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CONTENT

EFFECTS OF DIETARY N-3 POLYUNSATURATED FATTY ACIDS AND ANTIOXIDANTS ON BEEF FATTY ACIDS AND LIPOPEROXIDATION IN MEAT-PRODUCING CATTLE

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INTRODUCTION

Consumers are more and more aware of the putative link between meat lipid consumption and human health. An increased intake of n-3 (ω3) fatty acids (FA) (from animal products) together with a ratio of polyunsaturated fatty acids (PUFA) n-6/n-3 close to 5 could be beneficial for the human health (AFSSA, 2001). Since feeding of finishing cattle with linseed-supplemented diets increased ω3 PUFA of lipids in both fresh and cooked meat (Bauchart et al., 2005, Normand et al., 2005), beef can be a possible additional source for n-3 PUFA in human nutrition.

Here are presented some main results of the Program "Lipivimus" financed by the French National Research Agency (ANR, 2007-2009) concerning the impact of n-3 PUFA and antioxidants (vitamin E, plant extracts rich in polyphenols) added to the diets of finishing cull cows on beef FA characteristics and the relative sensitivity to lipoperoxidation of plasma and muscles at slaughter or of beef following technological treatments.

The main objectives of this program are a better understanding of feeding and genetic factors (and their interaction) underlying the variability of lipid content in the skeletal muscle and its fatty acid composition in relation of meat quality traits. The effects of various lipid and antioxidant enriched diets were compared to determine the optimal feeding conditions and its duration for a production of meats with improved sensorial and nutritional qualities.

Additionally, experiments were conducted to find the equilibrium between higher amount of unsaturated FA (especially n-3 PUFA) in meat and the lack of deleterious effect on animal health and on sensory and nutritional properties of their meat lipids.

In this context, an adverse effect of lipid peroxidation can be stimulated not only by the high sensibility of PUFA to reactive oxygen species attacks during oxidative stress but also by a non adequate status in dietary or endogenous antioxidants. Moreover, the lipoperoxidation intensity is potentially stimulated by a pre-slaughter stress of cows and by technological processes applied to beef.

EFFECTS OF N-3 PUFA AND ANTIOXIDANTS ON BEEF FATTY ACIDS

Lipids deeply control the nutritional/health value and the sensorial qualities of meats, especially in ruminant animals (Geay et al., 2001).
Factors linked to animals (age, gender, genotype) and their feeding conditions [characteristics of ingredients and their proportions in diets, fatty acid (FA) composition and physical form of lipid supplements] can more particularly modulate during the fattening period, characteristics of beef lipids such as i) their quantity in muscles and associated adipose tissues, ii) the distribution of their major [triglycerides (TG) and at a lower extend, phospholipids (PL)] and minor classes (free and esterified cholesterol, diglycerides and free FA), iii) their FA composition.
Thus, the impact of dietary n-3 PUFA sources (grass, oleaginous seeds, fish oil) given in the finishing period to improve the nutritional value of beef FA has been extensively described in young (<2 years-old) bovines (bulls, heifers, steers) of which muscles were relatively poor in lipids (Bauchart et al., 2005; Scollan et al., 2005). On the other hand, little is known on the effect of dietary n-3 PUFA rich lipids on beef FA in oldest and fat cattle.
The aim of this study was to analyze, on beef lipids and FA, the impact of extruded linseed (rich in n-3 PUFA) given alone or with rapeseed (rich in n-3 and n-6 PUFA and in 18:1 n-9) in association or not with antioxidants given to Normand (fat breed) cull cows during the finishing period.

Experimental aspects

Animals, diets and beef collection

The experiment was performed with 72 Normand cull cows for a 100 day feeding study (9 treatments; n=8 for each diet/treatment) to analyse the interactions between lipid supplements, antioxidant supplements in diets and stressful situation of animals.
Animals were assigned at random to rations composed of concentrate (70%) and straw (30%) without any lipid supplements (C diet) or with extruded linseed (n-3 PUFA source) (L diet) or the mixture of extruded linseed (1/3) and rapeseed (2/3) (40g oil/kg diet DM) (RL diet) without or with antioxidants composed of vitamin E alone (155 IU/kg diet DM) (LE diet) or associated with a mixture of plant extracts rich in polyphenols (PERP, 7g/kg diet DM) (LEP and RLEP diets) from rosemary, grape, citrus and marigold (provided by Phytosynthèse Society, Riom, France). Some animals were exposed to a stressful situation (transport in a cattle truck for 15 min followed by a forced run for 30 mn and, finally, a 15 mn transport in truck to the abattoir) just before slaughter (2 treatments; n=8 for each treatment).
Diets for both experiments were calculated to be nearly similar in terms of energy and nitrogen contents and to allow a mean live gain weight of 1150 g/d. Animals were slaughtered by weight block and body fat score (3.0-3.5) at the experimental abattoir of the INRA Centre of Theix. Zootechnical parameters (feed efficiency, live weight gain, colour, carcass marbling grading, carcass pH and temperature, carcass dressing percentage) were determined for all experimental animals.
Samples (100g) of *Longissimus thoracis* (LT) and of *Semitendinosus* (ST) muscles were collected 1d *post mortem*, cut into small pieces and was ground into a fine homogenous powder in liquid N_2 and finally stored at -20°C until lipid and FA analysis.

Lipid and fatty acid analysis
Total lipids of LT and ST muscle tissues were extracted by mixing 6g of meat powder with chloroform /methanol (2/1, V/V) and determined gravimetrically. Their different lipid classes were separated by HPLC (Kontron, Switzerland) on silica 5µm column (150 mm long, i.d. 4.6mm) with a ternary solvent gradient and quantified by evaporative light-scattering detection (Sedere, France) as described by Reynolds et al., (1998).
Fatty acids (FA) were extracted from total lipids and transmethylated with BF3-methanol. Their detailed composition was determined by GLC analysis (Perichrom. France) using the CP Sil 88 glass capillary column. Response coefficient of each individual FA was calculated by using the quantitative mix C4-C24 FAME (Supelco, USA). Results were expressed as mean values. The effects of dietary treatments on lipids and FA have been analysed by the Student's T test.
Beef total lipids and their fatty acids (from Habeanu et al., 2008 and 2009, Bispo et al., 2009).
Distribution of lipid classes
Dry matter and lipids contents were 6 and 31% higher in LT muscle than ST muscle respectively (data not shown). Total lipid contents varied with that of triglycerides (TG) while phospholipids (PL) were stable (Table 1). Minor lipids (free cholesterol, cholesteryl esters, diglycerides and free fatty acids) represented only 9.8 and 8.9% of total lipids in LT and ST muscles respectively (Table 1). Unsaturated lipid supplements did not altered significantly total lipids and their major classes of muscles. On the other hand, free fatty acids and diglycerides tended to be higher in the lipid supplemented groups for the two muscles probably because of an hydrolysis of TG (lipolysis).

Table 1. Effects of lipid supplements on the major and minor classes of lipids in Longissimus thoracis (LT) muscle of cull cows (from Habeanu et al., 2008 and 2009). ^{a,b,c} *, P≤0.10.*

Diets	**C**	**L**	**RL**	***SEM***	***P***
Dry matter	26.93	26.73	27.13	*0.42*	*0.81*
Total lipids	4.70	3.29	3.46	*0.46*	*0.79*
Triglycerides	3.79	3.03	2.87	*0.41*	*0.69*
Phospholipids	0.70	0.68	0.69	*0.01*	*0.61*
Cholesteryl esters	0.03[a]	0.05[b]	0.01[a]	*0.04*	*0.03*
Free cholesterol	0.07	0.06	0.06	*0.005*	*0.52*
Diglycerides	0.05	0.07	0.05	*0.02*	*0.38*
Free fatty acids	0.06[a]	0.19[b]	0.05[a]	*0.03*	*0.03*

Long chain fatty acid composition:
Dietary unsaturated lipid supplements from linseed (L) and the mixture rapeseed + linseed (RL) significantly increased proportions of 18:3 n-3 (+56% and +36% respectively), total *trans* 18:1 (+ 66% and + 105%); and of 9*cis*,11*trans* 18:2 (CLA) (+ 50 % and + 41%) in LT muscle (Table 2) of which 18:3 n-3 and CLA are known to be beneficial for the human health (Table 2).

Such increases were mainly to the detriment of *cis* monounsaturated FA. The effects of dietary lipids were also observed in the ST muscle (data not shown), this muscle having higher levels of 18:3n-3 (+25%) and CLA (+27%) than that of the LT muscle. Similar significant stimulating effects concerned total n-3 PUFA leading to a decrease of the ratio n-6 PUFA/n-3 PUFA beneficial for the human health.

Table 2. Effects of lipid supplements on fatty acid composition of total lipids in Longissimus thoracis (LT) muscle of cull cows. (from Habeanu et al., 2009) ^{a,b,c}, *P≤0.05;* ^{A,B,C} *, P≤0.01*

Fatty acids	**C**	**L**	**RL**	***SEM***	***P***
	% total fatty acids				
18:3 n-3	0.39^{A}	0.61^{B}	0.53	*0.05*	0.05
*cis*9,*trans*11-18-2 (CLA)	0.32	0.48	0.45	*0.05*	0.08
Σ Saturated FA	47.01	48.22	48.29	*0.80*	0.44
Σ *cis* MUFA	45.38^{A}	41.56^{B}	40.90^{B}	*0.59*	<0.0001
Σ *trans* MUFA	2.46^{A}	4.08^{Bb}	5.04^{Cc}	*0.32*	<0.0001
Σ n-3 PUFA	0.90	1.23	1.02	0.11	0.17
Σ n-6 PUFA	4.03	4.05	3.80	0.26	0.77
n-6 /n-3 ratio	4.54^{A}	3.32^{B}	4.10	*0.28*	0.028
P/S ratio	0.11	0.12	0.11	*0.009*	0.80
Total FA (g/100 g tissue)	***3.95***	***3.64***	***3.63***	***0.41***	***0.64***

These results confirmed previous observations in LT and RA muscles of bulls given a similar linseed supplement with a paralleled increase in 9*cis*,11*trans* 18:2 (CLA) and in its precursor the 18:1 Δ11*trans* (Bauchart et al., 2005), both being beneficial for human by their hypocholesterolemic action (Bauchart et al., 2007). On the other hand, such increases observed in our present fat and relatively old bovines were lower in intensity than that observed in the young males, suggesting a decreased reactivity of muscles and its connected fat tissues towards n-3 PUFA-rich lipid supplements.

Separate GLC analysis of the FA composition of beef polar (dominated by PL) and neutral (dominated by TG) fractions (purified by preparative solid phase extraction using silica cartridges activated by aminopropyle groups) clearly showed a specific composition as reported earlier (Bauchart et al., 2005).Thus, the PL fraction was 10 times higher in PUFA (25% vs 2.5%) than the TG fraction dominated by saturated and monounsaturated FA (data not shown). The FA composition of these lipid fractions were affected by the lipid supplements, especially the n-3 PUFA of PL in the ST muscle in cows given the L diet (+55%) (Habeanu et al., 2009).

Addition of antioxidants (vitamin E and PERP) reinforced the stimulating effect of lipid supplements on proportions of the three considered FA (18:3 n-3, total *trans* 18:1, 9*cis*, 11*trans* 18:2) in the total lipids of the two muscles (data not shown). These effects of antioxidants were previously reported in young Charolais bulls given similar linseed plus vit E supplements during the finishing period, suggesting a modulation of the microbial fatty acid metabolism by the dietary antioxidants in the rumen (Bauchart et al., 2005).

Trans isomers of 18:1:
Analysis of the different isomers of trans 18:1 (from beef total trans 18:1 purified by preparative HPLC) by gas-liquid chromatography-mass spectrometry (GLC-MS) clearly showed that with a basal diet rich in cereals, lipid supplements can diversely modified the health value of beef *trans* 18:1 on the basis of the Δ9*tr* and Δ10*tr* (detrimental) and Δ11*tr* 18:1 (beneficial) (Table 3). They had a positive effect when 18:3n-3 was mainly provided (diet L) or a rather negative effect when 18:3n-3 was associated to 18:1n-9 (diet RL) (Bispo et al., 2009).

Table 3. Effects of lipid supplements (L= linseed; R = rapeseed) on the distribution of trans 18:1 (in % total trans 18:1, mean ± SD) in lipids of bovine LT muscle determined by GLC-MS (, P< 0.05) (from Bispo et al., 2009).*

***Trans* 18:1**	**6*tr***	**7*tr***	**8*tr***	**9*tr***	**10*tr***	**11*tr***	**12*tr***	**13*tr***	**14*tr***	**15*tr***	**16*tr***
Diet C	1.3 ± *0.9*	0.5 ± *0.1*	1.9 ± *0.3*	8.5 ± *1.5*	33.7 ± *18.6*	36.1 ± *14.4*	4.3 ± *1.2*	3.4 ± *1.0*	4.0 ± *1.3*	3.4 ± *1.8*	2.9 ± *1.9*
Diet L	0.6 ± *0.5*	0.4 ± *0.1*	1.6 ± *0.4*	5.0* ± *0.8*	15.6* ± *6.7*	33.2 ± *11.8*	6.1* ± *0.3*	8.7* ± *0.8*	9.1* ± *0.9*	10.9* ± *9.0*	8.9* ± *2.7*
Diet RL	0.5 ± *0.4*	0.6 ± *0.1*	2.3 ± *0.6*	6.4* ± *0.9*	41.1 ± *16.4*	25.0* ± *12.4*	4.9 ± *9.2*	5.8 ± *1.8*	5.5 ± *1.6*	5.0 ± *1.8*	3.1 ± *1.1*

EFFECTS OF DIETARY AND PHYSIOLOGICAL FACTORS ON PLASMA AND BEEF LIPOPEROXIDATION

Beef having improved nutritional qualities (higher content of n-3 PUFA associated to a better protection of PUFA by antioxidants) by breeding factors are marketed with a high added value such as the French Label Rouge procedure. In this context, it is necessary to precise the impact of specific meat technological treatments to preserve their qualities until the sale of products. Indeed, technological treatments as storage and processing may favour peroxidation processes and lipolysis which entail negative consequences on nutritional and sensorial qualities (Min and Ahn, 2005). In this context, PUFA of phospholipids are the primary substrates of lipid oxidation in muscle foods whereas triacylglycerols would play a minor role. Incorporation of PUFA (from seeds or fresh grass) into bovine muscle lipids did not activate muscle antioxidant enzymes, the protection against the peroxidation process being strictly dependent on the amount of dietary antioxidant molecules stored in muscle cells (Durand et al., 2005).
The effects of technological treatments on beef lipoperoxidation stability were studied on PUFA rich beef varying in their antioxidant content (from cows given the L, LE or RLEP diet). The impact of beef ageing and packaging treatments were more especially analyzed.

Experimental aspects

Animals and diets

The study was performed with the animals given the same diets (concentrate/straw based diet, lipid and antioxidant supplements) and stress breeding conditions than for the analysis of beef lipids and fatty acids (cf. Part I Effects of n-3 PUFA and antioxidants on beef FA). Intensity of the animal stress was evaluated by plasma cortisol determined i) at the end of the finishing period, ii) just after slaughter during the bleeding.

Beef technological treatments

LT and ST muscles were collected just after slaughter, stored under vacuum for 12 d at 4°C and finally cut as steaks as on the market and packaged at +4°C in a tray overwrapped with a film either i) under air for 4 d. (A), ii) under modified atmosphere (70:30, O_2/CO_2) for 7 d. (MA), or in a bag iii) under vacuum for 14 d. (V). Beef samples were immediately ground into a powder in liquid N_2 and stored at -80°C until analysis.

Methods for lipoperoxidation characterization

The intensity of lipoperoxidation in plasma (jugular vein) and in beef samples at slaughter was determined by the measurement of malondialdehyde (MDA) production (after its extraction by hexane) by HPLC (fluorescence detection) using a tetraethoxipropane calibration curve. Plasma and tissue vitamin E was determined by HPLC (UV detection).

The *in vitro* susceptibility of plasma to lipoperoxidation was evaluated by measuring the kinetic production of conjugated dienes (CD) indicating the degree of resistance to lipoperoxidation (Lag phase), the lipoperoxidation velocity (Tx max) et the maximal amount of produced CD (Q max). The Peroxidizability Index (PI) of beef FA was calculated from the FA composition of total meat lipids according to the equation reported by Hu et al. (1989) as follows: IP = (% dienoic x1) + (% trienoic x2) + (% tetraenoic x3) + (% pentaenoic x4) + (% hexaenoic x5). This index estimated bis-allylic hydrogen atoms in PUFA and therefore their susceptibility to peroxidation. Plasma free 4-hydroxy-2-nonenal (HNE) and free 4-hydroxy-2-hexenal (HHE), respective specific markers of n-6 and n-3 PUFA oxidation, were evaluated by GC-MS.

Effects of dietary n-3 PUFA and antioxidants on plasma and beef lipoperoxidation (Gobert et al., 2008a and b, 2009a to c)

Plasma lipoperoxidation

Dietary n-3 PUFA supplements reduced the resistance capacity of plasma against lipoperoxidation (-11%) favouring conjugated diene (x 1.75) and MDA (x2) formations (Table 4).

Only the combined supply of antioxidants (vit E and PERP) effectively protected plasma lipids against peroxidation (LEP diet) that was favoured by dietary n-3 PUFA supplements, the plasma MDA and lipoperoxidation velocity Tx being reduced by -80 and -33% *(P=0.01)* respectively in the LEP diet compared to the L diet (Table 4).

Such protection by the mixture vitamin E and PERP would act not only during the propagation phase of the lipoperoxidation reaction (by vitamin E that acts as a chain breaker), but also during the initiation of the reaction by the uptake of free radicals by the plant polyphenols (EVRP) as earlier demonstrated in rats and sheep (Gladine et al, 2007) and in lactating cows (Gobert et al., 2008c).

Table 4. Effects of n-3 PUFA and antioxidant supplements on markers of plasma lipoperoxidation in cull cows in the finishing period (P<0.1, ** P<0.05, *** P<0.01) (from Gobert et al., 2008b)*

Diets	C	L	LE	LEP	Prob.
MDA (µg/mL)	0.05 ± 0.02 [a]	0.10 ± 0.06 [b]	0.05 ± 0.04 [ab]	0.02 ± 0.02 [a]	**
α-tocopherol (µg/mL)	1.82 ± 0.80 [a]	4.11 ± 1.15 [b]	9.65 ± 5.65 [c]	11.05 ± 5.36 [d]	***
Lag (min)	13.2 ± 0.8 [a]	11.7 ± 0.8 [b]	11.8 ± 2.0 [ab]	15.2 ± 4.3 [ab]	**
Tx max (A_{234}/min)	8.04 ± 2.51 [ab]	9.52 ± 0.12 [a]	8.89 ± 2.58 [ab]	6.32 ± 1.38 [b]	***
Q max (CD max)	217 ± 58 [a]	379 ± 27 [b]	347 ± 115 [b]	375 ± 52 [b]	*

In cull cows submitted to emotional and physical pre-slaughter stress, plasma lipoperoxidation evaluated by the MDA level (Table 5) was not increased in the stress groups compared to the groups without stress. However, specific markers of n-3 and n-6 PUFA peroxidation (HNE and HHE respectively) (Table 5), tended to decrease in cows given dietary vit E and PERP (LEP group) (*P*=0.07 and NS, respectively).

Table 5. Effect of dietary (L vs LEP) and pre-slaughter (no stress vs with stress) treatments on lipoperoxidation intensity indicators and antioxidant status in plasma of cull cows collected just after slaughter (from Gobert et al., 2009b).

Diets	L		LEP		*P*-values		
Treatments	No stress	With stress	No stress -	With stress	Antioxidant	Stress	Antioxidant x stress
MDA (µg/mL)	0.06	0.07	0.06	0.05	NS	NS	NS
HNE (ng/mL)	3.5	2.5	1.9	2.1	0.07	NS	NS
HHE (ng/mL)	0.45	0.42	0.38	0.34	NS	NS	NS
Vit E (µg/mL)	3.17	2.91	7.75	8.77	<0.0001	NS	NS

Beef lipoperoxidation

Effects of meat processing on lipoperoxidation.

At slaughter, meat MDA values were similar in animals given the control diet (C group) or the lipid enriched diet (L diet), the PI value being not increased by the addition of n-3 PUFA (from linseeds) in diet. Due to the contact of beef slices with a high level of O_2, lipoperoxidation was far higher in beef of the L group stored under air packaging (A,

x10.4, P<0.05) or under modified atmosphere packaging (MA) (x21.8, P<0.05) when compared to that of the same beef samples after slaughter ($D_{0)}$ (Table 6).
Even with a similar PI value of beef samples, lipoperoxidation intensity (estimated by the MDA tissue level) tended to be higher in meats stored under MA packaging in the L group (2.96 µg/g of tissue) compared to that of the C group (2.19 µg/g of tissue (*P*=0.1). This could be the result of a higher sensitivity of meats (from the L group) to n-3 PUFA, confirming previous results in meats from steers given PUFA-rich diets (Campo et al., 2006).
Effect of dietary antioxidants.
Dietary vitamin E provided with PERP effectively protected beef against lipoperoxidation since MDA values were always lower than 1µg/g tissue, considered as threshold of tolerance for meat rancid flavor acceptability (Campo et al., 2006), in all treated meats (*P*<0.05). On the other hand, these dietary antioxidants protected n-3 PUFA enriched beef from deleterious packaging conditions, that were A (*P*<0.09) and especially MA packaging (*P*<0.0001) (Table 6).
As early shown (Gatellier et al., 2000), vitamin E alone was not enough efficient against lipoperoxidation in PUFA enriched beef. As in rats and sheep (Gladine et al. 2007) and in lactating cows (Gobert et al. 2009a), vitamin E associated to plant polyphenols would exhibit synergic actions as the result of their respective lipophilic and hydrophilic properties. Vitamin E would act as a chain breaking antioxidant and PERP as free radical trappers, explaining their efficient protection of beef against lipoperoxidation, even in the most deleterious storage conditions.

Table 6. Malondialdehyde (MDA) concentration (µg/g of tissue) in Semitendinosus muscle of cows given C, L and LEP diets. Beef MDA was determined at slaughter (D_0), after 12 d meat ageing (D_{12}), and after storage for 4d. in a tray under air packaging (A), for 7d. under modified atmosphere packaging (MA) (70:30, O_2/CO_2) and for 14d. under vacuum packaging (V) (from Gobert et al., 2008a). $^{a, b}$ *P<0.05*

MDA (µg/g tissue)	**Meat treatments**				
	D_0	**D_{12}**	**V**	**A**	**MA**
C	0.16 ^{a} ± 0.05	0.15 ^{a} ± 0.01	0.16 ^{ab} ± 0.04	1.10 ^{b} ± 0.38	2.19 ^{c} ± 1.88
L	0.14 ^{a} ± 0.04	0.19 ^{a} ± 0.09	0.19 ^{a} ± 0.09	1.41 ^{b} ± 0.72	2.96 ^{c} ± 1.36
LEP	0.16 ^{a} ± 0.05	0.14 ^{a} ± 0.03	0.14 ^{a} ± 0.02	0.65 ^{a} ± 0.49	0.93 ^{a} ± 0.82
Diet effect					
C *vs* L	0.9	0.9	0.9	0.5	0.1
C *vs* LEP	0.6	0.7	0.8	0.3	0.01
L *vs* LEP	0.6	0.8	0.8	0.09	<0.0001

Effects of dietary n-3 PUFA and antioxidants on beef colour (Parafita et al., 2008)

The aim of this study was to analyze in processed beef, i) the possible detrimental effect of the dietary lipid supplement rich n-3 PUFA from linseeds on colour of beef from cows subjected to an emotional and physical pre-slaughter stress, ii) to determine the

protecting effect of antioxidants provided during the 100 d finishing period. Colour measurement was determined with spectrophotometer equipped with an integrating sphere. Colour coordinates were calculated in the CIELAB system. From the reflective spectra in the visible (360 to 760 nm), results were expressed as lightness (L*), redness (a*) and yellowness (b*). Differences of reflectance were used to calculate the oxygenation index (R630-R580; myoglobin oxygenation) et the % of metmyoglobin (MetMB) responsible for the brown colour of meats.
Results showed that, with beef under vacuum packaging (V), no effect of feeding and stress conditions were noted for beef colour parameters. Beef under air packaging (A) from cows given lipid supplemented diets had a higher redness value than that from control cows. In the case of stressed animals given lipid supplemented diets, beef under air packaging (A) present lower redness than animals given the antioxidant supplements. Beef under modified atmosphere packaging (MA, favouring the oxygenation of myoglobin) had higher values of redness than A packaging (data not shown), but present a low redness value in beef of stressed cows given only lipid supplements (Figure 1).

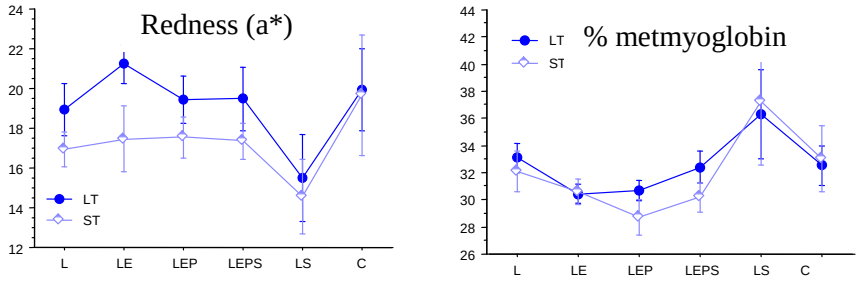

Figure 1. Effects of antioxidant supplements (E, EP) and of emotional stress (S) of cows given control (C) or linseed enriched diet (L) on colour parameters of LT and ST beef packaged under modified atmosphere (from Parafita et al., 2008)

Indeed, these last animals were more sensible to oxidation and their percentage of MetMB was higher (P>0.0001). However, whatever the colour parameters (redness, oxygenation index, percentage of MetMB), supplementation of diets with vit E and PERP led to beef colour characteristics at least similar to that of beef from the control cows and, therefore, could inhibit oxidation phenomena generated in stressed conditions (Figure 1).
This work clearly showed that dietary vitamin E supplement improved colour intensity of beef packaged under modified atmosphere. Moreover, colour of meat from stressed cattle was more deeply altered, especially in meat from bovine animals given n-3 PUFA enriched diets. In these conditions, dietary supply of vitamin E associated to PERP represented a very efficient mean to prevent alteration of beef colour as for beef lipoperoxidation (Gobert at al., 2009b).

CONCLUSION

In meat producing cattle, addition of n-3 PUFA (from extruded linseed) in diets improved the nutritional value of beef by favouring incorporation of 18:3n-3 and other LC n-3 PUFA (EPA, DPA) in muscle tissues, especially in phospholipids of cell membranes. Moreover, such a nutritional strategy would also favour deposition of conjugated linoleic acid (CLA) especially the rumenic acid (9*cis*,11*trans* 18:2) and its precursor, the vaccenic acid (18:1Δ11*trans*), beneficial for the health of human by their hypocholesterolemic action.
On the other hand, addition of rapeseed (rich in 18:1 n-9cis) together with linseed (rich in 18:3n-3) would limit incorporation of 18:3n-3 in muscles and favour deposition of trans isomers of 18:1 known to be detrimental for human health (18:1Δ9*trans* and especially 18:1Δ10*trans*).
High levels of dietary n-3 PUFA absorbed by animals stimulated lipoperoxidation i) in its whole body, leading to a risk of alteration of tissue and organ functions (oxidizing stress) with negative consequences on the animal welfare and health, ii) in their muscle tissues where oxidized products (MDA, HHE, HNE) were deposited, altering the nutritional value of beef products for consumers. To avoid lipoperoxidation processes in animals given n-3 PUFA from oleaginous seeds, the present work clearly showed that vitamin E added to diets was less active than the dietary mixture composed of vitamin E and plant polyphenols (PERP). The dietary strategy combining n-3 PUFA and the antioxidant mixture added to diets would avoid major risks of lipoperoxidation in beef products whatever i) the conditions of meat ageing and packaging (especially those with contact with oxygen), 2) the level of stress of animals prior slaughter.
Additionally, such dietary strategy based on the use of dietary vitamin E and plant polyphenol supplements would also avoid alteration of beef colour (notably in stressed animals) which paralleled tissue lipoperoxidation. It would play a positive role in the stability of meat, an element very beneficial for meat processing industries to better define their strategies in terms of meat production and transformations .

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DUST EXPLOSION PROTECTION IN FEED MILLS ACCORDING TO THE EUROPEAN ATEX DIRECTIVES – RISK ASSESSMENT AND NEW FINDINGS

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ABSTRACT

The formation of explosive atmosphere in form of dust-air clouds in plants for grain processing or grain handling cannot be prevented completely against the materials and processes. Strains at milling, mixing, sieving and conveying lead to air movements and generation of dust-air mixes. Depending on the handled materials, their relevant material properties as well as the process and plant technique, the occurrence of explosive dust-air mixes in the interior of production plants as well as in their surrounding can be expected with a different likelihood. The period of the existence of an explosive dust-air atmosphere as well as its changing in concentration over the duration determine the hazard assessment and zoning of hazardous places as required in ATEX Directive 1999/92/EC as well as the hazardous machine expenditure specified in ATEX Directive 94/9/EC [1, 2].
The actual dust concentrations in the interior of the plant components and in their surrounding, the frequency of explosive dust-air atmosphere and their changing in concentration over the duration are not always known or the coherence between material and technological influences and the generation of explosive dust-air clouds could not be solved finally [3]. For this reason, experienced data and estimations are consulted for the working risk assessment (Directive 1999/92/EC). According to the present lack of knowledge on the likelihood and the duration of the occurrence of hazardous explosive atmosphere and their duration, uncertainties in the estimation and evaluation of risks as well as the derivation of necessary measures on part of the responsible plant operator can result. For improving the state of knowledge concerning the evaluation of explosion hazards at grain and feed plants, the IFF Research Institute initialised a research project. The target research results shall enable the mainly small and medium-sized enterprises (SME) of the grain processing and feed industry to carry out a scientifically assured analysis of dust explosion hazards and to conduct the required zoning on basis of reliable and objective results.
Key words: *Dust explosion protection, ATEX, risk assessment, dusting behavior, explosive atmosphere*

DUST EXPLOSION RISKS AND ASSESSMENT OF EXPLOSION RISKS AT GRAIN PROCESSING

Grain and feed dusts are combustible and can generate explosive dust-air mixes when dispersing in air. The average natural dust content in grain is between approx. 0.1 – and

3 %. Up to 80 % consist of organic grain content [3]. Dusts are defined to be solid particles with particle sizes of 1 – 500 µm in gases which sediment caused by their own weight. However, they remain as air-dust mix in the atmosphere for some time [4, 5, 6]. Explosive areas are characterised by dust concentrations within the lower explosion limit – for grain and feed dusts respectively > 30 g/m³ – and the upper explosion limit (normally 2 – 4 kg/m³).
For avoiding cases of damage as a result of dust explosions, operators of plants in which explosive dust-air mixes can occur are obligated to implement the contents of ATEX Directive 1999/92/EC and the national legislation respectively. If there are any areas at a plant where the occurrence of explosive dust-air mixes cannot be excluded, a systematic assessment of explosion risks must be carried out. The assessment of explosions risks initially focuses on:

- likelihood that explosive atmosphere will occur and their persistence,
- likelihood that ignition sources, including electrostatic discharge will be present and become active and effective,
- the scale of the anticipated effects.

Adequate measures must be taken to attain the aims of the Directive [1].
The generation of explosive atmosphere has to be prevented with priority, for example by replacing explosive materials or applying inerting measures. If this is not reliably possible the risk of an explosion has to be minimised by absence of ignition sources as well as further technological measures for explosion protection [1].
At the processing of grain to feed and food, an avoidance or prevention of the generation of explosive dust-air atmosphere is possible with limits only. Therefore the likelihood of their occurrence has to be evaluated as basis for protection measures meeting the requirements.
In accordance with the Directive 1999/92/EC a classification to zones of places where explosive atmosphere may occur has to be carried as following:

- Zone 20 a place in which an explosive atmosphere in form of a cloud of combustible dust in air is present continuously, or for long periods,
- Zone 21 a place in which an explosive atmosphere in form of a cloud of combustible dust in air is likely to occur in normal operation occasionally and
- Zone 22 a place in which an explosive atmosphere in form of a cloud of combustible dust in air is not likely to occur in normal operation but, if it does occur, will persist for a short period only.

If the occurrence of hazardous explosive atmosphere can be reliably excluded for a place, so this is free of zones [1].
On the basis of classification of zones, the requirements for equipment have to be fulfilled according to the prescriptions of the ATEX Directive 94/9/EC [2]. In accordance with the three zones, three device categories are defined which are subdivided according to the relevant ignition-source reliability or absence:

- Category 1: Ignition sources are avoided by two independent protection measures or safety despite two independent errors also at rare breakdowns

- Category 2: Ignition sources are avoided in the normal operation and at frequent breakdowns
- Category 3: Anticipated ignition sources are avoided in the normal operation.

13 different ignition sources are known [8]. The following ignition sources can be available and effective in grain processing plants:

- electrical apparatus,
- hot surfaces,
- flames and hot gases,
- mechanically generated sparks,
- stray electrical currents,
- static electricity,
- lightning,
- chemical reactions.

In many cases, it is not possible to avoid explosive atmosphere or sources of ignition with a sufficient degree. Measures must then be taken to limit the effects of an explosion to an acceptable extent. Such measures are

- explosion resistant design,
- explosion relief,
- explosion suppression,
- prevention of flame and explosion propagation.

These measures generally relate to migitation of explosions within installations e.g. bins or filters. Equipment and protective systems must comply with Directive 94/9/EC.
The comprehensive hazard analysis as well as the necessary measures for achieving the aims of explosion protection have to be compiled in an explosion-protection document. This document has to be kept up-to-date.

NEW FINDINGS FOR DUST EXPLOSION RISK ASSESSMENT AT GRAIN PROCESSING

As the previous state of knowledge on the likelihood of the occurrence of explosive dust-air atmosphere was not satisfactorily, the Research Institute of Feed Technology initiated a research project. The aim of this project was the minimisation of dust-explosion risks when handling combustible dusts in grain processing plants. An essential pre-condition for a reliable risk assessment is the knowledge of diffuse and deducted dust concentrations, their frequency, duration and changing in concentration over the duration. Topical findings point out that the dusting behaviour of bulk materials can have an influence on the duration and changing in concentration over the duration of a dust-air mix so that its characterisation for typical compound feeds, macro and micro

components as well as semi-finished and finished products is a necessary supplementation of the available data records (e.g. BIA-GESTIS-DUST-EX database)

METHODS FOR CHARACTERISING THE DUSTING BEHAVIOUR

The requirements for characterising the dusting behaviour of bulk materials (in the laboratory scale) result from operational processes during which dust is raised and dispersed. For the grain processing, those are mainly impact and shear stresses due to conveying and transport processes.
For characterising the dust generation by impact stresses, a single-drop process (design Palas DustView) was used in the frame of the research project. The sample (sample size 30 g normally) drops through a downpipe (length 500 mm) into a dust chamber. Lasers and detectors measure the reduction of the initial intensity of the laser (opacity). The measured result is given as dimensionless index from the values 0.5 s and 30 s [Palas].
The rotation-drum method according to Heubach (Heubach Dustmeter) is used for determining the dust generation from powder by rotating under mechanical stress. Here shear stresses are mostly efficient. The construction of the used rotation drum is illustrated in **Figure 1** as standard process.

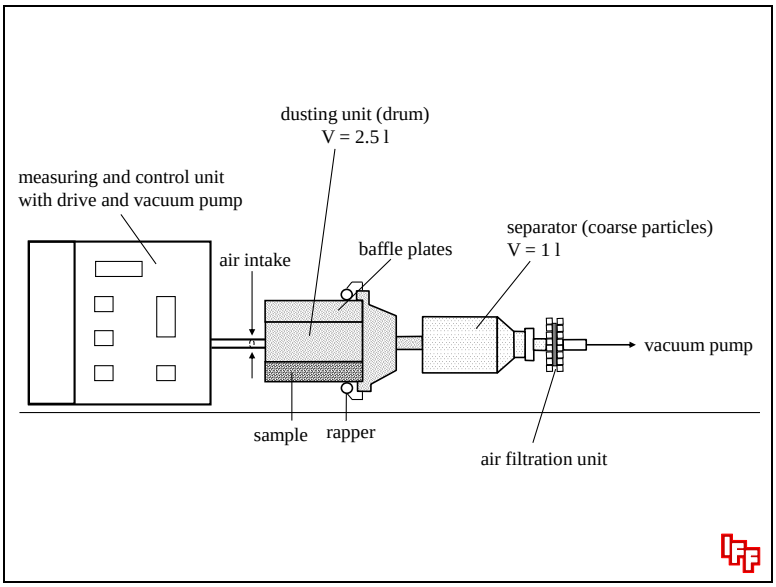

Figure 1. Rotation-Drum-Method, design Heubach Dustmeter (standard)

The material sample (sample size 100 g normally) is added into the rotation drum (volume 2.5 l). The drum rotates for 5 min at a speed of 30 min^{-1}. An air throughput of 20 l/min is necessary. Dispersible dust contents are released by the rotation movement. They are captured by the air flow and deposited on a filter with defined porosity. The amount of the deposited dust is determined gravimetrically. The declaration of the

dusting behaviour is given as dimensionless dust index SR which is calculated by subtracting the starting from the ending weight.
Macro and micro components as well as semi-finished and finished products were investigated and belong to the following material groups:

- grain, legumes, oilseeds
- meal and oil-mill by-products
- mash compound feed
- waste products (cleaning waste products, filter dusts).

For a relevant characterisation, the particle-size distribution, bulk and tap density as well as moisture are also determined.
The statistical evaluation of the dust indexes SF (drop method) and SR (rotation method) for selected material systems (pig fattening feed, wheat, barley) showed that they have a positive linear correlation. In future, the results on the dusting behaviour shall be used for predicting the frequency of the occurrence of explosive dust-air mixes.

MEASURING THE MATERIAL CONCENTRATION AND THE TIME-BASED CONCENTRATION CHANGE IN DIFFUSE DUST-AIR CLOUDS

The processes for measuring diffuse dust concentrations which are already known from the work safety usually do not allow an evaluation of explosion hazards. Methodical investigations for determining diffuse dust releases when handling bulk materials in the laboratorial scale are the basis for the development of a measuring system which can measure disperse diffuse dust concentrations spatially (two-dimensional) and temporally. Base is a photometrical emission measurement. The measured variable is the light attenuation by particles (extinction). A scheme of the build-up of the measurement system as well as used system components is illustrated in **Figure 2**.

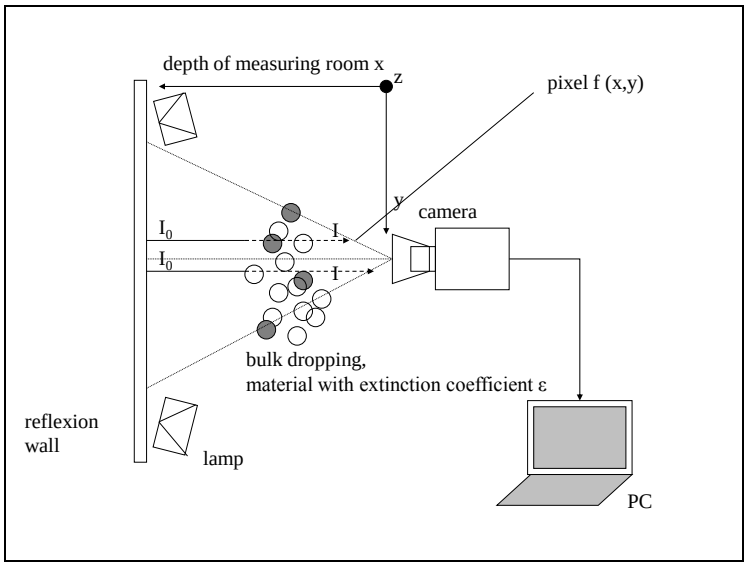

Figure 2. Schematic build-up and system components of the developed measurement system for measuring the dust concentrations in dust-air clouds

The knowledge that a standard photo consists of 256 grey-value levels was used for evaluating the photo captures. The change of grey-value levels by a dispersed material amount is equivalent to the light attenuation or the opacity respectively. By means of this measured variable and by knowing the material specific extinction coefficients, the concentration of a dispersed material can be determined by the Lambert-Beer law.
After the measurement system was calibrated and the basic operativeness could be documented in the relevant application range under pilot conditions, measurements on diffuse dust concentrations were done at a grain bulk chute for side-dump trucks. The bulk chute had a dust barrier. Dust-separation equipment was not available, however, there was a natural exchange of air. When recording the measured values, precleaned wheat was delivered. The current general risk assessment emanates from a distinct minimisation of the dust releases and explosion hazard if grain is available in precleaned form. This general hazard assessment shall be verified by the measurements.
The concentration profiles of the whole dropping process are illustrated in the following **Figure 3** as an example for four of totally eight measuring points.

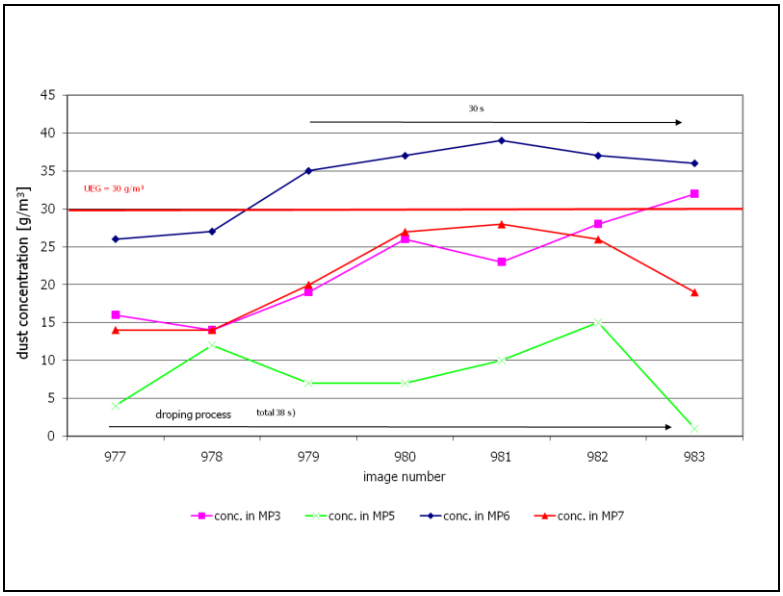

Figure 3. Dust concentrations at the bulk chute at the receipt of grain

The concentration profiles illustrate that during the dropping process there are both measuring points with explosive dust concentrations that are above the lower explosion limit, i.e. > 30 g/m³, and measuring points with dust concentrations that are below the lower explosion limit. Explosive dust concentrations especially occur at measuring point MP6 directly above the bulk chute. On the whole, the distribution of the dust concentrations in the occurred dust-air cloud is inhomogeneous and characterised by local concentration fluctuations. After the investigated dropping process is finished, the dispersed particles sediment relatively fast so that there are concentrations below the lower explosion limit already after some seconds.
The lower explosion limit is expected to be exceeded permanently in areas above the grid at intervals of up to approx. 1.5 m during the duration of the considered dropping process. If the result is related to the whole operation period of the plant under usual operation mode, the occurrence of a hazardous explosive dust-air cloud can be occasionally expected according to the definition of zones. This result is contradictory to the current hazard assessment when handling cleaned or precleaned grain as well as recommendations for the classification of zones in the raw-material receipt of the grain and feed industry. This shows a further need of research for the assessment of explosion risks.

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STARCH IN ANIMAL FEED

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ABSTRACT

This study presents the starch contents in grain of the most widely grown Zemun Polje (ZP) maize hybrids, then chemical structure and functional properties of maize starches, digestibility of different maize starches obtained by the alpha-amylase of the pancreas of swine and *in vitro* digestibility of maize grain.
The described results show that there are not only differences in regard to the grain maize starch content, structure and functional properties, components that mostly affect grain yield, as basic components in the animal feed production, but that there are differences in the digestibility of maize grain that depends on the genetic background, i.e. hybrid.
Key words: *maize, starch, amylose, amylopectin, gelatinisation, viscosity, digestibility*

INTRODUCTION

The animal feed production is traditionally one of the greatest maize consumers. Not only in our country, but in many others, maize has suppressed almost all other cereals. The most important and unique reason for this are its chemical properties and energy values. As an essential component of the animal production, maize covers from 50 to 90% of energy necessary for functioning of the organism and for the maintenance of a body temperature [2]. Starch is in a principal carbohydrate and at the same time is an essential chemical constituent of maize grain. Its content averagely amounts to approximately 70% [1].
Besides crude fibre, starch is globally the most distributed organic matter. It is the most important reserve material that is accumulated in fruits, seeds, roots and tubers, in the form of starch granules. Depending on its botanical origin, starches differ over their chemical structure, size and form of the starch granule, and therefore by their functional and sensory properties. In recent times the advance in studies of a fine structure of starch granules has been made [4, 5].
Starch is composed of a mixture of two polysaccharides: amylose and amylopectin. Amylose is a linear fraction of starch composed of α-D-glucose joined by α-1,4-glucoside bonds. Amylopectin, a highly branched polysaccharide, whose linear macromolecule-polymers α-D-glucose are joined by α-1,6-glucoside bonds. Some starches are composed of the third polysaccharide bond that is usually in literature refer to as the intermediate component. Natural starches contain 20-30% amylose and 80-70% amylopectin. Amylose forms a colloidal dispersion in hot water (which helps to thicken gravies) whereas amylopectin is completely insoluble. Starches of certain maize, barely and rye species designated as waxy, contain over 90% amylopectin, while the purist starches contain only amylopectin. On the other hand, there are starches with a high

content of amylose, such as in high-amylose maize in which the amylose content ranges from 55 to 85%. Although both forms of starch are polymers of α-D-glucose as a monosaccharide component, they are significantly distinguishable from one another over their functional properties. The amylose to amylopectin ratio in the starch granules is one of the most important parameters that significantly affects functional properties of starch. Starches components of only one polysaccharide component have specific properties and as such broaden utilisation of starch, i.e. maize as a high yield carbohydrate plant. The unique chemical structure and functional properties of starch, as well as, its nutritive value distinguish it as a naturally renewable polymer from all other carbohydrates.
Molecular marker assisted selection with the aim of getting a desirable genotype is now routinely applied in maize breeding programmes, which enables genetic control of a molecular starch structure, whereby its properties can be altered [3]. The performed studies show that the starch granule structure and interactions with other components within the endosperm, as well as, processing conditions influence starch digestibility. However, in order to determine more detailed parameters of the starch structure that affect animal feed digestibility further systematic studies are necessary [13]. Considering directions of nutritive and technological observations, a special attention today should be paid to the development of inbreds and hybrids with specific traits and for special purposes. Studies of the structure and unique properties of starch of speciality maize hybrids are aimed at hybrids such as waxy, high amylose, high lysine and high protein hybrids, which, again, due to being a raw material for the sustainable animal feed production get their own great and deserved importance [6, 8, 14]. The objective of the present study was to perceptibly present our recent results on investigations on a starch role in animal feed and to describe the utility value of grain of different ZP maize hybrids as a raw material in its production.

MATERIAL AND METHODS

The basic chemical composition of grain, i.e. the content of starch, protein, oil, crude fibre and ash, was determined on the selected samples of the most widely grown ZP maize hybrids. The chemical structure and functional properties of maize starches were observed through the determination of contents of amylose, amylopectin, resistant starch, then the determination of thermo-chemical parameters of gelatinisation and viscosity. The digestibility of different native maize starches was observed via enzymatic hydrolysis by α-amylase pancreas of swine. The *in vitro* digestibility of maize grain dry matter was observed with the method after Tolley and Terry.
All methods used in this study were described in detail in previously published manuscripts [1, 8, 9].

RESULT AND DISCUSSION

The chemical content and structure, functional properties and the digestibility of starch and maize grain, as a basic component in animal feed production, are described and discussed in this study.
The content of starch and other basic chemical constituents of the grain, i.e. the chemical composition of maize grain is its most important property, not only for food

and feed, but also for the remaining of its uses. As a highly yielding carbohydrate plant, maize is very competitive in regard to other cereals in the animal feed production.
Table 1 presents the results of the determination of the basic chemical composition of 15 ZP maize hybrids [10].

Table 1. Chemical composition of ZP maize hybrid grain

Hybrid	Starch (%)	Proteins (%)	Oil (%)	Crude fibre (%)	Ash (%)
ZP 74b	70.7	9.6	4.8	2.4	1.3
ZP 243	70.2	10.6	5.7	2.0	1.5
ZP 300b	69.6	9.4	6.7	2.0	1.4
ZP 341	69.0	9.3	5.8	2.0	1.3
ZP 434	69.0	9.4	5.9	2.0	1.4
ZP 544	69.4	10.2	5.1	1.9	1.4
ZP 578	73.0	8.6	5.1	1.8	1.3
ZP 599	67.5	9.6	5.4	2.2	1.4
ZP 611k	68.2	12.7	4.8	2.3	1.5
ZP 633	72.8	9.9	5.1	2.8	1.4
ZP 677	70.2	9.6	5.1	2.1	1.4
ZP 684	70.5	8.8	4.8	2.1	1.4
ZP 704	70.6	9.6	5.0	2.1	1.4
ZP 704wx	71.2	9.4	4.7	2.3	1.4
ZP Rumenka	67.9	11.1	6.4	2.0	1.5
Average	70.0	9.9	5.4	2.1	1.4
Minimum	67.5	8.6	4.7	1.8	1.3
Maximum	73.0	12.7	6.7	2.8	1.5
SD	1.6	1.0	0.6	0.2	0.1

The grain chemical composition of observed hybrids ranged widely, especially for the content of starch, protein and oil. The content of starch, protein and oil varied from 67.5 to 73.0%, 8.6 to 12.7% and from 4.77 to 6.7%, respectively.

Chemical structure and functional properties of maize starch
Starch is one of the most important animals source of energy. The mechanisms of it degradation is very complex. The amylose to amylopectin ratio is important factor that affects the speed and the degree of starch degradation and its digestibility and

consumption by animals. Results obtained on contents of amylose, amylopectin and resistant starch in isolated maize starches are presented in Table 2 [8].

Table 2. Content of amylose, amylopectin and resistant starch in ZP maize starches

Hybrid	**Amylose content (%)**	**Amylopectin content (%)**	**Resistant starch content (%)**
ZP 74b	25,0	75,0	1.1
ZP 341	23,5	76,5	-
ZP 360	23,8	76,2	1.2
ZP 434	26,0	74,0	-
ZP 480	24.2	75.8	-
ZP 511	25.2	74.8	-
ZP 578	23.5	76.5	1.3
ZP 611k	23.2	76.8	1.5
ZP 633	24.0	76.0	-
ZP 677	23.9	76.1	-
ZP 680	21.7	78.3	-
ZP 684	23.3	76.7	-
ZP 704wx	1.0	99.0	0.6
ZP 735	24.8	75.2	-
ZP 737	24.0	76.0	-
ZP 750	23.7	76.3	-
ZP 808	24.0	76.0	1.6
ZP Rumenka	23.5	76.5	1.4
Average	22.7	77.3	1.2
Min	1.0	74.0	0.6
Max	26.0	99.0	1.6
Sd	7.0	5.5	0.3

The amylose content in isolated starches of observed ZP maize hybrids was characteristic for standard that is waxy maize starches. Starches isolated from all hybrids except the hybrid ZP 704wx had the amylose content characteritistic for standard maize starches. According to the amylose and amylopectin content, the hybrid ZP 704wx can be classified into speciality hybrids designated as waxy maize hybrids. Foreign authors considered that these hybrids had significant nutritive advantages in ruminants [1].

In recent times resistant starches have been attracting the attention of many researches world-wide due to two reasons: potential positive effects on human health that can contribute to prevention of certain diseases, as well as, due to their functional properties. The resistant starch (RS) content was in a correlation with the amylase content. The lowest RS content of 0.6% was detected in waxy starch, while its highest content of 1.6% was recorded in standard starch of the hybrid ZP 808. Therefore, these starches are of an extremely great significance in human nutrition.

The differential scanning calorimetry (DSC) method was applied to study the most important functional property, gelatinisation, and to determine thermo-chemical parameters of selected samples (Table 3).

Table 3. Thermo-chemical parameters of gelatinisation of ZP maize starches[1] [11]

Hybrid	To (ºC)	Tp (ºC)	Tc (ºC)	ΔH (J g^{-1})
ZP 74b	62.1	66.6	70.6	13.6
ZP 360	63.3	68.3	73.4	15.0
ZP 434	65.0	69.5	74.2	15.6
ZP 578	62.7	67.4	72.2	15.5
ZP 611k	64.5	67.9	71.6	14.2
ZP 633	63.3	67.8	71.9	15.2
ZP 684	63.2	67.7	71.7	14.5
ZP 704wx	63.7	68.8	74.7	18.1
ZP 808	62.9	67.4	71.6	14.3
ZP Rumenka	63.7	68.3	73.1	14.8
Average	63.4	68.0	72.5	15.1
Min	62.1	66.6	70.6	13.6
Max	65.0	69.5	74.7	18.1
Sd	0.84	0.81	1.3	1.2

[1] T_o, T_p, T_c are the temperatures of the gelatinisation onset, peak and conclusion, respectively and ΔH presents the changes of starch gelatinisation enthalpy

The temperature of the gelatinisation onset varied from 62.1ºC (ZP 74b) to 65.0ºC (ZP 434). A connection between the amylase content and gelatinsation temperature was not detected in these samples. Waxy starch (ZP 704wx) expressed a significantly higher alteration of gelatinisation enthalpy (ΔH=18.1 J g^{-1}) in relation to standard maize starch (ΔH=13.6-15.6 J g^{-1}). The gelatinisation enthalpy change was correlated with the amylose content in isolated starches.
The Figure 1 presents amylograms of isolated starches of standard maize genotype ZP 434 and waxy maize genotype ZP 704wx. Amylograms of standard starches of all remaining studied ZP hybrids are similar to the amylogram of starch isolated from the grain of maize genotype ZP 434 presented in Figure 1 [8].

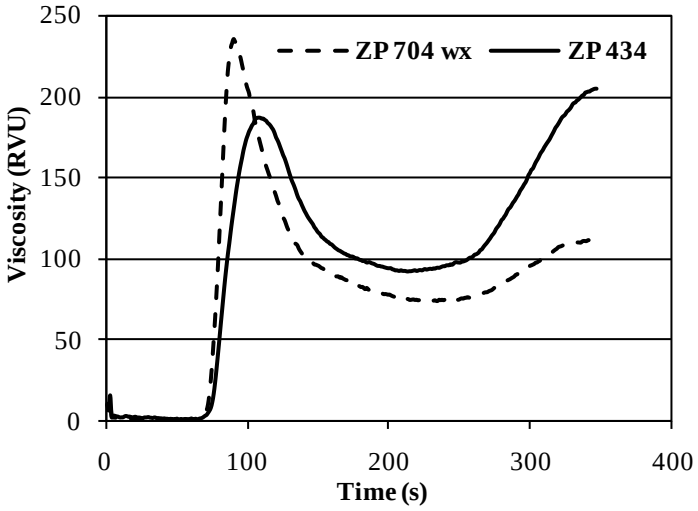

Figure 1. Amylograms of standard maize starch (ZP 434) and waxy maize starch (ZP 704wx)

The studies of gelatinisation and viscosity of maize starch are of enormous practical significance for the animal feed production, especially feed that is made by the application of a modern procedure of thermal processing of raw materials, for both the production process itself and for usability of nutrients in the animal organism.

Maize starch and grain digestibility

Not only the starch structure and its functional properties from the aspect of a starch role in the animal feed, but also its digestibility is also important. Results on the determination of the digestibility of six different types of native maize starch by α-amylase of pancreas of swine are presented in Figure 2 [9].

