficient number of animals or trials.

A possible explanation for the lack of microbial proteosynthesis response to the nature of dietary energy would be (beside complexity of factors involved in the ruminal ecosystem) the fact that, although the quality of the Sudangrass hay was low, it still accounted for a large part of the dietary nitrogen and this was a source of slowly degradable nitrogen, whose peak of availability was shifted comparing to availability of sugars. The increase in readily available carbohydrates was beneficial only when matched the protein from sunflower meal, which accounted for only a quarter of dietary protein.

CONCLUSIONS

Partial replacement of grains (wheat, barley, corn) with molasses in diets of growing rams based on low quality Sudangrass hay did not increase the outputs of purine derivatives in urine (the diets gave similar values - 5.6-5.7 mmols/d) or the levels of microbial protein synthesis in rumen (15.34g/d for the grain group and 16.08 for the molasses group). On the other hand, molasses can replace large part of the energy ingredients of the diet, without adversely influence microbial proteosynthesis via rumen pH or other ruminal parameters.

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Index of authors

A

Adamović Milan, 196
Alberti Francesca, 130
Almaši Radmila, 183
Andrić Goran, 168
Anghel Laura, 105
Antunović Zvonko, 137
Arapović Željko, 295
Audenaert Kris, 30

B

Bacić Dragan, 454
Barnić Saša, 254
Bendikas Petras, 86
Beuković Dejan, 234
Beuković Miloš, 234, 273
Bilandžija Nikola, 150
Bjedov Siniša, 295
Bliznikas Saulius, 86, 209, 447
Boboš Stanko, 454
Bočarov-Stančić Aleksandra, 196, 254
Bodroža-Solarov Marija, 183, 209
Bojkovski Jovan, 454
Božičković Aleksa, 215, 273, 302
Brlek Tea, 150

C

Chrastinová Ľubica, 1
Celano Gaetano Vitale, 130
Celano Maria Palma, 130
Chrenková Mária, 1
Cornescu Gabriela Maria, 97
Criste Rodica Diana, 97
Cvetković Biljana, 222

Ć

Ćirković Miroslav, 400
Ćupić Željko, 345

Č

Čabarkapa Ivana, 288, 374, 405, 432
Čolović Radmilo, 158, 383, 432, 447

D

Dam Hans, 54
Dapčević Tamara, 327
Dario Marco, 130
De Baets Bernard, 30
Di Modugno Girolamo, 41
Djorgovska Nataša, 144
Dragomir Catalin, 158, 483
Drăgotoiu Dumitru, 483
Džinić Natalija, 439

Đ

Đidara Mislav, 137
Đorđević Nenad, 215, 234, 273, 302
Đorđević Vesna, 400
Đuragic Olivera, 383, 471

E

Eissen Jaco, 54

F

Fasano Francesco, 130
Filipčev Bojana, 183
Formelová Zuzana, 1

G

Giannenas Ilias, 9
Glamočić Dragan, 234
Grbić Jasna, 339
Grubić Goran, 215, 273, 302

Gubić Jasmina, 405

H

Habeanu Mihaela, 17, 80
Habrun Boris, 137
Hadnađev Miroslav, 327
Haesaert Geert, 30
Hebean Veronica, 17
Heijden Maarten, 54

I

Ikonić Predrag, 439
Ivanc Aleksandar, 458
Ivanov Dušica, 158, 383
Ivanović Lana, 411
Ivetić Aleksandra, 302
Ivetić Vojin, 320

J

Jakić-Dimić Dobrila, 248, 269, 320
Janković Saša, 400
Jeločnik Marko, 411
Jeremić Dragomir, 432
Jevtić-Mučibabić Rada, 339
Jojić-Maličević Ljiljana, 269
Jokanović Marija, 439
Jovanović Rade, 196, 418, 471
Jurcoane Stefana, 105
Jurišić Vanja, 150

K

Kaluđerović Dragana, 282
Kapetanov Miloš, 242
Kečkeš Jelena, 248
Kinčeš Ibolja, 333
Kirchner Alexandra, 25
Kljajić Petar, 168
Kokić Bojana, 209, 288, 374, 383, 447
Koprivica Gordana, 158, 339
Kormanjoš Šandor, 209, 262
Kosovac Olga, 333
Kostadinović Ljiljana, 282
Košutić Milenko, 222
Krička Tajana, 150
Kuljanin Tatjana, 339
Kureljušić Branislav, 320

L

Landschoot Sofie, 30
Laporta Luca, 41
Laudadio Vito, 41
Lauková Andrea, 1
Lefter Nicoleta, 17
Lević Jelena, 254
Lević Jovanka, 66, 209, 215, 282, 288, 374, 383, 392, 447
Lević Ljubinko, 158
Levkov Vesna, 144
Lilić Slobodan, 262
Ljubojević Dragana, 400
Lojanica Mladen, 215, 302
Lückstädt Christian, 45
Lukić Miloš, 227