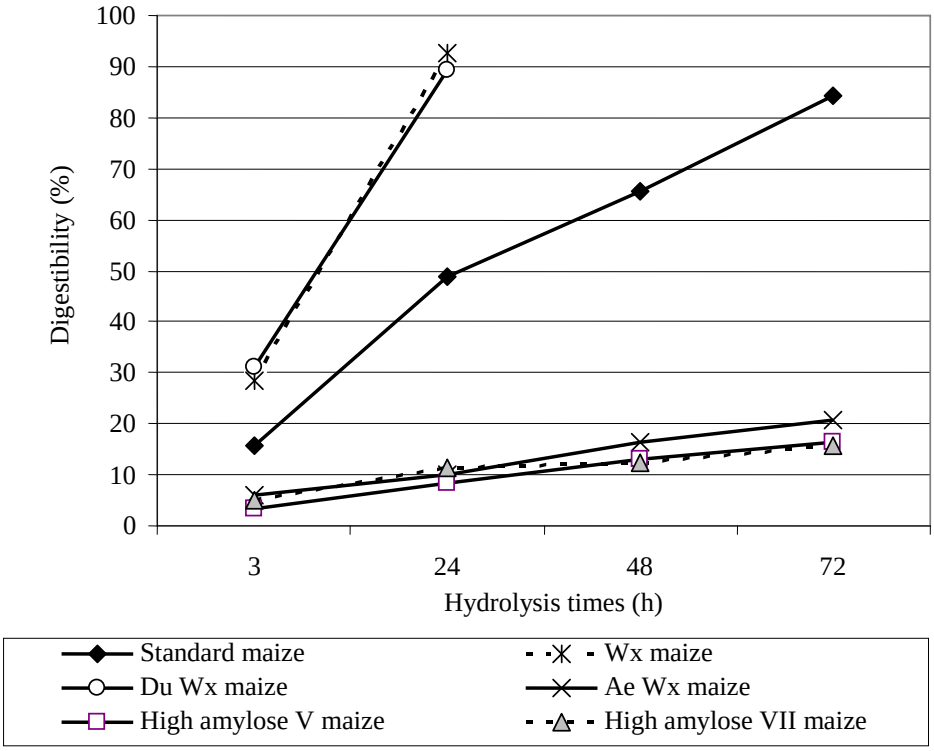

Figure 2. Digestibility of native maize starch by α-amylase of pancreas of swine

The digestibility of different native maize starches was analysed by α-amylase of pancreas of swine. The degree of hydrolysis was expressed as a percentage of digestible carbohydrates in relation to an initial amount of starch.
The normal amylose and amylopectin starch (27.3% and 72.7%, respectively) was registered in standard maize starch. Waxy maize starch was composed of almost only amylopectin (99%), while amylose maize starches were characterised by the increased amylose content (62.4 and 81.0%). After 24 h of α-amylase activity, the degree of hydrolysis for observed starches ranged from 8.4% (high-amylose starch) to 92.8% (waxy starch). Results show that the degree of hydrolysis obtained by α-amylase of pancreas of swine was determined by the ratio between amylose and amylopectin as a basic parameter of native starch in a way that the degree of hydrolysis was higher for starches with a lower amylose content (starch of standard, wx and Du wx maize) and vice verse this degree was lower with a high amylose content (starch of Ae wx, high-amylose V and high-amylose VII maize). Factors affecting the starch digestibility in different animals have not been yet sufficiently studied and presented in the available literature. Therefore, these scientific studies are necessary to be continued [12, 13].

Results gained for the *in vitro* digestibility of the dry matter of studied grained of observed hybrids are presented in Figure 3 [7].

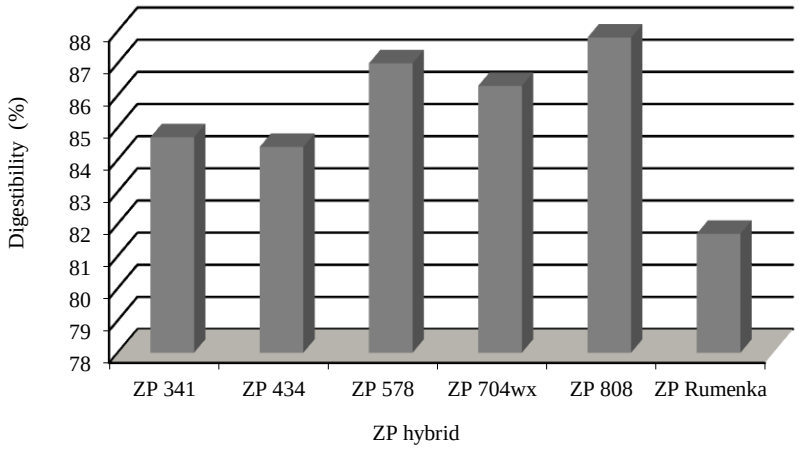

Figure 3. Digestibility of ZP maize hybrid grain

The variation of the digestibility of the grain dry matter from 81.7 to 87.8% for observed hybrids ZP Rumenka and ZP 808, respectively, indicates that these values significantly differ among observed hybrids. These differences are caused by the different genetic background, i.e. hybrid.

CONCLUSION

Results described and discussed in the present study show not only the existence of the differences related to the maize grain starch grain, its structure and functional properties, components mostly affecting maize yield as a basic component in the animal feed production, but also the existence of differences in the digestibility of different maize starches and the digestibility of maize grain that depends on the genetic background, i.e. hybrid. These results are comparable with results obtained in leading research centres all over the world.

Considering the role and the application of starch in animal feed, its chemical structure, functional properties and the digestibility represent unavoidable and very fundamental parameters. The most important chemical characteristic and the reason for maize advantage over other cereals in animal nutrition is certainly its high starch content, i.e. the high energy content. These studies are a contribution and the starting point for further studies of the fundamental and practical importance within the field of the utilisation of maize, the most important natural renewable raw material for our country in the sustainable animal feed production.

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USING MODERN BIOTECHNOLOGIES IN ANIMAL NUTRITION

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ABSTRACT

This paper reviews the application of modern biotechnologies from the aspect of using certain animal feed additives with the goal of obtaining better production, reproductive and health parameters. These additives can be used as animal feed either separately or within concentrate mixtures. Some of the additives are used on purpose in order to ensure their presence in products like milk and eggs which enhances their quality for human nutrition, which is the final goal of modern nutrition experts.
The paper considers the effects of live yeast cells, certain microelements in gelatin and organic forms and poly-saturated fatty acids i.e. omega-3 and omega-6 and their significance in animal nutrition and indirectly in human nutrition as well.

INTRODUCTION

Animal feed science has a responsible task, because animal feed needs to combine multiple goals at the same time. Besides ensuring good animal health and good productive and reproductive performance conditions, reduce the environment pollution and provide materials that preemptively strengthen of the human organism and raise the natural disease immunity through milk, meat and eggs.
Such a complex task can be most efficiently realized by applying modern biotechnology methods which include the use of specific feed types which were created through the recent scientific disciplines such as molecular biology and genetic engineering. Through research in these areas, grain and leguminous types with improved nutritive properties. On the other hand, the need to use natural substances (pro-biotic, enzymes, inoculants chalets etc.) which help to more thoroughly use certain nutritive materials from the food. In this way, a significantly lower portion of the undigested materials is excreted into the soil that lowers the pollution levels. The undigested feed portion that is excreted into the soil not only seriously endangers the micro climate but also uses up large amounts of oxygen for the subsequent fermentation which endangers the atmosphere.

This pollution is most drastically present in the most developed countries that have polluted the environment and the soil so much, that they now must revise their agricultural production structure.
Principally, the use of specific additives in animal feed is usually applied in those cases when certain problems connected to some production level or the animal health need to be overcome. This includes a group of non-nutritive ingredients which regulate the pH value, the growth, modifies metabolism processes etc. (Hutjens, 1991). For example, if such additives are used in the feed of dairy cows, the following factors must be considered in order to justify their use: obtaining of the appropriate effect, economic and obtained rationale and the desired effect. When some of the additives is included into cow feed a change in the a certain performance is expected: larger milk production, a change in the milk contents, better dry matter consumption, stimulating the synthesis of micro-biological proteins or the evaporable fatty acids, improved digestion, pH stabilization, improved growth, reduction of heat stress, health improvement in the form immunity improvement.

Using live yeast cells in domestic animal feed

Yeast represents a food additive which is getting increasingly more attention lately. It is becoming increasingly present in commercial vitamin and mineral mixtures. Its high nutritive value is reflected in the rich presence of enzymes, fatty acids, B vitamin, unknown growth factors and amino acids (more than 40% of the dry matter). Including yeast in ruminant and non-ruminant meals, more efficient use of celluloses and other nutritive substances as well as a higher daily growth (Sretenović and associates 2001).
Yeast cells also absorb micro-toxins from feed and help with the absorption of minerals such as phosphor, magnesium, calcium, copper, potassium, zinc and manganese (Hutjens, 2005).
Having in mind the fact that many countries prohibit the use of non-absorptive antibiotics for domestic animal feed, yeast is being increasingly used in ruminant feed as pro-biotic. Yeasts can stimulate the development of other microorganisms in contrast to antibiotics which inhibit the growth of microbes. The importance of yeast higher for ruminants than non-ruminants due to their specific digestive tract which is characterized by the presence of various microorganism populations. Yeast is a safe, natural product whose useful effects are notable for the *Saccharomyces cerevisiae* culture which is widely used as additives in animal feed. Besides the numerous positive effects, yeast regulates the pH of the rumen, uses oxygen as aerobes and stimulates the anaerobic activity of the rumen, creating a source of nutritive materials and vitamins for rumen microorganisms. Yeast has a specifically positive effect on the improvement of cellular activity which is highly important for ruminants. Besides, yeast helps with the stabilization of bio chemical processes in the rumen and thereby positively influences the general health of the animals and their production results.

Using yeast in ruminant feed

Yeast is used for the feed of highly productive ruminant animals, especially dairy cows. Live yeast cells are used: in early lactation period in order to better use the meals, during stress when milk production and milk fat production is reduced, for dry cows in order to better utilize the nutritive and during the use of low quality forage. Yeast also has a positive effect when fed to heifers because it speeds up their growth. Yeast was shown to be very useful in the nutrition of dry cows as well.

Within the research papers of *Sretenovićе and associates (2006)* the effects of the application of the "Yeasture" product in meals of dairy cows were tested. This product is composed of live yeast cells, selected from three types of Saccharomyces cerevisisiae in combination with pro-biotic bacteria and enzymes (*Lactobacillus casei*, *Streptococcus faecium*, *Aspergillus oryzae*, *Lactobacillus acidophilus*, 1,3-b and 1,6 D-Glucan, hemicellulase, Protease, cellulase, Alpha amylase). in order to overcome the problems generated in the dry period.

The experiment examined the digestibility of dry matter, provender, use and milk contents as well as somatic cells. The application of the aforementioned product has influences the increase of digestibility in Lucerne hay ($P<0.01$) as with the silage of the entire corn crop and sugar beet silage ($P<0.05$). Using this additive has also caused an increase of milk production by 2.57 kg 4%MKM or 10.86% ($P<0.05$). The "Yeasture" product has reduced the count of somatic cells by 7.3 percentile points which is connected with a better health condition of the udder.

Significant discoveries were made by Yoon and associates (1998) as well as by Wang and associates (1999). Those discoveries show that rumen microorganisms increase the digestive capabilities of provender if the meals contain yeast, which has led to an increase of digestibility of dry matter, celluloses unsolvable in neutral detergents (NDF), celluloses unsolvable in acidic detergents (ADF) as well as hemi-celluloses for silage maize. Based on the observed effects, it can be concluded that using yeast cells combined with pro-biotic and enzymes in diary cow meals during the periods of dryness and early lactation is fully justified.

The research of the impact of yeast cells on the milk amount and composition was also conducted by Chinese researches. For example, Wu Zilin (1996) showed that including yeast into dairy cow meals increases the consumption of dry matter by 3.94%, dry milk corrected by 3.5% milk fat by 7.07%, milk fat by 5.77%, but has a significantly lower effect on the contents of milk proteins and lactose.

In a series of experiments conducted by Hutjens (1991) cows which received yeast in meals had increased their milk output by 4% from 23.5 to 25.1 kg. It has also been conducted that adding yeast during the early lactation period showed significant results while adding it during the middle of the lactation period had almost no effect. Dann and associates (2000) concluded that the milk composition concerning the fat and protein

entail was very variable, while the Canadian researches Robinson and Garrett (1999) found that including yeast into meals leads to a significant increase in the consumption of dry matter during the transitional period for cows and lessens the body weight loss after giving birth.
Schingoethe and associates (2004) have included yeast into cow meals in order to test their effects during the summer period when the average temperature was 33^{o}C (28 to 39 ^{o}C). Yeast cells have increased the milk amount by 4% from (31.2 to 32.0 kg/d), while the milk fat (3.34 to 3.41%) and protein entail (2.85 to 2.87%) remained similar in both treatments. Wu Zilin (1996) noted that yeast cells significantly increase the body mass and average daily growth in the test group of cows. This has a large influence on the quick recovery of body mass after giving birth and overcoming heat stress during the summer period.

Utilizing microelements obtained by using modern biotechnology in animal nutrition

Microelements as necessary ingredients of all types of domestic animal meals are traditionally used in the form of inorganic salts, mostly sulfates, chlorides, oxides or carbonates. However, as the largest number of factors which influence their usability are known, constant efforts are being made to improve their usage in the human and animal organisms. It was discovered that metals such as Cu, Zn and Mn in the form of chelate (i.e. tied to amino-acids and peptides) can be much better used within the body and thereby improve growth, productivity, reproduction, and give positive effects in milk production, reduce the number of somatic cells in milk and the appearance of mastitis, raise the body's immunity, improve food usage and ease stress states.
Microelements such as Mn, Cu and Zn are produced in chelate form. Chelatinization refers to a special type of forming complex metal ions and ligands. Ligands can be defined as molecules which contain an atom with free electron steam. Metal ions are bound to ligands in the complex over donor atoms such as oxygen, nitrogen or sulfur. Chelatinization represents a connection of such ligands with metal ions over two or more donor atoms where the complex which contains one or more hererocyclic rings (connections) which contain the metal atom are formed. It is known that the usability of microelements from inorganic salts (sulfates, carbonates, oxides and chlorides) is very low and that applying organically bound microelements (chelates) in animal nutrition represents a relatively new technique, although it was known to science for some time.
On the other hand, selenium is used in organic form and applied to beer yeast where it is bound to the amino acid metionine or cistine. The characteristic of these compounds is that they are electrically neutral and do not form unsolvable complexes with other food components in the digestive tract so they arrive unaltered to the re-absorption spot.
Selenium and chrome are organically bound microelements. They are obtained by a solution of yeast on a foundation enriched with selenium or chrome.

The importance of organically bound selenium in animal nutrition

Today it is known with a certainty that selenium is one of the microelements which play an essential role in the organisms of both humans and animals. An element is considered as essential if defects arise when it is removed from the diet and are resolved again when it is restored.

Selenium is found in over 200 proteins and plays a primary role as a co-factor in the glutation-peroxidase system (GSH-Px) the activity of which is connected to the erythrocyte function, the destruction of peroxides which are formed during normal lipid metabolism as well as the formation of alpha-tokopherol. The symptoms of selenium deficiency in animals are: increased mastitis appearance, increased number of somatic cells in milk, late ovulation, quiet estrus, poor conception and fertility, reduced immunity. The primary organic form of selenium is selenium-metonine which uses the amino acid mechanisms to build into selenium-proteins. Selenium-metonine is the main form of selenium present in kernels, seed of oil plants and other plant materials. The concentration of selenium in feed types varies widely according to the selenium content within the soil.

Until recently, selenium was used in inorganic form as selente or selenid but it was proven that it acts as a pro-oxidant rather than an antioxidant in those forms and can destabilize cell membranes, and has significantly less bio-usability. If sodium selenium is taken as the 100% mark, then the bio-usability of cobalt selenium is 105% selenium-metonine 245% and selenium yeast 290% for ruminants. The explanation for these differences lies in the fact that rumen microbes convert selenite and selenate into unsolvable components which are inefficiently absorbed through the intestinal tract, while rumen microbes do not attack selenium in organic form such as selenium-metionine.

With the appearance of yeast enriched with selenium the possibilities of adding organically bound selenium to animal feed appeared which gave significant effects in pig and poultry feed. For example, the integration of selenium into muscle tissue depends on the level and the form of the selenium within the meal. With the increase of inorganic selenium in food its concentration within the muscle practically remains constant, organic form leads to linear increase of selenium within muscles which remains nearly constant. This is important for improving the quality of meet which can become a good source of selenium for human nutrition.

The advantages of organic selenium compared to its inorganic varieties are that in this form it is often encountered in nature, can be easily absorbed, is held in tissues as a reserve, can easily be transferred onto the offspring over colostrums and milk. Furthermore, the larger concentration of selenium within the colostrums ensures the better health status of calves.

The significance of vitamin E and selenium in the nutrition of dairy cows should be especially noted. It is known that Se and vitamin E act as in synergy as components of the anti-oxidant system which protects the animal of various deficit diseases such as rash growth, muscular dystrophy etc. The meals need to contain an adequate amount of vitamin E in order to ensure the selenium use (Nicholson and associates 1991.)
A large number of research papers has confirmed the positive effects of using organic selenium on the production, reproduction and health condition of the animals that is connected to better absorption and utilization within the body. Sodium selenite acting as the primary source of selenium in animal feed brought in through premixes, is transfused into insolvable compounds within the rumen through the micro flora and is passively absorbed from the intestines, becomes chemically reduced to selenide and is transported to the liver where it is synthesized into selenium-metonine, the biologically active form of selenium or to the kidneys where it is extricated into urine, so that ruminants use it poorly.
It has been confirmed that the selenium content in milk increases 4 to 5 times when selenium is added in organic form. A higher concentration of selenium in milk is the result of better retention of organic selenium compared to the selenite because selenium-metonine incorporates itself into all of the body's proteins. The milk gland extracts large amounts of metonine for building milk protein. A large increase of the metonine concentration in milk through selenium yeast nutrition is caused by the constant integration of selenium-metonine into the casein during milk synthesis.
During selenite intake the largest part of the absorbed selenium enters the inorganic pull and is likely incorporated into specific selenium-proteins but not into proteins such as casein. A higher concentration of Se in milk products means more selenium intake for humans which has positive implications (Sretenović and Vukić-Vraneš, 2004).
In order to receive high quality milk, two key moments are important: adequate nutrition and adequate health status of the milk gland, which can be prospered by a regular intake of selenium in organic form. The recommendations for the daily intake of selenium are 0.3 and 0.1 mg/kg of dry matter per dairy and beef cows. Further additions to not grant any additional benefits.
The positive effects of selenium on the prevention of clinical mastitis is likely connected to selenium's influence on neutrophiles and other immunity cells. In the research of Bolanda (2002) adding selenium in combination with other micro elements into cow meals has led to the reduction of the number of somatic cells by 40%. In the second experiment of the same author, where cows were equally divided in accordance to the somatic cell count, the average milk yield was higher by 1.08 kg/day compared to the control group ($P<0.05$), and the number of somatic cells lower by 38%.
In the research of U Popović and Marina Vukić Vraneš (1998) adding organically bound selenium (Sel-Plex 50) in combination with live yeast cells and organically bound zinc (Bioplex Zn) into cow meals during a period of 100 days, it has been determined that the milk amount increased by 171kg or 7.6%, the milk fat amount increased by 6.14 kg or 7.8%, and the protein amount increased by 7.63 kg or 10.42% ($P <0.05$). In the same experiment carried out for first calvers has a significant increase in protein amount from 3.32 to 3.46 ($P <0.05$). For lactating cows, a higher percentage of mastitis has been

noticed in the control group (9%) than in the experimental group (4.6%), which can be partially explained by the influent of the treatment.

In the works of Sretenovićeve and associates (1994) the effects of organically bound selenium in the form of selenium-metionin were compared with inorganic selenium effects in the meals of dairy cows. The inclusion of selenium into meals was started 15 days prior to calving and lasted for the next 100 days of lactation. In the experimental cow group the milk amount was increased by 0.83 kg or 3.5% ($P<0.05$), and the selenium content in the blood was increased by 2.1%. The same authors have researched the effects of adding organically bound selenium and other microelements in 1999 compared to the inorganically bound selenium with the 30:70% ratio, toward the productive and reproductive traits of dairy cows. The selenium treatment was started during the high gravidity phase. Results indicate that the amount of milk from 4% in the start group was higher by 1.43 kg or 7.22% ($P <0.05$). The number of somatic cells was reduced in the experimental group compared to the control group by 13.78%. The fertility in the experimental group was higher by 10.34% than in the control group (64% toward 58%). These results undoubtedly indicate the justifiability of including organically bound selenium into cow meals, from the aspect of improving their health status as well as from the excretion of selenium into milk because that is the most natural way of introducing selenium into the human body.

If there is no sufficient amount of selenium animal meals, it is necessary to add it through premixes into concentrate mixtures but care must be taken of the chemical form of the compounds because the absorption depends on it (the true absorption of Se for dairy cows is 10-15% of the consumed amount).

In the research of Donoghue and associates (1995) cows from the test group which received organically bound Cu, Zn and Se in 100, 250 and 2 mg per day have produces a slightly larger amount of milk compared to the control group (24.75 : 24.50 kg). The conception period from the first seeding of the experimental group toward the control group was 57.7 : 65.2%; the service period was 75.5 : 68.8 days and the number of somatic cells was 575000 : 317000. Concerning the amount of selenium in dairy cow meals, the experiment results indicate that the Se absorption depends on its chemical form. Peter and associates (1982) state that the Se absorption in the form of selenium-metonine is higher by 12-13% compared to selenite (inorganic forma).

The positive effects of adding organic selenium have also been confirmed in the experiments with other animals. For example, giving organic selenium to laying hens increases the selenium amount in eggs by 20% compared to the Na-selenite (Payne and associates 2005).

It isn't clearly determined which amount of selenium is necessary for the daily consumption of humans. Many medical research papers indicate that selenium plays a role in preventing cancer. Clark and associates (1997) in his 10 year study indicate that the possibility of cancer (especially the colorectal and prostate types) is reduced by 50%

when 200μg of organic selenium is taken each day. The daily intake of selenium in the largest part of the population is lower than 200μg. One of the ways of introducing selenium into the human organism is through milk and meat.
The higher selenium concentration in milk products means a higher intake of selenium into the human body, which has a positive influence as selenium is a microelement with powerful anti-oxidant capabilities. Milk enriched with selenium is considered as functional food which can help prevent many diseases.

Omega-3 fatty acids in milk and meat and their significance for animal and human nutrition

When taking about healthy food and its influence on the life quality and length, it is often said that a person is as old as his blood vessels. It is proven that heart and coronary diseases and the appearance of arthrosclerosis are a direct consequence of the intake of food of animal origin with a high lipid and saturated fatty acid content, especially certain types of cholesterol which is the main cause of these diseases.
Considering this fact, programs of healthy nutrition which propagate food enriched with non-saturated fatty acids, mainly omega-3, because it was undeniably proven that they have a positive influence on the human health (Sretenović, 2005). Besides the absolute content of omega-3 fatty acids in the meal, the relationship between omega-3 and other types of non-saturated fatty acids namely omega-6 fatty acids.
The interest for omega-3 fatty acids is connected with the research conducted in Greenland in the 1970s. Bang and associates (1980) have determined through their research that the Eskimo population of western Greenland has an astoundingly low percentage of coronary illness, which was traced back to their nutrition habits which is traditionally rich with fish and other see food.
This type of food is rich with omega-3 fatty acids which are called eiko-pentanoic (EPA) and docoza-hexanoic acid (DHA). Other human communities like the Alaska and Japan population which have a similar diet also have a lower coronary disease rate.
Omega- 3 and omega- 6 fatty acids which are poly-unsaturated and which are found in certain food types (the so called traditional foods) or are added to food making it functional, are now in the focus of scientific research. The natural sources of omega-3 (lanoline acid) are: fish oil (a rich source which commonly contains 30-50% omega-3 fatty acids compared to the total weight), fish from the northern seas, tuna, trout, salmon, crabs, lint seed, green vegetables, and leguminous. The low content of omega-3 fatty acids is characteristic for beef, pig and chicken meat. Regardless of the trace amounts of these acids in these products, consuming high amounts of meat in the meals can satisfy the daily needs. The sources of Omega-6 or lanoline acid can be found in meat, vegetables and vegetable oil (sunflower, soy, cotton seed) as well as milk.
It has been determined that nutrition can partially influence the composition of fatty meat tissue. This has brought animal nutrition into focus, especially the nutrition of pigs and poultry with such energy sources which will influence the acquisition of high quality final products with the reduced content of hazardous lipids. Sunflower, sugar beet and fish oil are used as energy sources and carriers of unsaturated fatty acids and sugars,

primarily glucoses etc. The full-fat soy been is an excellent source of energy and high quality proteins. Soy with a reduced content of anti-nutritive materials has shown significant advantages in use because it contains a favorable ratio of unsaturated and saturated fatty acids. The results obtained from including 10% of full-fat bean of oil beet of the Leo ”00” sort into the concentrate mixtures used for fattening pigs, which led to a decrease of the total cholesterol content and the poly-saturated fatty acids in the tissues of fattening pigs.

Unsaturated fatty acids reduce the cholesterol levels, so the widespread view is that saturated fatty acids should be completely replaced with unsaturated fatty acids. Later, this stance was revised, as poly-unsaturated fatty acids can have a hazardous effect if they are taken in too much through food and can even influence a decrease of the immunity, carcinogen effects, osteoporosis, increased creation of peroxides and the reduction of HDL cholesterols. The recommended daily intake of the poly-unsaturated fats is 10% (7-8%), and 1 g/day per lanolin.

Considering all this, healthy food programs are getting an ever increasing place in the world, particularly for food which is enriched with unsaturated fatty acids, primarily omega-3, because it is undoubtedly proven that they are essential to human health (Sretenović, 2005).

Understanding the role of the omega-3 fatty acids in health maintenance starts by understanding the chemical structure of certain fatty acids. Omega-3 fatty acids are poly-unsaturated fatty acids with a long chain (LC-PUFAs) which include: alpha-linolein acid (ALA) which is the most famous omega-3 fatty acid in human nutrition. ALA has 3 double helixes at atoms 3,6,9 from the CH3 terminal end. The human body cannot synthesize ALA, which makes it an “essential fatty acid”. This proves that it needs to be taken in via food. Poly-unsaturated fatty acids with a long chain (LC-PUFAs) represent about 20 of the brain’s dry matter and their deficit is dangerous for the development and the function of the brain (Belz i sar.,2007).

This acid is mainly found in plant oils such as nuts, leguminous, vegetables, lint seed, certain vegetable and grain oils. Lint seed is the richest source of alpha-linolein acid with a ration of -50% in the total fatty acid content. Fish, fish oil and algae oil are the richest sources of the other two omega-3 fatty acids EPA-20:5, and DHA-22:6.

In order to overcome many health problems, the increasing presence of a large number of food which are not traditional sources of omega-3 fatty acids, such as dairy and bakery products, meat, baby food, etc. are being enriched with small amounts of these fatty acids. The demand for such products rises because the beneficial health effects of the fatty acids are now more widely known (Sretenović i sar., 2007).

An average meal consists of various components, including a fat and oil mixture the basis of with are made of fatty acids. It is assumed that one meal contains about 20 different fatty acid types which are classified as saturated, mono-unsaturated and poly-unsaturated.

The fats contained within the meal are not used in the same manner, and different fatty acids have different roles in the body. Some of these include: beta oxidation for energy creation, storing in deposits for future integration into phosphor-lipids, which form the main structural components of all cell membranes. The scope of the integration into cell membranes depends on the consumed amount. Enriching cell membranes with omega-3 can alter the cell signal, the function of cell proteins and the expression of genes.
As the human organism doesn't have a enzyme system which is necessary for the synthesis of omega-3 fatty acids, they must be taken in via meals (this is why they are called "essential fatty acids").
Modern biotechnologies offer various ways of enriching products with omega-3 acids. Products of animal origin such as milk, meat and eggs are enriched with omega-3 fatty acids by introducing these acids into the meals of the animals which incorporate them into these products. The increase of the omega-3 fatty acid content can also be achieved by the application of modern biotechnological methods in plant selection and cultivating varieties which synthesize a larger amount of ALA, or fatty acids which are similar to EPA and DHA.

SUMMARY

This paper reviews the application of modern biotechnologies from the aspect of using certain animal feed additives with the goal of obtaining better production, reproductive and health parameters. These additives can be used as animal feed either separately or within concentrate mixtures. Some of the additives are used on purpose in order to ensure their presence in products like milk and eggs which enhances their quality for human nutrition, which is the final goal of modern nutrition experts.
The paper considers the effects of live yeast cells, certain microelements in gelatin and organic forms and poly-saturated fatty acids i.e. omega-3 and omega-6 and their significance in animal nutrition and indirectly in human nutrition as well.

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PREDICT THE ENTEROBACTERIACEAE DECONTAMINATION OF FEED BY PELLETING SYSTEM

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ABSTRACT

This study was set up following discussions between the DGAL and the animal feed industry. It has three principal objectives. This first is to show the impact of granulation on the bacteriological quality of feeds. The second is to set up minimum decontamination conditions in order to obtain less than 1000 enterobacteria per gram of feed and a decrease of at least 3 log via the granulation process. The third objective consists in introducing and then circulating a protocol for validating a treatment line to test its decontamination efficiency.

Two phases were set up to meet these objectives. Phase 1 consists in studying the decontamination occurring with the granulation of four feeds (pig/laying hen with a fine granule size distribution and pulverulent aspects with little fat, and pulverulent, very fat chicken/turkey) using a pilot press with a rotating tubular die plate. These trials test the impact of two factors: firstly, the temperature at the processor exit (45 to 90°C), and secondly, die plate thickness directly correlated with the retention time in the die plate (2 to 6 sec.). Samples are taken at the hopper entry and the processor and pilot cooler exit during granulation. These are then used to do analyses to count enterobacteria (standard NF VO8054 at 37°C) and total flora (standard NF VO8 051). The results have shown that the four feed types all have a decontamination that is more or less significant depending on the granulation parameters. For the chicken and turkey feeds, it appears that the processor temperature is more of a factor than the die plate used. However, for the pig and laying hen feeds it appeared that the use of more constricted die plates caused the feeds to heat up in the die plate and simplified their decontamination at processing temperatures that are not always very high. Following these trials, statistical treatments (multi-linear regression) were done to establish decontamination tables. For each feed, two tables (one allowing a safety margin and the other none) associate the minimum processing temperature with a given retention time to ensure that decontamination objectives are met.

During phase 2, eighty four industrial trials were conducted with the same type of feeds to check the correlation between the tested lines and the tables (with or without margin) and validate them. Samples taken at the mixer exit and after processing at the cooler exit showed that the tables with no margin were generally sufficient to reach the decontamination objectives.

Key words: *granulation, decontamination, temperature, die plate tickness*

GENERAL DESCRIPTION OF FORBERG STEAM HEAT TREATMENT SYSTEM

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ABSTRACT

In the first step of the process the conditioner/mixer is charged with the feed, which has to be treated. The feeding can take place out of a single bin or different components can be mixed with the Forberg high speed mixing process. Then steam is injected into the mixer to heat up the product to the desired temperature. Now the temperature is maintained over a certain period of time to kill salmonella and certain kinds of bacteria. In this stage of the process liquids, which are not initially hygienic like molasses for example, can be treated as well. After the heat treatment the mixer discharges directly into a dryer/cooler. The mixer and the chutes are heated to maintain a temperature over the dew point at all surfaces. As soon as the dryer is charged the conditioner can already produce the next batch. In the dryer, warm air is first introduced to extract some moisture from the product. In the second step ambient air is blown in to reduce the product temperature further. After the product has reached the desired temperature and moisture content, additives such as vitamins, enzymes, flavors, pharmaceuticals, etc., which are sensitive to heat can be added. The additives can be applied in powder or liquid form. They will be mixed to perfection with low CV values. The product is now ready to be stored, packed or transported. There is no segregation that has been observed like in many other processes because transport of the final mixture is very short. The air of the process goes through a filter unit before being released clean to the environment, or alternatively it is circulated to a water-cooled air cooler and used again in a closed loop system. After drying and cooling in a Forberg dryer/ cooler/mixer the process ends. The mixture is now ready for packaging, bulk loading or intermediate storage. The system is based on standard Forberg systems, which are modified to enable them to perform the process requested by the customer. Standard units include the conditioner and the Forberg dryer.

Key words: *steam heat treatment, mixer, warm air*

CURRENT ISSUES IN PELLETING IN RESPECT TO PHYSICAL PELLET ANALYSES

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ABSTRACT

Thermo-mechanical treatments are applied in order to achieve certain specific goals such as gelatinisation of starch, denaturation of proteins, inactivation of anti-nutritional factors (endogenous enzymes, trypsin inhibitors, etc.), drying/cooling and product shaping. These changes have influence and/or overall beneficial effect on physical characteristics, digestibility and nutritional value of the feeds.
Present study was conducted to investigate the influence of cereal starch exposed to various processing techniques on physical pellet quality. Diets with equal amounts of oat hulls, rape seeds and fish meal were cold-pelleted or steam conditioned and pelleted with or without inclusion of 200 g/kg pure wheat starch. Durability of the pellets was reduced ($p<0.05$) for the starch containing diets compared to non-starch diets under both processing conditions. Despite a significant improvement ($p<0.05$) in pellet quality within starch containing diets as a consequence of gelatinised starch addition, pellet durability was lower ($p<0.05$) for gelatinised starch containing diets compared to non-starch diets.
Key words: *gelatinisation, cold-pelleting, steam-pelleting*

INTRODUCTION

A majority of the 600 million tons of compound feed produced annually are pelleted [2]. The pelleting process was introduced to the feed industry at beginning of the 20th century with an overall purpose of the feed particle unification, and consequent increase in density and flowability of the product. By the agglomeration of the various feed particles into pellets, challenges of the mash feeding such as selective eating and/or segregation of the ingredients could be solved. Supplementary benefits of pelleting includes: reduced dust problems, decreased feed wastage, destruction of pathogenic organisms, less energy spent for prehension, and eventually improved animal performance. The physical quality of the pellets is instrumental in achieving these goals. If the durability and hardness is insufficient, pellets may break during storage and transport resulting in dust problems and feed loss. Although a lot still remains to be elucidated on the causes for varying physical pellet quality, composition of the diet and processing conditions have been shown to be major factors.
Starch is a major constituent of cereal grains and an abundant energy source for domestic animals. In addition, processing of the starch leads to paste-like properties as a consequence of gelatinisation [7]. Thus, starch may have binding properties in pelleted feeds. Large part of the investigations on starches, however, has been conducted on relatively high water systems (>300 g/kg) and thus their application to rather low

moisture feed processing conditions are questionable. Although a number of investigations from the feed industry suggest that binding capacity of starch containing feed ingredients increases with heat treatment and starch gelatinisation [4], the role of starch for physical pellet quality is not clearly defined. Results by Svihus et al. [9] even indicate that starch may not be a major contributor to the pellet quality, and Gilpin et al. [3] found a negative correlation between degree of gelatinisation of starch and pellet durability.

MATERIAL AND METHODS

All diets were processed at Centre for Feed Technology, Norwegian University of Life Sciences (Ås, Norway).
In present experiment a diet composed of equal amounts of rapeseeds, fishmeal and oat hulls was made. The ingredients were selected so that the starch content was minimized (Table 1), and so that physical quality would be poor after pelleting, thus assuring that effects of starch on physical quality could be revealed. The oat hulls were added as unground oat hulls from the dehulling process, whereas rapeseeds were ground in a hammer mill (Münch-Edelstahl, Wuppertal, Germany licensed by Bliss, USA, 18 kW, 3000 RPM) fitted with a 3 mm sieve before processing. One part of the diet was diluted with 200 g/kg of a wheat starch (Raisio Grain Starch Ltd, Raisio, Finland). Each of these two diets was thereafter divided into 6 batches which were either cold-pelleted or steam-pelleted. In addition, one part of the diet was diluted with 200 g/kg of gelatinised wheat starch prior to division into three batches and steam-pelleting. Batch size was 100 kg and a sample was collected after temperature had become stable. The gelatinised wheat starch was produced by long-term conditioning, and was measured to have an extent of gelatinisation of 796 g/kg.
Cold-pelleting was achieved by by-passing the conditioner and pressing mixed ingredient mash through the pellet press die (Pellet press, Munch Wuppertal - Germany, 5t/h, 2x17.5 kW, RMP 350.100) at a production rate of 700 kg/h. Steam-pelleting was performed by heating the material with steam at 75˚C for approximately 20 to 30 seconds in a pellet press conditioner prior to pelleting. Dimensions of the pellet die were 5 mm diameter and 60 mm thickness. The large pellet diameter was chosen to assure a low pellet durability of the diets without starch. In order to achieve similar moisture content among processing methods 30 to 40 g/kg of water was added prior to cold-pelleting using a nozzle Teejet 4003.
A sample was collected at the exit of the pellet press in an insulated box fitted with a thermometer (Electronic Thermometer, TESTO 110, GM 295 14 770, Germany) to measure post-pelleting temperature.
After processing, all pelleted diets were cooled in a Miltenz counter-flow cooler (Miltenz, Auckland, New Zealand, 2000 kg/h capacity) for ten minutes. Moisture content after cooling was 130 g/kg, with maximum 10 g/kg variation between diets.
Fines percentage in pelleted diets were measured by sieving approximately 100 g of carefully homogenized pelleted feed through a 4.0 mm sieve while vortexing (amplitude 1.5 mm) on a Retsch AS 200 sifter (F. Kurt Retsch GmbH & Co., Haan, Germany) for one minute. Durability of the pellets was measured using a Holmen pellet tester (Holmen Chemical Ltd., Borregaard Group, Norsolk, UK) and a Ligno pellet tester (Borregaard

Lignotech, LT 110). For the Holmen test, samples consisting of 100 g of unbroken pellets, collected after 30 seconds sieving through 2.5 mm sieve, was pneumatically conveyed around in a closed cylinder loop for one minute. For the Ligno test, pellets as described above was agitated for one minute in a closed chamber against a perforated surface by the use of pressurized air. Pellet durability index was recorded as the proportion of unbroken pellets remaining on the sieve after sieving as described for quantifying fines.
Energy consumption (kW) during pelleting was calculated as described by Payne [5]:
Energy Consumption (kW) = (IxUx1.73xPf)/1000; where I is the average pellet press motor amperage, U is the voltage, Pf is the power factor (ratio between the actual load power and the apparent load power drawn by an electrical load).
Feed samples (ground to pass through a 0.5 mm sieve) were assessed for gelatinisation by Differential scanning calorimetry. One part sample was mixed with two parts of water. After 30 minutes, approximately 130 mg sample was heated from 0 to 160°C with an increase of 5°C per minute on a Mettler DSC 30 S (Mettler Toledo AG, Schwerzenbach, Switzerland). Silicon oil was used as reference. Determination of enthalpy values was carried out by computer integration of inverse peaks. Degree of starch gelatinisation (g/kg) was calculated based on the difference in enthalpy between sample before and after feed processing. The measurements were conducted in duplicate.
All statistical analyses were conducted using the general linear models procedure of the SAS [6]. Two-way analysis of variance with the class variables process and starch was performed in. Significant differences between treatments were determined by using the Ryan-Einot-Gabriel-Welsh F-test. The pairwise comparisons included the diet containing gelatinised starch, and were based on a one-way analysis of variance. The level of significance was 0.05.

RESULTS

As expected, fat and protein content decreased when diets were diluted with starch (Table 1). Extent of starch gelatinisation (Table 1) was doubled when steam-pelleting was used as a processing method in comparison to cold-pelleting.
Durability of the pellets was lower ($p<0.05$) for the starch containing diets compared to non-starch diets, but the magnitude of the difference was particularly large for cold-pelleted diets, which explains the significant interaction effect (Table 2). Although a clear improvement ($p<0.05$) in pellet quality was observed when native starch was replaced with gelatinised starch, pellet durability was significantly lower for diets containing gelatinised starch compared to non-starch diets. Pellet durability was higher ($p<0.05$) and amount of fines was lower for steam-pelleted compared to cold-pelleted diets. As expected, post-pelleting temperature increased ($p<0.05$) as a consequence of steam-pelleting instead of cold-pelleting.

Table 1. Chemical properties of the diets

	Experimental diets[a]				
	Non-Starch (CP)	Starch (CP)	Non-starch (SP)	Starch (SP)	Gel-Starch (SP)
Starch, g/kg	91	263	77	231	245
Protein, g/kg	576	435	614	445	456
Fat, g/kg	168	159	169	152	156
Dry matter, g/kg	907	907	881	882	889
Ash, g/kg	68	57	65	55	54
Starch gelatinisation[b], g/kg	-	53	-	102	892

[a] CP and SP denote cold-pelleting and steam-pelleting, respectively
[b] Denotes extent of starch gelatinisation in processed diets

Table 2. Processing and physical properties of the diets

	Experimental diets[a]						***P - values***		
	Non-Starch (CP)	Starch (CP)	Non-starch (SP)	Starch (SP)	Gel Starch (SP)	SEM	process	starch	proc*starch
Energy consumption (kW)[b]	8.6	8.6	8.1	8.0	7.7	-	-	-	-
Post-pelleting temp. (ºC)	49.5^{C}	50.4^{C}	78.5^{A}	79.8^{A}	72.4^{B}	0.69	0.000	0.146	0.798
Fines, g/kg	416^{B}	530^{A}	168^{C}	217^{C}	168^{C}	2.08	0.000	0.004	0.157
Pellet durability index H[c], %	5.9^{D}	0.5^{E}	46.8^{A}	21.5^{C}	39.3^{B}	1.26	0.000	0.000	0.000
Pellet durability index L[d], %	4.5^{D}	0.8^{E}	56.8^{A}	33.1^{C}	48.6^{B}	1.32	0.000	0.000	0.000

[a] CP and SP denote cold-pelleting and steam-pelleting, respectively
[b] Data not included in statistical analysis due to inconsistent variation in values
[c] Measured with Holmen pellet tester
[d] Measured with Ligno pellet tester
[ABCDE] Means without common superscript within a row are significantly ($P<0.05$) different in a Ryan-Einot-Gabriel-Welsh pair-wise F-test

DISCUSSION

Although starch has been considered to be an adhesive agent when processed [7], the current results show that addition of native starch does not improve pellet durability even for diet with very poor durability. Addition of pre-gelatinised starch did result in increase of pellet durability within starch containing diets, which is in agreement with Wood [12], but was still inferior to non-starch diets. The positive effect of pre-gelatinised starch indicates that starch has a potential to act as a binder if gelatinised [10, 12]. It is reasonable to assume that the binding properties observed for non-starch diets is caused by the proteins in the diet, as Briggs et al. [1] found a positive correlation between pellet durability and increased protein content in the diets. This is further supported by Winowiski [11] where a dramatic increase in pellet durability was observed by increasing overall protein content by addition of wheat.

A considerable increase in pellet durability for steam-pelleted diets compared to cold-pelleted diets is consistent with earlier research [8] where a positive effect of steam conditioning on physical pellet quality was observed. The starch gelatinisation data confirm previous observations that the limited water content and moderate temperatures during the pelleting process does not give any extensive starch gelatinisation [9].

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MANUFACTURERS OF ANIMAL FEED IN SERBIA: PAST, PRESENT, FUTURE

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ABSTRACT

The aim of this study is to demonstrate the position of animal feed manufacturers in agriculture and industry in general, after more than half the century in existence. Currently, as one of the members of the International (from year 2004) and European Association of Animal Feed Manufacturers (from 2009, with observer status), the Serbian Association of Animal Feed Manufacturers operates according to numerous directives of EU in this field. The main task of the Association is to promote local products in a market economy, with a focus on the quality of ingredients grown on controlled, local soil. Establishing norms for high quality feed requires also a thorough feed analysis. By grouping analytical-methodological processes, based on reference laboratories, the readiness of animal feed manufacturers for participating in development programmes increased, which is significant for overall economic development in Serbia. Animal feed manufacturers have a task to identify a mutual interest and establish a vision for the exposure of closely related and geographically-adjacent companies, organizations and institutions into the region. Mutual support of animal feed manufacturers and encouragement of creativity can systematically influence the advancement of processes in the food chain, for which is essential the development of livestock industry and the advancement of processes in the food industry through placement and innovation regarding products of animal origin. By analyzing the prospects of various sectors of agriculture and food industries, it can be concluded that animal breeding as well as the production and processing of meat are the "subsectors" with the highest potential in Serbian agriculture. It is essential to send a message also from this conference that satisfactory results in livestock and agriculture depend largely on safe and quality animal feed.

Key words: *animal feed manufacturers, advancement, competitiveness, safety and quality of animal feed*

INTRODUCTION

All attempts to monitor the number of animal feed manufacturers in Serbia indicated a wide-spread production with many small, often unregistered manufacturers of animal feed. In addition, outdated and incompatible statistical nomenclature in the area of animal feed poses a question of whether the statistical reports regarding the total capacities in Serbia are correct.

PAST

The collapse of the former state and especially the sudden shift and opening of our country towards Europe and the rest of the world, required the local manufacturers of animal feed to adapt to new market conditions as soon as possible. It was expected that the standards imposed by developed countries will be fulfilled in order to allow uninterrupted functioning of the entire agricultural marketplace. This occurred as a result of a „transitional shock" originated mainly through the advent of competition at the beginning of this century. The choice was clear: either enter a marketplace or stay faithful to the basic, traditional, manufacturing practice.
The period from 1991 to 2004 was characterized, with some insignificant oscillations, by the decrease in local animal feed production. According to official statistical results, the negative trend in the production of animal feed was followed by a decrease in the number of animals, which negatively affected the results in Serbian agriculture. This period also featured a large number of animal feed manufacturers, which is an indicator of the involvement of agrare sector which requires special attention. The production of animal feed was monitored regarding increased productivity and animal breeding, and later on as because of the conditions for gaining access to the free market. However, what is far more important is the influence of animal feed on the health of animals (which had been neglected in last few decades) and its indirect effect on human health and environment. For example, animal feed contained medications and herbal substances either for faster fattening or for better feed utilization. But, the effect of their usage was that animal health was masked, while not enough attention was paid to the properties of those preparations. Also, not enough consideration was given to the damaging effect of molded raw materials in manufacturing of animal feed, which presented problem in animal nutrition. The bigger problem represented residues which are a health risk to humans (presence of mycotoxin residues in meat, milk and products of animal origin). Official reports regarding the impact of animal nutrition and disarray in the feed industry on decrease in animal production, did not exist. Deepening of problems and additional barriers in livestock industry development continued at the beginning of the new millennium, when new pallets of animal feed, produced by foreign manufacturers, appeared on the domestic market. That transitional period in our society forced our local animal feed manufacturers to create conditions for growth and development of production. However, outdated and inaccurate regulations presented obstacles that could not be avoided and were limiting the development of new manufacturing technologies.

Decision for joint appearance

Association of Animal Feed Manufacturers was constituted in 2004 at the Serbian Chamber of Commerce (SCC), with the aim of participating in all required legislative procedures and actions, which will contribute to the survival of the production process, increase the competitiveness in the free market and provide high quality animal feed products. Importance is given to initiation of the development of livestock production. By following new world trends, by way of examining traceability of nutrition of live animals and processing industries obtaining of quality and safe products of animal origin, a higher level of self-control of these subjects was established, with the aim of creating positive influence on foreign trade in the areas of livestock, agricultural, food

and manufacturing industries. Association of Animal Feed Manufacturers of SCC is a member of International Feed Industries Federation (IFIF) from 2004. It is well known that by signing the Agreement on Stabilization and Accession to the EU, Serbia has accepted certain responsibilities which, among others, include aligning national legislation with the legislation of EU. This has enabled the Association of Animal Feed Manufacturers of SCC to initiate negotiations regarding future membership of European Feed Manufacturers' Federation (FEFAC). On 1 July 2009, in recognition of values, possibilities and knowledge of local manufacturers, the Association of Animal Feed Manufacturers of SCC became a member of FEFAC (with a status of observer), until the final accession of Serbia to EU.

PRESENT

This section, which covers the period from the founding the Association until present, has a goal of emphasizing the values of local animal feed manufacturers, by displaying quantitative parameters of this industry.

Business activities of animal feed manufacturers in Serbian economic system still face numerous limitations. Tax burdens are extremely high, which has a direct influence on the competitiveness of the local product in the marketplace. Although animal feed manufacturers (regulation from year 2005, application in January 2009) are faced with mandatory accreditation (HACCP, ISO), there are no clearly defined conditions for objects in manufacturing, while the area of manufacturing of additives for animal feed is entirely undefined. Numerous registered feed manufacturers (officially 369 of them in November 2008) and unequal coverage of the market, emphasises unfair competition in this area. It is true that we have fertile soil and plant resources, but we still have not produced a high-protein feedstuff of our own plant origin. A unique standard, and a ”brand” product for local animal feed, do not exist. Those are all indicators of neglecting the value of animal feed manufacturers in the economic stability of Serbia. On the other hand, it is expected is that feed manufacturers will get accreditation for Russian marketplace and a possible accreditation for muslim countries.

Based on official statistical data (Table 1), production of animal feed in 2008 has increased by 37.5% from the previous year. The total production of animal feed in 2008 amounted to 823.451 tons of which 759.116 tons comprised of compound feed, which was an overall increase of 37% compared to 2007. The total production of supplemental compound feed in 2008 was 49.659 tons, which is a 43% increase from the previous year, while the total amount of vitamin-mineral mixtures, premixes, amounted to 14.387 tons, which was an increase of 45% compared to 2007.

Table 1. Production of animal feed (in tons) [1]

	2004	2005	2006	2007	2008	First half of 2009
Premixes	8.601	8.837	9.231	9.900	14.385	7.162
Feed for pigs	186.473	200.905	239.525	235.348	287.427	122.744
Feed for cattle	108.876	105.332	131.794	139.723	157.439	88.292
Feed for poultry	178.296	205.689	222.849	197.962	345.473	187.926
Feed for other animals	9.281	9.323	14.508	14.499	17.609	11.397
Other animal feed	2.234	2.150	2.491	1.365	1.118	0.311
Total	493.761	532.236	620.398	598.797	823.451	407.987

[1]Source: Serbian statistical department

International obligations and official agreements of Serbia with other countries have a large influence on the position and business of animal feed manufacturers. They are directly influenced by the membership of countries of south-eastern Europe based on CEFTA Agreement from 2006, decision of Serbia for unilateral implementation of the Agreement on the Stabilization and Accession to the EU, obligations in the process of accession of Serbia to the World Trade Organization (WTO) and Free Trade Agreement with Russia and Belarus. Serbia had a deficit in the foreign trade of animal feed products, with the exception of 2007. The surplus in the trade of animal feed products was not a result of increased production, especially with decreased demand in the marketplace (poor status of the livestock production), but occurred primarily as a result of increased demand in the global marketplace. In 2008 slight increase continued in the foreign trade of these products.

As quality parameters of the work performed by the Association of Animal Feed Manufacturers of SCC could be considered numerous constructive suggestions, which have contributed to improved results of its members. However, it is certain that there are segments where significant improvements still can be made.

FUTURE

In order to stress the importance of this segment of local production, and in line with the needs of Serbian agriculture (July, 2007), it is essential to change the attitude of relevant institutions, as well as to start considering animal feed industry as essential link in the food chain. It is also necessary to define the base for national legislature, i.e. the first all-encompassing legislative act in the field of animal feed, as a pre-condition for the advancement of its production, survival and creation of conditions for competitiveness in the open market. In this regard, it is vital to consider the recommendations of animal feed manufacturers themselves, as well as their expectations.

Recommendations of local feed manufactures

With regard to the legislature, in a process which officially has lasted from 2004, intensive work was performed to generate and then support the adoption of laws in the field of animal feed. The Association of Animal Feed Manufacturers has also adopted various guidelines for future law acts that are expected to be put in place after the adoption of legislation. One of the recommendations of WTO and EU is to secure financial resources for official control of animal feed in order to allow work activities of inspection services and reference laboratories in this field, as well as to lower the financial expenditure of animal feed manufacturers, thus indirectly influencing the price of the final product. It is assumed that the national inspection services will have more "sense" in future with regard to the methods of sampling and the number of samples required for animal feed control.
The decision regarding the foundation of the Laboratory Information Management System, at the Association level, that specializes in the advancement of laboratory services in animal feed analysis, was the basis of the initiative for establishing the Panel of laboratories. Introduced as a future „quality club" and with the aim of protecting the feed manufacturers, a recognizable and active entity would be created, and it would play a key role in development of agriculture, as well as in the advancement of quality and competitiveness of local animal feed products. Consistent control and acquiring of high-quality animal feed through professional, constructive approach and effective business practices would accelerate adaptation to conditions and free flow of agricultural and industrial products in general.
An initiative for change and alignment of statistical nomenclature with that of the customs tariffe was proposed to the Serbian statistical department (April 2006). Today we can only confirm slow and low effects of the administration.
Numerous times the obstacles in conducting of business were brought to the attention of relevant financial and agricultural ministries and queries forwarded (requests for interpretation of disputed provisions of customs regulations, demands for reduction of VAT, recommendations related to facilitating business), but mostly without response.

Expectations of local feed manufacturers

Under the conditions of marketplace orientation, it important to legislatively regulate the field of animal feed production, and also the issuing of standards and alignment with EU regulations. The goal is to promote the local product – animal feed, which directly influences the quality of products of animal origin.
With regard to the advancement of production, it is important to monitor technological innovations in animal feed and possibilities for their application, and to regulate the status of local manufacturers. Therefore, it is expected from the national administration to implement incentives for export of animal feed products. Ensuring quality and safe feed for animals, in addition to the fulfillment of consumer expectations pertaining to high standards and health safety of food of animal origin, is the basis for the protection of health of animals and public health
It is expected that all necessary procedures will be implemented, which will contribute to the reduction of unfair competition and the «gray area» with regard to animal feed

manufacturers, which would increase the stability of the country. This is also significant for ensuring safe management of reproductive channels in which animal feed industry is important for life.
Increase in the competition and creation of „Serbian Feed Products“ brand, which is a national animal feed product, may have an impact on significant import substitution and improving of trade balance, that would enable active participation in creating of agricultural policies in the Republic.

….. FROM A THIRD ANGLE

Constant improvement and advancement of the system for animal feed production is essential. Manufacturers of animal feed have another challenge: the shift in management's awareness with regard to the importance of collective participation in the foreign marketplace and mutual respect regarding the exposure on the local markets. Implementation of individual, positive experiences in the country and abroad can make manufacturers of animal feed even more competitive, which would result in more successful results, but it is also a requirement for their survival on the local and foreign marketplace.

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FEED ADDITIVES-POTENT IMMUNOMODULATORS?

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ABSTRACT

The animal health is prerequisite for the quality and safety of foods for human consumption. Therefore we use feed additives and research active components for promoting health in animals (especially reinforcing the immune system). Ninty two commercial crossbred piglets weaned at 28th day and attended until 63rd day of life were divided into four groups of twenty three piglets. The first, control group had no supplementation in feed. The second and the third experimental group were added 0.2% Bio-Mos® and 0.2% Progut® preparation in the feed mixture, during the whole experimental period. Piglets from the fourth experimental group was treated with 10 ml orally given copolymer of polyoxyethylene and polyoxypropylene (POE-POP, USA patent No. 5.234.683/1993) on the weaning day (0 day). Body weight was controlled at the beginning of the trial, 21st and 35th day after weaning day and blood samples were taken from the animals in order to determine haematological values. At the end of the trial, number of leucocytes was significantly ($P<0.05$) higher in Bio-Mos® group in relation to the control one. The highest share of lymphocytes had piglets with addition POE-POP, but not significantly. Copolymer acted like strong growth promotor and all feed additives protected from the conditional diseases.

Key words: *pig, feed additives, Mannanoligosaccharides, copolymers, heamatological parameters*

INTRODUCTION

Last twenty years concepts in animal nutrition have been changing. Scientist all areas looking for the optimal nutrition which must be efficient in production, but also to promote health and protect against diseases. The animal health is prerequisite for the quality and safety of foods for human consumption. Therefore we use feed additives and research active components for promoting health in animals (especially reinforcing the immune system). What we can use as a feed additives? Feed additives are products used in animal nutrition for purposes of improving the quality of feed or to improve the animals' performance and health, e.g. providing enhanced digestibility of the feed materials. It is of the grate importance, especially for the weaning piglets. Weaning is the grate challange in the modern swine production. That physiological event takes place early in the piglets life (from 21st to 28th day of life), and could be the trigger for diarrhea

inccurence which slow down growing and could cause the death of the animals. Last decades scientist are looking for alternative feed additives which can block patogen bacteria whithout antibiotic usage. The aim of this paper is to confirm how different feed additives influence on growth and haematological indicators, especially with regard on immunohaematological status of weaned piglets. Therefore we use three experimental groups: mannanoligosacharides from the products Bio-Mos®, and Progut® and copolymers polioxyethilene (POE) and polyoxipropylene (POP).
Mannanoligosaccharides (Bio-Mos®), derived from mannans on yeast cell surfaces, act as high-affinity ligands and as a competitive binding site for the bacteria with mannose-specific fimbriae [4]. Is well known that oligosaccarides improved growth and feed efficiency of weaned pigs [1] associated with bacterial adhesions [4]and stimulated immunity in fish [12] and turkeys [2]. Additional methods processing long polysacharides chains, enchanes their activities. Hydrolyzed whole yeast (Progut®) is a mixture of mannans, β-glucans, nucleotides and peptides. It's hydrolyzed from inactivated yeast but unlike cell wall products it also contains the extract part of the yeast. Synthetic compounds termed nonionic block copolymers polioxyethilene (POE) and polyoxipropylene (POP) used like adjuvant in parenteral immunization. They act by adherence to lipids, promoting the retention of the protein antigen in local tissue and facilitating the uptake of the antigen by antigen presenting cells (Hunter et al. 1994). Now, we wanted to see how it works alone, without vaccine.

MATERIALS 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.
Ninty two commercial crossbred piglets (Swedish LandraceX Large White X Pietrein), progeny of eleven litters and three boars, weaned at 28th day and attended until 63rd day of life were used in the trial. Piglets were divided into four groups of twenty three piglets. Piglets were housed in isolation pens at 21 $\pm 2^0$ C and received a commercial weaner diet without antibiotics and had an unlimited acces to water. Piglets from all groups were fed on fodder mixture for weaned piglets containing 22% crude protein and 13.84 MJ ME/kg until 21st day after weaning (the first nutrition period, 1st NP; from 0 to 21st day) and on fodder mixture for growing pigs with 19% crude protein and 13.74 MJ ME/kg until they were 63 days old (the second nutrition period, 2nd NP; from 22nd to 35th day). The second and the third experimental group were added 0.2% Bio-Mos® and 0.2% Progut® preparation in the feed mixture, during the whole experimental period.
The fourth experimental group was treated with 10 ml orally given copolymer of polyoxyethylene and polyoxypropylene (POE-POP, USA patent No. 5.234.683/1993) on the weaning day (0 day). Body weight was controlled at the beginning of the trial, 21st and 35th day after weaning day. In the same time blood samples were taken from the animals in order to determine haematological values. Five millilitre samples were obtained from the *v. Cavae cranialis* using a Venoject(R) vacutainer into a test tube containing an anticoagulant (EDTA). The number of erythrocytes, leukocytes, levels of

haemoglobin and haematocrit were 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 leukocytes is given in percentages in relation to their total number. The data were analyzed using the general linear model procedure of the Statistica [14].

RESULTS AND DISCUSSION

Tables 1, 2, and 3 presented complete blood count, excluding number of leucocytes and differetial white blood, because we use them like immunohaematological parameters. Piglets of all groups on the beginning of the trial had similar number of erytrocytes and erythrocyte constants (MCV, MCH, MCHC, RDW), haemoglobine and haematocrit (Table 1).

Table 1. Haematological indicators of weaned piglets all groups at the begining of the trial (day 0)

	Control $\bar{X}$ **±sd**	**BioMos®** $\bar{X}$ **±sd**	**Progut®** $\bar{X}$ **±sd**	**POE-POP** $\bar{X}$ **±sd**
Erythrocytes $x10^{12}L^{-1}$	5,33±1,26	4,99±0,34	5,33±0,58	5,17±0,43
Haemoglobin, gL^{-1}	94,71±31,44	87,29±5,91	94,86±12,08	88±5,83
Haematocrit, %	30,29±11,38	26,71±2,29	29,29±5,47	32,14±9,49
MCV fl	55,57[a]±6,7	53,71±1,5	54,43±4,72	49,43[b]±14,3
MCH pg	17,29±1,7	17,29±0,49	17,57±0,98	17,14±0,69
MCHC gL^{-1}	316,43±16,5	324,43±8,18	323,29±20,54	312,14±10,73
RDW	25,14[a]±1,86	24,86[a]±3,39	24,57±3,05	27,57[b]±1,27

[a,b]=P<0.05;

There were no differences in the same parameters between the experimental groups on the 21st day of the trial (Table 2). But, at the end of the trial, significantly (P<0.05) higher number of erytrocytes had piglets with Bio-Mos® supplementation in relation to the control group (Table 3).

Table 2. Haematological indicators of the weaned piglets with addition different feed additives 21st day of the trial

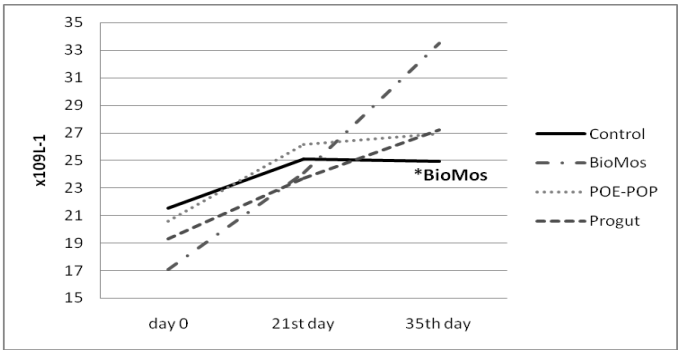
	Control $\bar{X}$ **±sd**	**BioMos®** $\bar{X}$ **±sd**	**Progut®** $\bar{X}$ **±sd**	**POE-POP** $\bar{X}$ **±sd**
Erythroc $x10^{12}L^{-1}$	5,15±0,57	4,96±0,21	4,89±0,8	5,56±0,65
Haemoglobin, gL^{-1}	95,17±13,14	89±5,32	88,71±10,86	98,43±11,18
Haematocrit, %	28,67±4,97	26,29±1,8	25,86±4,18	29,86±3,89
MCV fl	55±4,1	52,86±2,85	53,14±2,67	53,29±1,25
MCH pg	18,5±0,55	18±0,58	18,29±0,76	17,71±0,95
MCHC gL^{-1}	335,33±18,7	339±14,8	344,71±17,93	331±10,86
RDW	25,67±2,73	25,29±0,95	23,71±1,8	25±1,29

Table 3. Haematological indicators of the weaned piglets with addition different feed additives 35th day of the trial

	Control $\bar{X}$ **±sd**	**BioMos®** $\bar{X}$ **±sd**	**Progut®** $\bar{X}$ **±sd**	**POE-POP** $\bar{X}$ **±sd**
Erythrocy $x10^{12}L^{-1}$	$5,32^{a}$±0,94	$6,17^{b}$±0,73	5,76±0,55	6,01±0,45
Haemoglobin, gL^{-1}	99,57±14,27	113,43±16,07	105,57±9,05	108,43±6,27
Haematocrit, %	29,71±5,06	33,71±4,75	32,43±2,99	33,14±2,19
MCV fl	56±3,32	54,86±2,48	56,71±2,29	55,43±1,4
MCH pg	18,86±1,21	18,43±1,72	18,14±0,9	18±0,58
MCHC gL^{-1}	336,29±13,05	336,29±23,02	323,57±6,73	324,43±5,44
RDW	25,86±2,61	$24,14^{a}$±2,04	$23,14^{b}$±1,57	24,43±2,23

^{a,b}=P<0.05;

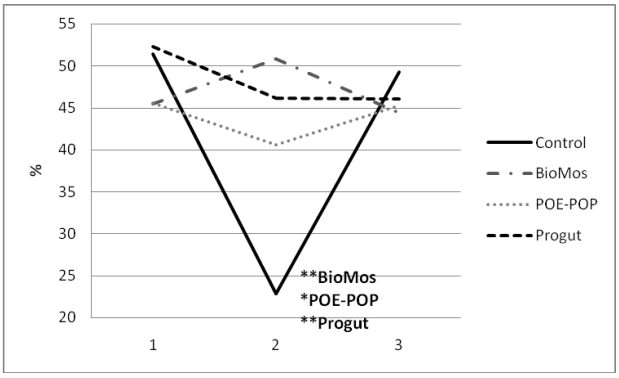
Haematological test revealed that total white blood cells did not differ between groups 21st day of the trial, although in the group with POE-POP supplementation was the highest (Graf 1). At the end of the trial, number of leucocytes was significantly (P<0.05) higher in Bio-Mos® group in relation to the control one.



*P<0.05

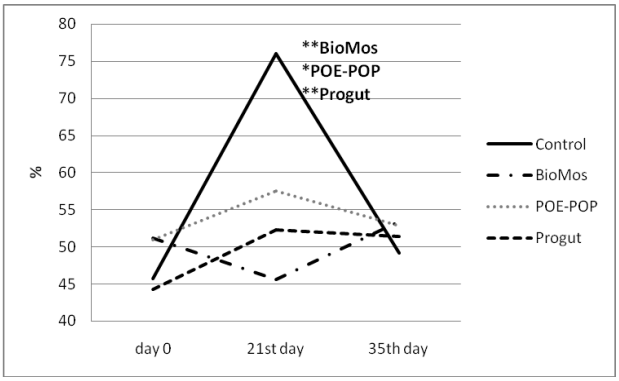
Graf 1. Number of white blood cells ($x10^9L^{-1}$) in the blood of weaned piglets with addition different feed additives

Share of the neutrophyls was significantly (P<0.05) lower in the control group 21st day of the trial in relation to the experimental groups (Graf 2), while at the end of the trial piglets all groups had about 45% share of neutrophyls which can we consider referent according to [3]. However, share of lymphocytes was higher in the control group of piglets in relation with POE-POP (P<0.05) and Bio-Mos® and Progut® (P<0.01), but only 21st day of the trial. At the end of the experiment the higher share of lymphocytes had piglets with addition POE-POP. This is in agreement with [16] who found copolymers' stimulatory effect on T and B lymphocytes, and natural killer cells in the lymphatic tissue of the gastrointestinal tract. But lymphocytes from the group with mannans were higher than in the control group, which also suggest that they had immunostimulatory effect, which confirmed [15] with proof of higher CD4 and CD8 lymphocyte subsets in the peripheral blood of the weaned piglets and [2] who found MOS elevate IgG and IgM levels in turkey. Neutrophils are important component of innate immunity involved in antibacterial defense. An increase in the number of neutrophils indicates either an infection or an influence of stress, exercise or glucocorticoid hormones [11] or may be indicator of the stressfulness of the handling experience [13] and [11]. Until the experimental groups showed normal leucogram, in the piglets' blood from the control group on the 21st day of the trial, neutrophile:lymphocyte reversal occurs, resulting in the predominant decrasing of neutrophils, that is typical for viral infections [3]. Piglets from the control group had diarrhea (data not shown) during the experimental period and needed individualy medication. It is important to know that commercial farm are positive of porcine reproductive and respiratory syndrome (PRRS) and rotaviruses.



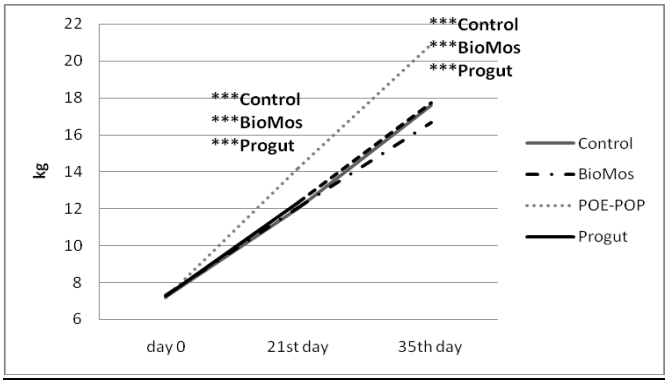
*P<0.05; **P<0.01

Graf 2. Share of neutrophils (%) in the blood of weaned piglets with addition different feed additives



*P<0.05; **P<0.01

Graf 3. Share of lymphocytes (%) in the blood of weaned piglets with addition different feed additives



*P<0.05; **P<0.01; ***P<0.001

Graf 4. Mean body weight of weaned piglets with addition different feed additives during 35 days of the trial

The heaviest body mass had piglets with POE-POP supplementation during whole experimental period, while piglets from the other groups had similar body mass as a control group. Copolymers are effective growth promoters in broilers, primary because of the ability to act as adjuvant to immune response [17]. But, there is no enough data about *in vivo* acting copolymers on swine growing. It is determined that relatively high concentrations of the block copolymer were delivered to the organs, where it remained accumulated for the long period [7]. That is the reason how it works so strong on the growing during 35 days on a one time basis. Mannans did not show strong effect on the growing. Dietary inclusion of the mannanoligosaccharides is the most effective immediately after weaning [10], our data show scarcely grater body weight in relation to the control piglets. It is known for a long time that yeast cultures have mainly been associated with yeast metabolites [5], now is clear that some of the positive effects of yeast in monogastrics might be associated with the yeast cell wall. Mannans can block attachment of ceratain bacteria to the intestinal wall and that is probably the main mechanism of positive effect that preparation on the health of the piglets. Therefore it is determined improved average daily gain (P=0.06) in piglets that were fed the supplemented diets with yeast culture and modified yeast culture + cell wall product [18]. Their effect is dependent on dose, so pigs supplemented with 100 and 200 ppm of β-glucan had lower average daily gain than pigs supplemented with 50 ppm [9]. Very high doses lead to increasing syntesis of proinflammatory IL-1β which is associated with decreased feed intake and growth performance [8].

CONCLUSION

Comparing different feed additives for weaned piglets we can conclude that POE-POP is the most powerful growth promotor. Live yeast culture preapared by different production methods stimulated immune system. Bio-Mos® upraised significantly number of WBC

count and piglets with feed supplementation did not show signs of virus infection, like piglets from the control group (without any feed additives). Piglets with POE-POP in addition did not have diarrhoea during the whole experimental period.

ACKNOWLEDGEMENTS

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REQUIREMENTS ON QUALITY FEEDS AND THEIR ASSESSMENT FROM VIEW OF ACTUAL KNOWLEDGE

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ABSTRACT

Quality of feeds is important not only for livestock efficiency but also for production of animal products. Therefore it is necessary to study the properties of feeds. Biological and chemical methods are used for this purpose and tests on animals are preformed to determine nutritive value, degradability and digestibility in feeds. Quality of feeds influences animal products, economy and environment. Therefore it is necessary to extend knowledge of nutritive value of feeds and nutrient and energy requirements by animals.
Key words: *requirements on feed quality, methods of feed evaluation and efficiency of their utilization*

INTRODUCTION

Production of sufficient amount of feeds in desirable quality and structure influences to a decisive degree not only livestock efficiency but also requisite quality of animal products for human nutrition. Quality of feed is represented by content of nutrients, concentration of energy as well as by dietary properties, properties influencing feed intake (physical form, course of fermentation, specific taste substances, degree of contamination, proportion of antinutritive or toxic substances, etc.). We can come to objective assessment of quality only after taking into account whole range of parameters, which are, however, not of the same value. Therefore it is necessary to assess each characteristic property of feed separately and to determine qualitative limits with regard to type of feed on the basis of minimum requirements on the studied parameters.
High and stable yields, high content of energy and other nutrients, high digestibility and utilization of energy and other nutrients, increased degradability of potentially utilizable structural saccharides, low content of antinutritive substances [2] are necessary for production of feeds for animal nutrition.

MATERIAL AND METHODS

Biological and chemical methods as well as biological and production tests on animals are used to determine nutritive value in concentrates and roughage, degradability and digestibility of feeds; mathematic-statistical modelling is used to formulate prediction equations of nutrient demands and development of information database systems of nutritive value in feeds.

RESULTS AND DISCUSSION

However, evaluation of feeds quality must be primarily founded with objective parameters assessed by chemical analysis of feeds. Although the classical analysis, the so-called Weenden's system, is still the basis for feed analyses, it is not sufficient for objective assessment of feed quality today (fig. 1). In new applied systems of feed and nutrients need assessment for individual species and categories of farm animals (Petrikovic and Sommer) is introduced a number of new quality and nutritive value parameters, which were not studied in practice till now. It is degradability of nutrients and intestinal digestibility of their non-degradable portion in ruminants.

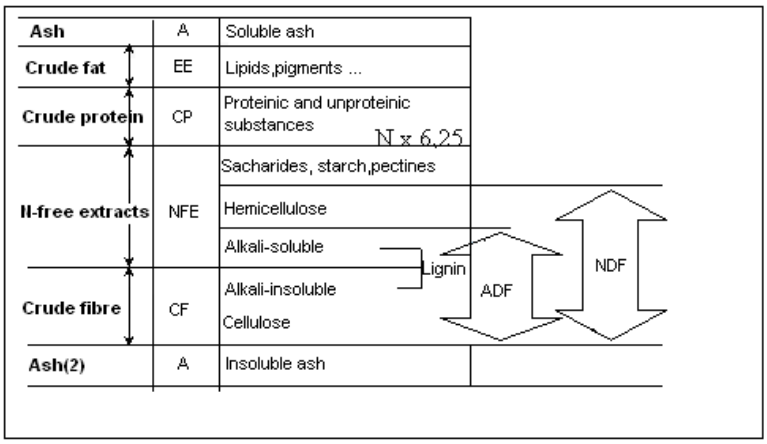


Fig. 1. Wenden's system of feed analysis

Development, introduction and tests of these systems of feed assessment are closely connected with intense increase in production intensity and conditioned by very intensive development of new experimental methods, techniques, analytical processes. More and more accent is laid on fractional composition of basic nutrients (saccharides, crude protein, fats). In the sphere of feeds evaluation a change to parameters takes place, which express better the real biological utilization of individual nutrients. All this necessitated principal revision of valid recommendations in order to correspond to present state of knowledge about processes, which are connected with change of nutrients in animal organisms, and about factors that influence it.

Gaining new knowledge in the sphere of biochemistry and physiology of nutrition is decisive for elaboration of recommendations of energy and nutrients needs for individual species, categories and production orientation of animals, with regard to physiological state, way of breeding and environment. Principal problem is to assess need of energy, because energy is necessary in all processes, which take place within metabolism in organism. Need for other nutrients (crude protein, amino acids, some fatty acids, macro- and micro elements, vitamins) are as a rule put in connection with feed (per kg SDM), animal (per day) or content of energy in feed (per 1 MJ). The matter of question in

ruminants is mainly new knowledge and possibilities to control physiological processes in the rumen, mainly:

- Decomposition of quickly fermentable saccharides, mainly starch and its flow through rumen into small intestine in an amount, which will enable maximum absorption of glucose.
- Continuous decomposition of cell wall parts fractions (cellulose, hemicellulose) and quick transport of non-utilizable particles from rumen; it can increase intake of feeds and energy. Post-ruminal utilization of cell walls by using enzymes can be also expected.
- Sufficient supply of crude protein and microbial proteins from feeds to small intestine.
- Influence of disbalance or synchronisation of digestive processes on efficiency and health in animals and on environment.
- Decrease in energy losses, mainly by decreased creation of methane in rumen.

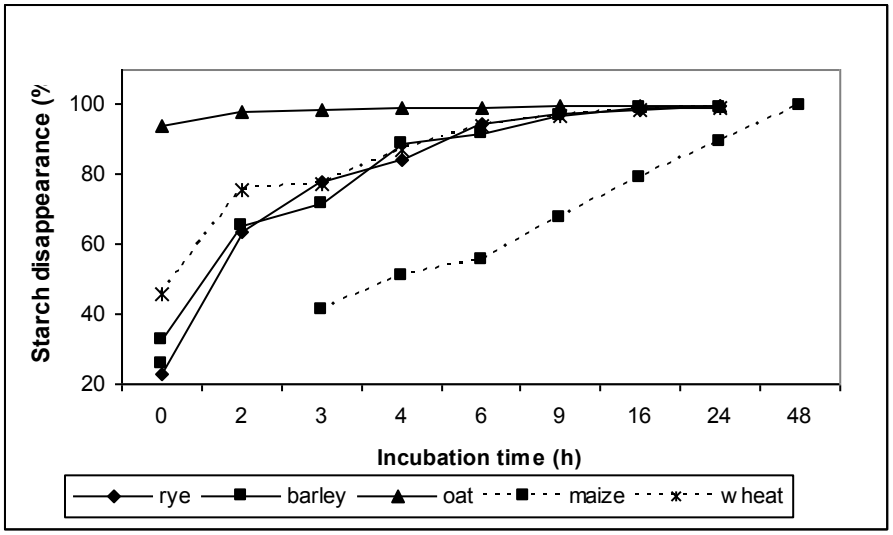
Knowledge of nutrients digestion in individual parts of digestive tract is necessary to recognize these processes. The criterion of nutritive value in feeds for ruminants is not total content of crude protein but the amount of really digested crude protein in small intestine, which depends on level of microbial proteosynthesis and crude protein degradability in proventriculi. Out of total amount of received crude protein from feeds are 70 – 80 % de-aminated to keto acids and ammonia by activity of proteases produced by bacteria and protozoa; ammonia is then at disposal for synthesis of bacterial proteins. Level of bacterial synthesis is closely connected to the change of saccharides and it is dependent on sufficient amount of disposable energy. Increased consumption of crude protein at high efficiency cannot be covered only by crude protein supplementation, which increases excess of ammonia in rumen. The problem can be solved using feeds with low degradability of crude protein in proventriculi only, increasing the flow of non-degraded crude protein from feeds directly into small intestine in this way [6].

It is possible to increase the amount of microbial proteins passing into small intestine by optimization of nitrogen and energy utilization in rumen. Synchronization of speed by which energy and nitrogen are released from feeds influences positively the synthesis of microbial proteins.

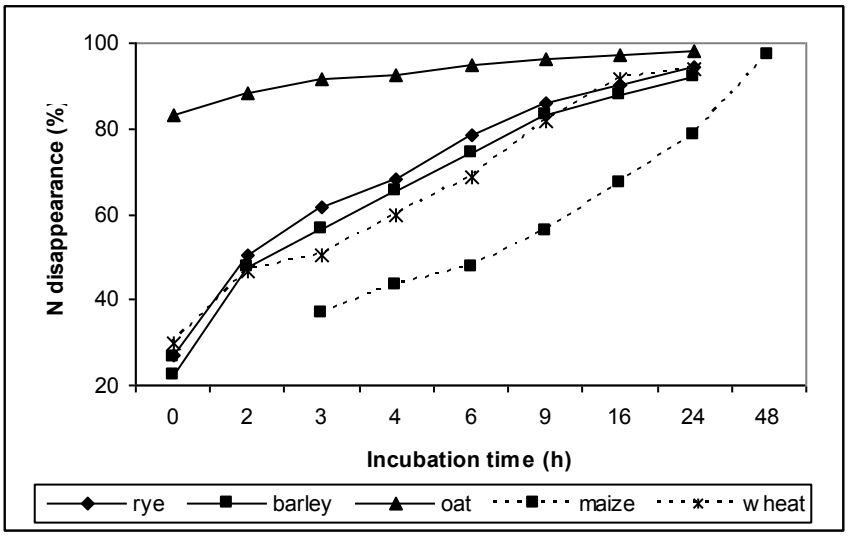
Table 1. True ileal amino acid digestibility of feeds (synthetic amino acid = 100% digestibility)

Tested feeds	Lysine	Threonine	Cystine + Methionine	Tryptophan
Wheat	84	85	90	88
Barley	78	81	85	80
Soybean meal	90	92	91	88
Fish meal	93	92	91	89
Wheat bran	72	69	84	80
Lucerne meal	51	59	50	50

Similarly in pigs and poultry is the evaluation of feeds quality on the basis of crude protein content insufficient already. The assessment of the basis of chemically determined crude protein did not take into account losses, which arise during digestion and utilization of feed in organism of animals [11]. Also for this reason it was necessary to broaden disproportionately the so-called security limits, by which the potential risks of deficiency could be eliminated, when defining standards of needs of total amino acids. These limits are mostly empirical, non-specific and can consequently cause non-effective utilization of nutrients. Expression of amino acid needs in biologically utilizable form is more accurate than data on need of total amino acids (table 1). Assessment and use of data on really digested amino acids, which have biological basis in difference to existing chemical-analytical data, enable to formulate and cover the need and to use more effectively the potential of feed at the same time.
Crude protein as well as saccharide component of feeds differs by its quality, which is connected with their chemical and physical structure and primary composition. It becomes evident by their consumption by animals, speed and range of degradation in rumen of ruminats; effectiveness of energy and crude protein utilization from feeds depends on it. Concentrates and roughage differ markedly in extent and speed of crude protein and saccharide component degradation (graph 1, 2).
Variability in speed of saccharides degradation influences the course of fermentation, causes changes in cellulolytic and amylolytic activity of rumen microflora and in the place of digestion, and in this way also utilization of nutrients by animal.



Graph 1. Saccharide component degradation in tested feeds



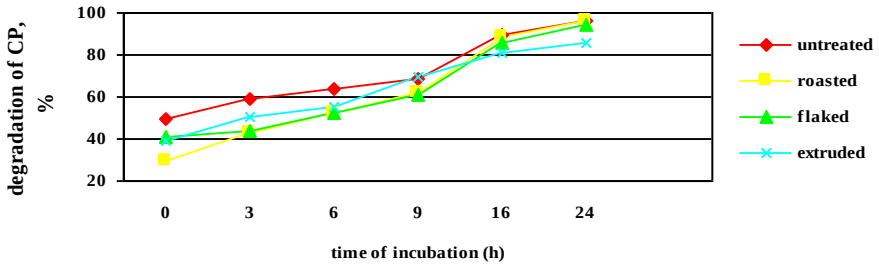
Graph 2. Crude protein component degradation in tested feeds

Precondition of optimization of feed rations and mixtures according to the mentioned parameters is determined choice and good knowledge of feed component properties. Therefore it is necessary to analyze the feeds more in detail and number of studied and evaluated parameters grows all the time, e.g. to characterize saccharide component necessitates not only to assess the content of crude fibre but also the content of individual fractions (acido- and neutral detergent) of fibre, lignin, content of starch, saccharides and proportion among these components. Great differences between individual species of grain crops, but within a species as well, influenced by variety, site and season were found in the content and proportion of amylosis and amylopectin, ratio of total and soluble non-starch polysaccharides, arabinose and xylose as well as proportion of protoplasmic proteins of albumins and globulins (table 2). This great variability results in great differences in utilization of nutrients by monogastric animals (they can cause antinutritive effect) and it influences effectiveness of animal production [5].

Table 2. Content of non-starch polysaccharides and pentosans (%)

		Rye 1	**Rye 2**	**Rye 3**	**Wheat**
NSP	total	15.8	15.5	15.5	10.7
	insoluble	10.8	11.3	10.5	8.1
Pentosans	total	8.4	8.3	9.0	5.8
	insoluble	6.0	6.3	6.0	5.2
	soluble	2.4	2.0	3.0	0.6
Xylose/arabinose		1.62	1.68	1.54	1.45

Utilization of decisive feeds in production depends on content of nutrients, which is variable and is result of genetic, agro-technological and environmental influences as well as on ways of processing. Their nutritive value is limited mainly by digestibility of proteins, degradability of crude protein, composition of amino acids, utilization of amino acids, and by presence of anti-nutritive factors, which can be inhibited by thermal treatment, mainly extrudation (graph 3). It will be necessary to pay more and more attention to utilization of these methods of treatment and improvement in feed components in connection with increase in production intensity in our country also. It is necessary to determine suitable, art specific and perhaps also variety specific, technical and technological parameters of physical treatment to obtain optimum parameters of degradability, digestibility and utilization of crude protein and energy from these feeds by animals.



Graph 3. Changes in degradation of crude protein by various thermal treatments of pea

Plant breeding and genetic techniques, which were aimed mainly at protection against pests, minimization of utilization of protective substances and tolerance of plants to unfavourable conditions, should contribute significantly to more effective utilization of feed resources. This generation of crops brought advantages mainly to their producers. A number of experiments were performed with GM crops during the recent years [8, 9, 10, 7]. We have at disposal results from experiments with GM maize [1, 3, 4]. Their growing represents important saving of energies as well as chemical preparations for plant treatment, mainly herbicides and insecticides. Reduced costs for their production are in turn reflected in relatively lower prices, and decreased need of chemical protection represents also a certain contribution for environment, not to say the targeted insecticide protection, when only certain groups of insects are affected.

Present trends in the world show that the development will advance certainly very markedly in spite of opponents and no bans will prevent the progress in utilization of technology for GMO preparation. Today we are able to grow genetically modified plants on saline or very dry soils or in places soiled during previous very intensive utilization of chemical fertilizers and pesticides. Some transgenic plant species are modified to be used at phytoremedities, i.e. at removal of pollutants as for instance heavy metals from environment.

In the future we envisage also changed content of nutrients in plants, which will influence significantly also the nutritive value of feeds. We expect mainly: increase in content and utilization (digestibility and absorption) mainly of starch and other highly

digestible saccharides, proteins (or selected amino acids), fats, and/or selected fatty acids (non-saturated fatty acids); decrease in content of anti-nutritive substances (glucosinolates, alkaloids, tannins, glycosides, saponins, phytohormones, and other), decrease in lignification of vegetative parts in plants and thereby increase in microbial degradability of utilizable substances, which is precondition of higher intake of roughage; as well as breeding of plants, which will have higher concentration of special nutrients, e.g. some enzymes (phytase and other), macro- and micro-elements, vitamins, in certain parts. Crops with such added value will create part of the common food chain.
Generally it is reported that 50 % of nitrogen and 40 % phosphorus in flowing waters originate in agriculture, emissions of ammonia in atmosphere coming to more than 90 % from animal production. It is possible to decrease markedly the excretion of nitrogen and phosphorus into the environment by purpose modified measures:

- phase feeding – by adaptation of crude protein, energy and phosphorus to requirement of animals
- decrease in crude protein content by supplementation of commercial amino acids or
- assessment of need of amino acids on the basis of ileal digestible amino acids
- preserving constant ratio between content of lysine and net energy in feed mixtures
- assessment of ratio among individual amino acids after the ratio in so called ideal proteins
- improvement of P digestibility in plant feeds by addition of plant or microbial phytases at decrease of phosphoric salts in feed mixtures; organic form of phosphorus (phytate) in plants can be used by monogastric animals only to 30 -35 % (table 3).

Table 3. Phosphorus and calcium content in feeds for pigs

Tested feeds	**P (g/kg)**		**Share Ca (g/kg)**		**Available**	
	total	phytate	(%)	total	Ca(g/kg)	P (g/kg)
Rye	3.70	2.70	73	0.40	-0.80	1.85
Wheat	3.40	1.87	55	0.50	-0.75	1.90
Barley	3.90	2.54	65	0.51	-0.82	1.85

0.65 g Ca per 1g phytate-P

New methods of granulation, extrusion, expansion, as well as other physical treatment of feeds will be a contribution to inactivation of undesirable substances in feeds, and to better utilization of some nutrients, e.g. crude protein, starch, non-starch polysaccharides, etc.

CONCLUSION

The quality of feeds for animal production and their change in the process of digestion influence production of animal products, economy as well as environment. Effective utilization of feeds is possible only by extension of knowledge of nutritive value of feed on one hand and nutrient and energy requirement by animals on the other.
It appears from this that for animal nutrition is necessary to take following requirements into consideration when growing feed crops:

- sufficient feed production of high quality with minimum inputs (e.g. lower consumption of water, nutrients, areas, etc.)
- increased resistance to pests, tolerance to drought and higher content of salts in soils
- decreased content of undesirable (antinutritive) substances in feeds, remnants of undesirable contaminants (e.g. mycotoxins) and protective substances
- increased content and utilization of nutrients from plants (amino acids, fatty acids, vitamins, enzymes) and in the end increased digestibility, thereby increased utilization of energy and other nutrients. As a result it is expected that animals will excrete lower amounts of excrements.
- positive effect on quality of animal products.

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ANIMAL HEALTH AND QUALITY OF ANIMAL PRODUCTS INFLUENCED BY NUTRITION: RESEARCH EXPERIENCES IN IAS KOSTINBROD, BULGARIA

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ABSTRACT

Nutrition is a science that examines the relationship between diet and health. We are scientists who specialize in this area of study, and are trained to provide safe, evidence-based dietary advice and interventions.
Our objective was to examine biological effect of more both Bulgarian and not Bulgarian additives and to observe data on their influence on the health of animal, level of production, reproductive capacity and quality of products from animal origin. Also, we pay attention on the sustainability of the observed results with different type and kind of animals.
Herbal additives have aroused much scientific interest over the past few years. Most of the studies have been performed on the extracts of herbs and used in the feed of different type and categories monogastrics and ruminants. Because of that many herbs show prophylactic and therapeutic effects by regulating the functioning of the internal organs of animals. Bulgarian scientists have recently discovered the potential of a plant extract mixture of seven components in dairy cow nutrition to improve the production and quality of milk and to get better its composition. Substances found in herbs have stimulating and regulating effects on metabolic processes and could reduce environmental stress. Feeding herb mixtures seems a more beneficial approach due to cumulative effects that individual herb types can have when fed in combination. The physiological effect of the Bulgarian nutritional additive *OVOCAP*® is based on the seven alkaloids known under the common name CAP (Methyl-vanillyl-nonenamide-capsaicin), carotene and other biologically active substances. While the action of carotene is well studied, the action of CAP is not fully cleared. Our experiments with lactating cows showed that CAP has observed effects on: levels of HDL cholesterol were significant lowered ($P<0.05$); levels of LDL and VLDL cholesterol as well as triglycerides and total lipids were significant lowered ($P<0.001$).
Tribulus Terrestris dry extract contains biologically active substances as steroids, saponins, flavonoids, alkaloids, unsaturated fatty acids, vitamins, tannins etc. The main active components are saponins of the furostanol type: protodioscine and protograciline. Bulgarian product has trade name of *Vemoherb-T*, produced by firm of Vemo 99 Ltd., Sofia, Bulgaria. Up to now, Bulgarian experimental works showed that *Vemoherb - T* has: significant positive effect on production (poultry); sure positive effect on reproductive capacity and spermatogenesis (rams, White Plymouth Rock mini cocks and Guinea fowls); 29 % negative effect on serum testosterone (broiler parents); significant negative effect on the blood level of Ca (poultry); significant hypoglycemic effect (poultry); sure negative effect on blood triglycerides and total cholesterol (poultry);

contradictory effect on blood serum protein (poultry); no sure effect on egg yolk lipids and fatty aids composition (laying hens).
The comparison of experimental results from feeding the additive *BIOPRO* as probiotic in sires` diets showed that: the great relative effect was obtained on the blood level of Mg; both the levels of blood total protein and P were increased; no effect was obtained on the level of Ca and the blood glucose had tendency to lower; there was positive correlation between levels of Zn both in the blood and sperm.
The shortage and continuous price enhancement of traditional nutritive substances in the animals diets calls for search of their alternative. In Bulgaria the research in this area is insufficiently. There are not investigations for rabbits. In this respect is interesting to study the dry distillers grain of wheat (DDGW), produced by Geo Milev Ltd, Iskar Station, Sofia, as a source of crude protein (CP) - 26% and crude fiber (CF) -13% as well as the meadow hay and wheat straw as main sources of crude fibers in the growing rabbits diets. Our experimental work goes on with other kind of dry distillers` grain.
Iodine, a requisite substrate for the synthesis of thyroid hormone, is known to cause pathological conditions when its intake is excessive or deficient. We examined biological effect of Iodine in biological active form from *Jodis concentrate* produced by Ukraine. Our experimental results by poultry and rabbits showed sure effects on level of production, mortality, reproductive capacity and biochemical changes of the blood parameters.
Our conclusions here are in agreement with biological sensible concept of animal health and quality of animal products influenced by nutrition.
Key words: *animal, nutrition, additives, BIO-PRO, OVOCAP, DDGW, plant extract, Tribulus terrestris, Jodis concentrate*

INTRODUCTION

Although the world`s population is expected to grow by only 25% in the next 20 years, an increase of 50% in the demand for food of animal origin is projected. The decrease in absolute and per capita land area available for agricultural use will result in less area available for producing feed and to increased competition between humans and animals for energy and nutrients. In this context it is an important task of the science of animal nutrition to discover and exploit new resources, particularly product which cannot be used directly by humans (e.g. high-fiber organic substances) or which are considered undesirable or unsuitable for human consumption (offal and other food industry by-products). Whereas the utilization of conventional feedstuffs and nontraditional byproducts in animal nutrition was once an extremely economic model for recycling, in the recent years doubts have arisen, due to the occurrence of BSE and the discovery of dioxins in the feed. In EU agricultural policy has in part responded to these issues with new regulations. But it is very doubtful it can be considered ecologically responsible, economically reasonable or in the long time politically justifiable to use byproducts considered valuable by nutritionists as fuel or to dispose of them as waste. Such a trend may be tenable for short tine in Europe, but it will not be acceptable worldwide, particularly in regions where feed supplies are scare.

RESEARCH EXPERIENCES IN IAS KOSTINBROD

HERBAL ADDITIVES

Herbal additives have aroused much scientific interest over the past few years. Most of the studies have been performed on the extracts of herbs and used in the feed of different type and categories monogastrics and ruminants. Because of that many herbs show prophylactic and therapeutic effects by regulating the functioning of the internal organs of animals. Bulgarian scientists have recently discovered the potential of a plant extract mixture of seven components in dairy cow nutrition to improve the production and quality of milk and to get better its composition. Substances found in herbs have stimulating and regulating effects on metabolic processes and could reduce environmental stress. Feeding herb mixtures seems a more beneficial approach due to cumulative effects that individual herb types can have when fed in combination.

OVOCAP

Presentation

The physiological effect of the Bulgarian nutritional additive *OVOCAP*® (Kitanov, 1998) is based on the seven alkaloids known under the common name CAP (Methyl-vanillyl-nonenamide-capsaicin), carotene and other biologically active substances. While the action of carotene is well studied, the action of CAP is not fully cleared. Our experiments showed that CAP has: bacteriostatic effect on some bacteria (poultry); no deviation from normal values of transaminasic activity of the liver, hydrolytic activity of mycosis and histostructures of the jejunum, stomach, liver and kidneys (swine); 4-5% effect on real digestibility of the amino acids in standard compound feeds (geese); effect on the laying rate (pheasants); effect on the reproductivity (hens, turkeys, ewes and cows); effect on milk fats (sheep).

The aim of this study was, taking into consideration the above, to study the action of OVOCAP® administrated per os, on production and quality of milk and its physicochemical composition of cows.

Material and Methods

Animals: A total of twelve lactating American Brown cows (BW = 695 ± 28 kg; at the beginning of the lactation) were separated into two equivalent treatments for 1,5 year, started in the autumn to the winter feeding period to prevent so different pasture conditions.

Feeding: Cows were fed typical diets during the winter (corn silage, meadow hay, straw, wheat bran, potatoes and compound feed) and summer (pasture and compound feed). Experimental cows received in addition to concentrate part of daily ration per 2x22 ml *OVOCAP*® every 28 day post partum, as first doses were on 3/4 days post partum. (Table 1)

Methods: Analytical methods of feed used – in accordance with international standards (AOAC).The statistic processing of the obtained results – Microsoft Excel.

Controlling parameters: Milk yield, (average per day), milk quality (somatic cells count, SCC and bacterial count, BC) and composition of milk in % (dry matter (DM), fats, protein, solids-non-fat (SNF), lactose); levels of cholesterol (HDL, LDL and VLDL), triglycerides and total lipids. For more details see Petkova et al. (2008a, b) and Petkova et al. (2009).

Table 1. Experimental design with lactating cows and the additive OVOCAP®

Components of the diets	Control winter diet	Control summer diet	Experimental winter diet	Experimental summer diet
Compound feed, g/kg milk	350	350	350	350
Corn silage, kg	10	-	10	-
Meadow hay, kg	4	-	4	-
Brewery pulp, kg	15	15	15	15
Wheat bran, kg	2	2	2	2
Straw, kg	4	4	4	4
Potatoes, kg	5	5	5	5
Pasture	-	Ad lib.	-	Ad lib.
OVOCAP®, ml to a respective scheme	-	-	22	22

Main results

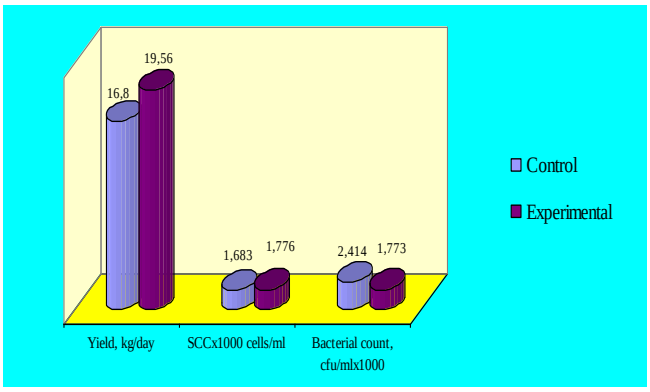

Fig.1 Yield and quality of cow milk

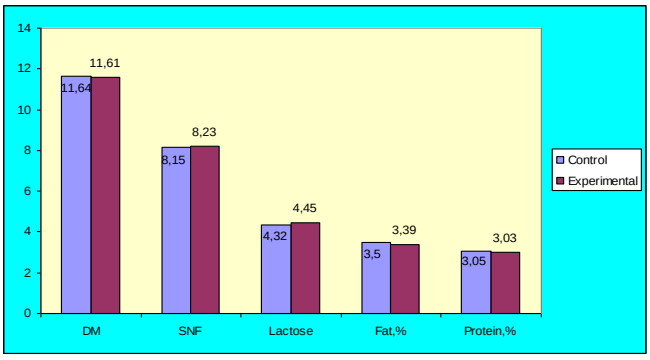

Fig. 2 Composition of cow milk in %

Table 2. Blood lipid profile in lactating cows (mmol/l) (n=20)

Items	Control	Experimental
HDL cholesterol	2,89	2,42*
LDL cholesterol	2.14	1,30***
VLDL cholesterol	0,15	0,07***
Triglycerides	0,21	0,16***
Total lipids.	5,38	3,95***

* P ≤0,05
*** P ≤0,001

Table 3. Reproductive indexes in the cows

Items	Control				Experimental			
	min	max	x*	SE	min	max	x*	SE
Open days	62	288	180.5	47.8	27	120	74.8	15.1
Rest days	26	50	37.5	6.4	27	67	40.4	7.2
Wasted days	36	241	143.0	45.2	0	86	34.4	14.7
Intercalving period, days	354	592	470.5	49.9	313	364	338.8	8.2
Pregnancy length, days	280	303	287.5	5.0	274	296	286.0	3.7
Days in the Milk (DIM)	293	531	409.5	49.9	214	331	281.4	20.2

CONCLUSIONS

Feed intake was similar in two groups.

No differences were observed for milk yield under the influence of OVOCAP®.

Overall, both milk composition and quality were influenced to better. There were tendency toward to decrease content both of milk fat (3,14%) and bacterial count (26%) and to increase content of lactose (3%).

* Observed levels of HDL cholesterol were significant lowered (P<0,05) under the influence of OVOCAP®.
* Observed levels of LDL and VLDL cholesterol as well as triglycerides and total lipids were significant lowered (P<0,001) under the influence of OVOCAP®.
* Intercalving period was reduced (by 28%)
* DIM was reduced (by 31%)
* Independans period was reduced by 105 days
* As whole, wasted days was reduced by 108 days.

This experiment demonstrated that OVOCAP® is adjusted to use in lactating cows with sure benefit on milk composition and quality, blood lipid profile and clear positive effect on reproductive indexes of females.

Tribulus terrestris dry extract

Presentation

Bulgarian scientists have recently discovered the potential of a dry plant extract from annual herb *Tribulus terrestris L* (*Zygophylaceae*) in animal nutrition as ecological additive to improve the production, reproductive functions and quality of products from animal origin, so as to get better their composition and the health in general. The folk medicine uses this herb in human as aphrodisiacs, diuretics and as a medicine decreasing blood pressure, cholesterol and glucose. Substances found in herbs have stimulating and regulating effects on metabolic processes and could reduce environmental stress. *Tribulus terrestris* extract contains biologically active substances as steroids, saponins, flavonoids, alkaloids, unsaturated fatty acids, vitamins, tannins etc. The main active components are saponins of the furostanol type: protodioscine and protograciline. Bulgarian product has trade name of *Vemoherb-T*, produced by firm of Vemo 99 Ltd., Sofia, Bulgaria. Up to now, Bulgarian experimental works showed that *Vemoherb - T* has: Significant positive effect on production (poultry); Sure positive effect on reproductive capacity and spermatogenesis (rams, White Plymouth Rock mini cocks and Guinea fowls, Nikolova et al., 2009); 29 % positive effect on serum testosterone (broiler parents, Kashamov, 2007); Significant positive effect on the blood level of Ca (poultry); Significant negative effect on the blood level of glucose (poultry); Sure negative effect on blood triglycerides and total cholesterol (poultry); Contradictory effect on blood serum protein (poultry); No sure effect on egg yolk lipids and fatty aids composition (laying hens, Grigorova, 2008). There exist no data concerning the effect of dry extract of *Tribulus terrestris*, with trade name of *Vemoherb-T* produced by firm of Vemo 99 Ltd., Sofia, Bulgaria in bulls of service. This study was conducted to asses the biological effect of *Vemoherb -T* on the blood biochemical changes and sperm both quality and quantity in bulls of service.

Material and Methods

Material: Animals: 9 bulls of service from three different breeds and different ages; Start of experiment: 06.11.2008; End of Experiment: 26.01.2009; Duration of experiment: 2 x 40 days and after-effect control (40 days); Feeding: daily rations, by meadow hay (65%) and compound feed (35 %);Additive: *Vemoherb T* (3 mg/kg body weight/daily)

dissolved in the compound feed; Compound Feed composition (in %): Barley – 12.0; Corn – 15.0; Oat – 35.0; Wheat bran – 25.4; Sunflower meal – 8.0; Chalk – 1.6; Premix – 3.0;
Controlling parameters: Feedstuffs - chemical composition; Blood from *V. jugularis* - once/day for the treatment and at end of the experiment, morning before feeding; Sperm by artificial vagina two times per week for quality parameters: volume, concentration, motility, survivability.
Methods:
Analytical: Chemical composition of feedstuffs was determinate in accordance with international standards AOAC; blood and sperm parameters were estimated in clinical laboratory Cibalab; Statistical: Values are expressed as means ± SEM. Statistical analyses was performed by *Student`s t-test.* For more details see Petkova et al. (2009).

Main results

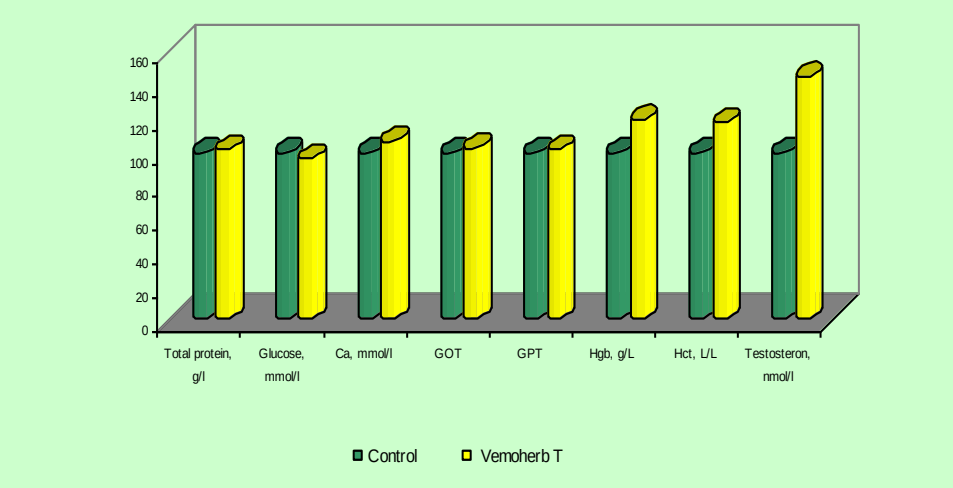

Fig.3. Relative effect of Vemoherb on biochemical changes in the blood of sires

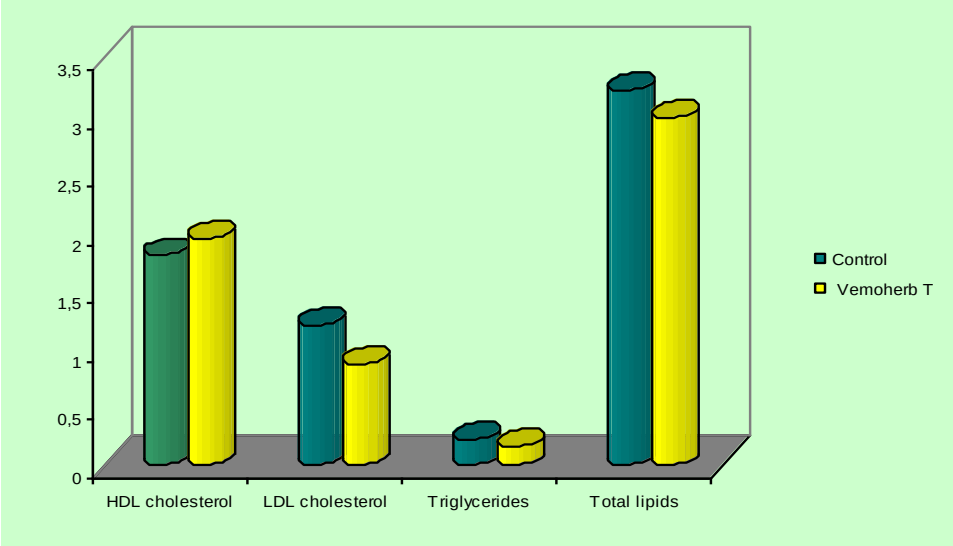

Fig. 4. Blood lipid profile in sires (mmol/l) (n=9)

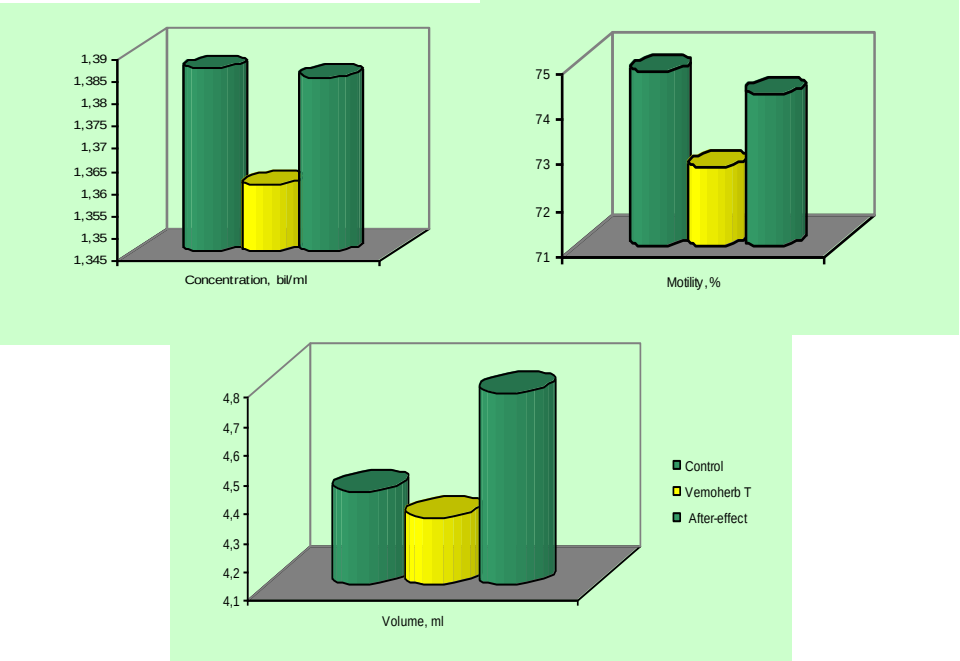

Fig. 5. Spermograms of the ejaculates from sires

CONCLUSIONS

The observed results are original on the biological effects of dry extract of *Tribulus terrestris* in amount 3mg/kg body weight with bulls in service.
The main effect is on the blood level of testosterone, which was increased by 45% under the influence of the additive.
The blood lipid profile was positive reversed:
* LDL cholesterol was decreased by 27%
* Triglycerides were decreased by 23%.
* HDL cholesterol was increased by 8%.
* Total lipids as a whole were decreased by 6.9%.
Spermograms showed after-effect of the additive: ejaculates volume were increased by 8% during the 7 weeks after the end of experiment.
This experiment demonstrated suitability of *Vemoherb T* to improve bulls` reproductive capacity and gives opportunity to use greater amount from this additive with experimental aim.
This experiment demonstrated that *Vemoherb T* is adjusted to use in bulls of service with sure benefit as a whole.

PROBIOTICS

Bio-Pro

Presentation

It is developed special Bulgarian additive on the basis of a brewers yeast dried and choline-chloride. The main arguments to compose BIOPRO were the common composition of compound feeds for monogastric and ruminants, which include corn, as main component, and barley and wheat. As is well known, corn grain has a deficit of choline and the other grains have a deficit of biotin. Having use BIOPRO with the compound feeds, animals receive rations with eliminated deficiency both of choline and biotin and/or using them at synthetic form. Except this, BIOPRO consists of all 8 vitamins from group B.
There were experiments, carried out to asses the effect of BIOPRO as probiotic with lambs, piglets and laying hens. Experimental results show that BIOPRO has equivalent to American probiotic effects. At the same time, the cost effectiveness is significant higher, because of price is four time lower. Overall, BIOPRO allows to preventing disorders and trouble with health and decrease the losses, origin of them.
Chemical analyses show that BIOPRO has the next energetic and nutritional value:

ME, kcal/kg – 1654	Ca, % - 0,26
Moisture, % - 10,70	P, % - 1,21
Crude Protein, % - 27,20	Lysine, % - 1,89
Crude Fibre, % - 7,50	Methyonine, % - 0,48
Crude Fats, % - 2,46	Meth + Cystine, % - 0,86

This study was conducted to asses the effect of Bulgarian nutritional additive BIOPRO on the biochemical changes of ingredients of the blood of sires.

Material and Methods

The study was carried out in two different Stations for Artificial Insemination (SAI 1 and SAI 2) during the 3 year period. 108 samples of blood from *V. jugularis* were analyzed for content of total protein, blood glucose, Ca, P and Mg. Levels of Zn both in the blood and the sperm were estimated at 32 and 24 samples respectively.

Feeding: The experimental protocol shows that during the years there were periods with or without additive (table 1); to daily rations, composed by meadow hay (65%) and compound feed (35 %). During the experimental periods sires received in addition to concentrate part of daily ration per 0,5 % BIOPRO.