M

Makarinskaya Alla, 116
Mandić Anamarija, 288, 312
Marin Daniela, 17
Maslovarić Marijana, 418
Matei Petre Stelian, 105
Matekalo-Sverak Vesna, 262
Matić Jovana, 288
Matin Ana, 150
Memiši Nurgin, 352, 360, 367
Menkovska Mirjana, 144
Mihaljev Željko, 189, 242, 345
Mikulec Željko, 137
Milenković Ivan, 418
Milovanović Ivan, 312
Milošević Niko, 295
Milošević Nikolina, 400
Mimica-Dukić Neda, 312
Mišan Aleksandra, 312
Mišljenović Nevena, 158, 339
Mościcki Leszek, 59

N

Naeherer Karin, 74
Nešić Ksenija, 248, 269
Nježić Zvonko, 222

O

Obradović Saša, 392, 458
Okanović Đorđe, 262
Ondruška Ľubomír, 1
Orlić Dušan, 242

P

Palić Dragan, 262, 432
Panić Marija, 196
Pantić Vladimir, 254
Parkányi Vladimír, 1
Pavkov Sava, 282
Pavlović Ivan, 320
Pavlović Nikola, 269
Pavlovski Zlatica, 227
Pedrosa Katia, 74
Petkova Mariana, 66
Petrović Ljiljana, 439
Pisinov Boris, 248
Plachá Iveta, 1
Plavša Nada, 295
Plavšić Dragana, 374, 405, 432
Pojić Milica, 418
Polačiková Mária, 1
Pop Smaranda, 483
Popović Zoran, 273
Poslončec Danijela, 183
Potkonjak Dubravka, 242
Prodanović Radiša, 320
Prunić Bojana, 189, 242
Psodorov Đorđe, 312
Puvača Nikola, 295

Q

Quaglia Nicoletta Cristiana, 130

R

Radivojević Mihailo, 196
Radojičić Biljana, 454
Radović Č., 333
Radutoiu Daniel, 97
Rafay Ján, 1
Ristić Milutin, 262
Rodrigues Inês, 74
Ropota Mariana, 80

S

Saftić Mirko, 333
Sakač Marijana, 312
Salma Nataša, 254
Samardjioski Vlado, 144
Savić Božidar, 248, 320
Sedej Ivana, 312
Selvaggi Maria, 130
Spasevski Nedeljka, 209, 447
Spremulli Luigina, 130
Sredanović Slavica, 215, 383, 471
Stanaćev Vidica, 295
Stanaćev Vladislav, 295
Stanković Slavica, 254
Stavrakakis Sophia, 9
Stefanović Raško, 392
Stevanović Jasna, 203
Stojanović Bojan, 215, 273, 302
Strompfová Viola, 1
Subić Jonel, 411
Surdu Ioan, 105
Szabóová Renáta, 1

Š

Šarić Ljubiša, 374, 405
Šarić Marija, 144
Šimurina Olivera, 183
Škrbić Zdenka, 227
Šperanda Marcela, 137
Šperanda Tomislav, 137
Špoljarić Danijel, 137
Švirmickas Gintautas, 447

T

Tamborrino Carmela, 130
Taranu Ionelia, 17
Tarvydas Vytautas, 86
Tasić Tatjana, 439
Theobald Peter, 45
Todaro Enzo, 130
Tomić Zorica, 352, 367
Tomović Vladimir, 439
Trbović Dejana, 400
Tufarelli Vincenzo, 41

U

Uchockis Virginijus, 86
Untea Arabela, 97
Urbsienė Danguolė, 86
Ušlinovska Irina, 144

V

Valpotić Hrvoje, 137
Vandepitte Jan, 30
Vasilachi Andreea, 483
Vasiljević Ivana, 288
Vătuiu Daniela, 105
Vătuiu Ioana, 105
Vlassa Mihaela, 483
Voća Neven, 150
Voicu Dorica, 109
Voicu Ilie, 80, 109
Vries Peter, 418
Vukašinović Marija, 392, 458
Vukić-Vranješ Marina, 196
Vukmirović Đuro, 158, 383, 432, 447

W

Waegeman Willem, 30

Y

Yegorov Bogdan, 116, 122

Z

Zdjelar Gordana, 288

Ž

Živkov – Baloš Milica, 189, 242, 345
Živković Branislav, 333
Živković Jasmina, 222
Žujović Miroslav, 352, 360
Žutić Milenko, 320

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