Main results

Table 4. Biochemical changes in the blood of sires (x)*

Items	SAI 1		SAI 2	
	- Biopro	+ Biopro	- Biopro	+ Biopro
Total protein, g/l	71.06	76,8	69,0	78,63
Relatively effect	100,0	108,08	100,0	113,96
Glucose, mmol/l	3,03	2,90	2,90	2,60
Relatively effect	100,0	95,71	100,0	89,65
Ca, mmol/l	2.21	2,31	2,35	2,36
Relatively effect	100,0	104,5	100,0	100,42
P, mmol/l	1,75	1,88	1,72	1.95
Relatively effect	100.0	107,43	100,0	113,37
Mg, mmol/l	0,89	1,18	0,93	1,10
Relatively effect	100,0	132,58	100,0	118,27

*every x is average from 31 estimations in SAI1 and 45in SAI2

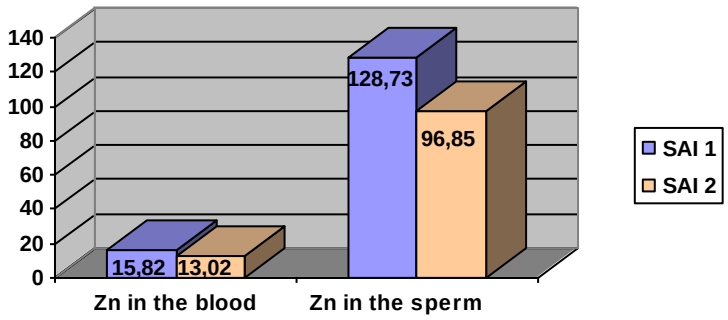

Fig. 6. Level of Zn in the blood and the sperm of sires, µmol/l

CONCLUSIONS

The comparison of experimental results from feeding the additive BIOPRO in sires` diets shows that:

* The great relative effects was obtained on the level of Mg – 32% (SAI 1) and 18% (SAI 2).
* Obtained levels of total protein were increased with 8% (SAI 1) and 13% (SAI 2).
* Obtained level of P were increased with 7% (SAI 1) and 13 % (SAI 2).
* No effects were obtained on level of Ca and blood glucose had a tendency to lower.
* There was positive correlation between levels of Zn both in the blood and sperm.

DDGS

Presentation

The meadow hay, wheat straw and DDGW are a cheap source of CF. DDGW can not be used as a unique source of CF. It contains only13% CF, therefore it is necessary to combine by roughage's. On the other hand it's a cheap source of protein - 26-30%. (Marinov, 2008; Todorov et al., 2008).

Iliev and Kozelov (2008) used dry distillers` grain of maize in the lambs' diet. Also Kanev (2009) used the same product in compound feed for growing pigs. In dairy cow's diets Todorov (2008) substituted successfully compound feed by DDGW. There aren't dates for the using of this waste product of the spirituous industry in non-ruminant animals. Marinov (2008) recommended including DDGW in their diets not more than of 15%.

The purpose of our study was to investigate the effect of alfalfa hay substitution in the with a meadow hay and weath straw combined with DDGW, produced by Geo Milev, Ltd, Iskar St., Sofia, Bulgaria in growing rabbits diets on their productivity.

Material and Methods

In the experimental base of Institute of Animal Science- Kostinbrod was conducted a growth experiment with a total of 60 rabbits (New Zealand White) at the initial age of 45-50 days and average live weight of 1.3 kg. The animals were distributed into three groups- a control group and two experimental groups. The animal were raised in wire cages lined in a single layer and fed *ad libitum* with granulated TMR twice daily- at 8 and at 15h . Water was supplied via nipple watering trough. The trial lasted 42 days.

Three types of TMR for growing rabbis were formulated. All diets contained 20% oats, 15% barley, 16.40% wheat bran, 10% soybean meal, 5% sunflower meal, 1.50% calcium phosphate, 1% limestone, 0.40% salt, 0.50% Premix 6645, 0.05% cicostat, 0.10% DL-methionine, 0.05% lysine. The main source of CF for the diets were: for the control group- 30% alfalfa hay, for the I experimental group – 20% meadow hay + 10%DDGW; for a II group: 15% wheat straw + 15% DDGW.

The total chemical composition was determined by the conventional Weende analysis.

The TMR were granulated with a pellets mill, die 4, pellets length 2 cm. During the trial were controlled once weekly the rabbit's body weights (kg) and the feed intake (kg). The vitality, eventful incidences of digestive disorders and mortality in the investigated groups were daily observed.

The obtained results statistically processed by Excel 2000, single factor, ANOVA program. For more details see also Grigorova et al. (2009).

Main results

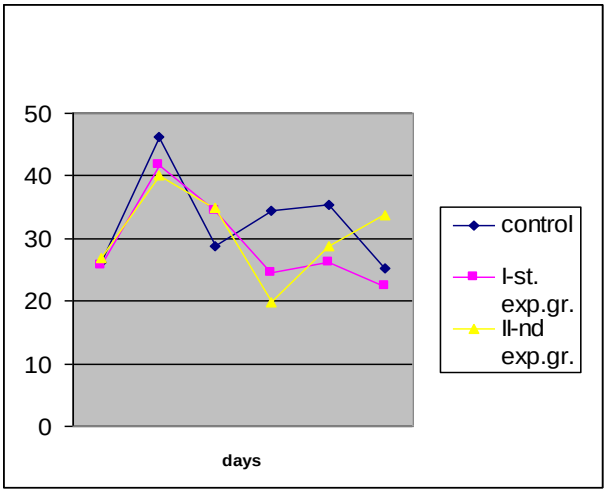

Fig.7. Growth curve of the male rabbits, g/day

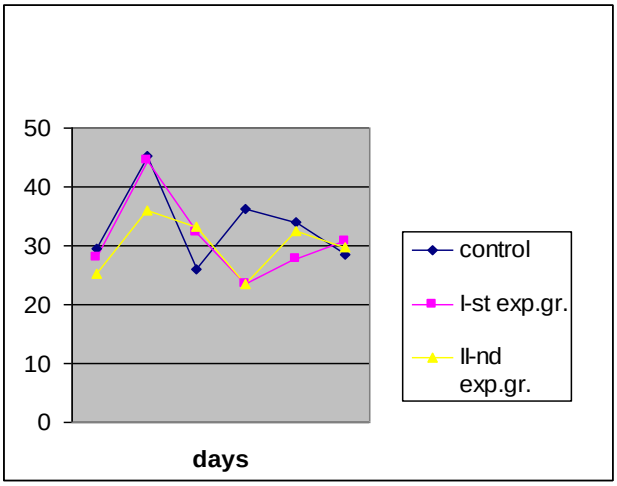

Fig.8. Growth curve of the female rabbits, g/day

Table 5. Body weight growth, feed conversion and mortality at growing rabbits

Groups / **Parameters**	**Control**		**I-st group**		**II-nd group**	
	♂	♀	♂	♀	♂	♀
Number animals	10	10	10	10	10	10
Period, days	41	41	41	41	41	41
Initial weight, kg	1.308± 46.3	1.269± 50.12	1.336± 33.24	1.265± 35.35	1.289± 45.67	1.292± 41.84
Final weight, kg	2.684± 66.29	2.646± 61.29	2.561± 76.77	2.572± 80.87	2.562± 110.44	2.555± 65.89
Average daily gain, g/day	32.74± 1.04	33.99± 0.93	29.16± 2.08	31.12± 1.72	30.17± 1.95	30.13± 1.72
- %	100	100	89.1	91.6	92.2	88.6
Feed intake, g/day/head	128.60± 7.56	130.06± 8.31	124.82± 5.55	125.09± 5.63	121.96± 6.51	115.99± 5.95
Feed conversion ratio, g/kg	4053± 389	3992± 395	4422± 572	4182± 374	4158± 423	3917± 248
- %	100	100	109.1	104.8	102.6	98.1
Mortality	0	0	0	0	1	0

CONCLUSIONS

The tested total mixed rations with: 30% alfalfa hay, 20% meadow hay + 10% dry distillers grain of wheat and 15 % wheat straw + 15% dry distillers grain of wheat, have equivalent effect on: growth, feed utilization (P>0.05), rabbits vitality and deficit of digestive disorders.

The use of meadow hay and wheat straw combined with DDGW in the rabbits` TMRs as alternative sources of crude fibers is expedient, because their price is lower in comparison with the price of alfalfa hay.

JODIS CONCENTRATE

Presentation

Iodine, a requisite substrate for the synthesis of thyroid hormone, is known to cause pathological conditions when its intake is excessive or deficient. According the International Council for Control of Iodine Deficiency Disorders (ICCIDD) (Bohac et al., 2009; Gerasimov, 2009), many country of all over the world, including Bulgaria are in the list of the countries which compulsory have to use iodized salt in the processed food. We used product *Jodis concentrate* which has a patent N PCT/UA, 990020/22.08.2001, Geneva, Switzerland. The product contents 20mg/l Biological

Active Iodine and was produced by Ukraine, "MPK – Yark – Kiev", Kiev district, v. Petrushki.

Material and Methods

In the experimental base of poultry farm "Ameta" in town Razgrad was conducted a growth experiment with a total of 2x18300 chickens from Pure Line at the firs day initial age and average live weight of 0,040kg. The birds were distributed into two groups- a control group and experimental group. The animal were raised in freedom and fed *ad libitum* with compound feed. Water was supplied via nipple watering trough. The trial lasted 37 days.

The total chemical composition was determined by the conventional Weende analysis.

During the trial were controlled once weekly the chickens body weights (kg) and the feed intake (kg). The vitality, eventful incidences of digestive disorders and mortality in the investigated groups were daily observed.

The obtained results statistically processed by Excel 2000, single factor, ANOVA program.

Preliminary results

*Table 6. Results of analysis of the slaughtered chickens, with the addition of Jodis concentrate **

Indicator	**Control group, n = 10**	**Experimental group, n=9**
Set chickens, number	18 300	18 300
Average live weight, kg	1.833	1.983
Died, the number	1789	1416
%	9,33	6,87
Transmitted, the number	16 270	16 655
Slaughtered for analysis, number	10	10
Live weight at slaughter, kg	1,898	2,079
Hot carcass weight, kg	1,254	1,353
Yield,%	66,05	65,08
Weight of internal organs, g		
- Liver	47,46	51,12
- Heart	9,48	10,01
- Gizzard	16,16	16,28
- Spleen	2,19	2,11

* A total amount of used Jodis is 50 liters for 37 days.

Table 7. Chickens` meat chemical composition (in % of DM) (x±SE)

Parameters	Control group, n = 10		Experimental group, n=10	
Thigh muscle				
Dry matter, %	24,98	0,45	25,52	0,33
Protein	80,63	1,65	80,13	1,47
Fat	18,18	1,45	19,52	1,43
Ash	4,18	0,14	4,36	0,11
Chest muscle				
Dry matter, %	26,55	0,18	27,67	0,15
Protein	92,74	0,67	89,07	0,51
Fat	5,53	0,54	6,90	0,52
Ash	4,62	0,10	4,30	0,06

CONCLUSIONS

According the used additive to determine the productivity indices and meat quality of chicken from Pure Line hybrid on all parameters growth, dry matter, protein, fat, ash, simultaneously, in the two groups studied, we made the next conclusions:

1. The mortality, a great problem of this hybrid on practice in Bulgaria, was decrease by 2.50%.
2. The meat quality was no influenced by the additive.

GENARAL CONCLUSIONS

Using the animal nutritional additives is on the focus of many scientists. We have to have not only a sound knowledge of fundamental nutrition and background but also farm conditions on practice and good understanding of statistics. Institute of Animal Science in Kostinbrod, Bulgaria, with its working group on nutrition and feed technology has a great experience on using additives in animal nutrition. So often our trial results are inconclusively. They sometimes could not be published under the scrutiny of scientific journals. But our experience up to now is significant. We have a gained database of more Bulgarian and not Bulgarian additives by different type and kind of animals. It could allow us to go on our experimental work with sure benefit influence for both animal science and husbandry to optimizing animal nutrition, the health of animal, level of production, reproductive capacity and quality of products from animal origin.

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MYCOTOXINS IN FEED

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ABSTRACT

Despite years of research of the occurance and the presence of mycotoxins in feed and food, as well as contemporary possibilities for their prevention, the problem of poisoning by these secondary metabolites of molds is still very current in the world. Yet it is estimated that about 25% of world production of cereals and other grains is annually contaminated by the identified species, and probably a larger percentage of still unknown mycotoxins.
It is believed that over 220 species of molds have the characteristic of mycotoxins production. Among large number of mycotoxins which have been identified so far, only a small number are of medicinal, nutritive and economic significance. They are mainly products of *Aspergillus*, *Penicillium* and *Fusarium* species.
Mycotoxicoses pose a nutritive-medical, but also a diagnostic problem, because certain mycotoxins cause changes in a number of organs. Diseases caused by mycotoxins are not contagious, they are connected with food and/or feed, they are similar to avitaminoses, thay can not be treated with antibiotics or other medicines and they do not cause immune response in the organism because they are of the small mollecular mass, so that animals are permanently not protected from their effects. The content of mycotoxins in feed more often causes the appearance of chronic mycotoxicoses, and the effects of smaller quantities over a longer time period are the same as of bigger quantities over a short period.
Timely detection of the presence of mycotoxins in feed and the subsequent elimination of the contaminated feed from use, can aleviate the negative effects, but a certain time is required for the elimination of the resorbed quantities of mycotoxins and the disappearance of the detrimental consequences. That is why constant and multilevel feed monitoring must be practiced in order to secure efficient reaction for the successful prevention of harmful effects of mycotoxins.
Key words: *feed, mycotoxins, mycotoxicoses*

INTRODUCTION

Despite years of research of the occurance and the presence of mycotoxins in feed and food, as well as contemporary possibilities for their prevention, the problem of poisoning by these secondary metabolites of molds is still very current in the world. Yet it is estimated that about 25% of world production of cereals and other grains is annually contaminated by the identified species, and probably a larger percentage of still unknown mycotoxins [13].
Mycotoxins are secondary metabolites of saprophytic fungi, which enter the body of animals and people mostly through contaminated feed/food. They induce a toxic

reaction. Economic losses caused by contamination with mycotoxins are almost invaluable. They occure in different stages of production of cereals.
Fusarium species attack crops during growth and produce the so-called polish mycotoxins, or toxins present in the grain growth. *Aspergillus* and *Penicillium* species usually develop after harvest and therefore they make storage mycotoxins. Mycotoxins contamination is limited by moisture content in grain, which should not exceed 15%.
Opportunities for fungal contamination of grain increases due to stress from drought. Practical experience shows that in the infected feed different mycotoxins could be found, and their type and concentration depend on the climate and storage conditions. Moderate continental climate, which is characterized by high humidity, such as in Canada, USA and Europe, were favorable for the development of *Fusarium* and *Penicillium* species, and DON, ZEA, OTA, and T-2 toxins.
Mycotoxins lead to health disorders in all animals, but the effects are more noticeable in highly productive animals in the farm keeping due to significantly greater consumption of concentrated feed, although other feedinstuffs may be also contaminated with mycotoxins. Acut mycotoxicoses rarely occur in conditions of modern livestock production, however, small doses of mycotoxins can lead to decrease of productivity, immunity and create a disease. Another problem is that in the organism of animals that have taken contaminated feed can be found residues (mycotoxins and their metabolites) in different concentrations, which may be a cause of harmful effects for people.
Disturbing data on the situation in our country indicate that the problem of permanente contamination of feed with different mycotoxins is still present [31, 1, 17, 9].

FUNGI - PRODUCERS OF MYCOTOXINS

Conditions for the fungi growth and mycotoxin production depend on the type of fungus, but also on the presence of spores, organic substrate and the appropriate humidity, the presence of oxygen, temperature and pH. For the growth fungi need moisture over 12%, and the activity of water over 0.7. Temperature for the production of toxins can range from 4-60^{o}C and the electrochemical reaction of 5 to 7. *Aspergillus* species grow in conditions of low water activity and at higher temperatures than *Fusarium* species that require higher water activity, but can grow at a lower temperature. *Penicillium* species grow at relatively low water activity and low temperatures. It is necessary to highlight that the optimal conditions for growth and development of molds are not identical to optimal conditions for the production of toxins [14].
It is important to emphasize that the presence of fungi in feed need is not always related with the presence of mycotoxins [29]. On the other hand, due to stability of mycotoxins, feed may contain mycotoxins although mycologic test gave a negative result. Also, by common analytical procedures, it is impossible to detect mycotoxins which are chemically modified ("masked" mycotoxins) under the influence of the interaction of plants, microflora and toxinproductive fungi. However, in the gastrointestinal tract binded mycotoxins are released and exhibit their primary harmful action. In our climate most mycotoxins are secondary metabolites of mainly *Aspergillus*, *Fusarium* and *Penicillium* mold.

MECHANISM OF ACTION

Mycotoxins cause health disturbances of all animals, but the effects are more noticeable in highly productive animals in the farm keeping due to significantly greater consumption of concentrated feed, although other feedinstuffs may be also contaminated with mycotoxins in considerable degree. Changes caused by mycotoxins depend on the type and quantity of mycotoxins in feed, the time of entering the body, as well as genetic (species, breed of animals), physiological (category, age, diet, health status) and external factors (climate, keeping conditions) [14].
In the body mycotoxins cause a range of disorders and the biochemical changes, from functional and morphological damage to the appearance of clinical signs of mycotoxicoses and subsequent mortality. Biochemical changes occur first, and are based on disorders of resorption of nutrients, lack of protein transfer or the existence of competition for the same receptor between nutrients and mycotoxins, and the existence of a specific metabolic block of reserves of nutrients.
Biochemical disorders of metabolism of certain nutrients primarily cause functional damage to certain target cells and organs. Functional impairment primarily affect the biological membranes, which causes disruption of permeability. Metabolic disorder causes in some organelas ultrastructural first, and then histological changes [26]. Larger amounts of toxins and/or longer use of contaminated feed increases the level and intensity of pathohistological alteration progressing in patoanatomic changes that affect most of the target organ. These disorders in the first stage go relatively unnoticed, and then nonspecific signs of health disorders, in the form of reduced consumption, retardiranog growth, reduced productivity and increased feed conversion could be observed. In the second stage of disease there are no clinical signs that have special importance for differential diagnosis, and symptoms are similar to diseases caused by different etiologic factors. In the final stage, clinical signs are related to the manifestation of disorders of certain organs and/or system, and some mycotoxin may cause clinical signs that have certain differential diagnostic significance. Lethal outcome arises as a result of biochemical, metabolic, functional and morphological damage.

TOXICITY

Different mycotoxins exhibit varying degrees of toxicity, which depends on gender, age, species and breed of animals, quality of meals and the presence of other mycotoxins [14]. According to the degree of toxicity, mycotoxins are tentatively divided into three groups. Extremely toxic mycotoxins (cyclohlorotin, rubratoxin B) cause lethal outcome in quantities of less than 1 mg/kg BW, a very toxic (aflatoxin B1, trichotecens, citreoviridin) cause the same effect in quantities between 1-10 mg/kg BW and other mycotoxins cause a lethal outcome in quantities greater than 10 mg/kg BW.
Ruminants are generally resistant to most harmful effects of mycotoxins [24] due to rumen microflora which effectively converts entered mycotoxins in less toxic or non-toxic compounds. Among monogastric animals, pigs are extremely sensitive to the effect of zearalenone, while chickens are practically insensitive. Even within the same class (Aves) there are significant differences in sensitivity to certain mycotoxins (chickens are insensitive to the F-2 toxin, but turkeys are sensitive). In the same animal species, some

race and/or strains are much more sensitive than others to certain mycotoxins. Generally, in relation to the age - young animals are more sensitive, according to gender - male animals. Production and physiological status plays an important role, where animals with better production results or animals whose hormonal status is high (pregnancy, growing, lactation) are more sensitive. Harmful effects of mycotoxins emphasize deficiency and/or imbalance of nutrients, the presence of other diseases, as well as stressful situations. Special influence on manifestation of harmful and toxic effects of certain mycotoxins has the presence of other mycotoxins in feed (synergism).
During metabolism mycotoxins change in the organism [23]. Some intermediate metabolites become less toxic (AFM1 less than AFB1), and some much more toxic (zeralenol of zearalenone) and carcinogenic (aflatoksikol).

MYCOTOXICOSES

Mycotoxicoses are nutritionally-medical [22], and diagnostic problem, because some mycotoxins cause changes in multiple organs. Diseases caused by mycotoxins are contagious, related to feed and/or specific feedingstuffs, similar to vitamin deficiencies, can not be treated with antibiotics and other drugs and in the organism does not cause immune response because they are of low molecular weight, so that the animals are permanently unprotected from their effects. Low content of mycotoxins in feed and/or food in practical terms often causes the appearance of chronic mycotoxicoses [8], but the effect of lower amounts over a long time has the same effect as larger quantities during a short period.
The most frequent are aspergilotoxicoses (aflatoxins, ochratoxin, sterigmatocystin, citrulin, patulin), fusariotoxicoses (zearalenone, trichotecens) and peniciliotoxicoses (citrinin, citreoviridin, luteoskirin, ciklohlorotin), while stahyobotritoxicoses, dehondrotoxicoses and mucorotoxicoses have less importance and are mainly related to crude feedingstuffs.

SIGNS OF MYCOTOXICOSES

Symptoms of illness depend on the target organs, as well as the character, intensity and size of changes caused by the mycotoxin. Hepatotoxins cause damage of morphological and functional structure of the liver cells and stimulate the development of liver cancer (aflatoxins, rubratoxin, sterygmatocystin, fumonisin, sporodesmin etc.). Nephrotoxins cause morphological and functional damage followed by renal failure (ochratoxin, citrinin). Neurotoxins cause nervous system damage and bleeding in the brain (patulin, pentrem, citreoviridin, fumonisin). Cytotoxins damage epithelial cells of the skin and mucous membranes of the digestive tract and the endothelium of blood vessels causing necrosis (necrotoxins) and bleeding (trichotecens). Estrogens develope hyperestrogenism genitals problems (zearalenone, zearalenol). Photosensible toxins cause redness of the skin and the appearance of hepatotoxic manifestations (sporodezmin). Factors of feed refusal cause decrease of consumption and vomiting with all the consequences on the production results and animal health (DAS, DON, trichotecens), and respiratory toxins damage the respiratory mucosa (fumonisin, trichotecens).

Different mycotoxins (aflatoxin, T-2 toxin, vomitoxin, ochratoxin, fumonisins) significantly damage immune system performing its suppression. Immunosuppression is based on the direct effect of mycotoxins on cellular and humoral immune responses caused by atrophy of immunocompetent tissues and organs (bone marrow, lymphnodes, thymus, bursa Fabricii, pancreas, spleen), reducing the number of T and B lymphocytes, as well as reducing the concentration of protein, and especially the globulin in blood. A number of mycotoxins (aflatoxin, ochratoxin, citrinin, sterigmatocystin, patulin) shows carcinogenic effects and disruption of normal transcription by binding with molecules of protein, DNA and RNA. In addition, it was found that a number of mycotoxins (aflatoxin, zearalenone, sterigmatocystin, patulin) exert the character of mutagens. Some mycotoxins are embriotoxic or exhibit teratogenic effect. Most of mycotoxins which are potent inhibitors of protein synthesis (aflatoxin, ochratoxin, T-2 toxin, rubratoksin B) express teratogenic effects [14].
The specificity of the effect of mycotoxins on certain organs is related to the specific distribution of mycotoxins in target organs, the sensitivity of different target cells, depending on the stage of development the cells themselves, as well as the activation of mycotoxins in more toxic intermediates and/or different permeability of cells of certain organs for different types of mycotoxins.
A number of different mycotoxins are known so far [30] of which only a small number have medical, nutritional and economic importance (aflatoxins, ochratoxin, trichotecens, zearalenone).

FUMONISIN

Until now, there are isolated and identified fifteen different fumonisin placed in series A, B, C and P (3). The most common are the B series fumonisins and molecular form of B1, a product of mold *F. verticilloides* (formerly F. *moniliforme*). Later it was discovered that also other *Fusarium* species can be producers of these types of toxins (*F. proliferatum, F. nygamai, F. anthophilum, F*. and *F. Dlamini napiforme*), as well as molds of the genus *Alternaria*, which synthesize FB1. However, in naturally contaminated corn could be found only forms of B1, B2 and B3 [11].
Fumonisins are toxic to the central nervous system, liver, lung, pancreas and kidneys of various animal species, particularly horses and pigs. The mechanism of action is based on the inhibition of biosynthesis of sfingolipids [12]. It is proved that they are cancer promoters causin leukoencephalomalacy in horses [15] and edema in swine [7], while in ruminants they are considered to be far less potent. However, Whitlow and Hagler [35] state that fumonisin is also toxic for cattle, manifested by reducing the production of milk and disorder of enzymes levels in serum as an indication of liver disease. Milk excretion of fumonisins is negligible [25]. Based on the results of testing the impact of fumonisin on poultry it was found that this toxin is not a particular threat to the health and/or productivity of this species. Minimum effective dose of 75 ppm FB1 in the feed is about 150 times higher than the highest published level of contamination in feed for poultry [12].
Mycotoxicological testing of feed samples from different countries showed that the content of fumonisin B1 is in the range of 0.055 to 5.0 ppm and usually less than one third had a positive result on the presence of this toxin [12]. Although so far it has not

been registered and described a single case of fumonisintoxicoses in our country, but presence of these types of *Fusarium* fungus in our country, and the isolation of fumonisin B1 from several samples of corn and soybean meal, it is considered that the occurrence of this mycotoxicoses in our area is also possible [16].

DEOXYNIVALENOL

Deoxynivalenol (DON), often called the vomitoxin, is mainly isolated from corn and other cereals, oil meals, hay and silage, and produced by fungi genus *Fusarium* (*F. graminearum, F. semitectum, F. Nivala* and *F. poae*). For his presence in feed for pigs the phenomenon of feed refusal, diarrhea, vomiting, reproductive disorders and mortality are linked [35]. DON in cattle does not cause serious health disorders due to the activities of rumen microflora, which transformes it into a less toxic metabolites, which then is largely excreted from the body. Deoxynivalenol is often the cause of poor feed consumption [34] and reduced milk production in dairy cows. At the beginning of lactation cows with high milk production, which are under greater stress and lower immunity, and cattle in nutrition deficiency with the weaker degradation of mycotoxins in the rumen, are more sensitive. Fattening cattle and sheep tolerate higher concentrations of deoxynivalenol in feed, without affecting consumption, growth and feed conversion [2, 36].

T-2 TOXIN

T-2 toxin occurs as a toxic product of the fungal genus *Fusarium* (*F. tricinctum, F. roseum, F. poae, F. sporotrichioides*) that synthesize it at lower temperatures (2-40^{0}C), while at temperatures above 32^{0}C they lose this ability.
After ingestion T-2 toxin is rapidly resorbed in front parties of the digestive tract, and already after 1 h reaches its maximum concentration in blood. Then follows the slower phase and the distribution of T-2 toxin and its metabolites in some tissues, while after 3-4 h the highest amount can be found in the liver, kidneys, stomach and bile, and in the muscle and skin reaches a maximum after 12 h. After 24 h the highest concentration of T-2 toxin in the excreting organs and in the bile duct, liver, kidneys and intestines. Enterohepatic recirculation delay excretion and increases toxicity (phenomenon of delayed effects). Elimination of T-2 toxin and metabolites occurs via the urine during the first 24 hours, and then the remaining part is excreted via feces. Ingested T-2 toxin is left as a deposit significantly in any organ, and the residues are effectively eliminated during the few days after the termination of consumption of contaminated feed. During the consumption of contaminated feed, T-2 toxin is excreted by eggs, and his concentration is higher in albumen than in yolk (0.04 vs. 0.13%). During the long-term intoxication, eggs excrete about 0.1% of ingested toxin [27].
T-2 toxin inhibits the replication of DNA, and causes changes in DNA that have non-reversal character, but nevertheless T-2 toxin is considered, with prolonged action, that can induce mutagen, teratogenic and carcinogenic effects. Many trichotecens inhibit protein synthesis by blocking elongation of polypeptide chain at the position of peptidil-transferase in polysome subunits 60S. It was also noticed the possibility of irreversible inhibition of initiation of protein synthesis that leads to decay of polysomes. This toxin

shows and immunosuppressive and cytotoxic effect, and also causes the disorder of the mechanism that regulates the use of vitamin A [27].
Trichotecens are considered to be significantly more toxic than other metabolites of *Fusarium* fungi, and within them, type A is significantly more toxic than B-type trichotecens. Toxicity depends on the animal species, sex and age, and the amount of toxins. The effect in different animals, expressed as the relative toxicity, ranges 1-10 mg/kg of BW, so therefore T-2 toxin belongs to a very toxic mycotoxins [21].
T-2 intoxication is characterized by lower growth, with increased feed conversion, while the intensity of the disorder of production results is proportional to the amount of mycotoxin and length of exposure [32]. Decrease in production is emphasized if there are present other mycotoxins in feed. Clinical symptoms are manifested by depression, lethargy and breathing difficulties. Local effects are epithelonecrotic stomatitis, oral ulceration and necrosis. Early occurrence of vomiting may be accompanied by diarrhea, which is often bloody. Extension of protrombin time and potential vascular damage cause widespread haemorrhage and haemorrhagic diathesis. Also, there is fever, anemia, growth inhibition and poor utilization of feed. Chronic toxic effects of T-2 toxin primarily reflect on the production results [21].
T-2 toxin causes pathomorphologic changes in the liver, digestive tract, kidneys, bone marrow, skin and lungs, as well as in the heart, reproductive organs, spleen and nervous tissue [27].

ZEARALENONE

Zearalenone (F-2 toxin) is a secondary metabolite of *Fusarium* fungi genus (*F. graminearum, F. roseum, F. Nivala, F. tricinctum, F. sporotrichoides, F. oxysporum, F. moniliforme, F. lateritium*) [22]. The major growth of mold occurs when relative humidity is over 70%. The optimal temperature for development of fungi ranges from 18 to 24 ^{o}C, with the greatest production of zearalenone noted during changes of medium and higher temperatures [14]. Zearalenone belongs to the group of phytoestrogens. So far it has been registered over 15 different derivates that have different biological activity. Basically, they have a similar configuration (phenolic core) as estrogenic substances.
Zearalenone is resorbed from the digestive tract and by portal bloodstream is transported to the liver where it accumulates and metabolized by the action of and reductase and esterasa enzymes. Metabolic half time of zearalenone is 86.6 hours and in 4-5 days it is mostly secreted from the body. Zearalenone and its derivatives are mostly eliminated in the form of glucuronides via feces (40-60% of ingested amount), and to a lower extent via the urine (only 2-4% of whole amount). This toxin is excreted in milk, even 42-44 hours after ingestion of contaminated feed and continues during the next 5 days after the termination of its consumption. Zearalenone residues and its derivatives can be found in the edible parts of animals which were fed contaminated feed, but usually in the liver and muscles. Even in the meat of clinically healthy animals could be determined 10 μg/kg. Zearalenone was determined in the egg yolk also. Residues are carcinogenic, and their biological effects are compared with the effects of dietilstilbestrola or estradiol [10, 33].

Zearalenone and its metabolites have anabolic effects, similar to estrogen and foliculostimulative hormone. Biological activity can be explained by competition with 17-β-estradiol for specific binding sites on estrogen receptors and interference with enzymes involved in metabolism of steroides. The mechanism of action is based on binding to estrogenic receptors in the cytosol, and the mycotoxin-receptor complex is transported into the cell nucleus. F-2 demonstrates the effects of toxins in all the metabolic processes affected by estrogen hormones [18], but usually in the genital organs and in the process of reproduction. The consequence is, primarily, premature sexual development in immature female and inhibition of normal development of the testecles in males.
Zearalenone is characterized by slightly less toxicity, compared to other metabolites of *Fusarium* fungus. Toxic effect in different animal species, or the relative toxicity (LD50) ranges from 1-10 mg/kg BW zearalenone and therefore belongs to a very toxic mycotoxins. Pigs are more sensitive to its effects [6] then other animal species, such as ruminants and poultry, especially chickens. F-2 toxin has cytotoxic and genotoxic effects [4].
The type and intensity of symptoms of zearalenontoxicoses depends on the animal species, age and gender. In the clinical picture is dominant estrogen syndrome which is manifested by edema and vulvar hyperemia, with slightly muculagineus vaginal discharge. In severe cases, prolapsus of vagina and rectum could be seen. F-2 toxin at low doses increases the size of the milk glands and reproductive organs and/or causes early maturation. In higher doses, negatively affects the conception, ovulation, implantation, fetal development and vitality of newborn animals. In addition, in the clinical picture also may occur diarrhea, vomiting, feed refusal, loss of weight and hemorrhages. Characteristic pathomorphologic changes in the external genital organs in often are observed in the form of edema and hyperemia [20].

AFLATOXIN

Aflatoxins are a mixture of similar chemical compounds labeled by fluorescing colours as B (blue), which are significantly more toxic, and G (green). They are products of secondary metabolism of molds *Aspergillus flavus* and *Aspergillus parasiticus*, although there are opinions that other strains and species also have the ability of synthesis of these toxins in the traces. *Aspergillus* fungi are capable of growth and sporulation in the temperature range 6-54^{0}C, while the optimal are temperature 30-35^{0}C, minimum of 0.78 aw for growth and optimal pH is 5.5.
Ingested aflatoxin is effectively resorbed in the digestive tract and reaches the bloodstream for 30 minutes and the hepatocytes for 1 hour where its biotransformations starts. It is believed that among the metabolites aflatoxicol is the most mutagen agent that causes carcinogenic changes in the liver. Excretion is mostly done via bile and urine and to a lesser extent by milk (aflatoxins M). After 24 hours in the body remains 20-30% of the entered aflatoxin. The biological half-time decay is 72-96h, and 7 days after use of contaminated feed aflatoxin residues and its metabolites were found only in eggs and milk. Aflatoxin tends to be deposited in all soft tissues and fat depots, mostly in the liver and kidneys. Detection of residues is relatively difficult and possible only for the original form of toxin and its metabolite M1 [24].

Primary toxic effects of aflatoxin are formed at the level of interaction with genetic material. Molecule penetrates the cell and its nucleus, and enters between the base pairs of DNA. Create errors in the transcription of DNA, which greatly slows down the process of transferring information, a consequence is the inhibition of protein synthesis and synthesis of "wrong" protein. Immunosuppressive effects of aflatoxin are also proven in chickens and turkeys, as well as in some laboratory animals [12].
Aflatoxins are very toxic compounds, for most animal species. The relative toxicity (LD50) for the most sensitive ducks and turkeys is 0,3-0,6 mg/kg BW, pigs 0.6 mg/kg BW and ruminants 0.2 mg/kg BW, while most resistant are chickens 6,5-16,5 mg/kg BW [12].
The clinical signs of acute aflatoxicoses are characterised by depression, anorexia, icterus and hemorrhages. Steatorhea is a common finding, and hemorrhages in many parts of the body of pigs, in lactating animals milk production reduction to the complete cessation, and mortality in birds for 7-14 days in most opistotonusu and legs extended backwards. In broilers this disorder [22], manifests as retarded growth and lower feed conversion (0.25 kg/kg), impaired imunogenesis (0.6 mg/kg) or egzitus (about 1 mg/kg). In laying hens the most striking changes are in some biochemical parameters, and decrease of egg production and weight. In chronic aflatoxicoses clinical picture is atypical and is manifested by reduction of feed consumption, increase conversions, decrease body weight, milk and egg production, as well as the significant immunosuppresion. The consequences are carcinogenic, mutagen, embriotoxic and teratogenic effects.
Pathomorphological changes develop in multiple organs, and one of the indicators of aflatoxin intoxication is increased relative weight of internal organs. As it belongs to hepatotoxic group of mycotoxins, the most common and the most visible changes are observed in the liver, in the form of discoloration, necrosis, hemorrhages, fatty infiltration and atrophy. Also hemorrhages and necrosis are present along the entire intestinal tract with proliferation of biliary channels. Aflatoxin B1 causes the kidneys nefroses, and the toxin amount in feed greater then 0.2 ppm causes testicular atrophy, interruptions in germinative epithelium and spermatogenesis termination, as well as slower development of the ovaries with reduced relative weight and expressed folicular atresis [24].

OCHRATOXIN

Ochratoxin is synthesized by storage fungi of the genus *Aspergillus* (*A. alutaceus, A. sulphureus, A. melleus, A. alliaceus*) and *Penicillium* (*P. viridicatum, P. verrucosum, P. cyclopium, P. commune*) in conditions of humidity higher then 16%, while the optimal time for the creation of toxin is 7-14 days. Maximum production occurs at temperatures 20-25^{0}C, but it is possible in the range from 2 to 31^{0}C.
Ochratoxin is mostly resorbed in the front parties of the digestive tract and via bloodstream it reaches the kidneys and liver, primarily, but to a lesser extent to the muscles also where it deposites. Excretion is mainly carried out through the urine and feces, while the elimination by milk is minimal. Excretion by eggs is also noted [19].
The mechanism of action of ochratoxin is based primarily on the effect of the enzymes involved in the metabolism of phenylalanine and mitochondria function [28]. The

secondary mechanism is linked to the increased lipid peroxidation in microsomes of liver and kidneys [5]. The third mechanism of toxicity of ochratoxin is based on inhibition of respiration in mitochondria, which acts as a competitive inhibitor of protein transmitters localized on the inner membrane and mitochondria. It is teratogenic agent with different potency in various animal species, as the result of different possibilities of placental transfer. Mice, rats, hemsters and chickens are sensitive, while pigs are resistant. Carcinogenic properties of ochratoxin exhibit a high frequency of renal adenomas and cancer, especially in males, with frequent metastases in the liver and lymph nodes, and in females the multiplication of fibroadenoma in milk glands. Ochratoxin A exerts immunosuppressive action [12].

Toxic effects depend on the species and animal age, gender, health status, degree of contamination and the length of ingestion of contaminated feed. Toxicity is significantly affected by the form of toxins (OA, OB, OC, OD, OA methyl ester, methyl ester OB, OB-ethyl ester, Oα and Oβ). LD50 for ducks is approximately 0.5 mg/kg of BW (to 3 mg/kg BW), for goats 3 mg/kg BW, pigs 6 mg/kg of BW, in Beagle dogs 9 mg/kg BW, cattle 13 mg/kg BW, for female rats 21.4 mg/kg of BW and for males 30.3 mg/kg of BW.

Ochratoxicoses is usually manifested by anorexia, fever, diarrhea, and uremia. Typical symptoms, especially in pigs, are polidipsia and poliuria with glucozuria. It is believed that changes of the total protein level and albumin are the most sensitive indicator of acute ochratoxicosis. Also, by urine testing, besides the presence of OA and its metabolites, lower pH and the presence of protein may be determined [19]. For chronic ochratoxicoses nonspecific clinical picture is characteristic with retard growth, decreased body weight, lower feed consumption and increased feed conversion. Pathomorphological changes are primarily in the kidneys and liver. The kidneys are enlarged and pale (like boiled) and liver fatty degenerated.

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A SURVEY OF OCCURRENCE OF TOXIGENIC FUNGI AND MYCOTOXINS IN PIG FEED SAMPLES FROM DIFFERENT PRODUCTION AREAS IN SERBIA

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ABSTRACT

It is considered that human and animals are permanently exposed to effects of various mycotoxins at the time, usually at low concentration. Therefore, the aim of the present work was to evaluate the contamination of pig feed samples, taken from different production areas in Serbia, by aflatoxins (AFT), ochratoxin A (OTA), deoxynivalenol (DON) and zearalenone (ZEA). Moreover, we focused on the contamination of pig feed samples by the associated toxigenic fungi and influence of environmental factors such as moisture content (%) and water availability (aw) on both the growth and mycotoxins production. The results showed that majority of samples were contaminated by the two mycotoxins. The ochratoxin A (OTA) and deoxinivalenol (DON) was detected in 50 and 55% of examined samples, respectively, while zearalenon (ZEA) was found in 44,4% of samples originating from investigated regions. Presence of aflatoxin was not found. The average concentrations of mycotoxins in complete feedmixes were: OTA-0,06 mg/kg, DON-0,78 mg/kg while the average ZEA content was 0,85 mg/kg. Total mould count (CFU/g) in three samples of complete feedmixes was up to 10^5, in nine samples it was around 10^5-10^6, while in six samples, level of contamination from moulds was higher than 10^6. During a mycological analysis of complete feedmixes intended for fattening swine, a total of six genera and 14 species of moulds were identified. The main isolated fungi belong to the genus *Penicillium* (94,4%) while the moulds from *Fusarium* and *Paecilomyces* genera isolated in 55,5% and 44,4% of the samples from investigated localities, respectively. Other fungi from the genera *Aspergillus* (22%), *Mycor* (11,1%) and *Alternaria* (5,5%) were represented in a less amount.
Key words: *pig, feed, moulds, mycotoxins*

INTRODUCTION

The contamination of agricultural commodities with fungi able to produce toxic metabolites is one of the main worldwide concerns. Discoloration, quality deterioration, reduction in commercial value and mycotoxin production has been linked to mouldy contaminated foods and feeds. It not only generates great economic losses [10], but also represents a threat to human and animal health, particularly through the synthesis of

mycotoxins. Mycotoxins are naturally occurring secondary metabolites of several toxigenic microfungi that contaminate the whole food chain, from the agricultural cultures to the plate of consumers. Mycotoxins occur sporadically both seasonally and geographically. In farm animals, mycotoxins exert their effects through four primary mechanisms: (1) intake reduction or feed refusal, (2) alteration in nutrient content of feed in nutrient absorption and metabolism, (3) effects on the endocrine and exocrine systems and (4) suppression of the immune system. In routine animal feed screening, mycotoxins are usually found at relatively low levels. Limited information exists regarding the effects of low levels of multiple mycotoxins in livestock. It has been suggested that combinations of mycotoxins at low concentrations may have negative effects on livestock, even though the concentrations of individual mycotoxins are well below concentrations reported to cause negative effects [26].

The main mycotoxins classes of concern produced by fungi in the genera *Aspergillus*, *Penicillium* and *Fusarium* include the aflatoxins, ochratoxin A, trichothecenes and fumonisins. Moisture and/or temperatures is the single most important factor in determining if and how rapidly molds will grow in feeds. Improper storage accompanied by too high a temperature and elevated moisture content in the grain favours further mycotoxin production and leads to reduction in grain quality [16, 22].

The aims of this work were: 1) to determine the mycobiota in pig feed samples, 2) to evaluate the feedstuffs' mycotoxins contamination, especially aflatoxins, ochratoxin, deoxinivlenol and zearalenone, 3) to correlate the mycobiota presence versus specific mycotoxins contamination in respect of the effects of the moisture content and water activity (aw), on the growth and mycotoxins production.

MATERIAL AND METHODS

Samples

The research materials consisted of 18 representative pig feed samples which were collected directly at animal farms from different provinces of Serbia during six month period. The samples (each about 1 kg) were stored at 4 °C and analysed the day after collection. On the same day, the moisture content of the feeds samples was determined by drying.

Reagents

Standards of AFT, OTA, ZEA and DON were purchased from Sigma-Aldrich Chemie GmbH. All other solvents and reagents were analytical grade.

Isolation and identification of fungal

Culturable fungal spore concentrations are presented in terms of colony-forming units (CFU)/g of samples. Isolation and quantitative enumeration of fungal propagules were done on solid media using the surface- spread method by blending a 10 g portion of each sample with 90ml of 0.1% peptone water solution. Serial dilutions, 10^{-1} to 10^{-6} concentration, were made from each material and 0.1ml aliquots were inoculated in triplicate on two media potato dextrose agar (PDA) and Czapek yeast extract agar (CYA) of fungal enumeration. After 3-7 days, growing fungal colonies were transferred to Czapek yeast extract agar (CYA) and incubated at 25 °C in the dark for 7 days. Macrofungi and moulds were identified to genera/species by their macro- and micromorphology features using appropriate identification keys [14, 17, 18, 24, 28].

Chemical analysis
All pig feed samples were analysed with validated methods and under quality assurance conditions.
Moisture content
The moisture content of the pig feed samples was determined by drying at 105°C until the weight did not change further [6]. The a_w value of the grain was measured in a hygroscope (*GBX Scientific Instruments FA-St/1, tastatura model MX 3700/ML 4700*) at a temperature of 20°C.
Analysis for aflatoxins (AFT), ochratoxin A (OTA) deoxynivalenol (DON) and zearalenone (ZEN)
The detection of these mycotoxins in all samples was performed by TLC using appropriate methodology [7, 8, 15, 19].
Statistical analysis
Differences in the mean levels of mycotoxins contamination across the three groups of positive samples was calculated by analysis of variance and then by a Student's t-test. Additional posttests were applied to evaluate differences between groups with statistically significant variation among means. The differences with *p* values smaller than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Mycoflora analysis
The results obtained from the mycoflora analysis in the samples are presented in Figure 1 and 2. Occurrence of mycotoxins contamination and mean contamination levels of mycotoxins (mg/kg) in samples originating from region where samples were collected are presented in Figure 3 and 4.
Total fungal counts ranged from 10^5 to $40x10^5$ cfu/g. In our study, the highest fungal count ($40x10^5$ cfu/g) and average total fungal counts was detected in pig feed samples collected from region Bogatić. From the 18 samples analysed, seven samples had cfu/g higher than the limit established by Serbian regulations ($3x10^5$) [20]. These samples included 3 samples originating from Vladimirci and 4 samples from Bogatić locality. Fungal count is an indicator of the quality of feeds and should not exceed $1x10^5$ cfu/g [2]. It is worth mentioning that in such a samples only *Penicillium* species mostly belonging to *F. equiseti* were isolated.
Species determination revealed great heterogeneity. A total of six genera and 14 species of moulds were identified. With the exception of *Alternaria alternata*, and *Mucor racemosus* which occurred only in one to two samples, the rest of the species were found in more than one sample in all locations surveyed. The most frequently isolated fungus was *Penicillium* species (Fig. 2), a mould present on 17 (94,4%) of the 18 samples examined, and comprised 30,6% ofthe total fungal population. Other frequently isolated moulds included *Fusarium* (55,5%) and *Paecilomyces* (44,4%) genera. *Fusarium* isolates were identified as *F. equseti, F. moniliforme, F. nivale F. semitectitum, F. tricinctum and F. avanaceum*, which are proven toxogenic moulds. Other fungi from the genera *Aspergillus* (22%), *Mycor* (11,1%) and *Alternaria* (5,5%) were represented in a less amount. Among fourteen species were identified, only *Paecilomyces* was not potentially mycotoxins produced fungi. The incidences of *Penicillium* species increased

throughout the winter months whereas the *Fusrium* did not show any well defined pattern of occurrence. *Penicillium* occurrence in the mixed feed samples is in agreement with the results reported by other authors [5, 9, 11, 12]. This shows that the incidence of these various species was important, as the produce was stored for prolonged periods of time.

Relationships between the moisture content, the storage duration and the incidence of moulds isolated

During the collection period, the moisture content observed per pig feed samples varied between 9,63 and 17,3% (Fig. 1B). The moisture content incresed during period september-december, and reached in the december (17.25%), whereas decreased values at the end of the monitoring period 9,63%. However, mean moisture content observed per region was similar, and varied between 12,2% (Senta) to 13,36% Vladimirci. On the basis of obteined results can conclude, as the storage period was extended, the moisture content reached. Additionally, positive and significant correlations between the level of contamination of samples by total fungal counts and the moisture content were found in all of exmined regions (r=0.475). Fungal colonization, growth and sintesis of toxins, results from the complex interaction of several factors (water availability, temperature and incubation time) and therefore, an understanding of each factor involved are essential to understand the overall process and to predict fungal spoilage in agricultural and food products [6]. Improper storage accompanied by too high a temperature and elevated moisture content in the grain favours further mycotoxin production and leads to reduction in grain quality [38-40]. Isolated species in our case are mostly storage contaminators, implicating that the high number of contaminated feed is most probably the result of manipulative mistakes during storage of feedstuffs or feed.

Occurrence of mycotoxins in samples

The results obtained from the analysis of mycotoxins in the pig feed samples are presented in Figure 3 and 4. The predominant mycotoxin for all analyzed samples was DON, while, on the other hand aflatoxins was not detectable. The incidence of DON and OTA in all the samples was 55.5% and 50%, respectively, while the median content of the positive samples was 0.78 and 0.06 mg/kg. The highest levels of DON were found in samples from region Vladimirci (2.5 mg/kg), therefore nine samples greatly exceeded the legally established DON limit (0.60 mg/kg) [20]. In respect to OTA content in only one sample from region Vladimirci OTA content (0.27 mg/kg) was exceeded the legally established OTA limit (0.25 mg/kg) [20]. The occurrence of ZEA was 44.5% and varied between 16.6% (Senta) to 66.6% (Vladimirci). Also, the ZEA contamination levels fluctuated in very wide range between 0.20 mg/kg (mean 0,03 mg/kg) (Senta) and 5.0 mg/kg (mean 1,92 mg/kg) (Vladimirci). The incidence of ZEA was significantly different in samples originatig from Senta and Bogatić ($p< 0.001$). Six pig feed samples was contaminated with ZEA above the legally established ZEA limit (1.0 mg/kg). No significant differences were found between the median DON and OTA contents for all feed items ($p>0.05$). Additionally, positive and significant correlations between the level of contamination of samples by total fungal counts and the OTA content (r=0.584) were found, as well as the moisture content and the OTA content (r=0.468). In respect to correlations between the level of contamination of samples by mycotoxins, between OTA and ZEA content and ZEA and DON content, significant correlations were found r=0.482 and r=0.358, respectively.

These results are comparable with the data reported by other authors and with recently publisched data in Serbia [1, 5, 9, 11, 12], and Europe [13, 21, 23]. The results shown the natural co-occurrence of both toxins in majority of samples (55.5%) at minimal concentrations of 0.057 mg/kg (OTA) up-to 5.0 mg/kg (ZEA). Differences between the incidence and contaminations levels of mycotoxins in pig feed samples obteined in the present study may be attributed, among others, to a different origin of basing corn, it is well known that cereal infection with moulds and toxin production depend strongly on environmental conditions (damp climate, cool temperatures). However, these data must be interpreted with caution as they were calculated from a limited number of samples.

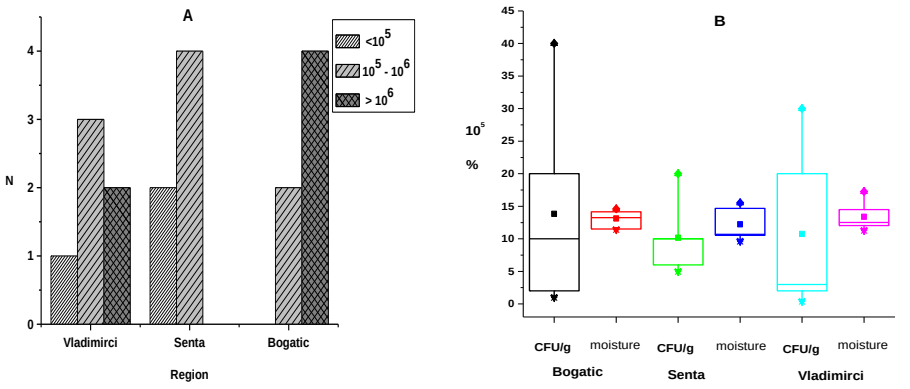

Figure 1. Total fungal counts (CFU/g) (A) and mean contamination levels of moulds and moisture content (B)

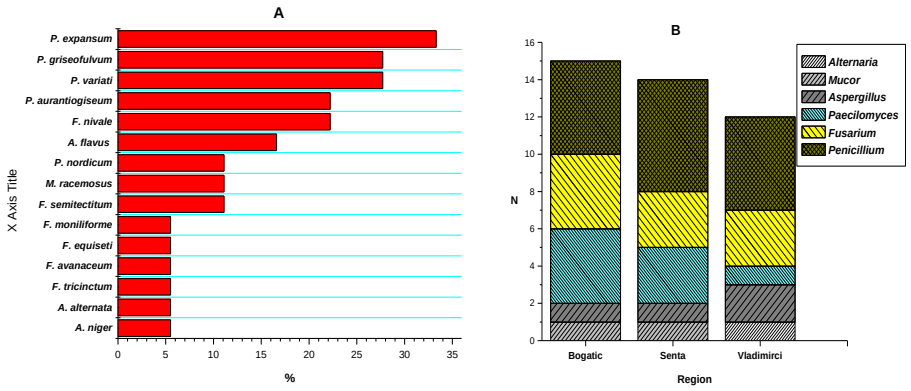

Figure 2. Relative frequencies of isolation of the dominant fungal species (A) and genus (B)

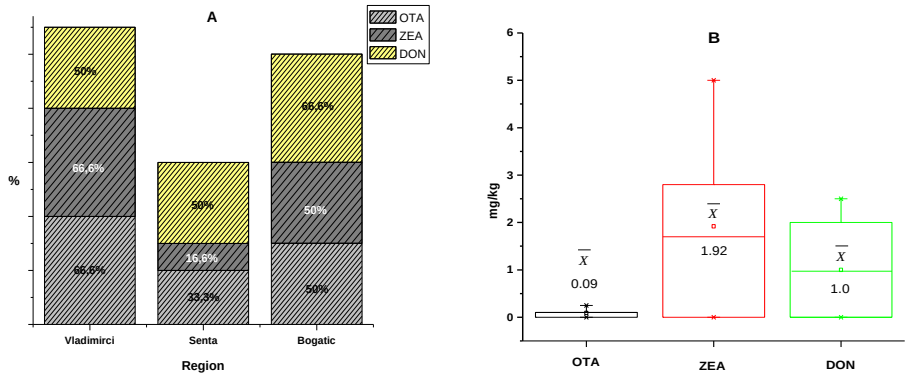

Figure 3. Occurrence of mycotoxins contamination in samples depending of regions (A) and mean contamination levels of mycotoxins (mg/kg) in samples originating from region Vladimirci (B)

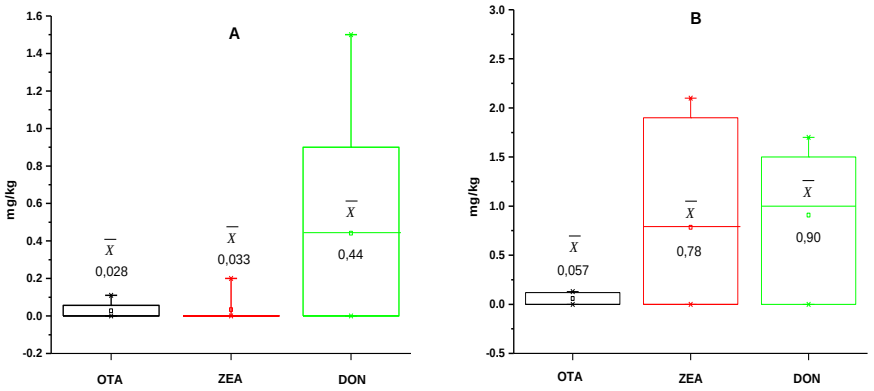

Figure 4. Mean contamination levels of mycotoxins (mg/kg) in samples originating from region Senta (A) and Bogatić (B), (ZEA p<0,001)

CONCLUSION

This study confirms the importance of continued surveillance of mycotoxigenic mould and mycotoxin occurrence in feeds in Serbia. More information is needed about why mycotoxins occur, when to expect them, how to prevent their occurrence and how to deal with their presence. The development of predictive models on mycotoxigenic mould activity and the conditions which will prevent mycotoxin production and which can give an indication of tolerances relevant to the legislative limits are important. Combined data

will enable us to realize the goal of developing realistic and accurate decision support systems for effective conservation of grain post-harvest. More data are needed about animal toxicity and about interactions with other mycotoxins, nutrients and stress factors such as disease organisms or environmental stress. Improved screening techniques are needed for monitoring mycotoxin occurrence, diagnosing toxicities and prevention and treatment.

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DYNAMICS OF MICROBIOLOGICAL AND MYCOTOXICOLOGICAL CONTAMINATION OF POWDERED AND PELLETED CALF FEED MIXTURE IN WINTER CONDITION STORAGE

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ABSTRACT

Changes in microbiological and mycotoxicological quality of powdered and pelleted calf feed mixture were investigated during 150 days storage period. The analysis were performed at the production day (day 0), after 45 days, 90 days and 150 days of the storage in calf farm conditions. Total count of bacteria was significantly higher in powdered mixture than in the pelleted one during the entire storage period. Regarding the yeast and mold count in powdered and pelleted samples the results were also significantly higher in the powdered one. After prolonged storage period (90 and 150 days) mold and yeast number decreased in powdered mixture on less then 10/g. Besides that, the number of identified fungal species was much lower in the pelleted calf fodder (6) than in the powdery one (17). The only common species found in the both types of tested samples were: *Absidia corymbifera, Aspergillus ochraceus, Cladosporium herbarum, Fusarium subglutinans, F. verticillioides and* one unidentified *Aspergillus* species.The dominant fungal genera in powdered calf feed mixture were: *Fusarium* (3 species), *Aspergillus* (5 species) and *Acremonium* (2 species).
Although potential producer of aflatoxin B1 – *A. flavus* was determined in powdered sample mycotoxicological investigations demonstrated the absence of this mycotoxin. Fusariotoxin zearalenone was found after prolonged storage period (90 and 150 days) in concentrations ranging from 0.368 to 0.736 mg/kg in powdered sample, and from 0.221 to 0.442 mg/kg in the pelleted one. The contamination of calf feed mixtures with this mycotoxin was expected because potential producers of zearalenone *F. oxysporum* and *F. subglutinans* were observed in powdered and pelleted samples. Type A trichothecene – T-2 toxin was detected in the analyzed fodder mixtures only at the production day (0.337 mg/kg).
Key words: *storage, calf feed, pelleting, microbiological and mycotoxicological quality*

INTRODUCTION

The main preoccupation of feed industry nowadays is the production of safe and hygienic correct fodder mixtures. It is conditioned by the quality of raw materials,

applied technological procedures and stability of the product in the storage conditions. Animal feeds can serve as a carrier for a wide range of microbial contaminants such as bacteria, yeasts, molds and their toxic metabolites (mycotoxins) [11]. Such contaminants have been shown to influence animal performance adversely and to compromise the safety of animal products.

The growth and sporulation of microorganisms as well as toxin production to a large extend are depending from moisture content of the substrates (feed ingredients and complete feed mixtures) as well as moisture and the relative humidity in the storage environment. It is known that minimum water activity (a_w) is: for bacterial growth a_w = 0.90, for yeast growth a_w = 0.85 and for fungal growth a_w = 0.65 [8].

One of the contemporary feed manufacturing processes for reducing presence of the microorganisms in feed is thermal processing (pelleting) [5]. This process involves mixing steam with the feed, pressing the mixture through a die, and cooling the pellets afterwards in order to remove heat and moisture. If the pelleting process is done correctly added 3-5% moisture in the form of steam is removed from the feed before shipment. However, if this excess moisture is not removed after cooling the pellets, mold growth will be encourage since pelleted feeds with higher water content are warmer and their storing in a cool bin will cause water condensation on the inside of the bin.

Although pelleting of fodder has been shown to reduce mold and bacteria count by the factor of 100 to 100,000 many fungal spores remain in the feed after it was pelleted. In right conditions the remaining spores can grow and produce mycotoxins. Thus, pelleting process delays, but does not prevent, the onset of fungal growth.

So, thermal processing alone will not guarantee the elimination of microorganisms. On the other hand, contaminated incoming ingredients, inadequate housekeeping measures, insects, rodents and food traffic through the feed mill can lead to recontamination of the fodder.

The goal of this investigation was to determine influence of pelleting procedure of calves mixture on microbiological and mycotoxicological proprieties of mixture during 150 days storage period.

MATERIAL AND METHODS

Samples. Investigated powdered mixture (11.29% moisture) was produced in Feed Mixture Industry, Padinska Skela. Components were mixed with horizontal mixer (Buhler), of 3000 t capacity. Pelleting of the same mixture was done by the press of the same manufacturer at 75^{o}C. Pellet diamether was 4 mm, lenght 4 to 6 mm, and moisture 11.13%. Composition of the mixture is shown in Table 1.

Immediately after the production of feed and pelleting, the samples for microbiological and mycotoxicological analysis were taken (day 0). Feed mixtures were kept in nylon bags during 150 days and sampled periodically (November 2008 – March 2009), at 20 cm above the floor, in ventilated, semi-dark and dry room. Average room temperature was 18 ^{0}C (7-22 ^{0}C).

Microbiological investigations were performed according to *Regulations on maximal quantity of harmful materials and ingredients in fodder* [17]. 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 [15]. Identifications of fungi were performed according to Domsh et al. [4], and Samson and van Reenen-Hoekstra [14].

Table 1. Composition of powdered and pelleted calf mixture

Component	**Percentual composition**
Corn (grounded)	34.30
Barley (grounded)	10.00
Soybean (full fat)	22.50
Sunflower meal (33% UP)	10.50
Wheat bran	16.50
Alfalfa flour	3.00
Limestone	1.20
Dicalcium-phosphate	0.40
Salt	0.60
Vitamine and mineral premix	1.00
Total	**100.00**

Mycotoxicological investigations
The presence of aflatoxin B1 (AFL B1) and zearalenone (ZON) was determined according to standard method [16], while diacetoxyscirpenol (DAS) and T-2 toxin were analyzed by applying the method of Pepeljnjak and Babić [13].
Fungal potential for fusariotoxins production (ZON, DAS and T-2) was investigated according to the rapid screening method of Filtenborg et al. [**6**] modified by Bočarov-Stančić et al. [3] on the following media: YESA (2% yeast extract, 15% sucrose and 2% agar, pH 6.5), PPSA (2% pepton-1, 15% sucrose and 2% agar, pH 6.5), and PDA (potato-dextrose agar, pH 6.9).

RESULTS AND DISCUSSION

Obtained results of the present investigation are presented in Tables 2-4.
Total count of bacteria in pelleted mixture at the production day (day 0) was 14.45 times lower than in the powdered mixture (1200,000/g). Similar results were obtained during complete storage period. The decrease of total count of bacteria in pelleted calf mixture, in comparison with powdered sample, was recorded constantly – it varied from 7.34 to 18.50 times (Table 2). The reduction of total bacterial count after 90 and 150 days of storage can be explained by the change of storage conditions. Probably the increase of surrounding temperature from February to March as well as the reduction of relative humidity during that period resulted in the decrease of the moisture content of both types of the mixtures, and consequently in the decrease of bacterial number. The differences in total count of bacteria in production day and after 45 days of storage were not statisticaly significant because they were inside the border of experimental error.
Other authors have also reported that pelleting process significantly reduced the total count of bacteria, molds and yeast. According to Sretenović et al. [18] in complete calf

feed mixtures the total count of bacteria decreased from 400,000/g to 20,000/g, and total count of molds from 12,000/g to 300/g, respectively. The simillar results were obtained by Lević et al. [9] – pelleting of the calf fodder was shown to reduce the total count of bacteria by the factor of 15, and the total count of molds by the factor of 8.25. Adamović et al. [1] observed also positive influence of the pelleting on the improvement of microbiological properties of feed mixtures with bentonite supplement.

Table 2. Bacterial contamination of calf feed mixtures during storage

Parameter	**Powdered mixture**				**Pelleted mixture**			
	Day 0	Day 45	Day 90	Day 150	Day 0	Day 45	Day 90	Day 150
Total count of bact/g x 10^3	1200	1000	740	360	83	77	40	49
Proteus spp./50 g	0	0	0	0	0	0	0	0
Escherichia coli/50 g	0	0	0	0	0	0	0	0
Coagul. positiv. *Staph.*/50 g	0	0	0	0	0	0	0	0
Salmonella spp./50 g	0	0	0	0	0	0	0	0
Sulphito-red. *Clostridium*/g	n.d.	n.d.	n.d.	100	n.d.	n.d.	n.d.	100

Legend: n.d. - not detected (<10/g)

Until 150 days of storage the number of sulphito-reducing clostridia in both types of calf mixtures was similar (below 10/g of sample). Increase in the number of *Clostridium* spp. after 150 days of storage in both types of samples (100/g) was probably a consequence of subsequent contamination. Other pathogenic bacterial species were not determined (Table 2).

Our results are in accordance with the findings of other authors that pelleting conditions do not eliminate all contamining bacteria, but colliforms are killed if pelleting is conducted over 80^{o}C [7]. Sretenović et al. [18] found that *Proteus* spp. were completly eliminated from mushes for lambs and laying hens after pelleting. On the other hand the thermal processing did not eliminate the sulphito-reducing clostridia.

After pelleting, more pronounced reduction was observed in the total count of molds and yeasts – from 26,000/g to 150/g (173 times) at the begining of the storage period (Table 3). The increase of total mold count after 45 days of storage of powdered sample can be explained most probably by recontamination. As in the case of bacteria, mold and yeast number decreased constanty in powdered calf mixture after 45 days until the end of the storage. Absence of molds and yeasts after 90 and 150 days of storage can be explained by the change in storage conditions i.e. lowering of moisture content in pelleted calf mixture and reduction of relative humidity of the air in the calf farm conditions.

Besides that, the number of identified fungal species was much lower in the pelleted calf fodder (6) than in the powdery one (17) (Table 3). It is interesting to notice that during storage period the number of species in powdered calf mixture was slowly decreasing

(from 12 to 10) while in the case of pelleted sample it was constant from the production day to 45 days (3), although different species were identified (Table 3).

Table 3. Fungal contamination of calf feed mixtures during storage

Parameter	**Powdered mixture**				**Pelleted mixture**			
	Day 0	Day 45	Day 90	Day 150	Day 0	Day 45	Day 90	Day 150
Total count of yeasts and molds/g	26 x 10^3	91 x 10^3	69 x 10^3	47 x 10^3	150	150	<10	<10
Identified molds								
Absidia corymbifera	+	+	+	+		+		
Acremonium fusidioides	+		+	+				
Acremonium rutilum	+	+	+	+				
Aspergillus flavus	+	+	+	+				
Aspergillus niger	+	+						
Aspergillus ochraceus		+			+			
Aspergillus versicolor	+							
Aspergillus sp.		+				+		
Cladosporium herbarum	+				+			
Fusarium oxysporum			+	+				
Fusarium subglutinans	+	+	+	+	+			
Fusarium verticillioides	+	+	+	+		+		
Mucor circinelloides f. *circinelloides*	+	+	+	+				
Penicillium aurantiogriseum	+							
Rhizopus nigricans	+	+	+	+				
Scopulariopsis brevicaulis			+	+				
Trichoderma sp.		+						
Total	**12**	**11**	**10**	**10**	**3**	**3**	**0**	**0**

The only common species found in the both types of tested samples were: *Absidia corymbifera, Aspergillus ochraceus, Cladosporium herbarum, Fusarium subglutinans,*

F. verticillioides and one unidentified *Aspergillus* species. The dominant fungal genera in powdered calf feed mixture were potentially toxigenic *Fusarium* (*F. oxysporum*, *F. subglutinans* and *F. verticillioides*) and *Aspergillus* (*A. flavus, A. niger A. ochraceus* and *A. versicolor*) as well as ubiquitous *Acremonium* (*A. fusidioides* and *A. rutilum*).
The dominance of these fungi is not surprising because minimum moisture content for their growth is ranging from 15% (*A. ochraceus*) to 18% (*A. flavus* and *Fusarium* spp.) [10]. Identified molds are common contaminants of fodder ingredients and complete mixtures in the region of Banat (A.P. Vojvodina, R. Srbija) [2].
The mycotoxin of greatest concern for dairyman is aflatoxin B1 (AFL B1), although others that are being studied frequently and which also appear to be of concern to ruminant animals are fusariotoxins: zearalenone (ZON) and type A trichothecenes (DAS and T-2 toxin) (Table 4).

Table 4. Presence of mycotoxins in calf feed mixtures during storage (mg/kg)

Mycotoxin (mg/kg)	**Powdered mixture**				**Pelleted mixture**			
	Day 0	Day 45	Day 90	Day 150	Day 0	Day 45	Day 90	Day 150
Aflatoxin B1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Zearalenone	n.d.	n.d.	0.736	0.368	n.d.	n.d.	0.442	0.221
Trichothecenes (T-2)	0.337	n.d.	n.d.	n.d.	0.337	n.d.	n.d.	n.d.
Trichothecenes (DAS)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Legend: n.d. - not detected (aflatoxin B1 < 0.0004 mg/kg, zearalenone < 0.0123 mg/kg, DAS and T-2 < 0.04 mg/kg)

Aflatoxin B1 (AFL B1) was not detected at all during present investigation, although *Aspergillus flavus,* potential producer of this mycotoxin, was continuously found in the powdered calf mixture from the production day (day 0) until the end of the storage (day 150) (Table 4). This finding can be explained by the fact that *A. flavus* isolates from Serbian commodities are not at all or are weak producers of AFL B1.
Zearalenon (ZON) was found only after 90 and 150 days of storage of both types of calf feed. Most likely, during storage the moisture content arised from initial 11.29% i.e. 11.13% to 17% or more – the minimal value necessary for mycotoxin production. The findings of this mycotoxin can be explained by the presence of molds *F. oxysporum* and *F. subglutinans* during 150 days of storage in powdered sample i.e. *F. subglutinans* in the pelleted sample at the production day (0 day) (Table 3). After 90 days of storage, higher concentrations of ZON in powdered sample (0.736 mg/kg) then in pelleted one (0.442 mg/kg) can be explained by the fact that probably higher inoculum of *Fusarium* conidia was present in powdered calf mixture. The absence of ZON until the 90 days of storage was according to our mind the consequence of longer time period necessary for the production of this fusariotoxin.
Type A trichothecenes (T-2 and DAS). Only T-2 toxin was detected during present investigation at the beginning of storage and in the same quantity in pelleted and powdered sample (0.377 mg/kg). This trichothecene was probably produced before

preparation and pelleting calf feed mixture by *F. oxysporum* and *F. subglutinans* species that were present in one of the feed ingredient, because these species are known as weak T-2 toxin producers in Serbia [12].
Pelleting did not positively affected on mycotoxicological properties of calf feed mixture.
From the production day (day 0) until the expiry date (day 90) pelleted as well as powdered mixture were microbiologically and mycotoxicologically correct according to *Regulations on maximal quantity of harmful materials and ingredients in fodder* [17]. Analysis performed 2 months after expiry date (day 150) showed, with the exception of sulphito-reducing *Clostridium* spp., that total count of other microorganisms (bacteria, yeasts and molds) as well as contamination with mycotoxin were even reduced (Tables 2 - 4).
In vitro experiments with two *F. verticillioides* isolates obtained from powdered calf mixture after 90 days of storage showed that this species did not have potential for ZON, T-2 and DAS biosynthesis. The similar results i.e. that *F. verticillioides* can not biosynthesize ZON and type A trichothecenes were presented in our previous investigation with different *Fusarium* cultures isolated from feed ingredients and complex animal mixtures [3].

CONCLUSION

It can be concluded that pelleting procedure of calf feed mixtures has had the positive effect on improvement of microbiological proprieties of investigated mixtures, but not on mycotoxicological once.
In the case of adequate storage conditions (moistute, temperature, housekeeping measures etc.) calf feed mixture can be used after expiry date because its microbiological and mycotoxicological correctnes can be preserved.

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Triticum aestivum ssp. spelta GRAIN MYCOPOPULATIONS IN ORGANIC PRODUCTION IN VOJVODINA REGION

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ABSTRACT

The investigations in this paper were based on investigating of mycopopulations of wheat that developed in weather conditions of growing during 2008-2009 at Backa Topola location. The investigations included three varieties of *Triticum aestvum* subsp. *spelt* and one variety of *Triticum aestivum* subsp. *vulgare* in the conditions of organic system production. Among causes of moulds, the most distributed were the varieties of *Alternaria* species which infection in unhulled spelt ranged up to 100%. The infection of spelt grain with fungi of *Alternaria* species decreased after the dehulling process and ranged from 16% in the variety Nirvana to 13% in the variety Ostro.
Key words: *grain of Triticum aestivum ssp. spelta, moulds*

INTRODUCTION

Spelt (*Triticum aestivum* ssp. s*pelt*) is an old hulled subspecies of wheat that has lately been grown more frequently in the system of organic certified food for humans and animals. The prerequisites that this wheat has for this very 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 pathogenes (1, 2, 6). Recently, there exist numerous international publicatios that compare the yield and nutritive values of spelt and bread wheat (3, 9, 10, 12, 15).

From the point of view of health safety foodstuffs obtained by the processing of grains of wheats, it is necessary to establish the mycopoulation of each seed party. Wheat grains in the field represent a valuable substrate for the development of great number of phytopathogenic and saphrophyte microorganisms that affect adversely the quality, with a significant number of them producing toxins that are harmful for the health of humans and animals. Fungi most frequently isolated from the grains of wheat come from the species *Fusarium, Alternaria, Mucor, Bipolaris, Epicoccum, Cladosporium, Penicillum* and others (7, 8, 16).

The aim of the investigation in this paper is investigating of mycopopulations of wheat that developed in weather conditions of growing production during 2008-2009 at Backa Topola location. The investigations included three varieties of *Triticum aestvum* subsp. *spelt* and one variety of *Triticum aestivum* ssp. *vulgare* in the conditions of organic system production.

MATERIAL AND METHODS

The following cultivars were included in the study:

- The samples of the three spelt cultivars (*Triticum aestivum ssp. spelta)* Ekö 10, Ostro and Nirvana were collected from Hungary, Austria, and Serbia, respectively, in order to establish organic field trials.
- The wheat cultivar Edevan *(Triticum aestivum ssp.vulgare)* for the organic production was selected in Austria.

The samples of the three spelt cultivars were dehulled by passing the grain between rubber-coated rolls and aspiration to remove the hulls.
Technology of plant growing procedure has been certified for organic production.
The trials were harvested during technological ripening. Grain mycoflora was evaluated following the Petri dish test (11), and the determination of mycopopulations was according to the method described by Barnett (5).
The data were statistically evaluated by the analysis of variance using Statistica 8 program.

RESULTS AND DISCUSSION

The intensity of the appearance of certain species of fungi on the seed of the investigated wheat varieties from the locality of organic certified area of Backa Topola are presented in the Tables 1,2, and Picture 1. Comparing the level of seed infection of different wheat varieties by certain moulds (Table 1), it can be noted that the absolute, thus also the highest infection (100%) is present in all the three varieties of *Triticum aestivum* ssp. *spelt* with hull. Significantly lower is the infection of the very grain of these three spelt varieties after the performed process of dehulling, ranging from 27% in the variety Nirvana (*Triticum aestivum* ssp. s*pelt*) to 32% in the remaining two spelt varieties. The lowest level of the total infection that tells about its genetic resistance to fungi was proved in the variety Edevan (*Triticum aestivum* ssp.*vulgare*) which has been recognized in Austria and selected for growing in organic system of production in only 16%.
Among causes of moulds during 2008/ 2009 vegetation period, the most present were the varieties of *Alternaria* species. Unhulled samples of all the three varieties of *Triticum aestivum* ssp. *spelt* were 100% infected, which represents a highly significant difference in comparison to grain infection after spelt dehulling, ranging from 16% in the variety Nirvana to 13% in the variety Ostro. On the basis of these results, we can say that hull in spelt represents an advantage when we speak of grain protection from moulds infection. High intensity of seed infection of wheats by moulds of *Alternaria* species were also proved by the other authors (7,14). It is known that also the varieties of *Alternaria* species produce secondary metabolites which can prove harmful for hunans and animals (4). High level of infection of wheat grains by moulds from this species points at the need for further investigation of toxicity of these varieties, as well as ecological prerequisites for forming of mycotoxins by *Alternaria* species.
Vegetation season 2008/2009 is characterized by unfavourable conditions for normal wheat development. Cooler weather conditions at the beginning of June 2009, as well as expected rains partially managed to alleviate the consequences of the longlasting spring drought. The quantity of precipitations for the whole territory of Serbia in June reached

128 mm, i.e. it is 60% higher than the many year level for this month (13). Plants that are weakened due to unfavourable environmental conditions represent an adequate substrate for inhabiting of parasites of weakness, like the varieties of *Alternaria* species, so that can represent an explanation for high intensity of the appearance of this fungus on the seed in the current year.

Table 1. Mean values and standard deviations for percentage of diseased wheat grain

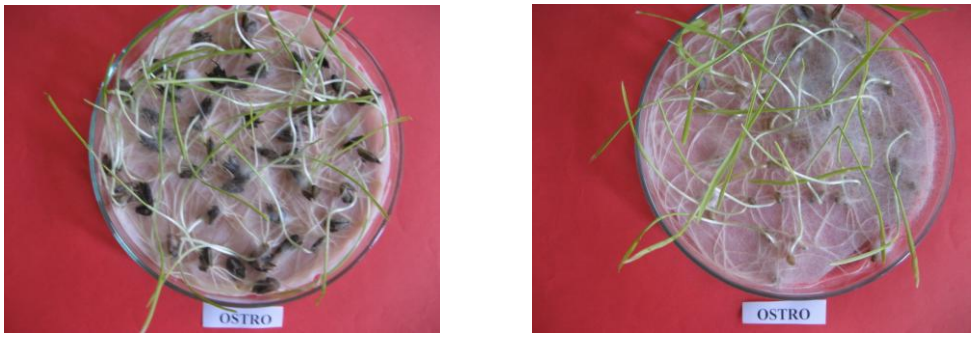
Wheat- cultivars		***Mycopopulation*(%)**				**Diseased grain (%)**
		Fusarium spp.	***Alternaria spp.***	***Mucor spp.***	***Penicillium spp.***	
hulled	*Triticum aestivum* ssp. spelta - Nirvana	6 ± 1,00ab	100 ± 0,0b	0	0	100 ± 0,00 c
	Triticum aestivum spp. spelta - Ostro	8 ± 1,15ab	100 ±0,00b	0	0	100± 0,00c
	Triticum aestivum spp.spelta-Ekö 10	4 ± 2,08 a	100 ±0,00b	0	0	100±0,00 c
dehulled	*Triticum aestivum* ssp. spelta - Nirvana	7 ±1,00ab	16 ± 0,00 a	0	4 ± 1,00	27 ± 5,27 b
	Triticum aestivum ssp. spelta - Ostro	10 ± 2,00b	13 ± 3,05 a	9 ±1,00	0	32 ± 3,00 b
	Triticum aestivum ssp. spelta -Ekö 10	8 ± 1,00ab	14 ± 1,57 a	8 ±1,15	2 ± 0,00	32 ± 2,80 b
	Triticum aestivum ssp.vulgare - Edevan	4 ± 1,00 a	12 ± 1,00 a	0	0	16 ± 2,51 a

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

Varieties of *Penicillium* ans *Mucor* species do not cause symptoms of the disease in wheats, but are considered to be saphrophytes that can inhabit substrates rich in carbohydrates and produce harmful mycotoxins in them (7). Among these fungi, the most distributed proved to be the varieties from *Mucor* species which were established in the variety Ostro (*Triticum aestivum* spp. *spelt*) in the intensity of 9%. (Table 1, Fig.1).

Table 2. Percentage of some fungus genus in cereal seed mycopopulations

Wheat- cultivars		***Mycopopulation*** (%)			
		Fusarium spp.	*Alternaria spp.*	*Mucor spp.*	*Penicillium spp.*
hulled	*Triticum aestivum ssp. spelta - Nirvana*	6,0	100	0,0	0,0
	Triticum aestivum spp. spelta - Ostro	8,0	98,0	0,0	0,0
	Triticum aestivum ssp.spelta -Ekö 10	4,0	100	0,0	0,0
dehulled	*Triticum aestivum ssp. spelta - Nirvana*	25,9	59,3	0,0	14,8
	Triticum aestivum ssp. spelta - Ostro	31,2	40,6	28,1	0,0
	Triticum aestivum ssp. spelta -Ekö 10	25,0	43,7	25,0	6,3
	Triticum aestivum ssp.vulgare - Edevan	25,0	75,0	0,0	0,0



a *b*

Fig. 1 Petri dish test for varietes Ostro (Triticum aestivum ssp. spelta)
a (hulled), b (dehulled)

CONCLUSION

Vegetation of the productive years 2008/2009 especicially favoured to mould development. Among causes of moulds in these years, the most present were the varieties of *Alternaria* species which infected the hull of unhulled spelt up to 100%. Hull in spelt is an advantage when speaking of grain protection from mould infection, because the infection of grains after spelt dehulling ranged from 16% in the variety Nirvana to 13% in the variety Ostro. The solution for growing plant species within organic production lies in selection to pathogens resistance. The lowest percentage of the total infection was established in the variety Edevan (*Triticum aestivum* ssp. *vulgare*) which has been recognized in Austria and selected for growing in the organic system production.

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PROGRAMS OF NATURAL FOOD AND ALTERNATIVE SYSTEMS IN PRODUCTION OF TABLE EGGS

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ABSTRACT

Industrial production of chicken meat provides meat for consumers throughout the year in large quantities and at relatively affordable price. Relatively poor quality of this meat (watery meat of not so distinct taste and aroma, high share of subcutaneous and belly fat, weak and breakable bones, etc.), as well as reduction of prices because of high supply, and certain nostalgia for „good old times", have influenced the perception of broiler meat as unhealthy and unnatural. On the other hand, changes in main principles of production of chicken meat in EU countries, as well as tendency for harmonization of our legislation with EU Directives relating primarily to preservation and improvement of farm animal welfare, environment protection and food safety, have caused intensive growth of numerous programs for production of natural (ecological, biological, organic) food in our country.
Key words: *table eggs, alternative systems, program of production, quality of eggs*

INTRODUCTION

Industrial production of table eggs began to develop rapidly in USA after the 2nd World War, and later also in West Europe. It is characterized by closed rearing facilities, artificial light and air conditioning, large number of highly productive hybrid layer hens in limited space, almost by rule in cage batteries, use of commercial food mixtures (with different additives – antibiotics, coccidiostatics, various supplements, hormones, etc.), application of numerous preparations for prevention and treatment of diseases and maintenance of hygiene in farm facilities. This production provides to consumers table eggs throughout the year in large quantities and relatively low price. Intensive, industrial egg production has very interesting development over last 20 years. Egg as feed stuff was unfairly accused of being harmful to human health. However, in recent years, this injustice is slowly corrected.

The fact that new organism is formed from the egg – chicken, is confirmation that egg is ideal feed stuff rich with nutritive substances. Egg is source of protein of the highest quality and it has unique combination of fatty acids which are necessary for human organism and these facts are used in promotion of egg as product of extremely rich nutritive composition and content.

Although 75% of total world production of eggs is performed in cage system with different housing density, in developed World countries considerable amounts of money are invested in new systems for housing of layers. Many participants in the chain of production of table

eggs have developed serious marketing of production and sale of eggs, in accordance with new legislative regulations of European Union (directives) relating to poultry welfare, aiming to increase the consumption of table eggs [17].
New systems in housing of poultry (improved cages, free range, semi-intensive system, deep litter, aviary system, organic production, etc.) provide primarily welfare of layers rather than improve the quality of table eggs. Considering that in countries of EU 92% of layers are still housed in cages it is normal to expect considerable decrease of egg production when new regulations/directives relating to minimum floor space per bird come into effect. Decrease of egg production in EU countries will result in decrease of profit, increase of price and reduction of number of employees.
Increased cost of alternative productions and at the same time of retail price of eggs from such production systems will certainly have effect on the decision of consumers regarding purchasing of this product. Question is whether the consumers are ready to pay higher price for poultry welfare or special guaranteed quality of eggs. Most of the polled consumers consider safety and freshness when purchasing eggs [24, 25]. Production system is also factor influencing the preferences of consumers, in other words entire concept which includes farm conditions, poultry welfare, nutrition, health condition, size of flock, etc. There are two categories of consumers, ″citizen of the world″ willing to pay more for specific product and the other category ″consumer″ who is expressing his concern about the production system but is not willing to pay more for such production.
New systems of production of table eggs, marketing and systems for monitoring of egg on their way to consumer and other way around from consumer to producer will be presented in this paper.

SYSTEMS OF PRODUCTION TABLE EGGS

In production of table eggs many different systems and methods are used which can be classified and categorized in different way. For this occasion the categorization in EU which is the simplest and most current will be accepted and presented briefly.
Battery (cage) system has been massively used in so called industrial production on farms with several tenths of thousands, even hundreds of thousands of layers, but also numerous farmsteads and agricultural households with small or medium flocks of layers. Hens are housed in cages grouped in so called batteries. According to number of tiers (cage levels) batteries are single tier or multiple tier (2 to 8 tiers). According to the position of cages, multi-tier batteries can be step deck, semi-step deck and vertical. In regard to the dropping/manure removal, they can be fitted with dropping channels, deep dropping lagoon and conveyers. One of the modern types of batteries combines ventilation of the facility with drying of manure on conveyers to the level of dryness of 70%. Some of the operations and procedures in batteries (feeding watering, collecting of eggs, manure removal) are manual, mechanized or automatic in different combinations (e.g. all manual procedures in simple batteries of small capacity, to fully automated procedures managed by PC). Number of hens

in battery cage can also differ: from one (individual cages to 2,3,4,5 and more hens (group cages). Mainly cages with 4 or 5 hens are used.
Battery system was created by the beginning of 20th century, and its rapid expansion started after 2nd World War and in countries with developed poultry production over 90% of hens in production of table eggs are housed in this system. However, many consumers, especially so called animal protection activists, perceive cage system as unnatural and inhumane. Therefore, this system was even prohibited in some countries, in Switzerland for instance it is still prohibited, and starting from 2012, in its present form, it will be prohibited in European Union. From that point on, in European Union only so called enriched cage systems will be allowed, i.e. significantly more spacious cages for larger groups of hens equipped with perches and sand troughs for scratching and plucking.
Non-battery systems in European Union include: a) production of eggs from free range, b) eggs from semi-intensive system, c) eggs from deep litter and d) eggs from aviary system.

a) **Eggs from free range** are produced by hens which have permanent access during day to free range predominantly covered with vegetation. This is type of pasture with maximum stocking density is 1000 hens per hectare (min. 10m² per hen). Interior of the hen house must comply with conditions stated under c) and d). Within this system **organic production** of table eggs is organized, where in nutrition of hens organically produced feeds are used without pesticides and in compliance with other conditions and requirements issued by competent organization and producer has to become member.

b) Eggs from semi-intensive system are produced by hens which have permanent access during the day to open grass range, of maximum stocking density of 4000 hens per hectare (min. 2.5 m² of pasture per hen). Interior of the hen house must comply with conditions stated under c) and d).

c) Eggs from deep litter are produced by hens housed in hen house with the maximum stocking density of 7 hens per 1 m². At least 1/3 of the floor space must be covered with litter of materials such as straw, sand, peat, etc. Sufficient part of floor surface available to hens (1/3 to 2/3) is used for collection of hen droppings/manure, i.e. it has to be covered with grids or wire net.

d) Eggs from aviary system are produced by hens housed in hen house with the maximum stocking density of 25 hens per 1 m² of floor surface. Interior of the hen house is equipped with perches, often in several levels, and for every hen perch of at least 15 cm of length has to be provided.
Poultry farms which produce eggs in stated non-battery systems are registered as such, regularly controlled by inspection of the competent government authority and are obligated to keep records issued by regulations (date of housing of hens, number and age of hens at housing and according to production systems, number of eggs produced and delivered by dates, date of delivery of eggs and name of the buyer, etc.). Centres for packaging of eggs are authorized to use the name of the system of non-industrial production on retail packages and on eggs, as well as to keep compulsory records on production of these eggs. Since eggs derive from non-battery systems they are more expensive compared to eggs from battery system and for potential misuse EU regulations have foreseen strict punishments.

In close relation to systems of production of table eggs which are based on housing systems are types of eggs which include some additional characteristics. These are i.e. fertilized eggs, eggs from hens which were fed mixtures without animal by products, eggs from hens whose nutrition consisted of high percentage of corn, eggs with high content of semi-unsaturated fatty acids and/or small content of cholesterol, eggs with high content of vitamin B, eggs enriched with Omega 3 fatty acids, eggs enriched with iodine, eggs with high content of vitamin D, eggs containing substances which have favourable effect against allergies and eggs with two yolks. Eggs whose content of certain nutritious substances has been intentionally altered are called designer eggs.

THE EFFECT OF THE HOUSING SYSTEM ON EGG QUALITY

Today, in production of table eggs, the cage system is mainly present or as it is popularly known as ″non-enriched cage″ and over 90% of layers of table eggs are housed in this system. New regulations of the European Union determine the minimum of 550 cm^2 of floor space per bird in cages. This minimum standard has to be applied until 2012 when battery system in poultry housing will completely be prohibited. It is expected that application of this directive will lead to considerable decrease in production of table eggs in EU and induce import of eggs from non-member states. Decrease in production of table eggs will result in reduced profit and number of employees. On the other hand, non-member states which are not in position to comply with these regulations and standards in housing of layers, due to lower production costs and higher production have opportunity to increase the sale of table eggs on EU market. Globally, application of new directives will have no general effect on improvement of poultry welfare, it will cause regionalization of the problem.

Production of table eggs used numerous strategies in order to create products of special quality and known origin (brand egg). New production systems have developed first in EU countries and later in other developed world countries aiming to provide poultry welfare and satisfy the requests of consumers of eggs of special and guaranteed quality. This relates first of all to eggs from free range, eggs from semi-intensive system, eggs from deep litter and enriched cages which provide primarily poultry welfare. On the other hand, enriched eggs appeared on the market, eggs enriched with omega 3 fatty acids, selenium, certain vitamins, etc. which satisfy specific demands of certain consumers. Eggs from organic production are specific since it is still not confirmed that these eggs have better quality than conventionally produced table eggs, however they have certain place on the EU market.

Many participants in the chain of table egg production have developed serious strategy of production and marketing of eggs based on new regulations of European Union relating to poultry welfare, origin of eggs and consumer opinions [9, 7, 3, 21, 18, 2]. Freshness and safety of product are still two main criteria for purchasing of table eggs, however production system as one of the important factors is also considered by consumers when purchasing the products.

Comparative investigation of different production systems ant their effect on egg quality was research topic of many researchers and obtained results are very different. In spite of

numerous researches there are no conclusions in favour of any system of poultry housing whether in relation to production results or egg quality [32]. Advantages and disadvantages are present in every system, but it is obvious that in alternative systems the welfare of layers is improved. Effect of housing system for layers on egg quality is minor, but presence of dirty eggs and microbiologically contaminated egg shell is higher in alternative systems [27, 4]. [29] established that there was no difference in quality of egg white expressed in HU between eggs from cage and free range system. In recent researches of the effect of housing system on production results and egg quality, in accordance with new regulations of European Union banning the cage system and regulating the standards for floor space per bird in cages, there are also opposite results which will be stated in this paper. [5, 28] haven't determined any significant effect of housing system on egg quality and [1] between quality of eggs from cage and aviary system. In two recent papers the quality of egg white (HU) was compared in eggs from cage, aviary and free – range system: in the first paper, better quality of egg white was established in eggs from aviary system [8], and in the other paper by [21] the authors have established better quality of egg white from free-range system. [10] investigated quality of egg shell of eggs from enriched cages and cage system and established higher percentage of dirty, cracked and eggs contaminated with bacteria, also that egg shell thickness was greater in eggs from enriched cages. Lower egg mass and better quality of eggs in aviary system compared to battery system was established by [6]. [30, 31] compared the percentage of dirty and cracked eggs in several models of enriched cages and established that there is difference between investigated models, but percentage of dirty and cracked eggs was lower than in any other alternative housing system for poultry. Quality of eggs was superior in layers housed in cages. [13] determined that eggs from layers fed only plant diet had 65.9 HU, eggs from layers from system respecting the poultry welfare 62.2 HU, eggs from layers fed non-standard diet 61.2 HU, fertilized eggs 61.2 HU and organic eggs 57.6 HU.

Research in our country

In our country, the research of the quality of eggs from layers housed in different systems started in 1967. Results of investigation of exterior and interior quality traits of eggs from conventional battery system, free-range system, deep litter and aviary system are presented in table 1. It can be concluded from data presented in table 1 that eggs from free range system had the best interior quality of egg white expressed through albumen height and HU and most intensive yolk colour. (Table 1)

Table 1. Some characteristics of eggs produced in different housing systems

Characteristics	Žigić et al. 1967 [33]		Pavlovski et al.1981 [22]		Pavlovski 1982 [16]		Pavlovski and Mašić 1986 [23]		Mašić and Pavlovski 1994 [12]			Pavlovski et al. 2001 [21]			Pavlovski et al. 2002a [19]	
	I	II	I	II	I	II	I	II	I	II	III	I	IV	III	I	II
Egg mass, g	63.0	62.8	57.7	59.4	66.3	59.6	64.4	57.5	63.1	62.2	61.3	63.7	62.5	61.2	67.7	60.7
Shape index, %	75.1	74.1	73.6	74.6	75.4	74.1	75.8	73.2	76.2	76.3	75.5				76.0	73.5
Shell colour, points	2.51	2.94	3.69	3.58	3.30	3.00	3.16	3.02	3.72	3.49	3.41	3.61	3.45	3.36	3.55	3.41
Albumen height, 0.1mm	51.3	59.9	49.9	56.2	47.3	56.4	72.6	77.6	70.0	64.1	66.1	77.2	77.1	77.3	72.6	77.6
Haugh Unit	66.6	73.9	66.7	71.6	60.6	70.2	82.6	88.1	79.8	75.9	78.2	85.5	84.8	87.2	82.5	88.0
Yolk colour, Roche	4.83	7.00*	9.44	13.6	8.80	11.0	9.74	12.8	9.84	9.98	10.2	10.5	10.3	11.2	9.74	12.6
Shell thickness, 0,1mm			31.9	34.6	36.0	34.3	35.1	32.7	35.5	35.8	36.7	37.2	37.7	36.2	35.1	32.7

I – cage , II – free-range, III – deep litter, IV – aviary

* - intensity of yolk colour, graded 1-12, was determined by comparosion with the colour of varying contrentracion of potassium bichromate solution (0.5-20 mg/ml water in tubes R 5 x 16 mm).

Marketing strategy and consumer attitudes - It is well known that in every production chain, the most important link is the final one – consumer. For each production, even production of table eggs, it is very important to know why consumers are purchasing specific product and what preferences consumers have in relation to that product. It is paradox that poultry production in many countries of the world, even in Serbia, over the period of several decades, has developed suddenly and become industrialized – revolution in livestock production, but almost no attention was directed to researches of consumer demands and marketing strategies. Even in countries with developed poultry production, first among few researches of the consumer relations to poultry products appeared in late sixties. However, in eighties these researches became very up-to-date and intensive, today on poultry scientific meetings researches of consumer attitudes and marketing strategies are given outstanding position. Favourable circumstance is that in our country considerable attention was directed to researches of the consumer relation to poultry products. Numerous researches directed in various directions have been carried out [15, 24, 25, 20]. It is interesting to mention that in year 1981 70,6% of questioned consumers thought that battery system was acceptable as production method, and one decade later this percentage was reduced to 54,6%, and two decades later to 35,6%. During this period the percentage of consumers in favour of banning of cage system almost doubled from 6,4% to 10,3% and 13,2%, respectively. In mentioned research years number of consumers which were willing to pay higher price by 10% for eggs of guaranteed and controlled quality or from free-range system increased from 46% to 63% and 71,5%. Obviously, number of consumers adherent to the free-range and banning of battery system on Belgrade market increased considerably which is in accordance with similar trends and new directives of EU.

PROGRAM OF PRODUCTION OF EGGS IN FREE-RANGE SYSTEM

The most serious and comprehensive program defined as Agrobiological system „Natura Vita"[14] at that time was member of International Federation of Organic Agriculture Movements (IFOAM). Starting from basic principles and rules contained in this system [11] developed the program of production of table eggs within Agrobiological system „Natura Vita*"*. Researchers of the Institute for Animal Husbandry, Belgrade-Zemun [26] developed the Program of production of table eggs of special quality, financed by several producers from Republic of Serbia. In this paper, because of limited space, we will present only basis of the stated Program.

SHORT REVIEW OF TECHNOLOGICAL PROCESS

Building and equipment- Segments of the grid floor are placed in the hen house, and subsequently 2/3 of the floor is covered with dry litter in a layer of 30 cm. Then, hanging feeders and nests with clean litter are placed and functioning of the light and watering equipment is checked, in order to eliminate or rectify potential mistakes and omissions in operation of the equipment.

Selection of layer hens – For production of table eggs in this Program hybrids are used which are not common or used in industrial production. In our conditions domestic indigenous hens can be used, naked neck hen, domestic populations of Rhode Island, New Hampshire, Amrok, Plimuth rock hens and crosses obtained from crossing of these breeds.

Housing of hens into hen house is done at the age of 18 weeks. First the distant parts of the hen house are housed and then parts closer to the door, and finally food and water consumption of birds is monitored. Two to three days after housing hens are released to free range – pasture. In the morning they are given food in feeders on the free range, and feeders are moved gradually away from the building and closer to the fence of free range.

Free range – pasture - At the age of 18 to 20 weeks to the end of laying (not longer than 72 weeks of age). On free range feeders and drinkers are placed. It is desirable to have trees within pasture or on its edges which would be used as shade for chickens to hide from hot Sun. If there are no trees it is necessary to make some kind of eaves. Pasture for poultry is source of protein, minerals and vitamins A, B, E and C. With additional nutrition using concentrated feeds, especially alfalfa meal with addition of vitamin D poultry can utilize free range exceptionally well.

Nutrition - Nutrition is process of digestion, adoption/absorption and conversion of food into tissues and energy in the hen organism. Therefore it is considered that nutrition, as well as housing, has decisive role and effect on production and quality of table eggs and poultry meat. For the purpose of production of table eggs of special and guaranteed quality it is necessary to provide special nutrition of poultry. Main nutrition principles are:

- Cereals are basis of the diet (minimum share of 70% in full mixtures except in starter mixture - min. 50%);

- No animal additives, feeds produced from GMO ingredients;
- Limited number of additives;
- Green and juicy (root-tuberous) feeds.

Change of the housing system requires adequate nutrition based on mixture of cereals and concentrate mixture, with usual mineral and vitamin additives. In accordance to principles of production of natural food and some requirements of European market, natural additives are introduced to these diets which are used to improve production results and quality of table eggs. These new additives are enzymes, probiotics, prebiotics, phytobiotics (medicinal herbs) and mycotoxin adsorbents.

By application of ***enzymes*** in poultry nutrition better utilization of certain nutritious substances is achieved for the purpose of obtaining of better production results (laying ability, number and mass of eggs), better quality of eggs and lower mortality. Special importance has enzyme phytase which improves utilization of phytine phosphorus (up to 30%) and reduced environment pollution.

Probiotics help development of useful to contrary to harmful (pathogen) micro organisms in digestive tract. By application of probiotics the health condition is improved, better production results are realized as well as better quality of eggs.

Prebiotics are non-digestible feed components (carbon hydrates, some peptides or lipids) which have beneficial **effect** on host through stimulation of development of desirable bacteria with simultaneous limiting of development of undesirable bacteria in digestive tract of the poultry. By application of prebiotics optimal production results are achieved, the food utilization is improved as well as the vitality of poultry.

Phytobiotics and their extracts have wide spectrum of utilization: they stimulate the food consumption and endogenous enzyme secretion, they have antimicrobial and coccidiostatic effect, they improve production results, they have beneficial impact on health of poultry and quality of products. Medicinal herbs which can be used as additives are camomile, lemon balm, mint, anise, yarrow, thyme, basil, etc.

Presence of mycotoxins in food for layer hens causes numerous problems, from negative effect on production results to different health problems which often end in death. They can be generated in contaminated food during process of production or storage. In strategy against mycotixins in poultry nutrition the best results were established when **adsorbents** were used. It is important for all adsorbents to have high specificity of absorbance, high affinity and adsorbing capacity and to protect as much as possible poultry from negative effect of mycotoxins. It is necessary to identify the mycotoxin and determine the most efficient adsorbent which would eliminate or at least alleviate the detrimental effect of mycotoxins on production results in poultry.

Water is essential nutritious substance, main component of the organism of poultry and necessary for all functions of the organism. Therefore, microbiologically and chemically safe water must at all times be available to hens.

Environment and production conditions

In order to realize satisfactory production results in extensive system of production of table eggs it is necessary to fulfil certain conditions.

Temperature: For production of table eggs optimal temperature is 22º C. Relatively high production efficiency is realized in wider spectrum of temperatures from 13ºC -26º C.

Ventilation/air flow and air humidity: Requirements of hens in fresh air are usually expressed per 1kg of their body mass – maximum is 4-5 m³ of air per hour and per kg. Relative air humidity in the space where hens are housed should be 50-70%. During very hot days corridors and litter can be additionally watered, and air mist sprayed in the hen house and on free range. High air humidity in cold days is very difficult to reduce.

Light program: Extensive system of housing of layer hens anticipates for prolonging of natural day light with artificial light, but total duration can not exceed 16 hours. Natural light is provided through open sides of the hen house, i.e. windows made of plastic foil. Artificial light is provided by electric bulbs of power of 4.5 W per m².

Floor surface and free range space: Program anticipates maximum 7 hens per 1m² of floor surface and access for hens to free range of at least 10 m² per hen

Feeding space: Program anticipates for hens at least 13 cm of feeding space on feeder or maximum 20 to 25 hens per one hanging cylinder feeder.

Drinking space: If drinkers (nipple) are used for water supply, one nipple is sufficient for 4 to 5 hens. In hen houses, along the corridor and on both sides above feeder troughs are water lines with nipples at distance of 24 cm. near feeders on the free range there should be drinkers, 4 with nipples, individually 2.5 m long with reservoirs of 50 l.

Nests: Program anticipates that hens are provided with group or individual nests with litter of natural material, where individual nest is for 5 to 7 hens, and 80 hens per 1 m² of surface of the group nest.

Health protection: Layer hens in extensive system, considering the open space available to them, must have very thorough and efficient protection. Health care is provided by application of series of protective measures and control of the health condition of hens and it is divided into general and specific. *General protection* includes those measures which are considered during planning of the building and production facility which contribute to safety and prevent introduction of infectants to the farm and their spreading within the farm or from farm to the surrounding environment. *Specific protection* relates to protection from diseases which occur on our geographical territory and it is realized through vaccination program. *Control of the health condition* is realized by clinical observation of hens and laboratory examination of died birds or their organs for the purpose of establishing of diagnosis and successful treatment of diseased flock.

Procedure with eggs: Eggs should be collected as often as possible, at least 4 times during laying period (from early morning to early afternoon). After collecting, eggs are placed in special storage room (chamber) with temperature of 10-15º C and relative air humidity of 70-75 %. Eggs with dirty or cracked shell, as well as eggs which are not for the market (bellow 45 g or above 75 g, of irregular shape, etc.) are consumed on the household or sold to local buyers. This Program determines minimum conditions in regard to quality of eggs. Eggs of

guaranteed and special quality are exclusively non-fertilized fresh eggs in egg shell that hasn't been washed or mechanically cleaned and categorized/sorted one day after production. In order for these eggs to have recognizable image, special packaging is designed in form of boxes or baskets. Beside adequate label, registered commercial name and all data issued by the Regulation, packaging should also contain information about the housing system of layer hens, composition of used food, max. period of time from laying to supply to the store, origin of eggs, minimum quality requirements, organization controlling the quality, storage conditions and shelf life at given conditions. In this Program it is determined that eggs have at the moment of delivery to the place of sale (average for entire package of eggs): minimum 70 Haugh units, egg yolk colour minimum 12 Roche units and egg shell thickness of at least 0.35.

CONCLUSION

Recommended Program of production of table eggs will result in product with slightly lower content of cholesterol and ideal ratio of unsaturated and saturated fatty acids, i.e. product of known and guaranteed quality, which will satisfy the demands of the domestic and foreign consumers who prefer natural food.

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INVESTIGATION OF THE EFFECT OF CITRUS FENNEL AROMA ON PRODUCTION, USE OF NUTRITIOUS SUBSTANCES AND SLAUGHTER RESULTS IN FATTENING CHICKENS

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ABSTRACT

The effects of addition of aroma Citrus Fennel in the nutrition of fattening chickens were studied. Obtained results showed that positive effects of use of investigated aroma are reflected in increase of body mass of chickens by 5.5%, increase of daily gain by 5.72%, increase of feed intake by 2.4%, improvement of feed conversion ratio by 3.1%. Addition of investigated aroma had no significant effect on protein and energy balance in chicken organism. Amount of abdominal fat decreased by 15.5% by adding of Citrus Fennel aroma to mixture for the chickens.
In general, obtained results showed that by introduction of aroma Citrus Fennel a positive effects in the nutrition of fattening chickens were realized.
Key words: *Citrus fennel, performance, balans, slaughter results, chickens*

INTRODUCTION

Limitation and prohibition of use of antibiotics as growth stimulators and promoters in animal nutrition have lead to need for application of additives of plant origin [4, 13], mainly extracts of medicinal herbs and spices.
Common trait of all plant extracts is their aroma. Due to differences in chemical composition it is plant specific. Chemical analysis of the composition of plant extracts indicates diversity of chemical substances which have not only aromatic ingredients, but also different biological effect.
Plant extracts and oils have antimicrobial, antivirus, antifungal, antioxidative and immuno stimulating properties [14, 3, 1, 4]. Also, there are data indicating that [18, 13] extracts promote secretion of bile and activity of the pancreas enzymes. Also, some plant extracts can reduce stress which has negative impact on immunological system [17, 16].
Addition of semi-synthetic aroma (sukran-810) to food which balances the sweet taste and aroma has positive impact on consumption of food and broiler growth [2].
Regardless of the realized research on multiple effect and action of plant extracts and oils, there are relatively few literature data on the effect of aroma citrus fennel on growth, utilization of nutritious substances and slaughter results in poultry.
Because of fore mentioned, objective of this work was to determine the effects of the introduction of stated aroma on growth rate, food consumption and feed conversion ratio, utilization of nutritious substances and slaughter results in fattening chickens.

MATERIAL AND METHODS

Trial was carried out on 200 one day old male chickens of 4-line hybrid Peterson, divided into the two groups. First, control group, of chickens was fed mixture without additive, and the second, trial group, received in the same mixture addition of 300 g/tons of food of Citrus Fennel aroma, produced in the production facility of the company Ireks Aroma, Zagreb, Croatia.

So called "three phase" nutrition system was used with starter, grower and finisher mixtures. From the day 1 to 17., chickens received starter mixture which contained 21.98% of crude proteins and 2.907 kcal/kg of metabolizable energy. From day 18 to 39, chickens were fed grower mixture with 20.02% of crude proteins and 3.069 kcal/kg ME. During final period, mixtures contained 18.30% of crude proteins and 3.163 kcal/kg ME (Tabela 1).

Table 1. The composition of diets in the experiment

The diets	**Starter**		**Grower**		**Finisher**	
Group	1 control	2 experim.	1 control	2 experim.	1 control	2 experim
Citrus Fennel aroma	-	0.03	-	0.03	-	0.03
	%	%	%	%	%	%
Corn	32.0	31.97	29.0	28.97	40.0	39.97
Wheat	20.0	20.0	27.0	27.0	19.0	19.0
Sunflower oil meal	11.0	11.0	14.0	14.0	14.0	14.0
Soybean oil meal	27.0	27.0	18.0	18.0	17.0	17.0
Fishmeal	4.0	4.0	4.0	4.0	0.5	0.5
Oil	3.0	3.0	5.0	5.0	6.0	6.0
Salt	0.1	0.1	0.2	0.2	0.25	0.25
Limestone	1.0	1.0	1.1	1.1	1.25	1.25
Dicalcium phosphate	1.33	1.33	1.1	1.1	1.45	1.45
Coccidiostatic Sygro	0.05	0.05	0.05	0.05	-	-
Enzyme	0.1	0.1	0.1	0.1	0.05	0.05
Vitamin-mineral premix	0.25	0.25	0.2	0.2	0.2	0.2
L-lysine	0.04	0.04	0.14	0.14	0.20	0.20
DL-methionine	0.13	0.13	0.11	0.11	0.10	0.10
Citrus Fennel aroma	-	0.03	-	0.03	-	0.03
Total:	100.00	100.00	100.00	100.00	100.00	100.00
The chemical composition:						
Crude protein, %	21.99	21.99	20.02	20.02	18.30	18.30
ME, kcal/kg	2907.0	2907.0	3069.0	3069.0	3163.0	3163.0
Lysine, %	1.20	1.20	1.10	1.10	0.97	0.97
Methionine, %	0.52	0.52	0.48	0.48	0.42	0.42
Calcium, %	1.00	1.00	0.98	0.98	0.92	0.92
Available phosphorus, %	0.44	0.44	0.40	0.40	0.39	0.39

As criteria for assessment of obtained results the following indicators were used: daily gain of chickens, daily food consumption, consumption of food for 1 kg of growth in different periods and mortality of chickens.

At the end of trial, on slaughtered chickens, 6 birds from each group, analysis of slaughter results was carried out.

Protein and energy retention was established by chemical analysis of 5 male chicken cadavers at the beginning and the end of trial according to method by [9].

Obtained results were processed statistically according to method by [21].

RESULTS AND DISCUSSION

The possibility for introduction of aroma citrus fennel into nutrition of fattening chickens was investigated.
Obtained results of average body masses obtained on 17th, 39th and 46th day are presented in table 2. Introduction of 300 g/t of Citrus Fennel aroma into mixture caused statistically significant (P<0,001) increase of body mass of chickens during the initial period. During the second and third fattening period, i.e. 39th and 46th day, body mass of chickens from the experimental group which were fed Citrus Fennel aroma in mixture, was higher by 4.6% (P<0.05) and 5.4% (P<0.01), respectively, compared to control group of animals fed mixtures without Citrus Fennel.

Table 2. Performance of chickens in the experiment

Group	**1 control**	**2 experimental**
Citrus Fennel aroma	-	0,03
Body weight of chickens:		
- at 17th day, g	349	404***
In the comparison at 1st group, %	-	+ 15.82
- at 39th day, g	1616	1691*
In the comparison at 1st group, %	-	+ 4.64
- at 46th day, g	1969	2077**
In the comparison at 1st group, %	-	+ 5.48
Average daily gain of chickens, g		
- from 1st – 17th day	18.19	21.43
In the comparison at 1st group, %	-	+ 17.81
- from 18th – 39th day	57.59	58.63
In the comparison at 1st group, %	-	+ 1.80
- from 40th – 46th day	54.70	63.90
In the comparison at 1st group, %	-	+ 16.81
- from 1st – 46th day	41.9	44.3
In the comparison at 1st group, %	-	+ 5.72

*) P < 0.05
**) P < 0.01
***) P < 0.001

Average daily gain of chickens in trial group during initial period was by 17.81% higher compared to control group (Table 2). During the second and third fattening period, the gains established in group of chickens fed mixtures containing Citrus Fennel were still higher, so during entire trial, the addition of Citrus Fennel had improved the gain by 5.72% compared to control group of animals.

Similar to gain, also daily food consumption during initial, the second and the third fattening period was higher by 13.9%, 1.5% and 1.5%, respectively (Table 3) in trial group fed mixtures based on Citrus Fennel. During the entire trial, addition of Citrus Fennel in the experimental group improved food consumption by 2.4% compared to the control group of chickens.

Table 3. Feed consumption of chickens in the experiment

Group	**1 control**	**2 experimental**
Citrus Fennel aroma	-	0.03
Average daily feed consumption, g		
- from 1st – 17th day	27.2	31.0
In the comparison at 1st group, %	-	+ 13.97
- from 18th – 39th day	118.50	120.24
In the comparison at 1st group, %	-	+ 1.46
- from 40th – 46th day	137.77	139.82
In the comparison at 1st group, %	-	+ 1.49
- from 1st – 46th day	85.83	87.87
In the comparison at 1st group, %	-	+ 2.38

In initial trial period, from day 1 to 17, no significant difference in feed conversion ratio between the investigated groups was established (Table 4). During the second and third fattening period, feed conversion ratio had improved, so for entire trial period food consumption for 1 kg of growth improved with the adding of Citrus Fennel by 3.1% compared to the control group fed mixtures without investigated additive.

Table 4. Feed conversion ratio of chickens in the experiment

Group	**1 control**	**2 experimental**
Citrus Fennel aroma	-	0.03
Feed conversion ratio, kg		
- from 1st – 17th day	1.495	1.502
In the comparison at 1st group, %	-	- 0.4
- from 18th – 39th day	1.949	1.936
In the comparison at 1st group, %	-	+ 0.67
- from 40th – 46th day	2.512	2.187
In the comparison at 1st group, %	-	+ 12.94
- from 1st – 46th day	2.045	1.983
In the comparison at 1st group, %	-	+ 3.03

Positive effects of adding of Citrus Fennel on the growth of chickens established in our study are similar to obtained results [8] who compared the effect of avilamycine and 150 ppm and 300 ppm XTACT, who established the increase of body masses by 4.7%, 5.4%

and 8.1% as well as improvement of food conversion by 5.8%, 3.1% and 7.1%. Addition of Digestovet to food after 42nd day caused increase of body mass of chickens by 7.6% with considerably more favourable food conversion [12]. Addition of the sage, thyme and rosemary extracts improves only the growth of chickens without the influence on feed intake and feed conversion ratio from 14th to 21st day [6]. Addition of ether extracts to the mixture (Crina Poultry) improves gain by 2% and food conversion by 5% and reduces the viscosity of the intestinal content and share of chickens with adhesive excrements. In two separate trial on young turkeys, live weight in 16th and 18th week increased by 3.9% and 4.4% due to the nutrition with Digestar [19]. However, other authors [11] point out that thymol and its isomer carvacrol have different effect on growth and triglyceride metabolism in the broilers.
In our study, addition of Citrus Fennel to the mixture caused certain decrease of dry matter in chicken organism. Content of crude proteins did not change, but crude fats decreased statistically significantly ($P<0.01$) in broiler carcasses (Table 5).

Table 5. Chemical composition of roasted chickens in the experiment

Group	**1 control**	**2 experimental**
Citrus Fennel aroma	-	0.03
In dry matter		
Dry matter	37.24	35.89
In the comparison at 1st group, %	-	- 3.62
Crude protein	53.85	53.13
In the comparison at 1st group, %	-	- 1.34
Ether extracts	34.32	26.35**
In the comparison at 1st group, %	-	- 23.22
Ash	7.28	7.24
In the comparison at 1st group, %	-	- 0.55

**) $P < 0.01$

Addition of Citrus Fennel caused no significant changes relating to intake of proteins. Noticeable is, however, the certain trend of decrease of retained energy in the organism compared to energy introduced to organism in chickens of trial group (Table 6).

Table 6. Bilans of protein and energy of chickens in the experiment

Group	**1 control**	**2 experimental**
Citrus Fennel aroma	-	0.03
Bilans of protein		
Protein intake by food, g	865.42	882.83
In the comparison at 1st group, %	-	+ 2,01
Protein retained in the body, g	486.27	493.15
In the comparison at 1st group, %	-	+ 1.41
Bilans proteina, %	56.19	55.86
In the comparison at 1st group, %	-	- 0.59
Bilans of energy		
Energy intake by food, kcal	11983	12202
In the comparison at 1st group, %	-	+ 1.83
Energy retained in the body, kcal	5696	5141
In the comparison at 1st group, %	-	- 9.74
Bilans energy, %	47.53	42.13
In the comparison at 1st group, %	-	- 11.36

Slaughter results indicate that the share of slaughter weight in the total weight was the same in both investigated groups, and in carcass ready to grill by 1.0% lower than in the control group, without Citrus Fennel, compared to the trial group of animals (Table 7). Share of thighs in carcass ready to grill was by 1.0% lower, and share of breast higher by 1.0% when Citrus Fennel was added to the mixture for fattening chickens. Amount of abdominal fat was by 15.5% lower in group fed Citrus Fennel in the mixture.

Table 7. Slaughter results of chickens in the experiment

Group	1 control	2 experimental
Citrus Fennel aroma	-	0.03
Body weight of chickens before slaughtering, g	2350	2536
In the comparison at 1st group, %	-	+ 7.91
Slaughter weight of chickens, g	1915	2054
In the comparison at 1st group, %	-	+ 7.25
Share of slaughter weight of chickens in the comparison at live weight, %	81.4	81.0
In the comparison at 1st group, %	-	- 0.49
Ready to grill roasted, g	1764	1868
In the comparison at 1st group, %	-	+ 5.89
Share of grill roasted in the comparison at live weight, %	75.0	74.0
In the comparison at 1st group, %	-	- 1.33
Drumstick weight, g	606	620
In the comparison at 1st group, %	-	+ 2.31
Share of drumstick in the comparison at grill roasted, %	34.3	33.2
In the comparison at 1st group, %	-	- 3.21
Breast weight, g	519	579
In the comparison at 1st group, %	-	+ 11.56
Share of breast in the comparison at grill roasted, %	29.4	30.9
In the comparison at 1st group, %	-	+ 5.10
Abdominal fat, g	41.4	35.0
In the comparison at 1st group, %	-	- 15.46

There is also stated [8] positive effect of XTRACT on appearance of chicken carcasses, where breast muscle was by 1.8% - 3.6% higher when XTRACT was added to food, and amount of abdominal fat was significantly lower when 300 ppm XTRACT was added. Favorable effects of plant extracts on chicken and geese carcasses were also established [7, 20].

Obtained results indicate that by adding of Citrus Fennel to the livestock food a positive effect on growth and utilization of nutritious substances is achieved.

Modern fast growing hybrids provide necessary nutritious substances through increased food consumption and its better utilization. Consumption of food depends on its edibility and taste. There is opinion that the chickens have no sense of smell. However, in order for animals to experience the sensitivity of taste, the source of chemical stimulation must be in direct contact with sense receptors. Although they are less compared to mammals, they are placed along the mucous membrane of the mouth and main role in transmission of stimulation has the saliva, since because out of door taste button is not in muoth area [Kralik et al., 2009]. Plant extracts and oils have positive effect on appetite of animals thank to their aromatic ingredients and other traits [13, 2].

Utilization of nutritious substances in the organism of chickens is closely related to digestion and reabsorption of nutritious substance.
Some authors [6] concluded that addition of oregano, cinnamon and paprika oil extracts as well as sage and thyme extracts have a positive effect on digestion of nutritious substances, especially secretion of bile which is necessary for digestion of fats and activity of pancreas enzyme [15, 18, 13].

CONCLUSION

The effects of addition of aroma Citrus Fennel in the nutrition of fattening chickens were studied. Obtained results showed that positive effects of use of investigated aroma are reflected in:

- increase of body mass of chickens by 5.5%,
- increase of daily gain by 5.72%,
- increase of feed intake by 2.4%,
- improvement of feed conversion ratio by 3.1%,
- addition of investigated aroma had no significant effect on protein and energy balance in chicken organism,
- amount of abdominal fat decreased by 15.5% by adding of Citrus Fennel aroma to mixture for the chickens.

In general, obtained results showed that by introduction of aroma Citrus Fennel a positive effects in the nutrition of fattening chickens were realized.

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THE QUALITY OF SILAGES FROM LUCERNE, WHOLE MAIZE PLANT AND MAIZE COBS PREPARED WITH VARIOUS ADDITIVES

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ABSTRACT

The aim of this experiment was to investigate effects of various additives on chemical composition and biochemical parameters of quality in silages prepared from lucerne, whole maize plant and maize cob. Additives used when lucerne was ensiled were: maize meal (50 g/kg) and homofermentative inoculant. Whole maize plant was ensiled with added urea (5 g/kg) and organozeolite (2 g/kg). Maize cobs were ensiled with added homofermentative inoculant and organozeolite (2 g/kg). Based on the obtained results it is found that the carbohydrate and biological additives had the highest influence for lucerne silage. Addition of urea significantly increased crude protein content in maize plant silages, but had no influence on the process of fermentation and silage quality. Addition of organozeolite decreased the degree of proteolysis in maize cob silages.
Key words: *additives, silage, lucerne, maize*

INTRODUCTION

At the beginning of the 21-st century ensiling technology received a new dimension of importance due to the widespread use of conserved feeds, especially silage, during the whole year, with the aim of achieving the maximally stabilized milk production [19]. The development of ensiling technology begins in the first half of 19-th century, and became much more developed during second half of 20-th century. The main factor that was limiting the distribution of this technology was lack of adequate mechanization and appropriate siloses, but also inadequate knowledge of basic principles of ensiling [15]. During the development of the ensiling technology there were many experiments whose results were successfully used in practice (chemical conservant based on organic acids, carbohydrate additives, wilting, biological agents), or completely discarded as inadequate (ensiling with electric current), or impractical (mineral acids). Today the biological preparations are favored and the main aim of the technology is obtain as good quality as possible, with the maximal aerobic stability and nutritive value [12, 14]. In our country the organozeolites are investigated as silage additives because they posses ability to adsorb mycotoxins, heavy metals and other substances potentially harmful for animals [9, 16]. The aim of this experiment was to investigate effects of various additives on chemical composition and biochemical parameters of quality in silages prepared from lucerne, whole maize plant and maize cobs.

MATERIAL AND METHODS

Experiment was organized as two-factorial (3 × 3), with three replications, where factor A was plant material and factor B type of additive (Table 1). Maize hybrid ZP-677 was ensiled in the wax phase of grain development, while lucerne of the NS Mediana ZMS 5 cultivar was used at the beginning of bloom and after short wilting. Urea that was used was chemically pure with the crude protein equivalent of 291.6%. The homofermentative inoculant that was used had according to the producer microcapsulated bacteria *Lactobacillus plantarum* (min. $1{,}0\times10^{11}$ CFU), *Lactobacillus acidophilus* (min. $1{,}0\times10^{11}$ CFU), *Streptococcus faecium* (min. $1{,}0\times10^{11}$ CFU) and *Pediococcus acidilactici* (min. $1{,}0\times10^{11}$ CFU). The amount of inoculant was used according to the recommendations, 250 g/10 t green mass of maize and 250 g/5 t for lucerne. Organozeolite was product of Institute for Technology of Nuclear and Other Raw Materials (Belgrade, Serbia).
All experimental silages after the treatment with the additives were compressed in plastic siloses with volume of 60 dm^3. After 56 days the siloses were opened and representative samples were collected for analysis. Parameters of chemical composition and quality of silages [1] were analyzed in the Laboratory for animal nutrition at the Faculty of agriculture, Zemun-Belgrade. Statistical analysis was done by analysis of variance with PC software StatSoft v. 6 [18].

Table 1. Additives used in experiment

Additive	**Material**		
	Lucerne	**Whole maize plant**	**Maize cob**
Maize meal, 50 g/kg	+	-	-
Inoculant, 250 g/10 t green mass of maize and 250 g/5 t for lucerne	+	-	+
Urea, 5 g/kg	-	+	-
Organozeolite, 2 g/kg	-	+	+

RESULTS AND DISCUSSION

Compared with the starting material all silages had somewhat more dry matter (Table 2). That was contribution of the additives, but also partly because some volatile substances were lost during the drying of samples. Silages were dried at 80°C and than the dry matter was corrected for the amount of volatile substances (acetic and butyric acid, ammonia and alcohol). The amount of such materials was very small [5].
Amount of crude protein in silages was lower compared with the starting material which is the result of some nitrogen loses in form of ammonia. The exception was whole plant maize silage with urea, where protein content was increased. Crude protein analyzed by Kjeldahl process reflects only the nitrogen contents, not the true protein [7].
Variability of lipid content is explained with the extraction of the part of the lactic acid with diethyl-ether [2], so that the highest amount of ether extract was detected in the

treatment with the highest amount of lactic acid. From the same reason silages had the higher amount of lipids compared with the starting material.
The additives used had no significant influence on crude fiber content in silages, although there was a decreasing trend in treatments with urea. Some previous investigations suggest that ammonia may partially degrade lignocellulose complex, which was what probably happened in such treatments in this experiment [8].

Table 2. Chemical composition of starting material and silages, g/kg DM

Parameters	Parameters, g/kg DM					
	Corrected DM, g/kg	Crude protein	Crude lipids	Crude fiber	NFE	Ash
Starting material						
Lucerne	332.54	188.65	68.37	256.32	378.30	108.36
Whole maize plant	360.20	72.04	63.86	170.24	647.92	45.94
Maize cob	562.35	78.98	37.26	41.03	694.63	148.34
Maize meal	896.37	81.24	41.75	20.42	842. 11	14.48
Silages						
Lucerne	338.67^{ab}	184.22^{a}	87.53^{ns}	260.21^{ns}	357. 59^{b}	110.45^{a}
Lucerne + 50 g/kg maize meal	340.37^{a}	182.53^{b}	90.42^{ns}	255.34^{ns}	364.00^{a}	107.71^{b}
Lucerne + inoculant	336.25^{b}	185.06^{a}	91.44^{ns}	258.13^{ns}	355. 74^{b}	109.63^{a}
Whole maize plant	362.94^{b}	68.72^{b}	87.63^{a}	176.54^{ns}	616.69^{b}	50.42^{ns}
Whole maize plant + 5 g/kg urea	365.93^{a}	87.43^{a}	50.57^{c}	170.73^{ns}	639.83^{a}	51.44^{ns}
Whole maize plant + 2 g/kg zeolite	366.27^{a}	70.36^{b}	62.38^{b}	174.95^{ns}	640.85^{a}	51.46^{ns}
Maize cob	567.54^{a}	76.27^{ns}	46.74^{ns}	43.31^{ns}	684.95^{ns}	148.73^{ns}
Maize cob + inoculant	565.26^{b}	78.93^{ns}	50.33^{ns}	42.15^{ns}	681.06^{ns}	147.53^{ns}
Maize cob + organozeolite	568.72^{a}	77.46^{ns}	48.82^{ns}	42.37^{ns}	680.03^{ns}	151.05^{ns}
Statistical significance (P)						
A	**	*	*	**	**	**
B	*	*	*	**	*	**

^{a,b,c} Values in same column for same plant material with different letter is significantly different ($P<0.05$) ns = no significance.

There was significant variation of NFE in lucerne silages was result of the carbohydrate additives used. Maize plant silage without additive had the lowest amount of NFE, which is result of the intensive fermentation and the use of fermentable carbohydrates for organic acid synthesis. For that reason all silages had lower NFE compared with the starting material.
Significant variations of mineral matters were observed only in lucerne silages because of carbohydrate additives.
The highest pH values were observed in lucerne silages (Table 3), which was expected concerning the high buffering value of that plant and its previous wilting [4]. When carbohydrate additive and inoculant were used there was significant decrease in pH value in lucerne silage. Adding urea to the whole plant maize silage resulted in significant increase of pH values. In spite of that, such silages had pH values within the optimum range [7].The use of inoculants in maize cob silages resulted in further decreases of pH values, below the optimum range.

Table 3. Parameters of biochemical changes in silages

Treatments	**pH**	**NH_3-N, g/kg ΣN**	**Acids, g/kg DM**						
			Lactic	**Acetic**			**Butyric**		
				F	B	T	F	B	T
Lucerne	5.02^{a}	156.17^{a}	41.45^{b}	9.53^{b}	42.58^{a}	52.11^{ns}	-	-	-
Lucerne + 50 g/kg maize meal	4.96^{b}	106.84^{b}	45.17^{b}	11.25^{b}	38.30b^{a}	49.55^{ns}	-	-	-
Lucerne + inoculant	4.78^{c}	107.52^{b}	58.72^{a}	16.32^{a}	32.18^{b}	48.50^{ns}	-	-	-
Whole maize plant	3.83^{b}	69.73^{b}	52.38^{a}	15.56^{a}	7.18^{ns}	22.74^{ns}	-	-	-
Whole maize plant + 5 g/kg urea	4.12^{a}	332. 25^{a}	36.04^{c}	11.87^{b}	8.25^{ns}	20.12^{ns}	-	3.88	3.88
Whole maize plant + 2 g/kg zeolite	3.85^{b}	24.41^{c}	41.28^{b}	10.13^{b}	8.34^{ns}	18.47^{ns}	-	-	-
Maize cob	3.88^{a}	56^{a}	28^{b}	7 ^{a}	3^{b}	10^{ns}	-	-	-
Maize cob + inoculant	3.63^{b}	42^{ab}	37^{a}	6^{a}	5^{a}	11^{ns}	-	-	-
Maize cob + organozeolite	3.96^{a}	47^{b}	30^{b}	3^{b}	6^{a}	9ns	-	-	-
Statistical significance (P)									
A	**	**	**	*	*	*	-	*	*
B	**	**	**	*	*	ns	-	*	*

^{a,b,c} Values in same column for same plant material with different letter is significantly different (P<0.05) ns = no significance. S – free, B – bonded, T – total.

The pH value and dry matter content are most important factors that dictate the proteolysis intensity, but they cannot completely stop it [3]. The amount of ammonia nitrogen in relation to total nitrogen is the first and fundamental indicator of the degree

of protein degradation. Presence of ammonia in silages which do not contain butyric acid is explained with the activity of plant enzymes [15]. All lucerne silages had ammonia nitrogen in the amount above 100 g/kg N, which is considered as the highest level for quality silage [11]. That is the result of high solubility of lucerne proteins [13]. Whole plant maize silage with urea added had the highest amount of ammonia nitrogen (> 320 g/kg N), which was direct effect of the additive used. In that treatment total ammonia N came from two sources: from degraded proteins of the ensiled material and from hydrolyzed urea **[**$CO(NH_2)_2 + H_2O = CO_2 + 2(NH_3)$**]**. Aside from increasing crude protein content, additives based on urea have importance because they provide aerial stability of silages. In was confirmed [17] that alkaline mineral additives bind part of the organic acids produced and stimulate fermentation and utilization of the remaining sugars, which decreases the possibility for later fermentation. This is very important in maize plant silages, which are very susceptible to secondary fermentations due to residual sugars. Ammonia also may have fungicide effect [6]. Addition of organozeolite significantly decreased amount of ammonia, which can be explained with the absorptive nature of zeolite [10].

The use of inoculants produced significant increase in lactic acid production, which was most evident in maize silages. On the other side, the use of ammonia significantly decreased activity of lactic acid bacteria. The variability in total acetic acid was not significant. Butyric acid was detected only in whole maize plant silage with added urea. Reduced fermentation in maize cob silages can be explained with the high dry matter content of the raw material, which is limiting microorganism activity.

CONCLUSION

In this experiment the highest significant importance was observed in the use of carbohydrate and biological additives for lucerne silages, because they had stimulating effects which were significant in all parameters of silage quality. The use of urea in the amount of 5 g/kg green mass increased significantly nitrogen content in maize plant silages, without negative effects on fermentation. The use of organozeolite had no marked effects on chemical composition and quality of silages, but it decreased degree of proteolysis.

Further investigations need to focus on interactions with simultaneous use of two or more additives, in order to determine cumulative positive effects and optimal doses of additives in those combinations.

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NEW RECOMMENDATIONS AND CONTEMPORARY ACHIEVEMENTS IN PREPARING FEEDS FOR GOATS FEEDING

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ABSTRACT

This research paper contains a synopsis of contemporary procedures and measures conducted in food preparation for goats as well as some specific characteristics in their nutrition. Reliance of the hay preparation on weather conditions in decreased today using conditioners and chemical preservatives. For feed ensiling, today the most recommended are additives based on biological agents that intensify and target the fermentation, also increasing the aerobic stability of silages. However, experience with grass silage and corn silage is different in nutrition of goats. The optimal fulfilling of the requirements for high product results should be given special attention in nutrition of goats. That is accomplished with the use of latest recommendations and software, which take into account a large number of nutritive parameters. The 1981 publication Nutrient Requirements of Goats (NRC 1981) was one of the first references to compile known research into a comprehensive document listing the nutritional requirements for goats in various stages of production. This document gained prominence and is still used as a reference for goat nutritional requirements. Two statements in the introduction of this publication illustrate the challenges that existed and still exist when attempting to address the nutritional requirements of goats. "It is increasingly evident that despite similarities to sheep and cattle, goats exhibit significant differences in grazing habits, physical activities, water requirements, feed selection, milk composition, carcass composition, metabolic disorders and parasites. The 1981 NRC has been an excellent source of information concerning goat nutritional requirements and should not be ignored. However, the tremendous amount of information, based on additional data, available in the 2004 publications referenced should be considered when evaluating or developing new goat feeding programs. Based on that research NRC published in 2007 new regulations in nutrient requirements of goats which represent a step forward compared to the former regulations from 1981. Based on that research NRC published in 2007 new regulations in nutrient requirements of goats which represent a step forward compared to the former regulations from 1981.

Key words: *nutrition, goats, preparing feeds, conserving, normative*

INTRODUCTION

Intensive and contemporary production in goat farming today is mostly based on tinned nutrients usage in order to prolong their usage period and also usage of concentrated nutrients which have been adapted for maximum use and requirements of different categories of goats by various methods of treatment (7). Usage of these nutrients prepared according to precise regulations and by using software for meals and compound combining, can achieve high level results which can express the genetic potential of goats to its fullest.

Goats can be fed with about 90 types of plants which make them the top domestic ruminant. They can be very choosy if given the chance but if their choice of food is limited, they are satisfied with lower quality food. However, that doesn't mean that high production can be achieved with such nutrition. For a solid and adequately large production, food should be of high quality and nutrition technology plays an important role in that process.

Goats are ruminant 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 (4). 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. 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 (23). 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. 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 (8, 9, 10). 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 (12).

GREEN FEED

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.
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. 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 (3).

Table 1. Goat performance on ryegrass pastures (1)

Stocking rate (n/xa[1])	8,0	10,4	13,6	16,4
Total weight/xa (kg.)	430	558	730	881
ADG (kg.)	0,31	0,20	0,22	0,18
Total gain/xa (kg.)	370	310	446	440

[1] Number of head on 0,4 xa

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 (5). 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 2. Intake level of green forages used as a sole feed in the basal diet (2)

Forage	State of lactation, days	Intake level g/kg BW* ^{0,75}
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.

PRESERVATION OF ROUGHAGE

Hey and silage are the primary types of preserved roughage. Because of the effect weather conditions have on the quality of hey, preparation and usage of hey has been replaced by the preparation and usage of silage. However in nutrition of goats in winter period hey is sometimes the basic and the only nutrient, especially for categories with lower production rate. Hey contains carbohydrates, protein, minerals and vitamins and it is very important in nutrition of goats for maintaining physiological state of the abdomen, it stimulates the movement of food through the animal's intestines, speeds up the development of the stomach with younger categories, prevents the decay of milk fat level etc (5, 9, 13). However, besides those beneficial characteristics, hey has certain deficiency. For example, it is the most variable nutrient compared to its chemical and nutritive structure; it limits the production in case it is the basic or the only nutrient etc. Nutritional value and quality of hey depend greatly on the type of plant it mostly consist of, period of hay making, method of drying, preservation and usage etc.

There were various attempts of improvement in mowed plants drying technology: additional drying with dry and cold air, artificial drying etc. (5) Because of the high cost of the energy-generating products, most of hey today is still prepared by drying on the ground all over the world. (13) However there has been a great improvement in hey preparation machinery. Accordingly, there is a big selection of machines today which handle the mowing, collecting and bale making and so all hey preparation procedures are completely mechanized.

Silage is very rarely used in nutrition of goats under the conditions in our region. The quality of silage primarily depends on the type and quality of the material in silos, which refers to dry matter content, degradable sugars and protein, with another significant step in the process which is probable additive adding during the preparation. Contemporary trends in silos' storage technology are based on chemical resources usage, biological additives usage, enlarged aerobe stability of the silage, micro toxins absorption etc

Silage for nutrition of goats can be prepared from various plants such as corn, barley, oat, rye, different types of leguminous plants and grass, sunflower, turnip, raw beet cuttings etc. Well prepared silage given to goats in a proper way can satisfy certain

nutritional value requirements in nutrition of goats. If some household uses silage as a goat feed, it is very important to additionally include a certain quantity of hey. Silage usually has diminished nutritional value because of a certain loss of it which appears during preparation and storage so it has to be combined with other nutrients. Usage of grass- leguminous silage in nutrition of goats which contains enough protein requires additional nutrients which provide energy, such as wheat. On the other hand, corn silage requires adding of a certain nutrient rich in protein (sunflower or soy additives) or leguminous hey, primarily containing alfalfa. (8, 9, 13)
Experiences are different with corn and grass prepared silage usage (13). Grass silage doesn't cause any significant trouble while corn silage can in some cases cause certain disturbance in digestive organs. Besides that, it can cause loss of appetite, loss of milk production and fattening of goats, especially in the last weeks of the gravid period. Reduction of intake occurs as a result of goats refusing to eat. Having that in mind, it is recommended to use it in nutrition in the first half of lactation period, and to combine it with 0, 4-0, 5 kg of hey per meal, like it's done with other silage. Because of that it is recommended to use it carefully in the nutrition of goats. It is very important not to use poorly prepared silage, moldy or defective silage or very sour silage in the nutrition. Silage should be gradually included into the nutrition, in smaller portions 2-3 times a day. Goats can be fed with silage and portion in a daily meal should be about 2-4 kg while the gravid head should be given smaller portions, 1-2 kg. Kessler (19) states that maximum corn silage intake should be 4, 5 kg per day. Well prepared grass silage can be fed to goats during the lactation period and during the entire gravid period. (3)

Table 3. Grass silage utilise in winter period for dairy goats (19)

Feeds	5. months of pregnancy	2. months of lactation, production of milk 5 kg	3. months of lactation, production of milk 3 kg
	Fresh supstance of feed, kg/day		
middling hay- aftergrass	0,5	0,5	0,5
grass silage good quality	1,7	3,1	3,4
Barley	0,3	0,3	0,5
Concentrate		0,8	

If beer trope silage is used in the nutrition of goats, Kessler (18) recommends a daily portion of 0, 9 kg, attending to its quality and gradual inclusion into the meal. When it comes to sugar beet root and leaves silage, it is recommended to use daily portions of 0, 5 kg when primary meal is hey. This silage contains small quantity of overall protein and cellulose.
Lower quality silage can cause a decrease of milk production by 5 to 15 %. Poorly prepared silage can also cause a disease in goats called listeriosis.

CONCENTRATED FEED

It is necessary to combine several nutrients in order to fulfill all the requirements of certain categories for intense and high production in goat farming. Concentrated feed used in nutrition of goats and their offspring include grainy nutrients, food industry debris products and additional industrial compounds. Grainy nutrients group includes corn, oat, barley, wheat and rye, leguminous plants such as soy, peas, lupines and other plant types such as sunflower, rapeseed, cotton etc. Concentrated feeds are much richer in nutritional value compared to roughage so they are mutually combined in the nutrition of goats (8, 9, and 13). Also in case roughage is of poor quality or when goats have a need for increased nutritional value intake, concentrated nutrients are unconditionally included in the meals (23).
Concentrated feed in nutrition of goats can be reduced to necessary quantities; they can be given as a roughage meal supplement in order to improve its nutritional value. They should be included in larger quantities in nutrition of high production goats. The quantity of concentrated feed used in nutrition of goats depends greatly on the production phase the heads are in and production level (20).
Not making concentrated feed the only element in nutrition of goats, for a longer period of time, should be given special attention because of the harmful consequences to the digestive organs (6). Goats prefer eating compounds which contain more concentrated feed. Concentrated feed compound can contain only grainy nutrients or grainy nutrients mixed together with food industry debris products and certain pellets (11, 13).

NEW RECOMENDATIONS FOR GOAT FEEDING

The optimal fulfilling of the requirements for high product results should be given special attention in nutrition of goats. In order to achieve that, contemporary regulations which consider a large number of parameters are applied. The 1981 publication Nutrient Requirements of Goats (16) was one of the first references to compile known research into a comprehensive document listing the nutritional requirements for goats in various stages of production. This document gained prominence and is still used as a reference for goat nutritional requirements. Two statements in the introduction of this publication illustrate the challenges that existed and still exist when attempting to address the nutritional requirements of goats. "It is increasingly evident that despite similarities to sheep and cattle, goats exhibit significant differences in grazing habits, physical activities, water requirements, feed selection, milk composition, carcass composition, metabolic disorders and parasites.
The 1981 NRC (16) has been an excellent source of information concerning goat nutritional requirements and should not be ignored. However, the tremendous amount of information, based on additional data, available in the 2004 (20, 21, 22) publications referenced should be considered when evaluating or developing new goat feeding programs. Based on that research NRC published in 2007 new regulations in nutrient requirements of goats which represent a step forward compared to the former regulations from 1981.

Table 4. Nutrient Requirements of goat (17)

Parameter	Body weight, kg	Dry matter intake, kg	Energy Requirements		Protein Requirements	
			TDN kg-d	ME Mcal-d	MP,g-d	DIP, g -d
Mature goats, early lactation, milk yield 2,06 – 3,22 kg						
	40	1,97	1,05	3,77	178	94
	50	2,30	1,22	4,41	205	110
Mature goats, mid lactation, milk yield 1,47 – 2,30 kg						
	40	1,96	1,04	3,74	156	93
	50	2,26	1,20	4,33	178	108
Mature goats, late lactation, milk yield 0,88 – 1,38 kg						
	40	1,69	0.89	3,23	121	81
	50	1,96	1,04	3,75	140	94
Mature bucks, prebreeding						
	50	1,36	0,72	2,59	58	65
	75	1,84	0,97	3,51	78	88

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. 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 (15) 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 (15).
As shown in Table 5, additional data has indicated a reduction of over 15% in the recommended protein intake for maintenance for mature goats from the 1981 NRC (16) level.

Table 5. Energy & protein requirements of a 70 kg mature goat (16, 20, 21, 22)

	NRC 1981	2004
ME mJ	10.26	11.18a
Crude Protein, g	96.3b	81.7c

a based on Luo et al. (20,21) value of 462 mJ ME/ kgW0.75
b NRC (16) C P requirement = Mcal DE * 32
c table 7 Sahlu (22) converted to crude protein utilizing NRC 1996

However, estimated energy requirements have been increased almost 9%. These changes can be attributed to a much larger database for calculating the values but, also a better understanding of the partitioning of nutrients to various body functions.
The nutrient requirements of growing goats (Table 6) show a different trend than those of mature animals. Protein recommendations increase by over 45% while energy recommendations increase very little. However, it must be remembered that

energy requirements are based on minimal activity needed to secure feed and the actual requirement may very (14).

Table 6. Energy & protein requirements of a 20 kg growing kid (16, 20, 21, 22)

	NRC 1981	2004
ME mJ	5.53	5.69b
Crude Protein, g	51.76	76.5c

a 50 g gain
b 2004 requirements for energy based on doelings and wethers
c metabolizable protein converted to crude protein utilizing NRC 1996

While the 2004 (20, 21, 22) data increases the information available to make decisions concerning expected performance from a wide variety of feeding conditions. It also enhances our understanding of goat biotype influence on energy and protein requirements it also highlights the need to better understand the feeds we are using if we expect growth performance from meat type goats. Recent published and unpublished data establishes this need more dramatically.

CONCLUSION

Contemporary achievements of science found their way to apply to food preparation area and nutrition of goats. Contemporary achievements are many and the most important ones apply to the effective preservation of roughage and treatment of concentrated feed, maximum compound homogenization in food factories and application of regulations and software in order to fulfill the requirements of various categories of goats with minimal cost of nutrition.

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EFFECT OF BENTONITE IN PELLETED FEED FOR CALVES

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ABSTRACT

Numerous researching show that bentonite except of using as binding material which increases pellets durability, act as buffer, also has favorable effect on ruminal acetate to propionate ratio, on ammonium N utilization in rumen, efficiently adsorbs mycotoxins in feed. Using of bentonite in feed for all category of cattle, can be of particular importance for high-concentrate rations, rations with high content and surplus of rumen degradable protein, as in diets that include feeds with presence of mycotoxins. Considering results of previously studies, and nonexistence of exactly results concerning on effects of using bentonite in diets for calves, this experiment was conducted with objective to determine effects of using of bentonite in concentrate for calves, on production performances of calves 30-120 days old. Twenty male Holstein calves were assigned in two groups: control group, fed feed without bentonite, and experimental group fed feed with added 1.5 % of bentonite. Mixture contained 18 % of CP, and in addition to feed, calves were fed high-quality alfalfa hay ad libitum. Experimental group show higher average daily gain (1.084 and 0.972 kg/day), higher average intake of feed (1.89 and 1.81 kg/day), better feed conversion (1.74 and 1.86 kg/kg of gain), and higher values of ruminal pH at age 80. and 120. day. In diets for calves with ad libitum intake of feed, it can be recommended using of bentonite for improvement of production performances.
Key words: *bentonite, feed, calves, nutrition*

INTRODUCTION

Bentonite is colloid clay of volcanic origin, and it is hydrated aluminum-silicate (montmorilonite) that contains sodium and calcium as changeable ion. Sodium-bentonite characterized distinct ability for hydration when increases mass and volume. Bentonite as binding material increases pellet durability and quality (*Stojanović et al. 2008*). In mixtures for animal nutrition, bentonite is used in proportion of 1-2 % (*Salari et al. 2006*). In water suspension particles of bentonite are charged with negative electricity, and this causes cation attracting (*Bringe and Schultz,* 1969). Using of bentonite in diets for rams in portion of 2 %, increased N retention, especially in condition of increased releasing of NH_3 in rumen (*Martin et al. 1969*). Authors emphasized the significance of

bentonite as binding material for pelleting of mixtures for animal nutrition. Cows fed high-concentrate ration (75:25 % of feed to hay ratio, DM basis) with mixture contained bentonite, showed increased ruminal concentration of acetate, decreased ruminal concentration of propionate, and higher concentration of acetate in blood serum (*Rindsig et al. 1969*). There were no decreasing of DM intake, characteristic for using $NaHCO_3$ and MgO. *Erdman* (*1988*) determined favorable effect of using bentonite in feed for lactating cows fed diets with high content of pelleted feed. Using bentonite in diet for lactating cows increased daily milk yield, increased concentration of ruminal acetate and decreased concentration of propionate, decreased lowering of ruminal pH, and had no effect on DM intake, that is usually for using other buffers. Using bentonite in rations for dairy cows results in slowing of ruminal digesta passage (*Rindsig et al. 1969*). The same assumption presented *Bringe and Schultz* (1969) for lactating cows fed high-concentrate diets, using fact that bentonite absorbs water and increases volume by 10-15 times, and in that way increases volume of ruminal content, reducing rate of ruminal passage. *Pasha et al.* (*2008*) reported that using bentonite in feed (0.5-1 %) affected longer retention time of feed in broiler digestive tract, enabled longer effect of digestive enzymes and increased digestibility of nutrients.
Considering results of reviewed studies, and nonexistence of exactly results regarding to effects of using bentonite in diets for calves, experiment is conducted with objective to determine the effect of using bentonite in pelleted feed for calves on production performances of calves 30-120 days old.

MATERIALS AND METHODS

Researching was conducted on 20 male Holstein calves on one of farms of dairy cows, of "PKB-Poljoprivredna Korporacija Beograd", in November/January period 2008/09. Selected 20 male calves at age of 30 days were assigned in two groups (experimental and control group), equalized for average BW, and housed in group-stalls (10 calves) with litter in closed-type object for calves rearing. Beside the liquid part of diet (scheme of liquid nutrition is presented in table 1) calves were fed pelleted feed contained 18 % of CP (composition of mixture is given in table 2) and high-quality alfalfa hay.

Table 1. Scheme of liquid nutrition of calves

Age, days	Fullfat milk	Milk replacer
5-29	6.0	-
30-39	3.0	3.0
40-69	-	6.0
70-80	-	3.0

Table 2. Feed composition

Component	Control group without bentonite	Experimental group with bentonite
Corn, ground grain, %	34.30	34.30
Barley, ground grain, %	10.00	10.00
Soybean, extruded, %	22.50	22.50
Sunflower mill, 33% CP, %	10.50	10.50
Wheat middlings, %	16.50	15.00
Dehydrated alfalfa, %	3.00	3.00
Limestone, %	1.20	1.20
Dicalcijum-phosphate, %	0.40	0.40
Salt, %	0.60	0.60
Vitamin and mineral premix, %	1.00	1.00
Bentonite, %	0.00	1.50
Totally	100.00	100.00

After age of 80. days, calves fed feed and alfalfa hay ad libitum. Control group fed feed without bentonite, while experimental group consumed mixture with 1.5 % of bentonite. Chemical composition of added bentonite is presented in table 4. Diameter of pellets was 4 mm. Calves consumed water ad libitum.

Table 3. Chemical composition of mixtures and feeds, %

Item	Feed without bentonite	Feed with added bentonite	Milk replacer	Alfalfa hay
Dry matter, %	88.71	88.87	96.12	87.46
Ash, %	3.96	5.13	10.70	10.14
Ether extract, %	5.42	5.36	16.11	1.35
Crude fiber, %	7.8	7.72	1.71	18.80
Crude protein, %	18.51	18.33	20.74	18.55
Calcium, %	0.72	0.74	1.03	1.42
Phosphor, %	0.60	0.58	0.89	0.22
NEL, MJ	6.86	6.78	10.09	3.96

Table 4. Chemical composition of bentonite

Component	Content, %
SiO_2	48.37
Al_2O	22.39
Fe_2O_3	4.73
CaO	5.86
MgO	1.81

Na_2O	0.07
K_2O	0.40
TiO_2	0.34
Particle size	< 50 μm

Body weights of calves were measured at the beginning of experimental period-30 days old, and at the end of period-120 days old. Amounts of consumed feed were recorded for complete groups, and whole experimental period, and average values for daily intake of feed were calculated. Ruminal pH was determined in samples of ruminal content obtained using stomach tube probe, and pH of blood was measured in samples obtained by punction *v. jugularis*, at age of 80 and 120 days. Determination of pH values of ruminal content and blood were done on five calves from each group.

RESULTS AND DISCUSSION

Determined production performances of calves were in appropriate with genotype, sex, age and diet composition (*Adamović et al. 2007., Radivojević et al. 2003., Grubić, 1995*). Results of analysis of average BW and average daily gain of calves (table 5) imply on favorable effects of using bentonite in feed for calves, in portion of 1.5 %. Although there were not statistical significant differences, between treatments, numerical differences were determined, and tendency for increasing of average daily gain and BW of calves, consumed mixture with added bentonite. Determined increasing of average daily gain for calves in experimental group was 11.52 %, compared with control group.

Table 5. Production performances of calves

Item	Control group without bentonite	Experimental group with added bentonite
Age at the beginning of experiment, days	30	37
Age at the end of experiment, days	126	122
Days in experiment	96	85
BW at the beginning of experiment, kg	54.55	54.05
BW at the end of experiment, kg	147.90	146.20
Total gain, kg	93.35	92.15
Daily gain, kg	0.972	1.084
Intake of feed, kg/day	1.81	1.89
Feed conversion ratio, kg of feed/kg of gain	1.86	1.74

It was found greater average daily intake of concentrate by 4.42 %, as better feed conversion by 6.45 %, for calves consumed experimental concentrate.

Table 6. Ruminal content and blood pH

Item	Control group without bentonite	Experimental group with added bentonite
Age at 80. day		
Rumen	6.28	6.54
Blood	7.40	7.45
Age at 120. day		
Rumen	6.14	6.39
Blood	7.40	7.49

Tendency for increasing of ruminal pH was found at age 80. and 120. day, for calves fed feed with added bentonite, even there were no statistical significant differences. Measured pH values were equal between groups. It was determined by organoleptic evaluation that pellets with added bentonite (1.5 %) characterized with shape that is more regular, were more compact and resistant on crumbling. These prevent losses of biologically active components added in concentrate in small quantity (vitamins and trace elements).

Results of this researching are appropriate with previously determined favorable effects of using bentonite in high-concentrate diets for cows (*Erdman, 1988., Bringe and Schultz,* 1969., *Rindsig et al. 1969*). Authors reported favorable effects of this buffer substance on ruminal pH and on increasing ruminal acetate to propionate ratio. This implies on positive effect of bentonite on maintaining optimal pH and preventing ruminal acidosis when high-concentrate diets characteristic for calves are fed. In addition, increasing of dietary DM intake and ruminal digestibility is provided, and stimulating of celulolitic processes in rumen increasing efficiency of fiber and DM utilization (*Stojanović et al. 2007*). Bentonite content in mixture (0.5-1 %) increases retention time of digesta in rumen and complete digestive tract, enables longer effect of ruminal microflora and digestive enzymes, and increasing digestibility of nutrients (*Pasha et al. 2008., Bringe and Schultz 1969*. Bentonite absorbs diluted NH_3, when ruminal concentration is high, and releases ammonia when concentration is low, enabling more efficient utilization of ammonia N for synthesis of microbial protein, in longer period. This decreases the peak of ruminal concentration of NH_3 and his absorption in blood, also decreases load of liver and using of energy for urea synthesis (*Martin et al. 1969*). It was determined that hydrated sodium-calcium-alumsilicates is characterized with high affinity for aflatoxin B_1 forming stable complex and decreases his inhibiting effect on animal growth. Bentonite binds up to 90 % of aflatoxin contained in feeds making unavailable for digestive tract (*Pasha et al. 2008*).

CONCLUSION

Results of this researching imply that using bentonite in proportion of 1.5 % in feed for calves at age 30-120 days, improved pellets quality, increased average daily gain, average feed intake, feed conversion ratio, and ruminal pH. In diets for calves, which

consume feed ad libitum can be recommended using of bentonite for increasing of production performances.

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PREVENTION OF HEAT STRESS IN POULTRY BY MODERN FEED TECHNOLOGY

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ABSTRACT

The aim of working is checking of possibility preventing heat stress, for poultry, using combination of selected supplements to fodder and describing pathological changing in surroundings high temperature conditions. Possibility of serious correction metabolism disorders by natural food supplements and sketching nutrition according to the metabolism is checking, too. Two groups of chicken older of 30 days were compared, in surrounding temperature of 33 to 38 C, one group was getting preparation ^Hot Pack^ Natura Point: Organic acid, extract plant Yucca (Deodorasa, Alltech inc.USA), mananoligosaharids (Biomos, Alltech inc. USA), triglicerids of fat acid middle of long chain, (AveMix MCT, Aveve Biochem, Belgium), Na electrolyte, K, Cl, Mg and the other group of chicken was control group. It was confirmed statistics important difference in mortality, conversion and growth increase. Pathological findings was characteristics for heat stress phenomenon for chicken whose did not get natural supplements mixture against heat stress. This results confirm possibility preventing consequences of heat stress of fattening chicken using certain combination of natural supplements in fodder and excuse of using it.

***Key words**: heat stress, poultry, natural supplements of fodder, prevention*

PRODUCTION OF NUTRITIONALLY VALUABLE AND SAFE FEED FROM A MIXTURE OF THE "OLD" BREAD AND CORN GRITS

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ABSTRACT

In animal feed production, feed quality is defined with its nutritive value, hygienic quality and physical properties. In this paper was examined quality of corn grits and old bread crumbs mixture which is extruded. Corn grits and old bread crumbs ratio was 60:40 and 50:50, respectively, and mixture was gained after mixing in contracurent mixer. Mixing time was 5 min and after mixing water was added to water content of 18%. Extrusion temperature was 95 °C.
In corn grits and old bread crumbs mixture were determined physical and chemical properties, hygienic quality, micotoxins content before and after extrusion.
Key words: *corn grits, bread crumbs, extrusion, hygienic quality, nutritive value*

INTRODUCTION

Food production represents extremely significant activity in the developed, as well as in the undeveloped parts of the World. Food shortage for the ever increasing population the existing problems makes even more significant.
The solution of increasing of production of foods and feeds could be found in the application of new technologies and of biotechnology, i.e. in the bioprocessing [6]. The basic orientations represent new technological procedures aimed to the increasing of the nutritive values of foods and feeds, as well as the valorization of by products from food industry and prom primary agricultural production. Contemporary in the world numerous processes for thermal processing of oil seeds and cereals are used: toasting, extrusion, hydrothermal treatment, micronisation, UHF treatment, dielectric heating treatment [7, 8], but in Serbia mostly extrusion process and hydrothermal treatment are applied [3, 9].
Adequate conduction of the thermal treatment results in the reduction of the thermo labile antinutrient contents to the tolerable levels, increasing of some nutrients (such as proteins, oil and carbohydrates), as well as the improving of sensory characteristics and microbial picture of the finished products [5]. Parallel with the reduction of the antinutritient contents, it is obvious to preserve nutritive valuable thermo labile components, and the applied process has to find the compromise between these two opposite claims.
Application of the extrusion process of corn, as the basic raw material for feed production, contributes to the better valorization of feeds during animal feeding [4].

During extrusion changes of carbohydrate complex of corn grits and of bread particles occur; starch content is diminished, owing to its degradation into dextrins. Such changes cause changes of the *in vitro*, as well as *in vivo* digestibility of starch, while the gelatinization of starch assures better accessibility of enzymes for degradation of starches and also cause the inactivation of amylase inhibitors [2].
The goal of these investigations is to find out the effects of extrusion on quality of mixtures of corn grits and bread crumbs.

MATERIALS AND METHODS

Basic chemical composition (moisture, crude proteins, crude fiber, crude fats and mineral substances contents) of the mixtures of corn grits and bread crumbs, were determined according to the AOAC methods (1980). Starch content, as well as the total and reducing sugars contents were determined according to the Rule Book on the methods of physical and chemical controlling of cereals, milling- and bakery products, pastry and rapid frozen dough (1988), ad the volume weight was determined according to the Rule Book on sampling methods and methods of conducting physical, chemical and microbiological analyses of animal feeds (1987).
The total counts of microorganisms, yeasts and molds, isolation and identification of *Salmonella* and of sulphite-reducing clostridia were determined according to the Rule book on the methods ob conducting of microbiological analyses and super-analyses of foodstuffs (1980).
For detection of the presence of coagulase-positive staphylococci, proteus sp. and *Escherichia coli* internal modified method was applied in the part of preparing of samples. 50 g of the examined samples was weighted in the Erlenmeyer flask and to it 450 mL of the prepared sterile nutritive medium was added. So obtained sample was gently homogenized and incubated 24 hours at 37ºC. Isolations and identifications were performed according to the Rule book on the methods of analyses and super analyses of the foodstuffs (1980).

EXTRUDING OF CORN GRITS AND OF BREAD CRUMBS

In the process of extruding of corn grits and bread crumbs, corn grits with 12% of moisture and bread crumbs were mixed in the ratios of 60:40 and 50:50 in the counter flow mixer, and after that, the obtained mixtures were moisturized to 18% of water content. For extruding of corn the extruder with the capacity of 900 kg/h was used. The installed extruder electromotor power was 100 kW. And that of the screw dosing unit – 1.1 kW. Extrusion temperatures were 90 and 95ºC, and nozzle diameter was 7.5 mm.

RESULTS AND DISCUSSION

Table 1 shows chemical and granulometric contents of the corn grits. These results indicate that the energetic proteinaceous feed contained 1544 kJ/100 g of energy and 75.23% of starch, 6.88% of proteins and 1.14% of fats. The granulometric analysis showed that the size of only 9.8% of particles was over 550 μm and 81% over 250 μm,

meaning that this feed component, with respect to its granulometric composition, belongs to finely granulated feeds, with the bushel weight of 654 mg/m^3.

Table 1. Chemical and granulometric compositions of corn grits

Quality parameter	**Content (%)**
Moisture content	13.36
Crude ash content	0.24
Crude proteins content	6.88
Total sugars content	2.23
Reducing sugars content	0.49
Starch content	75.23
Crude fat content	1.14
Energetic value	**kJ/100 g**
Energetic value determined with calorimeter	1544
Granulometric analysis	**Content (%)**
Particles > 550 μm	9.8
Particles 250 μm 550 mm	81
Particles < 63μm	9.2
Bushel weight (kg/m^3)	654 kg/m^3

Table 2 shows microorganism counts and mycotoxin contents in the corn grits.

Analyses results show that with microbiological analyses were not found *Salmonella* sp., coagulase-positive staphylococci, sulphite reducing clostridia, proteus spp., *Escherichia coli* In 1 g of sample count of molds was 80, and total count of microorganisms was 500. Mycotoxicological analysis showed aflatoxins content <3 μg/kg, ochratoxine A content <10 μg/kg and zearalenone content <25 μg/kg , indicating that this feed was hygienically correct with respect to requests of the Rule Book on performing of analyses and super analyses of foodstuffs („Sl. list SFRJ“, 1980).
In the Table 3, chemical analyses and granulometric composition of bread crumbs are shown.

Table 2. Counts of microorganisms and mycotoxin contents in the corn grits

Microorganisms	**Sample size**	**Count**
Salmonella spp.	50 g	Not found
Coagulase-positive staphylococci	50 g	Not found
Sulphite reducing clostridia	1g	Not found
Proteus spp.	50 g	Not found
Escherichia coli	50 g	Not found
Total yeasts count	1 g	Not found
Total molds count	1 g	80
Total microorganisms count	1 g	500
Mycotoxin content (ELISA)	**µg/kg**	
• Aflatoxins B1+G1+B2+G2	<3	
• Ochratoxine A	<10	
• Zearalenone	<25	

Table 3. Chemical and granulometric compositions of the bread crumbs

Quality parameters	**Content (%)**
Moisture content	12.87
Crude ash content	2.24
Crude proteins content	11.44
Total sugars content	2.72
Reducing sugars content	2.08
Starch content	63.34
Crude fats content	3.18
Energetic value	**kJ/100 g**
Calorimetric energetic value	1589
Granulometric composition	**Content (%)**
Particles > 550 µm	45.6
Particles 250 – 550 µm	40.2
Particles < 63µm	14.2
Bushel weight (kg/m^3)	415 kg/m^3

Bread crumbs represent a foodstuff obtained by additional processing of the not consumed bread. This product contains 63,34% of starch, 11.44% of proteins and 3.18% of fats. Its energetic value amounts to 1589 kJ/100 g, so that this feed in the animal feed production represent high-quality proteinaceous and energetic feed.
Microorganisms counts and mycotoxin contents in the bread crumbs are outlined in the Table 4.

Table 4. Counts of microorganisms and mycotoxin contents on the bread crumbs

Microorganisms	**Sample size**	**Count**
Salmonella spp.	50 g	Not found
Coagulase-positive staphylococci	50 g	Not found
Sulphite reducing clostridia	1 g	Not found
Proteus spp.	50 g	Not found
Escherichia coli	50 g	Not found
Total count of yeasts	1 g	Not found
Total count of molds	1 g	30
Total count of microorganisms	1 g	30
Mycotoxin content (ELISA)	µg/kg	
• Aflatoxins B1+G1+B2+G2	<3	
• Ochratoxine A	<10	
• Zearalenone	<25	

The obtained results show that the applied bread crumbs were hygienically correct from microbiological and toxicological points of view, having total number of microorganisms and molds of 30 in 1 g of crumbs, and aflatoxins were bellow 3 µg/kg, ochratoxine A bellow 10 µg/kg, and zearalenone content bellow 25 µg/kg.
Table 5 shows chemical and granulometric compositions of the extruded mixtures of bread crumbs and corn grits.

Table 5. Chemical and granulometric analyses of the extruded mixtures of corn grits and bread crumbs

Quality parameters	**Content (%)**	
	Extruded mixture corn grits : bread crumbs, 50:50	Extruded mixture corn grits : bread crumbs, 60:40
Moisture content	8.40	8.53
Crude ash content	9.25	9.81
Crude proteins content	1.25	1.03
Total sugars content	5.20	5.93
Reducing sugars content	3.46	3.40
Starch content	69.8	65.36
Crude fats content	2.64	2.12
Energetic value	**kJ/100 g**	
Calorimetric energetic value	1635	1642
Granulometric composition	**Content (%)**	
Bushel weight (kg/m^3)	119 kg/m^3	92 kg/m^3

From the obtained results it can be stated that the extruded products contained decreased water contents, decreased starch contents and increased total- and reducing sugars contents if compared with the starting raw materials. This can be explained by effects of thermal decomposition of starch that influences on the digestibility and utilization of starch [2, 12]. The increased sweetness, i.e. changes of sensory properties are exactly the results of the physico-chemical changes of the starch component. Also, the bushel weight of the extrudates, if compared with the starting raw materials, are lower, being 92 – 119 kg/m^3.
Microorganisms counts of the extruded mixtures of corn grits and bread crumbs age outlined in the Table 6.

Table 6. Microorganisms counts in the mixtures of corn grits and bread crumbs

Microorganisms	**Sample size**	**Count**	
		Extruded mixture corn grits : bread crumbs, 50:50	Extruded mixture corn grits : bread crumbs, 60:40
Salmonella spp.	50 g	Not found	Not found
Coagulase-positive staphylococci	50 g	Not found	Not found
Sulphite reducing clostridia	1 g	Not found	Not found
Proteus spp.	50 g	Not found	Not found
Escherichia coli	50 g	Not found	Not found
Total count of yeasts	1 g	Not found	Not found
Total count of molds	1 g	Not found	Not found
Total number of microorganisms	1 g	40	20

As can be seen on the basis of the obtained results, extrusion process as a consequence had almost total reduction of molds, in spite of the comparatively low extrusion temperatures 95 – 105°C and very short time of extrusion (6 – 10 s), but also, very high extrusion pressure, being 30 – 40 bar [5]. In the extruded mixtures, bacteria of the types *Salmonella* spp., coagulase-positive staphylococci, sulphite reducing clostridia, Proteus spp., *Escherichia coli*, with exception of the total microorganisms number, which was satisfactory according to the Rule book on the harmful matters and substances in the animal feed (1990).

CONCLUSION

The extruded mixtures of corn grits and bread crumbs in the feed processing industry represent highly valuable energetic feeds. The extrusion process upgrades nutritive value of the extruded mixtures of corn grits and bread crumbs because of the increasing of quantities of the total and reducing sugars contents, as well as because of changes in the starch complex of the extrudates, having a consequence the increases of the feed digestibility, what is followed with the increase of yields. The thermal treatment in the

cylinder and screw of the extruder, total count of microorganisms is suppressed, because temperatures above 90ºC and high pressure are being lethal for all species of microorganisms. The reduction of microorganisms assures hygienic correctness of the obtained feeds. With the extrusion of bread crumbs together wit corn grits it is possible on the high quality manner to solve problems connected with the return of the old bread in baking industry. The obtained extruded feeds can be recommended for feeding primarily of young categories of animals, in the production o fish feeds and the feeds for pets.

ACKNOWLEDGEMENTS

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INFLUENCE OF INDICATOR PARTICLE SIZE ON THE MIXER EFFICIENCY EXAMINATION RESULTS

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ABSTRACT

Achieveing mixture homogeniety is one of the basic objects in feed production. Now a days, usage of colored iron tracer particles is the most used method for mixer efficiency examination. Three different granulation of "microtracers" were used in this paper: FS, F and RF. Mixing efficiency of rotary drum shape additive mixer was examined for three different mixing time. The aim of this experiment was examination of influence of various tracer particle size on results of mixing efficiency testing. It is fortified that with increasing of mixing time, segregation of mixture appeared when FS and F tracers were used. With RF tracers, totally opposite results were obtained.
***Key words**: homogeneity, mixing, tracers, particle size distribution*

INTRODUCTION

Mixing is one of the most important unit operations in feed production process. Main objective in the mixing process is to produce a mixture in which probability of finding any component particle is the same in all positions in the mixture, but ideal mixing is not possible to achieve. If feed is not properly mixed, single portions will contain either more or less ingredients than it is formulated. These variations in feed composition can cause animal illnes, and, thus, economic losses to animal producers. Periodic routine mixer testing is very important from the aspect of safety, but also from economic and ethic point of view, especially for premix production. Different substances, including amino acids, microminerals and salt have been used as tracers to measure feed mixing efficiency. For many years, the colored iron tracer particles, which can be separated from the rest of feed magnetically, have been successfully used in practice. These iron-based products, from the Microtracers™ company, are including following tracer types, differed by size distribution and number of particles per gram: 1. Microtracer F (Colored Iron grit, 25.000 particles per gram); 2. Microtracer FS (Colored Stainless steel grit, 50.000 particles per gram); 3. Microtracer RF (Colored iron powder > 1.000.000 particles per gram). Microtracers can be detected and quantified by using Rotary Detector - laboratory magnetic separator, or by use of special magnetic probe [1, 2, 5, 6, 7]. The size uniformity of various ingredients that comprise finished feed can directly impact on final ingredient distribution. If physical properties of ingredients are relatively the same, then mixing becomes fairly simple, but if physical characteristics of ingredients differ more, blending and segregation problems become complex [4]. The aim of this paper is to point out importance of using tracer with proper particle size distribution in order to get trustful mixer efficiency results.

MATERIALS AND METHODS

In this paper, mixing efficiency of rotary drum shape additive mixer (model SYTH 0.25, Jiangsu Muyang Group Co. Ltd., Kina) was investigated, for mixing times of 5, 10 and 15 minutes. The volume of mixer was 70 dm^3 and the batch size was 5 kg. Corn meal, with particle size distribution similar to regularly used additives, was used as a carrier. Granulometric composition of corn meal was determined on laboratory sieve (model Minor, Endecotts Ltd., Great Britain) by "Test sieving method" (ISO 1591-1 1988 (E)) [8].

Homogeneity was determined by Microtracer® method [1], and following types of tracers were used: F (F-blue), FS (FS-blue) and RF (RF-blue). The concentrations of tracers were 10 g per tone of carrier which corresponds to mixing ratio of 1:100000. Every tracer has declared number of particles per gram, so it is possibble to count expected number of particles in sample: for tracers from group F - 25000, group FS - 50000, and group RF > 1000000 particles per gram. Granulometric composition of tracers was also determined with laboratory sieve. Formerly mixed particles of microtracer were separated from analyzed samples by magnetic separator (model Rotary detector, Microtracer Inc., San Francisco). Separated microtrasers from group FS and F were uniformly spreaded on surface of filter paper, and its color was induced in contact with 50% solution of ethanol. Afterward, number of particles was counted. On the other hand, surface color of separated microtracers from RF group was dissolved in 7% Na_2CO_3 solution, and its concetration was determined trough intensity of solution color, wich was detected by absorbance measuring with spectrophotometer (Janway Ltd., Great Britany).

Mixing uniformity for tracers FS and F group was statisticaly evaluated by using Poisson distribution. Criteria for good homogeneity or uniformity of mixture was given through probability P, for χ^2 (chi square) statistic. The mixture is uniform if P value is higher than 0,05 (5%), and it is not uniform if P value is lower than 0,01 (1%). There cannot be given any conclusions about uniformity for P values in the range between 1% and 5%. For the RF tracers coefficient of variation is basic criteria of homogeniety. Uniformity of premix is good when CV is less than 5%, for mixing ratio 1:100000.

RESULTS AND DISCUSSION

Table 1. shows results of granulometric analysis of carrier, and used tracers.

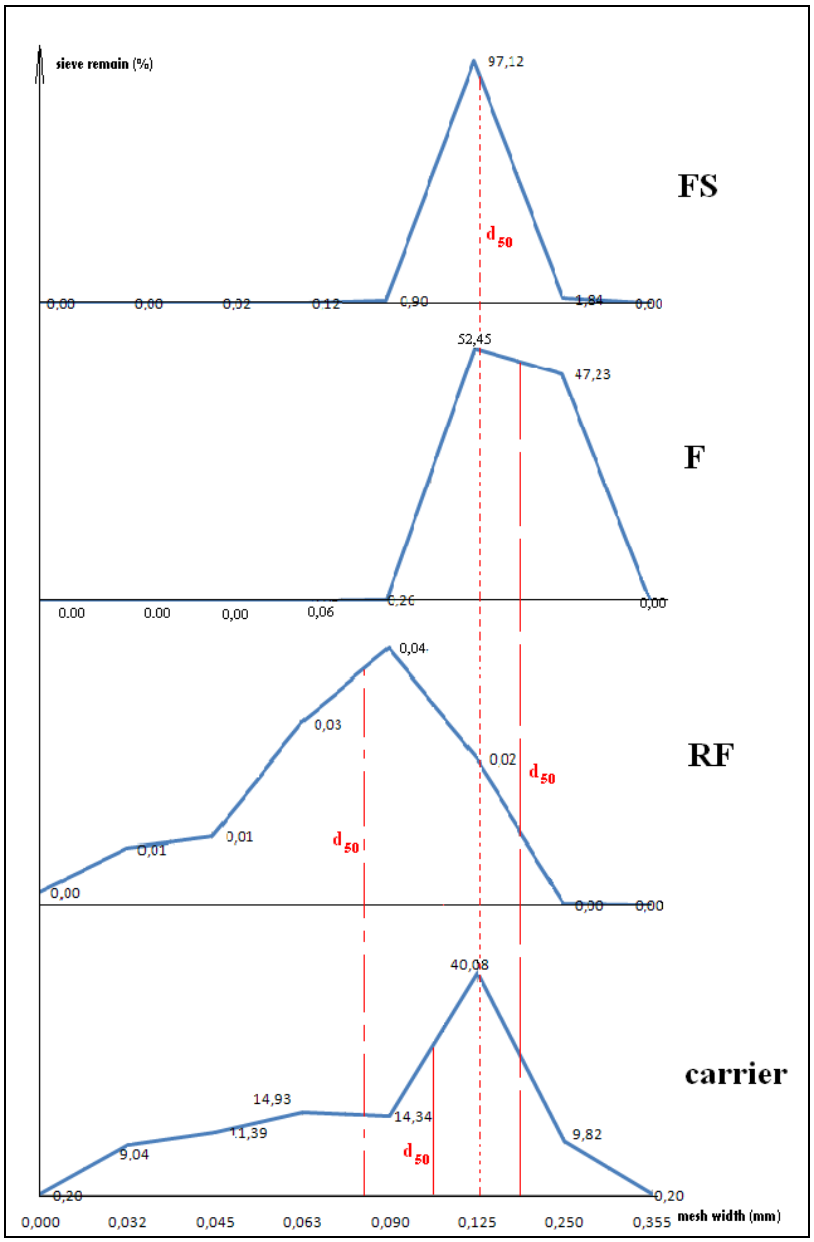
Table 1. Granulometric composition of tracers and carrier

Mesh width [mm]	Sieve remain, [%]			
	FS	**F**	**RF**	**Carrier**
0,355	-	-	-	0,20
0,250	1,87	47,23	0,12	9,82
0,125	97,12	52,45	20,18	40,08
0,09	0,90	0,26	35,55	14,34
0,063	0,12	0,06	25,07	14,93
0,045	0,02	-	9,52	11,39
0,032	-	-	7,83	9,04
Bottom	-	-	1,75	0,20
Total	100,00	100,00	100,00	100,00
Mean value, $\bar{x}$	0,13	0,19	0,08	0,11

Average particle diameters were counted on basis of granulometric analysis, where is:

$$d_{sr\ RF} < d_{sr\ Carrier} \approx d_{sr\ FS} < d_{sr\ F}$$

By comparing average particle diameters, it could be concluded that FS tracer was the best for examination of mixer efficiency. Never the less, comparing particle size distribution, it was noticed that RF tracer is the most similar to the carrier (Picture 1). On the other hand, FS and F tracers have narrow distribution of particle size, as well as small value of particle number per gram. That is what makes them inappropriate for examination of premix homogeniety.



Picture 1. Diagram of particle size distribution of tracers and carrier

Next results were obtained by examination of mixer efficiency by using FS and F tracers (Table 2 and 3):

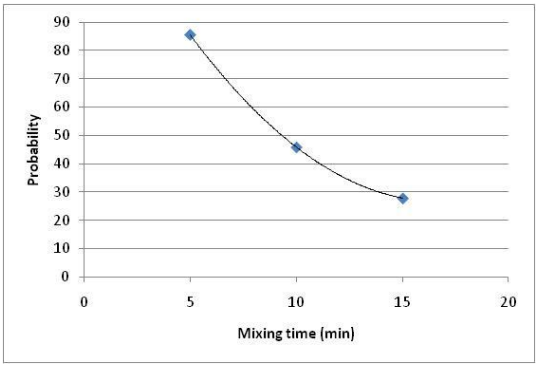
Table 2. Results of homogeniety examination by using FS tracer

	Sample mass, g			Particle number per 100 g		
	5 min	10 min	15 min	5 min	10 min	15 min
Sample 1	111,5	105,3	98,7	70,85	45,58	61,80
Sample 2	107,5	122,5	102,7	59,53	44,08	47,71
Sample 3	117,8	125,9	102,6	76,40	57,98	40,94
Sample 4	115,7	120,1	112,7	67,42	49,13	45,25
Sample 5	116	102,7	100,6	77,59	52,58	54,67
Sample 6	119,8	119,9	104,9	66,78	44,20	57,20
Sample 7	133	106,1	82,6	63,16	61,26	53,27
Sample 8	117,7	117,3	104,9	68,82	53,71	55,29
Sample 9	114,5		105	66,38		62,86
Sample 10	133,7		100,4	71,80		43,82
Mean value, $\bar{x}$				68,87	51,07	52,28
Standard deviation, σ				5,55	6,44	7,55
Coefficient of variation, %				8,06	12,62	14,44
Probability, %				85,4	45,83	27,85

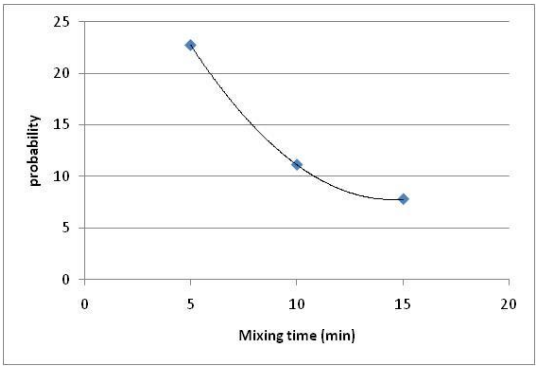
Table 3. Results of homogeniety examination by using F tracer

	Sample mass, g			Particle number per 100 g		
	5 min	10 min	15 min	5 min	10 min	15 min
Sample 1	109,8	104,4	117,1	36,43	24,90	24,77
Sample 2	119,2	114,6	115,5	23,49	22,69	28,57
Sample 3	112,5	114,5	111,9	40,89	25,33	11,62
Sample 4	104,8	103,6	101,7	27,67	24,13	15,73
Sample 5	102,5	119,4	116,8	30,24	17,59	20,55
Sample 6	106,8	106,5	115	28,09	12,21	13,91
Sample 7	110,4	123	107,3	29,89	13,82	19,57
Sample 8	112,2	124,1	100	22,28	15,31	19,00
Sample 9	112,9			33,66		
Mean value, $\bar{x}$				30,29	19,50	19,21
Standard deviation, σ				5,95	5,36	5,58
Coefficient of variation, %				19,65	27,51	29,06
Probability, %				22,77	11,14	7,79

Based on results from Tables 1 and 2, it can be clearly seen that usage of tracers FS and F gave results which show that probability P was higher than 5% (85,4%, and 22,77%) for mixing time of 5 minutes. Decomposition of mixture appeared as a result of mixing time increasing (Picture 2 and 3).

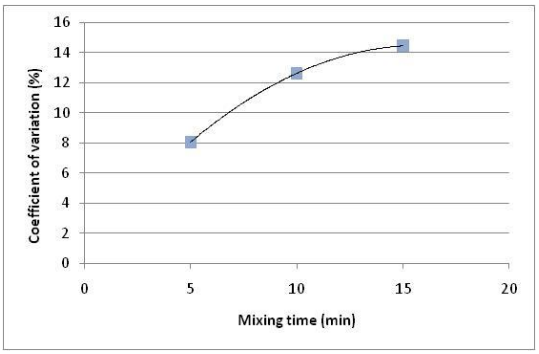


Picture 2. Diagram of probability P variation as a function of mixing time variation – FS tracer

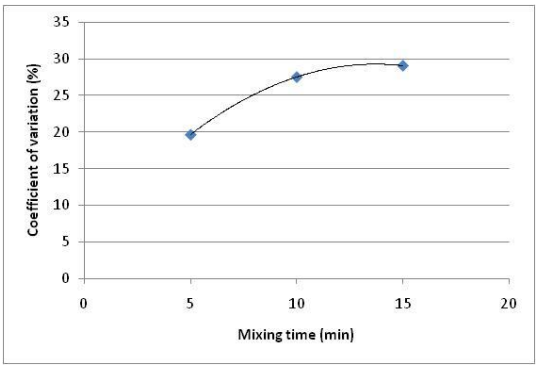


Picture 3. Diagram of probability P variation as a function of mixing time variation – F tracer

Coefficient of variation was used as a quality parameter for same tracers. It was done in aim to compare trend of its changing with trend of CV changing of RF tracer, due to the fact that probability P is criteria for homogeneity determination, through Poisson distribution (Picture 4 and 5). It can be seen that CV was higher with longer mixing time, which points to appearing of decomposition.



Picture 4. Diagram of CV changing as a function of mixing time variation – FS tracer



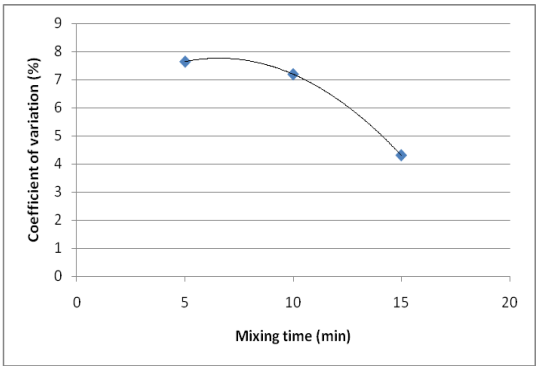
Picture 5. Diagram of CV changing as a function of mixing time variation – F tracer

Results of examination of mixer efficiency for additives by using RF tracer are shown in Table 4.

Table 4. Results of homogeniety examination by using RF tracer

	Sample mass, g			Absorbance (A) per 100		
	5 min	10 min	15 min	5 min	10 min	15 min
Sample 1	170,9	168,0	195,0	0,181	0,108	0,138
Sample 2	189,4	194,5	190,2	0,194	0,126	0,126
Sample 3	196,6	165,7	190,5	0,191	0,120	0,130
Sample 4	185,5	191,7	183,6	0,194	0,102	0,138
Sample 5	203,5	171,4	173,1	0,177	0,122	0,139
Sample 6	176,0	159,3	182,1	0,202	0,115	0,138
Sample 7	184,9	160,0	197,7	0,73	0,123	0,130
Sample 8	167,6	184,7	165,4	0,160	0,113	0,143
Sample 9	191,5			0,204		
Sample 10	175,0			0,201		
Mean value, $\bar{x}$				0,187	0,116	0,135
Standard deviation, σ				0,01	0,01	0,01
Coefficient of variation, %				7,7	7,2	4,3

From the results in Table 4 can be concluded that with usage of RF tracer for mixing period of 5 and 10 minutes CV results were higher than 5% (7,7% and 7,2%). This indicates that mixture had in adequate homogeniety. Prolongation of mixing time to 15 min caused decreasing of CV to level belowe 5%, which is recommended upper limit value in premix production (Picture 6).



Picture 6. Diagram of CV changing as a function of mixing time variation – RF tracer

CONCLUSION

Comparision of different tracers led to conclusion that choosing of appropriate tracer is very important and that tracer must have distribution of particle size that is similar to the distribution of particle size of mixture. FS and F tracers gave opposite trend of value changing, compared with RF tracer, which caused incorrect conclusions about homogeneity of mixture. This is result of difference between particle size distribution of FS and F tracers and carrier which, leads to decomposition during mixing process.

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PROTEIN DISPERSIBILITY INDEX AS AN INDICATOR OF THE DEGREE OF FULL FAT SOYBEAN HEAT TREATMENT

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ABSTRACT

Full fat soybeans (FFSB) intended for use in monogastric nutrition need to undergo heat treatment, so that present anti-nutritional factors can be inactivated. Under- or over-treatment will decrease the level of amino acids available to the animal. There are a number of laboratory methods that can be used to estimate the adequacy of FFSB processing, one of them being the Protein Dispersibility Index (PDI). In this paper, the results of an inter-laboratory study have been presented, with an aim to confirm the PDI globally accepted values of between 15 and 28 for adequately processed soybean. Seven FFSB samples were dry extruded at temperatures from 110°C to 164°C and assessed in the trial with chickens. Eight laboratories have been determining the PDI according to the official AOCS method. PDI range of 8.50 - 10.30 has been obtained for describing adequately extruded full fat soybean. Repeatibility limit r=2.11 and reproducibility limit R=7.73 were obtained.
Key words: *full fat soybean, extrusion, degree of processing, protein dispersibility index, inter-laboratory study*

INTRODUCTION

Full fat soybeans (FFSB) contain anti-nutritional factors (ANFs) which limit its use in monogastric diets (10). The ANFs can be inactivated by heat treatment. However, over-treatment will damage the protein and decrease amino acid availability (6). There is therefore an optimum range of temperatures where the ANFs are sufficiently removed without damage to reactive amino acids. Amongst other authors, Holmes (7), Ruiz et al. (8) and Zarkadas et al. (11) showed that moderate heating is necessary to increase the digestibility of soybean protein for non-ruminants. A comparatively mild heating leads to denaturation of tertiary and quaternary structures, allowing more effective penetration of digestion enzymes.
There are a number of laboratory methods that can be used to estimate the adequacy of FFSB heat treatment. Commonly used methods for assessing the processed FFSB quality are those for the determination of urease activity andex (UAI), trypsin inhibitor (TI), protein solubility in KOH (PSKOH), nitrogen solubility index (NSI) and protein dispersibility index (PDI). In a critical assessment of methods, Palic et al. (7) concluded that protein solubility is the best indicator for FFSB quality control and that therefore

PSKOH, NSI and PDI are the prefered methods. Batal et al. (4) reported that the PDI displayed the most constant response to heating of FFSB and that it may better indicate processed FFSB quality compared to other methods, and thus the PDI would be the first choice.

For adequately heat treated soybeans globally accepted values for PDI are between 15 and 28 (6). However, these values PDI are not specified in the description of the official AOCS method (3). In fact, no values were specified. Attempts to source original publication(s) which cited the PDI values of 15 - 28 for adequately-processed FFSB, have failed. It was therefore the aim of this study to confirm that globally accepted range.

MATERIALS AND METHODS

Raw soybeans were processed by dry extrusion at 7 temperatures: 110°C, 127°C, 136°C, 140°C 145°C, 151°C and 164°C and fed to chickens.

In vivo trial

A total of 384 male Ross broilers were randomly allocated to 48 pens, each containing 8 birds. On arrival all broilers were sorted into equal weight groups, and assigned at random to the different treatment pens, so that initial average weight and weight distribution were similar for all pens. They were allocated to one of seven dietary treatments containing the processed FFSB, with five replicates per treatment. The average body weight gain (ADWG) in the period from day 0 to 14 and feed conversion ratio (FCR) on day 14 were monitored as production parameters.
Data were analyzed using the statistical programme SAS/STAT (9).

Protein Dispersibility Index (PDI)

Protein dispersibility index (PDI) was determined according to the official AOCS method (3). It was subjected to an inter-laboratory study, which was conducted according to the Collaborative Study Procedures (1) and in which participated 8 laboratories. Seven FFSB samples which were assessed in the *in vivo* trial with chickens were analysed for PDI in duplicate. A "Hamilton Beach" Commercial Blender, model G936, (constructed according to requirements of the official AOCS method) was used by all laboratories. The speed of the blender was 8500 rpm. Repeatibility limit (absolute difference between two single results of analysis of the same sample obtained in one laboratory) and reproducibility limit (absolute difference between single results of analysis of the same sample obtained in different laboratories) were determined according to the AOCS procedure (2).

RESULTS AND DISCUSSION

In vivo trial

The results of the growth trial are shown in Table 1.

Table 1. Means of average daily weight gain (ADWG) in the period from day 0 to day 14 and feed conversion ratio (FCR) on day 14 of broiler chickens fed FFSB processed by dry extrusion at different temperatures

Treatment	ADWG (g)	FCR
110°C	87.8[bc]	2.081[d]
127°C	108.0[bc]	1.768[c]
136°C	138.3[a]	1.382[a]
140°C	132.0[a]	1.466[a]
145°C	123.0[a]	1.529[a]
151°C	97.2[b]	1.679[c]
164°C	79.8[c]	1.891[cd]
SEM[1]	7.94	0.081
LSD[2]	22.81	0.232
CV%[3]	19.1	11.5

[a,b,c,d]Values in the same column with different superscript differ significantly (P<0.05)
[1]**SEM** = Pooled standard error of the means
[2]**LSD** = Least significant difference
[3]**CV%** = Coefficient of variation

Statistical analysis of the results showed that the best performance was achieved by chickens that were fed the FFSB processed at 136^0C, 140^0C and 145^0C and that there was no significant difference between them (P>0.05). However, the difference between the groups that received the FFSB processed at 127^0C and 136^0C, as well as 145^0C and 151^0C, were significant (P<0.05). Based on the above-shown results, it has been concluded that 136^0C and 145^0C were the border temperatures of the range for adequately processed FFSB.

Protein Dispersibility Index (PDI)

Results of the inter-laboratory study of the PDI method are shown in Table 2.

Table 2. Results of 8 laboratories on determination of PDI in FFSB samples processed by dry extrusion at different temperatures

Lab No	PDI						
	110ºC	**127ºC**	**136 ºC**	**140ºC**	**145ºC**	**151ºC**	**164 ºC**
1	38.21	25.77	11.62	9.67	8.42	7.25	6.21
	36.66	22.1	9.22	10.55	8.89	7.82	6.75
2	37.26	21.32	10.34	9.07	8.74	7.77	6.74
	36.15	21.46	10.01	9.27	8.81	7.3	6.09
3	47.63	26.39	9.64	8.91	8.79	7.27	4.09
	47.18	28.2	10.01	8.36	9.39	6.8	4.23
4	44.72	31.1	14.69	10.87	9.1	3.96	1.62
	48.42	30.1	10.64	8.51	8.86	4.57	2.43
5	45.5	19.83	11.88	9.55	10.08	8.45	8.66
	47.62	18.17	12.54	9.5	9.9	8.26	8.38
6	46.24	27.79	10.17	9.12	9.02	8.89	7.78
	45.61	28.58	10.18	8.92	8.7	8.23	8.1
7	41.8	25.4	10.1	8.7	8.6	7.9	7.2
	41.9	25.25	10.7	8.8	8.1	8.2	7.1
8	35.86	11.84	6.15	6.9	5.01	4.73	4.66
	36.8	12.25	6.81	6.1	5.42	5.48	5.39
Average	*42.35*	*23.47*	***10.30***	***8.92***	***8.50***	*7.06*	*5.96*

FFBS samples processed at temperatures between 136^0C and 145^0C, represented adequately-processed FFSB (Table 1). The PDI values for these samples obtained in the inter-laboratory study, and shown in Table 2, were 10.30 and 8.50 respectively, which were, therefore, the boarder PDI values of the range for adequately processed soybean.
It should be stressed that the average PDI values for the range describing adequately processed FFSB of 8.50 - 10.30 (Table 2) are fairly different from those globally accepted (15 - 28). At this stage, there is no firm explanation for the difference between globally accepted values and those established in this study.
The repeatibility limit (r) was 2.11, whereas the reproducibility limit (R) was 7.73. These values are acceptable, but since it has been established in this study that the PDI range for adequately processed FFSB was between 8.50 and 10.30, the reproducibility value of 7.73 creates a concern with regard to use of the PDI method in practice.

CONCLUSION

The indications that the protein dispersibility index (PDI) might be the best indicator of the degree of FFSB processing, could not proven in this study.

Analysis of FFSB by the PDI method in this study generated values between 8.50 and 10.30 for adequately heat treated FFSB. Thus, globally accepted range of 15 - 28 could not be confirmed.
The PDI method had good repeatability limit (r=2.11), but the reproducibility limit was too wide (R=7.73) in relation to very narrow (8.50-10.30) range for adequately processed FFSB.

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A COMPARISON OF THE GRAVIMETRIC AND THERMOGRAVIMETRIC METHOD FOR ASH CONTENT DETERMINATION IN FEEDSTUFFS

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ABSTRACT

According to national and international regulations ash represents the mandatory parameter for feedstuffs labelling. It represents the total amount of the inorganic content used to estimate energy and calculate non-fiber carbohydrate content of feed. The gravimetric method is routinely performed in feed testing laboratories for ash content determination. Besides, it is official and widely accepted method for ash content determination in feedstuffs. Since it is characterized by different sources of uncertainties, the research on new analytical procedures has been stimulated. The thermogravimetric method (TGA) has already being utilized for ash determination in different kinds of food (such as coffee, milk powders, starches, flours, oil seeds etc.) since it requires shorter analysis times, smaller sample masses and no sample pretreatment. The values for ash content of feed samples selected for the study measured by the TGA method were higher than those measured by the gravimetric method. The TGA method was characterized by better precision in comparison to the gravimetric method. The MANOVA test ($P < 0.001$) and discriminant analysis ($P < 0.001$) pointed out that there were significant differences between the ash content values obtained by the TGA and gravimetric methods with clearly defined boundaries between the results of two methods. The differences in obtained results may be affected by equipment used, different measurement conditions, uncertainties of mass equipment and temperature controllers, computation effects, operator effects and random variation.
Key words: *ash, gravimetric method, thermogravimetric method, comparison*

INTRODUCTION

According to national and international regulations ash represents the mandatory parameter for feedstuffs labelling. It represents the total amount of the inorganic content used to estimate energy and calculate non-fiber carbohydrate content of feed [5, 6, 7]. The gravimetric method is routinely performed in feed testing laboratories for ash content determination. Besides, it is official and widely accepted method for ash content determination in feedstuffs. This method is characterized by extensive manipulation of sample by the analyst, large sample masses, long analysis times, and sample pretreatment before incineration, i.e. different sources of uncertainties. This fact stimulates research on new analytical procedures with more favorable characteristics for routine analysis, such as high analytical frequency, easy operation and better precision although the gravimetric method is still widely used as a reference method to check the performance of newly developed methods. The thermogravimetric method (TGA) has

already being utilized for ash determination in different kinds of food (such as coffee, milk powders, starches, flours, oil seeds etc.) since it requires shorter analysis times, smaller sample masses and no sample pretreatment [3, 9, 10, 13]. The application of TGA method for feed quality control has not been reported so far. Thermogravimetric analysis (TGA) is considered to be one of the five basic thermal analysis techniques. It involves the measurement of change of sample mass with change of temperature. In TGA, mass loss is observed if a thermal event involves loss of a volatile component. Chemical reactions, such as combustion, involve mass losses, whereas physical changes, such as melting, do not. The purpose of this study was to compare the gravimetric and thermogravimetric methods used for determination of ash content in feedstuffs.

MATERIALS AND METHODS

A total of 143 feed samples were collected for the study. Ash contents were determined by dry ashing in a muffle furnace at 550°C until the constant weight was reached. The thermogravimetric analyses (TGA) were performed using thermogravimetric analyzer TGA701 (Leco) with ceramic crucibles and sample mass of about 1.0 ± 0.1 mg, with dynamic air atmosphere at high flow rate (10 lpm) and ramp rate of 20ºC per minute in the temperature interval of 25-550ºC until the constant weight was reached. The measurements were carried out in replicates. The statistical approach applied in this study suggested by Pojić et al. (2009) [8] for the comparison of different analytical methods with each other included descriptive statistics (mean, standard deviation (SD), minimum and maximum, coefficient of variance (CV) and confidence interval), multivariate analysis of variance (MANOVA), discriminant analysis and analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Values for crude ash content measured by the TGA method ranged from 6.6 to 15.5 %, whilst values for crude ash content measured by the gravimetric method ranged from 5.6 to 13.2 %, being narrower in comparison to the TGA values (Tab. 1). The normal ash content of feed is from 7.0 to 13.0 depending on the type, so it was impossible to identify which method was more accurate [4, 6, 11]. The spread in ash content is representative of the values likely to be encountered in practice. The distribution of these results is shown in Figure 1 and 2.

Table 1. Central and dispersion parameters of determination of ash content in feedstuffs by the gravimetric and TGA method

Measurement	Mean	SD	Range	CV	Confidence interval	
Gravimetric method						
1	8.13	1.64	5.7 - 13.2	20.16	7.86	8.40
2	8.10	1.60	5.6 - 13.1	19.69	7.84	8.36
TGA method						
1	9.34	1.82	6.6 - 15.4	19.48	9.03	9.64
2	9.34	1.83	6.6 - 15.5	19.53	9.04	9.65

SD = standard deviation; CV = coefficient of variation.

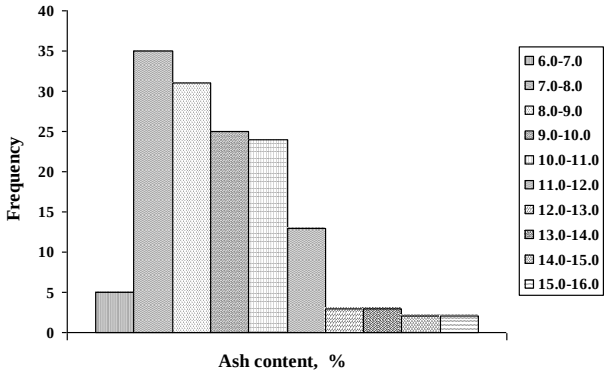

Fig. 1. Distribution of ash content of feed measured by the TGA method. Presented values correspond to mean (n = 2).

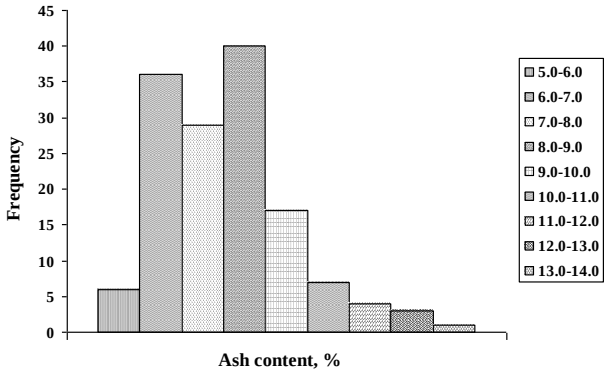

Fig. 2. Distribution of ash content of feed measured by the gravimetric method. Presented values correspond to mean (n = 2).

Central and dispersion parameters were estimated based on two repeated measurements performed by the TGA and gravimetric methods. Mean, SD, range, CV, and confidence interval values are presented in Table 1. The existence of a certain bias between these two methods is obvious, whereas the TGA method gave higher values for ash content than did the gravimetric method (Table 1, Fig. 3). The differences in obtained results may be affected by different equipment used, different measurement conditions, uncertainties of mass equipment and temperature controllers, computation effects, operator effects and random variation. Moreover, different subsamples were used to conduct the TGA and gravimetric analyses of crude ash content. The range, SD, and confidence interval values were smaller for the gravimetric method [2, 12].

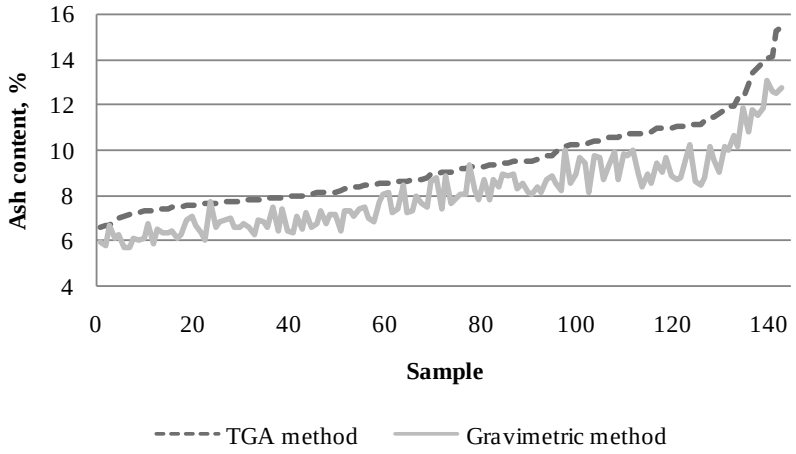

Fig. 3. Comparable overview of the results for ash content obtained by the TGA and gravimetric method

The MANOVA test ($P < 0.001$) and discriminant analysis ($P < 0.001$) applied pointed out that there were significant differences between the crude ash content values obtained by the TGA and gravimetric methods (Table 2). The position of confidence intervals (95% confidence limits) of the ash content results obtained by the TGA method (ellipse 1) and gravimetric (ellipse 2) methods plotted in two-dimensional space (Fig. 4) indicated the existence of clearly defined boundaries between the results of two methods. Also, the position of the ellipses confirmed the existing of the certain bias between the two methods. The orientation of the ellipses indicates that there is strong correlation between the two series of data, whilst the shape of the ellipses indicates that the TGA method yielded more precise results in comparison to those obtained by the gravimetric method. This was expected since there is less sources of measurement uncertainties in the case of the TGA method.

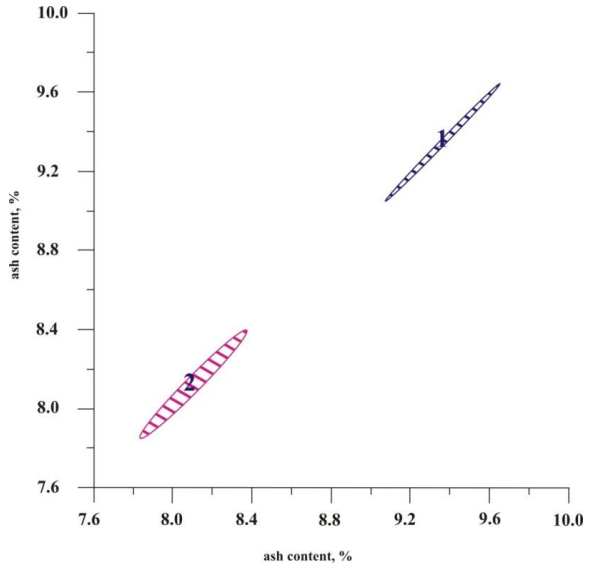

Fig. 4. Confidence intervals for the TGA method (ellipse 1) and gravimetric method (ellipse 2).

Table 2. Analyses of Differences Between the TGA and Gravimetric Methods with Regard to Ash Content Determination

Analysis	n	F	P value
Multivariate analysis of variance	2	20.269	0.000
Discriminant analysis	2	20.920	0.000

Repeated measurements have the great importance in providing more conclusive information on the characteristics of the analytical methods. Random deviations of repeated measurements are used to characterize the reliability of measurements and therefore their precision. They are often expressed as standard laboratory error and represent as a distribution of the results around the mean of the sample where the variation is randomly distributed to higher and lower values [1]. The SEL of the gravimetric method and TGA method was 0.22% and 0.05%, respectively showing that the TGA method was characterized by smaller SEL and better precision in comparison to the gravimetric method. Moreover, calculated values for P ($P > 0.1$) indicated that the significant differences between repeated measurements for the gravimetric and the TGA method as well, were not reported (Table 3).

Table 3. Differences between repeated measurements for the gravimetric and TGA method

Mean				
Measurement 1	Measurement 2	t	p	SEL
Gravimetric method				
8.10	8.13	0.13	0.894	0.22
TGA method				
9.34	9.33	0.04	0.969	0.05

CONCLUSION

Comparison of the results of the gravimetric and TGA methods used for the determination of crude ash content in feedstuffs showed that the results for the TGA method had demonstrated better precision compared with the reference chemical method. Furthermore, the results of this study showed the existence of a certain bias between the results of two methods, where the gravimetric method produced results that were lower than the results obtained by the gravimetric method. The differences in obtained results may be affected by different physical nature of the methods, different equipment used, different measurement conditions, uncertainties of mass equipment and temperature controllers, computation effects, operator effects or random variation. Further work should be performed in order to identify the level of accuracy of these methods.

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INFLUENCE OF LINSEED ENRICHED DIET ON OMEGA-3 FATTY ACIDS CONTENT IN PIGLET MEAT

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ABSTRACT

The aim of this study was to evaluate the influence of diet supplemented with linseed rich additive under commercial name Vitalan on omega-fatty acids content in piglet meat. The main ingredient in Vitalan is extruded linseed, which made the test diet rich in omega-3 acids. Twenty four piglets were divided in control and experimental group and grown to 32.5 kg of average live weight. Experimental group was fed the standard diet enriched with 2.5% of Vitalan. After the end of feeding period, the meat samples from both groups were analyzed for omega-3 and omega-6 fatty acids content in raw and oven- roasted meat. The ratio between omega-6 and omega-3 acids was established. Additionally, other parameters, such as weight gain, general health and appearance of piglets were monitored during the study. The treatment with linseed diet resulted in higher omega-3 acids levels, which lowered ratio between omega-6 and omega-3 acids in meat, thus making it better for a human consumption from a health perspective. Also, the treatment caused faster weight gain, better health and better general performance of piglets. The conclusion was that the diet enriched with extruded linseed had beneficial effect on the majority of monitored parameters in the study.
Key words: *piglets, extruded linseed, piglet meat, omega fatty acids*

INTRODUCTION

It is now generally recognized that dietary fats play an important role in human health. Among dietary fats major role belongs to polyunsaturated fatty acids (PUFA) with n-3 PUFA being most beneficial for human health. There is a great deal of evidence that n-3 PUFAs have anti-inflammatory, antithrombogenic and hypotriglyceridemic properties, they inhibit the formation of atherosclerotic plaques , prevent arrhythmias and have activity against some cancers such as breast, colon and prostate (Rose and Conolly , 1999; Connor, 2000). At the same time increased levels of n-6 fatty acids are associated with an increase in chronic diseases (Givens et al., 2006). Because of n-3 PUFA beneficial effects and the fact that western diet is very rich in n-6 fatty acids (Enser et al., 2000) the nutritional authorities have recommended the diet rich in n-3 polyunsaturated fatty acids and that n-6/n-3 ratio should be lowered to between 1 and 4 instead of the current 15-20:1 (Simopoulos, 2002). One way to improve this ratio is by modifying the fatty acids composition in meat, which is important part of human diet and natural supplier of fatty acids. Animal diet determines the fatty acid composition in

meat and by changes in the diet, fatty acids ratio in meat and its nutritional value can be modified. This is usually done by feeding animals with the feed which is enriched with fish oil or fish meal as sources of n-3 (omega-3) PUFAs or by feeding with meals containing seeds or oils rich in n-3 fatty acids (Raes et al., 2004; Kouba, 2003).
The aim of this study was to investigate the influence of supplemented linseed diet rich in n-3 (omega-3) polyunsaturated fatty acids on fatty acid composition and in particular omega-3 content and n-6/n-3 ratio in raw and roasted pork meat.

MATERIALS AND METHODS

Animals and diet
The study has been conducted with 24 pigs (50% Pietrain, 25% Landrace, 25% Great Yorkshire) at the pig farm 'Sabo Janos', Jermenovci, Serbia. The pigs were divided into two groups and fed with two types of diet, a standard diet and diet enriched with Vitalan (Vitalac, France) until reaching approximate live weight of 32.5 kg. One group was fed the control diet and experimental group was fed the standard diet enriched with 2.5% of Vitalan. Vitalan containes 85% extruded linseed and the rest are wheat bran and antioxidants. The diets composition is shown in Table 1. The piglets were fed from the weaning period, 35 days of age, until the age of 79 days, when they were slaughtered . Total feed consumption, daily weight gain and feed conversion ratio were monitored. The animals were fed *ad libitum*.

Table 1. Composition of experimental diets

	Control diet	**Experimental diet**
Vitalan		2,5%
Vitamins	8,0%	8,0%
Digestabl	2,0%	2,0%
Maize	38,0%	38,0%
Barley	31,8%	29,3%
Soybean meal	20,0%	20,0%
Agrotoks	0,2%	0,2%
Total	100,0%	100,0%

Slaughter and sampling
The animals were slaughtered and samples of meat (*M. Longissimua dorsi*), 6 pieces (200g each) from both groups were collected and kept in the refrigerator at 4^0C. A half of the samples were coocked in the oven at the temperature of 80-85^{0}C until the temperature in the centre of the meat reached 69^{0}C (about 1 hour). After 24 hours, the

samples were sent to the laboratories of Food Technology Institute in Novi Sad, where fatty acid analysis and sensory evaluation were performed.

Fatty acid analysis

The preselected meat samples were homogenized with food processor and fat was extracted from 1 g of each sample. From the extracted lipids fatty acid methyl esters were prepared with boron trifluoride/methanol solution. Obtained samples were analyzed by a gas chromatograph Agilent 7890A system with FID, auto-injection module for liquid and headspace sampling, equipped with fused silica capillary column (DB-WAX 30 m, 0.25mm, 0.50 um). The fatty acids were identified by comparison with standards from Supelco 37 component FAME mix and data from PUFA NO.2, Animal source BCR-163 beef/pork fat blend. Results were expressed as mg of fatty acids per 100 g of tissue (mg/100g) and as a ratio between omega-6 and omega-3 fatty acids.

RESULTS AND DISCUSSION

During the study the animals were readily fed and there was no illnesses or losses. All piglets were similar in size, healthy, with shiney and clean hair and vivacious. Results of monitoring the avarage weight, average weight gain, feed consumption and feed conversion ratio are shown in the Table 2.

Table 2. Average weight and weight gain of piglets, feed consumption and feed conversion ratio

	Average weight, kg			**Average weight gain, kg/day**		**Feed consumption, kg**	**Feed conversion ratio, kg/kg**
	0	35. day	79. day	1-35. day	35-79. day		
Control	1,70	9,00	30,00	0,209	0,477	45,00	2,14
Experimental	1,70	10,50	35,00	0,251	0,557	40,00	1,63

From the results shown in Table 2, it is evident that piglets from the treatment group fed with diet enriched with extruded linseed (Vitalan) consumed 40 kg of feed while the piglets from the control group fed with the diet without enrichemnet consumed 45 kg of feed. Even though they consumed less feed, the piglets from the treatmnet group, with Vitalan enrichment, reached higher weight (35 kg) than the piglets from the control group (30 kg).

The daily weight gain of piglets fed with Vitalan enrichement had higher weight gain (557 g daily) than the piglets from the control group (477 g).

Piglets from the experimental group that were fed the extruded linseed diet, had better feed utilization compared to the control group, as shown by feed conversion. The feed conversion ratio in the treatment group was 2.14 kg of feed per 1 kg of weight gain, while in the control group was 1.63 kg/kg.
The results of this study showed that piglets fed diet enriched with extruded linseed (Vitalan) performed better than the control group.
The use of different diets also affected the meat quality. Sensory evaluation gave unanimous opinion that baked piglet meat fed with enriched diet had pleasing colour, it was juicy, soft and of superb taste.
Results of the fatty acids analysis confirmed that this meat was of very good quality from the health perspective (Table 3.).

Table 3. Omega fatty acids content in piglet meat (mg/100g of meat)

		Omega-3		**Omega-6**		**Omega-6/Omega-3**	
		$\bar{x}$	Sd	$\bar{x}$	Sd	$\bar{x}$	Sd
Raw meat	Control	0,54	0,11	13,07	2,89	23,97	0,70
	Experimental	5,27	0,37	19,97	1,01	3,79	0,08
Baked meat	Control	0,62	0,14	12,58	1,47	20,77	4,25
	Experimental	3,96	1,37	17,92	2,23	4,77	1,12

From the results shown in the Table 3. it is evident that use of linseed enriched diet resulted in increased levels of omega-3 fatty acids in the treatment group (5.27 mg/100g of meat) compared to the control group (0.54 mg/100 g of meat). This significantly contributed to the more favourable ratio of omega-6/omega-3 in the treatment group (3.79) compared to the control group (23.97).
Similar results were obtained with baked meat. Omega-3 fatty acid content is higher in the baked meat fed with enriched diet (3.96 mg/100 g of meat) than in meat from the control group (0.62 mg/100 g of meat). Also, the omega-6 to omega-3 ratio was much more favourable in the treatment group (4.77) than in the control group (20.77).

CONCLUSION

Based on the results of this study, the following conclusions can be made.

- Piglets fed diet enriched with extruded linseed (treatment group) compared to the control group gained higher weight (35 kg compared to 30 kg) with lower feed consumption (40 kg compared to 45 kg).

- Piglets from the treatment group compared to the control group had a better feed conversion ratio (1.63 compared to 2.14 kg of feed per kg of weight gain) and better daily weight gain (557 g compared to 477 g).
- Levels of omega-3 fatty acids in the fresh meat of experimental group were much higher than in the control group (5.27 compared to 0.54 mg/100 g of meat) which made the ratio of omega-6 to omega-3 fatty acids much more favourable in the experimental group (3.79) compared to the control group (23.97).
- Levels of omega-3 fatty acids in the baked meat were much higher in the experimental group than in the control group (3.96 compared to 0.62 mg/100 g of meat) which also made the omega-6 to omega-3 fatty acids ratio much more favourable in the experimental group (4.77) compared to the control group (20.77).

From the data presented in this study it is evident that use of the extruded linseed enriched diet significantly elevated the levels of omega-3 fatty acids and improved the ratio of omega-6 and omega-3 fatty acids to the desired level of around 4.

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THE IMPACT OF EXTRUDED CORN ON THE ECONOMIC RESULTS OF CHICKENS PRODUCTION

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ABSTRACT

Aviculture enables acquiring of significant amounts of highly valuable products: meat and eggs, in the relatively short period of time. Production of fatty poultry practically presents the most intensive branch of the animal husbandry. The reproduction process is relatively short, which enables faster turnover of the engaged resources. Consequently, products which are cheaper compared to other kinds of meat are obtained, considering that production expenses have very important role. One of the ways to influence economic results is the use of extruded feed. This way enables the achievement of lower wastage and better production results, influencing directly the economic results of the production in such a way. Research of the economic results of the production of fatty poultry is based on the determination of the total production cost, value of the production and the financial result. Calculation of these economic categories is based on the data gathered on the selected households. The results obtained show measurable differences in the use of the extruded feed compared to the classic feeding system.
***Key words**: chickens production, extruded corn, price*

INTRODUCTION

Animal husbandry presents the most intensive branch of agriculture and has multiple significances, for both producers and consumers. The increase in the production of the meat, milk, eggs, among others is the foundation for the improvement of the nutrition structure of the population with highly valuable animal proteins.
Production of the fatty poultry presents a form of agricultural production, which is, by its nature, closest to the industrial production. Accordingly, great work productivity and control of production processes is achieved.
The solution of increased food production for people and animals is appliance and usage of new technologies in biotechnology, e.g. bio industry (Lazarević i sar., 2005). The main orientation is presented by new technological processes which aim at the increase of nutritive value of the food for people and animals. Nowadays, many ways of thermal processing of oilseeds and cereals are used in the world: toasting, hydrothermal refinement, micronisation, microwave treatment, bielectrical heat treating (Marsman et al., 1998), but in Serbia the most often used are the process of extrusion and hydrothermal process (Sakač et al., 2001; Filipović et al., 2008).

In the domestic production of forage mixtures, the corn has the leading position compared to other cereals, because of high energy contents (16.2 MJ/kg), starch, comparably big contents of oil and low level of cellulose. Corn, apart from the best digestibility, also has the best taste compared to other grains (Bekrić, 1999).
Proper conduction of the thermal process provides the reduction of thermo labile antinutritients to an acceptable level, increase of digestibility of some nutritients (proteins, oil, carbohydrates), as well as the improvement of sensory features and the microbiological picture of the final product (Filipović et al., 2003; Kormanjoš et al., 2007). Parallel with the reduction of the contents of the antinutritients it is necessary to preserve nutritionally valuable thermo labile components, so the process requires finding a compromise between the two efforts.

MATERIAL AND METHODS

Estimation of production expenses of fattening poultry in case of extruded and non extruded corn in forage mixtures is based on natural indicators determined based on the research conducted at the examined farm. It is an individual household which has its own food production for animals, with a farm capacity 3000 fattening chicken in turnus. During the fattening, feeding of one half of chicken was practiced with the food that contained extruded corn (experiment E), while the other half was fed with a mixture in which the corn has not been treated (control C). Calculation of the expense for food has been derived according to the standard of expenses for the preparation of animal food, based on market prices of certain kinds of food and experience normative. The expense for other material has been calculated according to the expenditure made on the observed farm and market prices. Investments into buildings and the equipment have been calculated based on the performed investments on the observed household. Expenses of the buildings' and equipment amortisation have been derived based on the assumed lifetime of the utilised means (Marko et al., 1998). Expenses for salaries have been calculated in accordance with realised expenses. Expenses for the energy consumption have been calculated on the basis of realised expenditure of the electrical power and fuel. Apart from that, the calculation includes expenses of veterinary and selection services. Calculation of the income is based on clarification of total income from the above mentioned production, whereby the financial result presents the income from the overall production (Andrić, 1998).

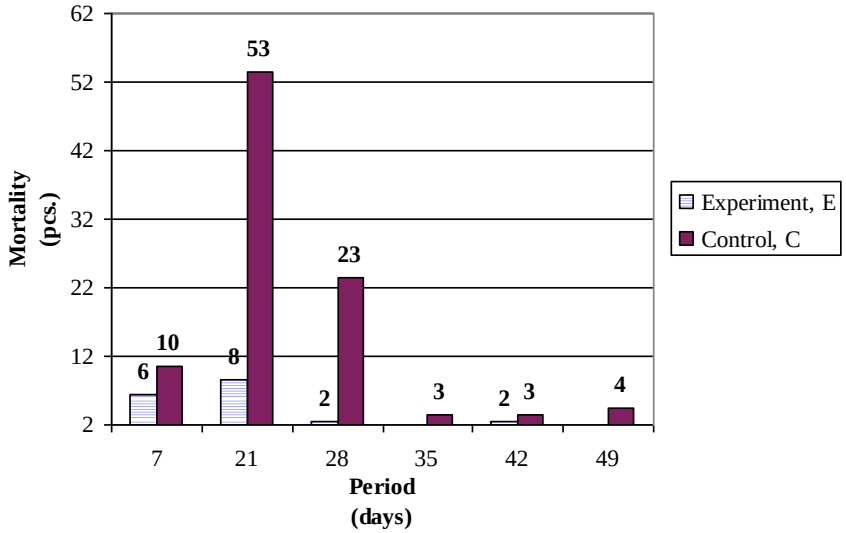
RESULTS AND DISCUSSION

During the analysis of the observed production, production results for both groups have been followed closely. The follow up was conducted on a weekly level. It is extended fattening in duration of seven weeks. Since the feeding regime has different effects depending on the age of the poultry, better insight into the overall effects of the food with extruded corn was enabled. The main production indicators are given in the Table 1. Lower mortality rate could be pointed as the most obvious result of the usage of extruded corn in feeding. In case of feeding with forage mixture with addition of non

Table 1. Basic production indicators of poultry fattening

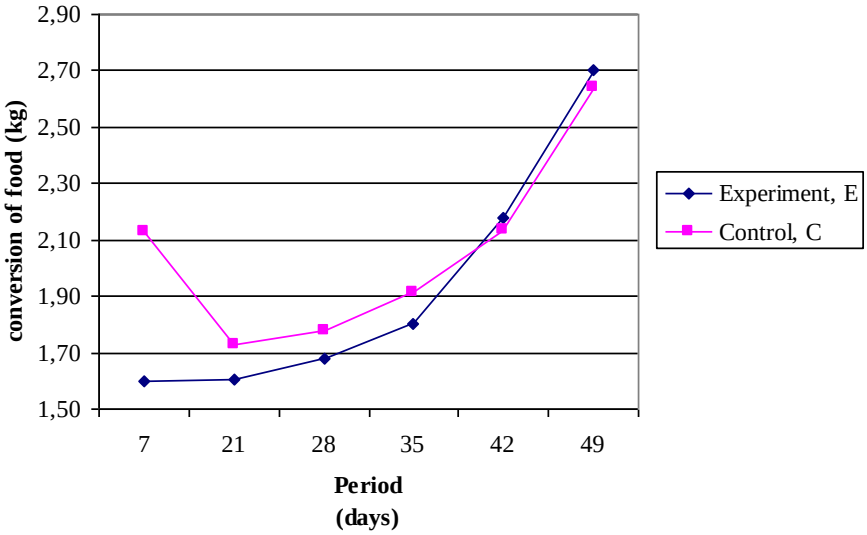
Period (days)	E				C			
	Body weight (gr)	Total food consumption (kg)	Mortality (pcs.)	Number of the flock in the fattening (pcs)	Body weight (gr)	Total food consumption (kg)	Mortality (pcs.)	Number of the flock in the fattening (pcs)
0	44,2			1.500	44,2			1.500
0-7	127,5	200	6	1.494	111,5	215	10	1.490
0-21	565,0	1.250	8	1.486	519,0	1.265	53	1.437
0-28	966,0	2.250	2	1.484	907,0	2.255	23	1.414
0-35	1.490,0	3.650	1	1.483	1.420,0	3.645	3	1.411
0-42	1.985,0	5.250	2	1.481	1.940,0	5.210	3	1.408
0-49	2.760,0	8.350	1	1.480	2.780,0	8.330	4	1.404

extruded corn, 96 chickens have died in total. If this is compared to the mortality in case of feeding with the mixture with extruded corn (20 chickens), it is possible to draw a conclusion about the great advantages to the usage of extruded corn in food for maintaining health condition of the poultry. Besides, provided results are specially expressed in the first four weeks of the fattening (Graph 1).



Graph 1. Mortality of chicken during the fattening

The second factor of significance for the overall production and the achieved economic result is the conversion of food. The calculation of the achieved conversion shows that the group fed with the extruded corn achieves better conversion whereby, on the level of the overall fattening makes 2,04 gr of forage mixture per kilogram of weight gain. Feeding with the forage mixture, the achieved conversion of the food is 2,13 kg per kilogram of the weight gain. The overview of the conversion change during the fattening gives a better insight into the food conversion. The overview has been made according to the available data and is given on the Graph 2.



Graph 2. Conversion of food during the fattening of poultry

It is obvious from the Graph that feeding chickens with extruded corn gives better results at the beginning of the fattening, whereby very beneficial effects on younger categories of poultry are shown once again. If we take a look at the Table 1 in the previous part of the paper, one can draw a conclusion about the almost same expenditure of food in both groups observed. Also, the group fed with food with the addition of extruded corn has bigger number of fattening chickens at the end of the fattening and achieves higher value of the production.

Total cost of the food makes RSD 286.335,00 for the group fed with the food with addition of extruded corn, or RSD 285.840,50 for the group fed in standard way. Accordingly, the cost of feeding per chicken has been calculated and it presents RSD 193,47 for the first group, and 203,59 for the second group, which is 5,23 % higher value that directly influences growth of the overall cost.

The analysis of the overall economic indicators of the observed production starts from the assessed investment into the farm, in other words investment into the buildings for breading with the following equipment. According to the assessment, the investment into the buildings and equipment amount to 135.000,00 €, or RSD 12.825.000,00.

Calculation of other expenses (energy, work and additional materials) of the production, has been derived per turnus and is given in the Table 2.

Table 2. Summary overview of other expenses per turnus

Description	Unit	Pcs / kg	Quantity	Total
Bedding	bale	120,00	100,00	12.000,00
Vaccine	pcs.	3.000,00	0,65	1.950,00
Vaccine	pcs.	3.000,00	1,35	4.050,00
Vitamins	pcs.	1,50	2.200,00	3.300,00
Revaccination	pcs.	3.000,00	0,65	1.950,00
Revaccination	pcs.	3.000,00	1,35	4.050,00
Electric power	-	-	18.500,00	18.500,00
Gas	-	-	30.000,00	30.000,00
Salaries	-	-	32.000,00	64.000,00
Total				**139.800,00**

The expenses presented refer to the both groups of fattening chickens observed. In the distribution, they were divided proportionally to the starting number of chickens, e.g. two equal groups. Accordingly, the given category of expenses amounts to RSD 134.025,00 per observed group, or RSD 44,68 per fattening chicken.
In accordance with the derived calculations, establishing of the total expense and the price of the fattening chicken has been derived. Calculation of these indicators is presented in the Table 3.

Table 3. Calculation of the total expense and the price, RSD

Expense category	E			C		
	Total expenses	**Price**	**%**	**Total expenses**	**Price**	**%**
Amortisation	64.125,00	43,33	15,3%	64.125,00	45,67	15,3%
Food expenses	286.335,00	193,47	68,1%	285.840,50	203,59	68,1%
Salaries	32.000,00	21,62	7,6%	32.000,00	22,79	7,6%
Expenses for the energy	24.250,00	16,39	5,8%	24.250,00	17,27	5,8%
Expenses of other and additional materials	13.650,00	9,22	3,2%	13.650,00	9,72	3,3%
Total	**420.360,00**	**284,03**	**100,0%**	**419.865,50**	**299,05**	**100,0%**

Calculation of the income includes the incomes the farm achieves and is based on the sale of fattening chickens. On sale, the price that was achieved was 120 RSD/kg. In accordance with the number of fattening chickens breed, average weight reached and the sale price, the calculation of the total income has been made. Calculation of the total income is give in the Table 4, according to the observed groups, and based on that the benefit has been calculated as the difference between the income and expense.

Table 4. Benefit calculation, RSD

Description	E	C
Total income	490.176,00	468.374,40
Total expense	420.360,00	419.865,50
Benefit	**69.816,00**	**48.508,90**

If the realised benefit is calculated per kilogram of produced chickens, we get RSD 17,89/kg for the group fed with the mixture with addition of extruded corn, and RSD 12,43/kg for the group fed with standard forage mixtures. The economy calculated from the ratio of total income and total expenses makes 1,17 for the group fed with the mixture with extruded corn and 1,12 for the group breed by the standard feeding system. Profitability of the production is obtained from the ratio of realised benefit and total investment. Total investment includes investments into the buildings and equipment and investment into the unfinished production within the fattening. Thereat, in total five turnuses are foreseen per year. Binding of means in the form of debits has not been calculated; instead the calculation has been derived with an assumption of advance payment. Profitability of the overall production process in the observed case is not hard to establish, since it is concentrated and monophase production. Realised profitability for the group fed with the mixture with addition of extruded corn was 2,67% and 1,86% for the group fed with standard forage mixtures. In both cases the obtained value is very low.

CONCLUSION

Lucrativeness and profitability of the production are the most important principles and the basis of rational business in the marker economy, which is all and more becoming an imperative for our production too. Economic results of the production of fatty poultry have in the paper been analysed from the narrower producers' perspective and what can be concluded is the following:

- The profit achieved per one turnus amounts to RSD 69.816,00 for the group fed with the mixture with addition of extruded corn and 48.508,90 for the group breed by standard forage mixtures, e.g. RSD 17,89 /kg and RSD 12,43 /kg per kilogram of produced chicken.

- Economy calculated from the ratio of total income and total expense makes 1,17 for the group fed with the mixture with addition of extruded corn and 1,12 for the group breed by standard feeding system.
- Detailed analysis of economic indicators shows very low profitability of the production. The realised profitability of the production makes only 2,67 % for the group fed with the mixture with addition of extruded corn and 1,86% for the group breed by standard forage mixtures. The obtained values in both cases very low and indirectly points to the need for the state subventions of investments in this sphere.
- Regardless of that, all presented indicators point out the justifiability of the usage of extruded corn in the preparation of food for animals.

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PRODUCTION OF FEEDS AND ADDITIONAL FEEDING OF GAME AS A MEASURE OF FOREST AND WILDLIFE PROTECTION

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ABSTRACT

Depending on the type of hunting ground and level of human activity, natural feed sources for game may be insufficient. When looking for food game may create various damages on crop, vegetable and fruit plantations, and also on domestic animals and bees. Natural feeding of game may be significantly improved with the amelioration of natural pastures, production of fresh green forages within the hunting grounds and planting fruit and other trees which produce edible outgrowth. Direct supplemental feeding of game (with concentrates or forages) is done usually in the winter time, and is done mainly to prevent game mortality and improve their body mass and the quality of their trophies. The mentioned measures are of importance for herbivore and omnivore animals. With the supplemental feeding of carnivores the better control of animals is achieved (particularly of rare species), the migrations are reduced and the harmful effects on domestic and other animals are reduced.
***Key words**: game, hunting ground, feeds, damages*

INTRODUCTION

In our hunting grounds game animals may have various harmful effects, or may be at various risks. There are solutions for both cases, and they may be more or less successful (17).

Damages that may be created by game on crops (maize, lucerne, wheat, barley, potato and others) are generally less than 1%, and therefore may be considered as negligible (13, 18). The biggest damage is created by wild boars and ruminants, while birds may be harmful only in period when maize is in early growth (10, 11, 12). Total damage in forests and orchards (distraction of young plants and bark cutting) may occur in periods when natural feeds are deficient, or if the number of animals becomes too high for the size of the hunting ground (25). That kind of damage is characteristic for hares and wild ungulates (16). Special type of damage in agriculture and domestic animals in our country is created by wolves and, rarely, other carnivores (24).

With the appropriate management measures such damages may be reduced or prevented in the hunting grounds (21, 22, 23). One of the most important measures is to increase feed production within the hunting ground, or supplemental feeding from other sources in times when damages occur (8).

FEEDING POTENTIALS OF SERBIAN HUNTING GROUNDS

Hunting association of Serbia is managing an area of 7.891.318 ha of hunting grounds where dominant species are small game and roe deer. The percentage of forests is one of the better parameters of suitability for life of most game animals. In that respect the Vojvodina is not in very good situation (only 7.07% of forests), while Zlatibor, Raška, South Serbia, Timok, Kosovo and Metohija are in better position (above 40% forests, Table 1).

Forests present suitable environment for game animals and provide natural feeds in the form of pastures, browse, seeds, mushrooms and small animals (for omnivores and carnivores), as well as water springs. In forest environment there is very little need for supplemental feeding, and may be considered only in cold winters with high snows (7). The lowland type of hunting ground, which is present in Vojvodina and in parts of Central Serbia, is much less abundant in natural feeds. The reason for that is intensive agriculture, the use of chemicals, high density of human and animal populations. In such circumstances the additional feeding of game is becoming indispensable, particularly during the winter (1).

Table 1. Overview of total hunting grounds in Serbia (14)

Name of the area	Area, ha	Forests and forest lands	Meadows and pastures	Arable land	Fruit and vineyards	Other land
Bačka	890975.60	40925.00	45078.50	703647.00	12188.00	89137.10
Banat	883169.00	42001.50	109705.00	635265.00	10175.00	86022.50
Srem	378491.00	69351.00	18684.00	241533.00	10961.00	37962.00
Vojvodina	2152535.60	152277.50	173467.50	1580445.00	33324.00	213121.60
Beograd	315685.00	36328.00	24864.00	199186.00	19835.00	35472.00
Podunavlje	499469.47	118802.47	67863.00	238566.00	30751.00	43487.00
Šumadija	691826.00	185420.00	110346.00	290347.00	59180.00	46533.00
Kolubara	573844.00	142224.00	70931.00	288631.00	35514.00	36544.00
Zlatibor	724339.00	281769.00	271713.00	113863.00	14335.00	42659.00
Raška	705913.67	292860.61	169298.69	163871.30	33072.00	45811.07
Timok	724269.45	302786.80	153614.50	205987.83	16481.33	45398.99
South eastern Serbia	722297.00	238461.00	174293.00	233667.00	31623.00	44253.00
South Serbia	625986.10	241498.60	155816.00	170804.00	24739.00	33128.50
Central Serbia	5583629.69	1840150.48	1198739.19	1904923.13	265530.33	374286.56
Kosovo	602287.00	257518.00	126476.00	183515.00	3459.00	31319.00
Metohija	489976.00	207825.00	126877.00	118953.00	8532.00	27789.00
Kosovo and Metohija	1092263.00	465343.00	253353.00	302468.00	11991.00	59108.00
Srbija	8828528.29	2457770.98	1625559.69	3787836.13	310845.33	646516.16

IMPROVEMENT OF QUALITY AND QUANTITY OF NATURAL FEEDS

The dominant plant species on our meadows are grasses and legumes of variable nutritional quality, also weeds and some harmful and poisonous plants. With the appropriate agrotechnical measures, like irrigation, plowing, cutting and fertilizing, the botanical composition may be changed in some areas of hunting grounds, and improved the quality and quantity of natural feeds (2). Natural pastures should be mowed two times per year, and plowed and fertilized once a year with 100 kg of mineral fertilizer per hectare (17). Those methods decrease the possibility for weeds to grow and produce their seeds, which is changing the floristic composition and nutritive value of the green mass.
It is also important to plant fruit and other trees that produce feeds for animals in the hunting grounds (15). Such trees are oak, beech, chestnut, plum, apple, pear, mulberry and similar.

PRODUCTION OF GREEN FORAGES ON MEADOWS AND ARABLE LAND

Aside from the improvement of natural pasture, it is possible to create new meadows in some areas. Usually they are made as mixtures of perennial grasses which have different life duration, morphological composition and nutritive value. Due to variability, such mixtures are suitable for various terrains and ecological conditions. The composition of those mixtures depends on many factors, especially on chemical composition of the soil (19).
One of the possibilities to produce green forages in hunting grounds. Most suitable for that purpose are legumes (lucerne and others), cereals (maize, ray, oats, triticale etc.), roots and tubers (beet, potato), cabbages (canola, kale, perko…) and others.
The mentioned plants may be used directly as green feeds, or as pasture, but also may be conserved for winter feeding – as hay, silage and haylage, and kept in other ways to be used when needed (3, 5). The feeds should be planted on several places in the hunting ground, so that animals wander searching for them. The culture production should be in a form of a "green conveyer" so that green forages are continuously available for animals (Table 2). Planted fields should have some fence so that animals are allowed to eat forages when they are in the optimal phase.

Table 2. Areas for deer feeding, ha/animal (17)

Type	Fenced hunting ground	Open hunting ground
Natural pasture	0,10	0,05
Artificial pasture	0,04	0,02
Arable land	0,05	0,03

SUPPLEMENTAL FEEDING

This is usually done during the winter, when the weather is cold and snows are deep. Beginning, amount and end of supplemental feeding depend mostly on the amount of available natural feeds, and those circumstances vary from year to year. Basic principle is to start with supplemental feeding earlier (October, November) so that animals became accustomed to it. This is also helping animals to produce body reserves for the winter. During other parts of the year supplemental feeding is done in extreme conditions, like drought or lack of feeds (for pheasants mostly).

It is necessary to prepare in advance the plan for supplemental feeding and to calculate the needs in concentrates, forages and fresh feeds. The most important are concentrates, in form of cereal grains or pelleted mixtures. The necessary minimum is to provide salt blocks in the hunting grounds (4, 6).

Supplemental feeding of carnivores is done with animal feeds (24). The exception is bear which can be fed also with fruit, cereals and pelleted concentrates. Supplemental feeding of carnivores particularly some rare species, it is possible to maintain their numbers and control of their population, the migrations are reduced and damages on domestic animals are reduced. Supplemental feeding of bears may create a habit in them to enter human settlements in search for the new feed sources (9).

CONCLUSION

It is possible to improve quality and quantity of natural feeds in the hunting grounds with various management measures, to produce feeds on meadows and arable lands and organize supplemental feeding of game animals with such feeds. As a result, it is possible to reduce damages produced by game on agricultural crops and damages on game itself. However, the only effective solution is to fence the hunting grounds and organize completely controlled management.

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CHEMICAL AND PHYSICAL QUALITY OF FORAGES FOR DAIRY COWS NUTRITION

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ABSTRACT

Forage quality is first determined by content of useable energy; crude protein, and his fractions: rumen degradable and undegradable protein; fibers: ADF, NDF and lignin. Besides the chemical composition, basic parameter that influence on nutritional value and quality of forages that compose diet is digestibility of DM, OM and nutrients. Significant part of forages in dairy cows diets is necessary for proper ruminal function and processes of rumen fermentation. The effect of forages in dairy cows' diets depends of plant species, stage of maturity, method of preservation and diet composition. Determination of forages and total mixed rations particle size is significant factor for lactating cows diet formulating. Optimal content of physically effective fiber-peNDF in diet and proper physical form of forages stimulate increasing of chewing activity, provide proper ruminal function and feed conversion, and eliminate appearance of milk fat depression and metabolic diseases.
Key words: *forages, quality, cows, nutrition*

INTRODUCTION

Energy and protein requirements of high-yielding lactating cows are difficult to supply, because of enormous high-energy requirements and in the same time limited capacity for diet dry matter intake. Considering these facts, formulated ration for lactating cows should contain high energy and protein concentration in dry matter, and should be high digestible. In practice, these could be providing by using the most quality forages as possible and with high portion of concentrate in rations. Quality of forages is first determined by content of available energy; crude protein, and his fractions: rumen degradable and undegradable protein; fibers: ADF, NDF and lignin. The significant portion of forages in lactating cows' diets is important for proper ruminal function, and processes of ruminal fermentation. The bottom line for forages portion in cows' diets is 40 % (*Grubić and Adamović, 2003*).

The ability of formulated optimal diet to supply nutrient requirements of high yielding lactating cows depends of his chemical and physical characteristics. The particle length of forages and total mixed ration is important parameter for diet formulation. Ration content of NDF-fiber (neutral detergent insoluble fiber), has substantially influence on DM intake, proper ruminal function, processes of microbial fermentation in rumen, digestibility of ration, feed conversion, chemical composition of milk (*Stojanović and Grubić, 2008*). Minimal dietary fiber content, considers through the concept of their physically effectiveness. Concept of physically effective fiber (peNDF) includes mean particle length of forages and total mixed ration, and forage and dietary content of NDF, and defines their physically effectiveness (*Mertens, 1997*). Optimal content of physically

effective fiber-peNDF in diet and proper physical form of forages stimulate increasing of chewing activity, provide proper ruminal function and feed conversion, and eliminate appearance of milk fat depression and metabolic diseases (*Stojanović et al. 2002*).

EFFECTS OF FORAGE CHEMICAL QUALITY IN DAIRY COWS NUTRITION

Meeting the energy and protein requirements of high-yielding lactating cows, depends of quantity and feed efficiency of consumed dietary dry matter and organic matter. Besides chemical composition, main parameter that influence on nutritional value and quality of forages is digestibility of DM, OM and some nutrients (*Grubić et al. 1996*).
The most important fact for nutritive value of grasses and legumes is stage of maturity at the moment of cutting (*Đorđević and Dinić, 2007*). With the maturing, the proportion of lives is being reduced, and proportion of steams with greater structural fiber content is being increased in the total yield. This causes decreasing of energy and protein content, increasing of fiber content and percentage of lignin, which is indigestible and inaccessible for animals (*Grubić et al. 1995*). Grasses is characterized with higher proportion of cell walls, and slower ruminal digestion of this material, than legumes, where is found that structural polysaccharides in cell walls have higher rate of digestion in rumen. Although with slower digestion, fiber from grass is more digestible and utilizable for dairy cows, according to lesser degree of lignification. Legumes are characterized with higher proportion of cell content (*Van Soest, 1994*).
It is found decreasing yield of 4 % FCM, with using first cutting grass-legume or alfalfa hay in diets for lactating dairy cows, with lesser quality because of cutting in later stage of maturity. There were no effects of second cutting alfalfa hay with different quality on yield of 4 % FCM (*Waldern and Baird, 1967*). The largest differences in dry matter digestibility of hay cut in different stages of plant maturity appear for first cutting hay, and are less for later cutting. Different nutritional value of grass hay cut at different stages of plant maturity did not have effect on milk production of dairy cows in early lactation, fed diets with high proportion of concentrate (*Steacy et al. 1983*). There were no effects of rations for dairy cows in early lactation with two different quality of alfalfa hay: early cut alfalfa (21 days interval, prebloom stage) and normal cut alfalfa (30 days interval, 10 % bloom stage) on DM intake and daily milk production, until the content of milk protein was higher (3.2 and 3.1 %) for cows fed ration with early cut alfalfa hay, and percentage of milk fat was higher (3.5 and 3.9 %) for cows on ration with later cut alfalfa hay (*DePeters and Smith, 1986*). *Miller et al.*(*1991*) report decreasing of DM intake (9.1 %), average daily gain (9.5-20.9 %), and content of NE for maintains (10.2 %), in diets for young fattening cattle with alfalfa hay (chopped and pelleted) prepared after cut of alfalfa in full bloom stage (36 days of vegetation), compared with alfalfa hay after cut at prebloom stage (25 days of vegetation). Using an alfalfa hay prepared after early cut (21 days of vegetation), instead of later cut alfalfa hay (30 days of vegetation), in diets for dairy cows in 2. phase of lactation, and dried cows, increases digestibility of: DM (67.4 and 61.5 %), energy (67.5 and 60.4 %), N (74.6 and 71.6 %), NDF (45.1 and 30.5 %), and ADF (44.1 and 31.0 %), (*Llano and DePeters, 1985*).
Merchen and Satter (*1983*) report increasing of lactic acid proportion and decreasing of acetic acid proportion in total acid content, as the least expressed decreasing (increasing

was found) of DM amino acids content, decreasing of soluble N, increasing of percentage of N insoluble in acid detergent (ADIN) in total N content, for ensiling wilted alfalfa (40 and 66 % of DM content), comparing with ensiling a fresh material with 29 % of DM. Better intake and total digestibility of OM is also found, as increasing of proportion of total digestible OM that is digested in the intestine, and decreasing of part of digestible OM that is fermented in rumen (probably as a result of depression of protein digestibility), for dairy cows in early lactation. In this study, increasing content of ruminally undegraded N, decreasing concentration of ammonium N in rumen, and increasing of flow of nonammonium N in duodenum is determined.

Table 1. Ruminal characteristics of some nutrients for lactating dairy cows fed alfalfa haylage or hay (Merchen and Satter 1983)

Item	**Dry matter, %**			**Hay**
	29	40	66	
Intake of OM, kg/day	16.3	18.5	17.6	16.5
NH_3- N in rumen, mg/100 ml	21.2	22.7	10.0	15.0
Digestibility of OM, %	69.8	73.6	71.5	68.7
Proportion of digestible OM (%), that is digested in:				
Rumen	70.9	69.7	57.9	65.2
Intestines	23.2	23.4	32.1	27.9
Colon	5.8	6.8	9.9	6.9
Flow of nonammonium N in duodenum, g/day	326	393	463	324
Flow of bacterial N in duodenum, g/day	257	314	287	229
Flow of dietary and endogen N in duodenum, g/day	69	80	176	95

Significant increasing of daily milk and milk protein yield as a result of using feeds that are sources of ruminally undegraded protein in the diets for lactating cows based on alfalfa haylage, points to limited duodenal flow of protein, with this kind of rations. This is more distinct for rations with alfalfa haylage as main forage compared with rations based on alfalfa hay (*Broderick et al. 2002*). Higher proportion of alfalfa haylage in diets for lactating dairy cows often is connected with increased concentration of dietary crude protein and increased content of rumen degradable protein. Comprehensive conversion of protein into NPN, during the ensiling process results with intensive releasing of NH_3 in rumen. Increased content of ruminally available energy in diet is significant for greater efficiency of utilizing of rumen degradable protein from alfalfa haylage, and synthesis of microbial protein (*Đorđević et al. 2001*). Greater content of ground high moisture corn grain in diets for lactating cows, increases synthesis and duodenal flow of microbial protein, as also a milk protein yield, to a greater extent for cows fed diets with alfalfa haylage (170 and 337 g/day, respective) then for cows on diets with alfalfa hay (100 and 100 g/day, respective), (*Vagnoni and Broderick, 1997*). Amount of protein, more accurate protein absorbed in intestine, and not energy, is main limiting factor in diets for dairy cows in the first half of lactation, based on high level of alfalfa haylage (*Dhiman et al. 1993*). There were no effects of replacing chopped alfalfa hay with alfalfa haylage in diets for dairy cows in late lactation, on DM intake, ruminal pH, ruminal concentration of volatile fatty acids, milk fat and protein, serum concentration of lactate,

but influenced on reducing of glucose concentration, and increasing of urea concentration in serum, and numerical decreasing of milk and milk protein yield (*Plaizier, 2004*).
Using of alfalfa haylage instead of raygrass haylage in diets for dairy cows in the second phase of lactation, increasing BW gain, (0.48 kg/day), and yields of milk (6.1 kg/day), 4% FCM (6.8 kg/day), milk fat (0.26 kg/day), milk protein (0.25 kg/day). Feed conversion (kg of milk/kg consumed DM) and conversion of consumed N (g milk N /g consumed N) were greater for cows fed raygrass haylage (27 %), as digestibility of dietary DM (16 %), NDF and ADF (53 %) too (*Broderick et al. 2002*). Raygrass and grass generally are characterized with higher digestibility of DM, NDF and ADF, than alfalfa, but with slower rate of degradation. Decreasing of dietary DM digestibility that is related with increased intake is more expressive for grass than for legumes.
Replacing of one half of corn silage with alfalfa haylage in ration for dairy cows in the second phase of lactation, increases percentage of milk fat (2.68 and 3.32%), and milk fat yield (1.17 and 1.45 kg/day). There were no differences for DM intake, and daily milk production, between treatments (*Onetti et al. 2004*). Replacing of one-half of alfalfa haylage with corn silage, decreases milk fat content from 3.35 to 3.04 %. Ruminal propionate concentration was higher, with decreasing chop length of forages, as also with replacing a part of alfalfa haylage with corn silage (probably because of increasing quantity of starch in ration). Acetate to propionate ratio was lower for diets with shorter forage feed particles, as also when alfalfa haylage were replaced with corn silage (*Krause and Combs 2003*).
According to *Ferreira and Mertens* (*2005*), DM digestibility of ground corn silage first depends of NDF and ADL (lignin insoluble in acid detergent) content (R^2=0.80). *In vitro* digestibility of corn silage DM that is not additionally ground depends of ADL content, portion of kernel particles larger than 4.75 mm, mean particle size of silage and DM content. Additional processing of kernel, treating chopped whole corn plant with onboard kernel processing rolls, increasing starch digestibility of silage. *Cooke and Bernard* (*2005*) report decreasing of corn silage starch digestibility for lactating cows, with increasing apart between kernel processing rolls from 2 mm to 8 mm. Cows in the second phase of lactation fed total mixed ration based on corn silage with theoretical chop length of 27.8 mm, consumed more DM (25.55 and 24.55 kg), OM (23.85 and 22.9 kg), showed higher ruminal (64.7 and 58.2 %) and total (96.65 and 96.0) starch digestibility, lesser daily yield of milk fat (1.21 and 1.32 kg), higher ruminal concentration of acetate (58.3 and 58.05 mg/dl) and lower concentration of propionate (25.85 and 26.3 mg/dl), greater intake of NE_L (171.33 and 163.18 MJ/day), than cows fed silage with theoretical cut length of 39.7 mm (*Johnson et al. 2003*). Kernel processing increased dietary starch digestibility (96.8 and 95.85 %). Additional kernel processing increased DM intake, milk yield and milk fat percentage (*Bal et al. 2000*). Authors explained these by higher ruminal and total starch digestibility. Decreasing of chop length of corn silage (1.90 and 0.95 cm) caused a depression of fiber digestibility. Digestibility of DM, starch and crude protein, were significant greater for cows fed rations based on corn silage with 32 % of DM content, compared with silage with 40 % of DM. Crop maturity and DM content of whole corn plant at the moment of ensiling, influenced at greater extent on fermentation and quality of silage, compared with additional kernel processing and using of inoculants. Increasing DM content of corn

plant during harvesting and ensiling provides greater content of NE_L in cows' rations (*Johnson et al. 2003a*). Higher cutting height of corn plant (12.7 and 45.7 cm) during harvesting and ensiling, numerically increased NDF digestibility (31.8 and 34.3 %), daily milk production (45.2 and 46.7 kg/day) and feed conversion (1.67 and 1.72 kg of milk/kg consumed DM) for cows in second phase of lactation (*Neylon and Kung, 2003*).
Rinne et al. (*2002*) reported depression of DM intake, digestibility of OM, CP, NDF, ADF, as also decreasing of milk yield (21.5 and 18.4 kg/day), for cows in middle of lactation, fed diets with grass silage (timothy-meadow fescue sward) ensiled after harvesting at later stage of maturity.
Increasing pH of corn silage by adding $NaHCO_3$, increased DM intake (3.91 and 4.58 kg/day) and OM intake (3.74 and 4.23 kg/day), in diets for young fattening cattle. Lowering acidity achieved using $NaHCO_3$ positively affected on intake of DM (6.95 and 7.73 kg/day) and OM (6.64 and 7.23 kg/day) for heifers fed corn silage, as also on intake of DM (7.03 and 8.22 kg/day) and OM (6.23 and 7.31 kg/day) for heifers fed alfalfa haylage. Higher content of moisture and lower of DM (38.0 and 29.7 %) in corn silage, depressed intake of DM (8.09 and 6,95 kg/day) and OM (7.80 and 6.64 kg/day) for heifers (*Shaver et al. 1985*).
Fiber digestibility is significant parameter of forage quality, because of large variations of ruminal NDF digestibility between feeds. Efficiency of forage NDF utilization at great extent affects on production performances of cows in lactation, from the reason of significant portion of fibers in dietary DM. Minimal recommendation for NDF content in dietary DM for lactating dairy cows is 25 %, and the greatest part (75 % of total NDF, or 19 % in dietary DM) should be from forages (*NRC, 2001*). Even that in rations for lactating dairy cows is necessary substantial portion of NDF, higher NDF content decreases DM intake, first of physical fill of rumen. Higher ruminal digestibility of NDF reduces physical fill of rumen and provides greater DM intake (*Mertens, 1997*). Higher ruminal digestibility of forage NDF statistically significant increases DM intake, by that energy intake and milk production. This effect is more expressive when physical fill of rumen limits dietary dry matter intake. Dietary NDF content and digestibility affects ruminal fill. Increasing of forage NDF digestibility by 1 %, increases DM intake by 0.17 kg and yield of 4 % FCM by 0.25 kg/day, for lactating cows (*Oba and Allen, 1999*). Milk yield (36.3 and 38.2 kg/day) and DM intake (19.4 and 20.4 kg/day) were significant greater for cows in early lactation, with increasing NDF digestibility (40 and 45 %) of alfalfa haylage (83 % of diet DM). Higher digestibility of dietary DM, OM and NDF, higher concentration of VFA in rumen and proportion of propionate in total VFA content, are also determined (*Dado and Allen, 1996*).

Table 2. Effects of alfalfa haylage NDF digestibility, on diet digestibility, characteristics of ruminal content, and production performances of cows in early lactation (Dado and Allen, 1996)

Item	LD	HD
DM intake, kg/day	19.4	20.4
Digestibility, %		
DM	62.6	65.2
OM	63.5	66.2
NDF	41.6	43.4
ADF	40.2	41.6
CP	73.8	74.8
Ruminal content		
pH	6.84	6.73
Total VFA content, mmol	137.5	143.9
Portion of acetate, %	65.0	62.6
Portion of propionate, %	19.0	20.7
Acetate : Propionate	3.4	3.0

LD-Diet based on alfalfa haylage with lower NDF digestibility
HD- Diet based on alfalfa haylage with higher NDF digestibility

Increasing of dietary NDF digestibility, increases content of NE_L. It was determined that alfalfa haylages with similar NDF content characterized with different digestibility of NDF, and these values were between 25 and 55 %, after 30 h of *in vitro* fermentation (*Allen, 2000*). This is equivalent with difference in concentration of NE_L for 1.38 MJ/kg DM of alfalfa haylage with 45 % of NDF content.
Physical and metabolically factors contemporary affect diet intake capability. Physical fill of rumen is able to limit feed intake, for diets with lower portion of concentrate, until for diets with higher concentrate content (more than 50 % of ration DM), metabolically (energy and nutrients requirements of lactating cows) at first than physical factors affect feed intake (*Allen, 2000*). Yields of milk and milk fat linearly increase with increasing of forage NDF digestibility, in total mixed ration for cows in the middle of lactation (*Robinson and McQueen, 1992*). *Miller et al.* (*1991*) determined that isoenergetic and isoprotein diets with lower digestibility of forage NDF decreased feed intake and milk production.

PHYSICAL QUALITY OF FORAGES

Capability for formulated optimal ration to supply nutrient requirements of high-yielding lactating cows depends of his chemical and physical characteristics *(Stojanović et al. 2008*). Adequate chop length of forages in total mixed ration (feed particle length) is significant parameter for diet formulation that should be considered equally with his chemical composition and nutritive value.
Level of physically effective fiber in rations for dairy cows affects chewing activity, flow of saliva rich in $NaHCO_3$ in rumen, pH of ruminal content, ruminal acetate to

propionate ratio, and milk fat content (*Grubić et al. 1999*). Concept of effective fiber includes chemical characteristics of forages and their chop length in diet, and expresses value of forages for chewing activity and ruminal function. Physically effective fibers (peNDF) are fibers in cows' diets that effectively stimulate saliva secretion and rumination (*Mertens, 1997*). Deficit of effective fiber in ration for high-yielding dairy cows, the most frequently causes these disturbances: milk fat depression, ruminal acidosis, ruminal parakeratosis, dislocation of abomasum, and laminitis (*Stojanović and Grubić, 2008*). This is especially characteristic for cows in early lactation with high requirements for dietary energy and protein concentration, as also for high digestibility of ration, because of limited ability for DM intake.

Forages, first corn silage and alfalfa haylage, with optimal chop length, provide adequate particle length and distribution of particles' fractions in total mixed ration (*Stojanović et al. 2009*). Recommendation for peNDF portion in diets for dairy cows in early and middle lactation is 20 % of dietary DM, for maintaining percentage of milk fat at 3.4 %. Concentration of peNDF in dietary DM should be 22 % to achieve pH 6 as ruminal average value (*Mertens, 1997*).

A simple method and device (system of sieves, Penn State Particle Separator-PSPS), developed for determination of particle size of total mixed rations for lactating cows, and some forages-components of TMR (corn silage and alfalfa haylage), as also for optimization of physical form of rations (*Kononoff et al. 2002*).

Yang and Beauchemin, (*2007*) researched effects of dietary peNDF concentration achieved with different chop length of alfalfa haylage (theoretical chop length 7.9 and 19.1 mm). Increasing chop length of alfalfa haylage, increased peNDF intake, but not DM intake. Digestibility of NDF was higher, caused by higher ruminal digestibility of fiber, and shifting of starch digestion from rumen to intestine. Increasing of forage particle size in ration, increases ruminal digestibility of NDF by 18 %, and DM digestibility by 6 %. Increasing particle size of alfalfa haylage, increased ruminal pH (6.36 and 6.16). This is result of higher intake of peNDF, increased total time of chewing activity and saliva buffer secretion, and shifting of starch digestion from rumen to intestine. Concentration of peNDF in ration is positively correlated with chewing activity (r=0.61), and negatively correlated with total time of ruminal pH value under 5.8 or 5.5 (r=-0.46). Larger forage particles form floating rough and dry layer of ruminal content that stimulates ruminal contractions. Without these movements, rumen becomes less dynamic system, with decreased efficiency for removing of VFA through absorption or fluid passage, and increased risk of acidosis appearance. Intake of larger forage particles reduces ruminal starch digestion, increases extent of intestinal starch digestion, decreases ruminal concentration of VFA, increases acetate to propionate ratio of molar concentrations. Authors emphasize significance of peNDF intake, more exactly forage NDF intake, for normal ruminal function, than consuming of total dietary NDF.

Yang and Beauchemin (*2006*) researched effects of different peNDF content in diets for lactating cows, with corn silage (theoretical chop length 28.6, 15.9 and 4.8 mm) as only forage. Increasing of average particle size of ration, did not affect DM intake, increased intake of peNDF, increased CP digestibility (65.7 and 61.3 %), showed tendency for increasing fiber digestibility (NDF-50.5 and 45.7 %, ADF-49.4 and 43.9 %) in whole digestive tract. Cows fed rations with higher content of peNDF showed tendency for

increasing milk yield, there were no effect on milk fat percentage, until total time of chewing activity (intake and rumination) were significantly greater.
Effects of chop length of forage and particle size of total mixed rations for lactating cows on milk fat content, appears when content of NDF is under minimal requirement, (25% NDF, and 19% forage NDF in dietary DM, *NRC,2001*).
Using of fine chopped alfalfa haylage (2.1 mm, theoretical chop length 0.48 cm) instead of coarse chopped haylage (3.1 mm, theoretical chop length 0.95 cm) in TMR (forage to concentrate 55:45 % in dietary DM) for Holstein cows in early lactation (3-8 weeks), depressed milk fat content (3.8 and 3.0 %), decreased daily yield of 4 % FCM, as also feed conversion (*Grant et al. 1990*). Rumination time and total chewing time were significantly decreased, as also ruminal pH and acetate to propionate ratio. It was determined increasing of glucose and insulin concentration in blood plasma and serum.

Table 3. Effects of different chop length of alfalfa haylage in TMR, on some physiological and production parameters of cows in early lactation (Grant et al. 1990)

Item	**Chop length**		
	Fine	Middle	Coarse
Intake of DM, kg/day	22.4	22.0	22.2
4% FCM, kg/day	27.52	30.28	29.49
Milk fat, %	3.0	3.6	3.8
Milk protein, %	3.0	3.0	3.1
Chewing activity, min/24 h			
Intake	195.3	204.4	204.7
Rumination	374.4	466.3	530.7
Total chewing activity	569.7	670.7	735.4
pH	5.3	5.9	6.0
VFA, mM/l			
Acetic	73.96	70.61	76.29
Propionic	39.19	30.80	26.09
Butyric	8.78	15.05	17.47
Acetate : Propionate	2.77	3.13	3.52
Glucose in blood plasma, mg/dl	65.9	54.0	44.9
Insulin in blood serum, ng/ml	0.30	0.26	0.20

Rustomo et al. (2006) reported that increasing chop length of corn silage and alfalfa haylage in ratio that characterized higher acidity potential of his concentrate part, showed tendency of increasing milk fat content by 0.32 % (3.87 and 4.19 %). Using fine chopped alfalfa haylage (3.02 mm) instead of coarse chopped alfalfa haylage (9.57 mm) in TMR for primiparous cows in early lactation, decreased milk fat percentage (3.73 and 3.41 %), as also production of 4 % FCM (26.54 and 24.81 kg/day). The same effect of depressed milk fat was determined for multiparous lactating cows (3.69 and 3.49 %), (*Fischer et al. 1994*).
Although intake of diet with deficit of effective fiber cause disturbance of ruminal function, and ruminal fermentation, excessive content of long and coarse forage particles

in total mixed rations for dairy cows, decreased feed intake and digestibility of consumed DM, and negatively affect on cows' energy balance (*Allen, 2000*).

CONCLUSION

Meeting the energy and protein requirements of high-yielding lactating cows, depends of amount of DM intake and feed efficiency of consumed dietary DM and OM. Forages make 40-60 % of DM of ration for lactating cows. Besides chemical composition, main parameter that influence on nutritional value and quality of forages is digestibility of DM, OM and nutrients. The effect of forages in dairy cows' diets depends of plant species, stage of maturity, method of preservation and diet composition. Forages, first corn silage and alfalfa haylage, with optimal chop length, provide necessary physically effectiveness of total mixed rations. Optimal content of physically effective fiber-peNDF in diet and proper physical form of forages stimulate increasing of chewing activity, provide proper ruminal function and feed conversion, and eliminate appearance of milk fat depression and metabolic diseases.

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THE POSSIBILITY OF USING SUGAR BEET MOLASSES, AFTER OSMOTIC DEHYDRATION OF APPLE, AS A COMPONENT OF ANIMAL FEED

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ABSTRACT

The paper examined the chemical composition of sugar beet molasses, before and after the osmotic dehydration of apple, to its possible use as component in the production of food for animals. Within the experiment, the osmotic dehydration of apple in the sugar beet molasses at a temperature of 55 º C and atmospheric pressure was performed. Dry matter content in apple was increased from 13.58% to 52.3%. During the experimental work, dry matter content, contents of sugar and some minerals (K, Na, Ca and Mg) in molasses before the process and after 1, 3 and 5 hours of immersion, were determinated. The results of this paper indicate that, during the osmotic dehydration, sugar beet molasses do not decrease in quality, moreover its enriched with some elements, and can be used as a component of food for animals. In this way, sugar beet molasses, as high quality raw material, could be used in two different technological process.
Key words: *sugar beet molasses, osmotic dehydration, food for animals, apple*

INTRODUCTION

Sugar beet molasses is a concentrated liquid extract that is a by-product of sugar refining. Molasses has a high content of solids (around 80%) and contains, in average, 51% saccharose, 1% rafinose, 0.25% glucose and fructose, 5% proteins, 6% betaine, 1.5% nucleosides, purine and pyramidine bases, organic acids and bases and pectines [9].

Molasses contains, in very small amounts (about 0.02%), amides, ammonia salts and nitrates. Of all amino acids, the most common is glutamic acid - about half of the total amount of amino acids. In the molasses there is the oxalic acid (0.01%), oxyglutic acid, lactic (0.5%), saccharic acid, humic acid, arabic acid and gliceric acid [2].

Apart from these ingredients, sugar beet molasses is a significant source of numerous micronutrients (vitamins and minerals), especially K, Ca, Na and Mg. Specially important is the fact that all mineral components of molasses are in the dissolved state and that the potassium is in much greater quantity than all other cations with share of 75% [9].

In addition to these minerals, in the molasses are also small quantities of the following elements expressed in mg / kg: Co = 0.59, B = 3.0, Fe = 115.0, Cu = 4.9, Mn = 18.0, Mo = 0.20 and Zn = 34.0.

Molasses also contains B-complex vitamins but does not contain fats and fibrous materials. Molasses has the humectant and antioxidant properties and influences on the activity of final products [10].
Molasses, as feed for animal food, is very tasty and very good source of energy. In addition to use as energy source, molasses also has the following purposes: 1) as appetizer (for better appetite), 2) to reduce pulverulent of the meal, 3) as a binder, 4) to stimulate microbial activity in rumen of ruminants, 5) for providing necessary biogenic compounds, 6) for supplying the animals with vitamins and microelements. Molasses contains over 250 important bioactive compounds, minerals and vitamins useful for human consumption. The pharmacology of natural and synthetic drugs there is no one product with so much nutritive valuable and biologically active substances such as sugar beet molasses [5].
Rajčan et al. (1971) state that molasses, combined with stodgy food, may have more favorable biological effect than grain has. Recommended amounts of molasses in mixtures for cattle were 5-10%, while for calves mixtures smaller amounts were recommended, since laxative effect of molasses. Molasses was usually given to pigs and poultry through molasses forage mixtures in quantities up to 6% [7].
Molasses is used for correcting of taste for rough and many other feeds. For this purpose, molasses dissolves in warm water in ratio 1:4. Dissolved molasses is using for spraying over the feeds. Feeds splashed with molasses become tastier and less powdered. So the animals consume more corn, straw, malt sprouts and other less tasty feeds [2]. Molasses is a good binder for pelleting industrial fodder mixtures in quantities up to 5%. It is also grateful as material for adding during silage [8].
The largest amounts of molasses (90%) are used in alcohol industry and in production of bakery yeast [2].
Due to high dry matter content in sugar beet molasses and diversity in chemical composition, imposed the idea of examining the possibilities of application of molasses as osmotic solution in the process of osmotic dehydration of fruits and vegetables as well as examining its impact on the nutritional profile and quality characteristics of osmodehydrated fruits and vegetable [3].
Osmotic dehydration is the process for the partial removal of water from fruits and vegetables dipping them in different hypertonic solutions. Osmotic dehydration is one effective way to reduce the water content with minimal negative effect on sensorial properties and nutritive value of final product. At the same time sugar beet molasses was enriched with valuable components from fruits and vegetables.
Driving force for diffusion of water is the difference in osmotic pressure between the plant tissue and the solution that surrounds it [4, 1].
The aim of this study was to determine changes in chemical composition of sugar beet molasses, after osmotic dehydration of apple, and to indicate the possibility of its application as a component in food for animals.

MATERIAL AND METHODS

Apples were purchased on a local market in Novi Sad, Serbia and stored at 4°C. Prior to the treatment, the apples were thoroughly washed and cut into cylindrical shapes, 20 mm in height and diameter, with a sharp apple corer**.**

Osmotic dehydration was carried out at 55°C under atmospheric pressure. The material to solution ratio was 1:4 (w/w). After osmotic dehydration the apple pieces were removed from osmotic solutions, washed with water and gently blotted to remove excessive water.
Finally, according to AOAC methods [6], we determined: content of dry matter, sucrose content, total content of reducing sugars, and invert sugar content, K, Na, Ca and Mg in the molasses prior to osmotic dehydration and after 1, 3 and 5 hours of immersion.

RESULTS AND DISCUSSION

Quantitative technological scheme of the osmotic dehydration process was shown in Figure 1.
The starting moisture content of apple samples was 86.42%, while the osmodehydrated product had dry matter content (SM) increased by more than 3.5 times. Table 1 shows the basic chemical composition of sugar beet molasses before and after osmotic dehydration (OD) of apple.

Table 1. Basic chemical composition of molasses during osmotic dehydration of apples

	Time (h)			
	0	**1**	**3**	**5**
Dry matter (%)	81,4	76,36	73,95	71,35
Saccahrose (%)	50,07	35,91	38,4	40,56
Invert sugar (%)	0,57	0,65	1,16	0,85
Total reducing sugars (%)	53,27	38,66	41,58	43,54
K (mg/100g)	4100,3	4112,84	4122,39	4129,11
Ca (mg/100g)	198,2	198,63	198,17	198,11
Na (mg/100g)	590,2	592,04	592,94	593,51
Mg (mg/100g)	95,6	95,73	95,65	95,42

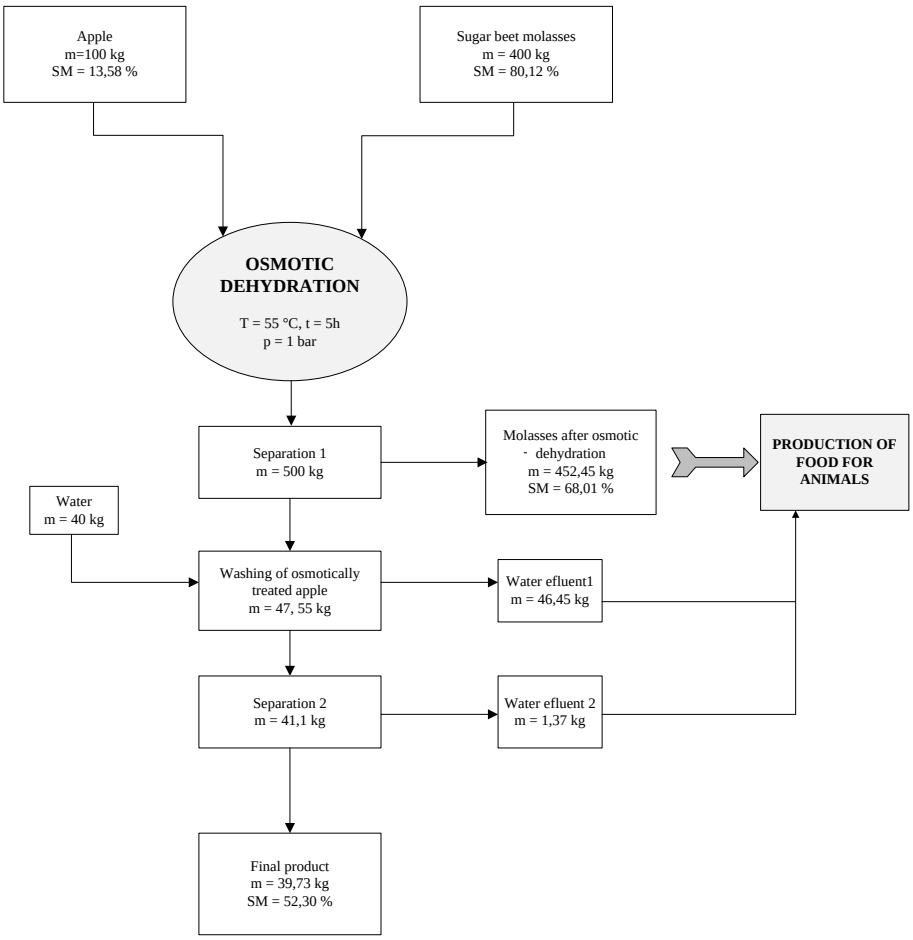

Figure 1. Quantitative scheme of osmotic dehydration of apple in sugar beet molasses

The purpose of the process of osmotic dehydration of apple is to reduce the moisture content, i.e. increase the dry matter content in the sample. At the same time with water diffusion, coming up to the diffusion of sugar and minerals from plant tissue in the osmotic solution, and vice versa. As a result we obtain nutritionally enriched apple which can be used as a supplement in functional food and the osmotic solution, slightly diluted sugar beet molasses, but very suitable for using in animal nutrition.
Molasses after osmotic dehydration has slightly altered mineral composition and therefore does not lose the quality. In silage it can improve tastefulness and increase its nutritional value. In mixture of food molasses reduces pulverulentes, it is good binder for pelleting feed and can be used as a carrier for various drugs. It is useful as a liquid

protein supplement. In many categories of food for animals it would be a significant source of potassium and sodium (Table 1) which are necessary for the regulation of osmotic pressure in body fluids and acid-base balance in the body of animals. Potassium plays an important role in nerve and muscle irritability, and participates in the metabolism of carbohydrates.

CONCLUSION

After using sugar beet molasses as hypertonic solution for successful osmotic dehydration of apples, it is nutritionally and sensory very good raw materials for animal nutrition. Molasses reserves color, smell, taste and other physical properties and from nutritive aspect is better quality, because some compounds diffuse from the plant cells into the osmotic solution during OD process. On the basis of specified chemically composition of used sugar beet molasses, for feeding animals it can be a good source of micronutrients, carbohydrates, and because of stickiness, and good binder.

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CHARACTERIZATION OF CHANGES IN SOYBEANS CAUSED BY TEMPERATURE TREATMENTS IN NUTRION OF HUMANS AND ANIMALS

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ABSTRACT

Soybean is, as a source of quality protein and energy, increasingly represented in manufacturing of food for humans and animals. Application of thermal processes in soybean leads to inactivation of anti-nutritive substances, primarily trypsin inhibitor, while the adoptability of proteins, oils and other nutritive matter increases. This study has examined the technological processes regarding thermal processing of soybean through extrusion and hydro-thermal treatment. The study also examined technological parameters of dry extrusion process, hydrothermal steam treatment and steam-free thermal treatment and data concerning the nutritive value of thermally treated soybean, as well as its hygenic safety.
Keywords: *soybean, extrusion, hydrothermal treatment, activity of urease, trypsin inhibitor, NSI*

INTRODUCTION

The production of food globally, as well as locally, is becoming increasingly important due to evident deficit in human nutrition. In order to find a solution to this problem, it is vital to increase primary agricultural production by utilizing new technologies in biotechnology, i.e. bioindustry [14].
Various technological processes are utilized to increase nutritive value of food intended for human and animal use, while in soybeans the thermal process – toasting, extrusion, hydro-thermal processing, micronization, microwave treatment are intended for this purpose [21, 16]. Extrusion processes and hydrothermal treatment are most widely used methods of thermal processing of soybeans [23, 8]. Locally, soybean is the main ingredient in local animal nutrition with regard to plant proteins, and it is regarded as a high-quality, energy feed because of its high oil content. European Union, where animal husbandry is very intensive, uses, among other raw materials, 20 million tons of soybean meal and over 2 million tons of thermally processed soybeans in manufacturing of animal feed [5]. In recent years, our country has increasingly been using nutrients from whole soybean obtained by using thermal treatments. Protein-energy nutrients from whole soybean contains roughly 38% proteins and 20% oils, and are used in manufacturing of animal feed, especially in animal feed compositions for younger categories of animals (pigs, calves, lambs, chickens) which require high energy content [11]. By using thermally treated soy – extruded full-fat soya and hydro-thermally treated

soy bean in manufacturing of animal feed, the technical problem of application of fat in feeding mixtures – the problem of greasing of animal feed, is resolved.

Utilizing soybeans in human and animal nutrition is subject to previous thermal treatment in order for inhibitory substances located in soybean, that is trypsin and himotrypsin inhibitors [6], hemagglutinins [27], fitates [28], saponins [25], anti-vitamins A, E and B_{12} [15] and others, to become deactivated or to diminish in content, and simultanous increase in the nutritive value of nutrients, hygenic safety and improvement of physical-chemical characteristics of soy.

Extrusion is a technological process in which soy is exposed to high temperature and pressure during which it is mechanically processed due to forces of friction, which causes changes in structure. Therefore, the final product is different in chemical composition, external appearance and form, and other characteristics [24] of the raw material. Reduction in anti-nutritive substances (trypsin inhibitors by 97-98%) and many physical-chemical processes in the soybean take place during the process of extrusion [26]. Changes also occur in the structure of protein complex which is reflected in increased digestibility of proteins. However, the negative effect of this process can be seen in diminished content of individual amino-acids, such as thermolable lysine [2]. Excessively intense thermic treatment leads to a decrease in protein content, diminished content of essential amino-acids and unwanted reactions – Maillard reaction, oxidations of lipids and other reactions [20]. During extrusion the digestibility of carbohydrates increases [17], which is reflected in changes of rheological properties of starch, which rises while extrusion takes place, its solubility is altered in cold water, viscosity decreases, while there is also a partial or complete release of amylase and amylopectin from starch granules [17]. Gelatin starch that forms during excursion process is irreversible and when starch cools off its volume is usually 2 to 3 times its original value.

Hydrothermic processing of soya bean is a technological treatment in which soyabean is placed inside of a special reactor (apparatus) and is exposed to saturated water steam under pressure for a specific period of time, after which the material expands rapidly. Due to heating up, increased pressure followed by rapid expansion in soybean, the cell membranes begin to break up, oil from sfrozome is released and inhibitory substances are deactivated [22].

Hydro-thermal processing of soyabean includes chemical changes caused by increased temperature and pressure, which occur as a result of rapid expansion, similarly to chemical changes in the process of excrusion. During hydro-thermal processing the soybean remains whole or is seperated into two halves, while extrudate that has consistency of flour is obtained during extrusion. Hydro-thermally treated soybean is light yellow in colour with the husk which makes the product suitable for manipulation, storage and transport in dispersed state and enables extended storing periods in relation to extruded full-fat soya.

MATERIAL AND METHODS

Extrusion of soy – dry process

Extrusion of soya was carried out using extruder type M2, model 1000 "Oprema zootehnička oprema" Ludbreg, Croatia. Soya was firstly dried in a dryer to a moisture level of 10% and then cleaned from impurities in silos. Extruder had a nominal capacity of 1000 kg/h, with installed 77 kW electromotor and a screw feeder which had a 1.5 kW electromotor. Extruder structure is made up of four barrel segments and four screw segments with different pitch length. Extruder screw segments are hauled onto extruder axle and the steam lockers are placed in between segments in order to regulate the pressure in extruder. Used steam lockers were 5 3/2", 5 3/2", 5 ½" and 5 ½". The extruder head with a nozzle is placed at the very end of the screw. The first segment of the extruder contains a connector for water and technological steam.

The process of extrusion begins by transporting soya using a screw carrier which brings it from floor storage room into the extruder basket. The basket contains an indicator of the upper level, which deactivates the screw carrier when the basket becomes full. When the bean becomes empty, it it activated again and it automatically doses the soy into the basket above the extruder. Permanent magnet is located in the basket in order to prevent iron that has "strayed" from reaching the extruder. From the bean, by use of the screw feeder, soya gets into the first segment of extruder head and the extrusion process begins. Soya passes between the barrel and the screw and, by action of flight, steam lockers and rings, gets through a 1-1.5 mm gap, which results in high pressure and temperature, which at the exit from the extruder (extruder head where the nozzle is located) ranges between 120-130 ^{o}C . Extruded soya enters a vibration cooler where vibrations enable its movement on coller trays towards the top, and intense airflow produced by a ventilator allows for the extruded mass to cool off. Exdrudate goes into the basket, located above the transport screw, which carries cooled extruded soya into bulk cells. From the silo, the extruded full fat soya is transported by screw conveyers to a scale where the bagging is done.

Hydro-thermal treatment of soyabean

Hydro-thermal treatment of soyabean is performed in a special reactor (device) where soya is exposed to water steam under specific pressure (6-8 bars), within a particular time period (7-15 min), after which the material expands rapidly.

Soybean is transported to the surge bean with a volume of 5 t. It is transported from the bean by screw conveyer to a bulk elevator and further to the receiving bean, where the raw material is stored temporarily. Soya is then evenly fed into the feeding bean using the slide gate and from the bean gets into a reactor, in which the hydro-thermal treatment is taking place by steam action during a specific time period. Upon the completion of the cycle, the slide gate opens and the finished product is transferred from the reactor into a dryer – cooler.

The start of the next cycle can take place after the slide gate has been closed. Intensive drying-cooling of processed soybeans is performed using airflow supplied through a channel and a collector by using ventilators. Processed material is placed into a transport vehicle by screw carrier. Water steam which is vital for thermal treatment of raw soybeans is transported from the boiler to the reactor. Operational mode is maintained

without time limits in addition to achievement of constant operational parameters – pressure and temperature. The potential for full utilization is connected to degradation of sferozome walls where oil is stored. Destruction of the sferozome structure is achieved through rapid, but controlled expansion from reactor vessel to expanded vessel. Rapid mechanical change of state in soybeans which is created by quick opening of the vessel under pressure, results in the destruction of soybean structure at the end of the process.
Given the fact that the process is performed with mild thermal preparation and the moistening up until mechanical destruction does not occur, the soybean remains whole or divided into two halves. The soybean that has been thermally treated must be finalized into an end product due to the moisture content in treated soybean after thermal processing, which ranges between 16-18%. In order to ensure the stability of the end product, the content of surface moisture must be decreased by drying. Direct drying of soybeans after completion of the cycle by implementing well-known systems, such as fluid or rotational dryer, does not provide optimal results. Namely, high, end temperature of soybean which is approximately 80 ^{o}C, causes the temperature of the drying agent to rise, in which case the mass would be exposed to additional thermal shocks and inconsistencies, similarly to toasting. The retention time of the mass during drying would be short in the above-mentioned systems, that is, it would require a long rotating drum. This would be too expensive and would create unfavourable conditions with regard to sudden cooling and drying. In order to avoid these types of problems, a principle of thermal processing of the mass is implemented during which the vertical vibrational movement along the helicoidically shaped channel occurs. Described technological line for hydro-thermal treatment of is able to process up to 3 t/h of soya. The process is based on batches and is intermittent which gave rise to the concept of "block" – devices for hydro-thermal treatment where processing is performed semi-continuously. In practice, the implementation of this system is equal in simplicity to all widely used systems.

Chemical methods for determining soya quality
Basic chemical composition (moisture content, raw proteins, raw fat, raw cellulose and minerals) of soybean, extruded full fat soya and hydro-thermally treated soyabeans is determined according to methods A.O.A.C. [4]. The content of trypsin inhibitors in soyabean, full-fat soyabean and hydro-thermally treated soya bean is determined according to the methods of Hamerstand and associates [10]. Activity of urease in analysed samples is established according to the method prescribed by International standard ISO 5506 [13].
Nitrogen Solubility Index (NSI) is determined according to the A.O.C.S. method [1].

Microbiological analyses
Total number of micro-organisms, yeasts, molds, isolation and identification of *Salmonella* and sulfido-reducing clostridia was established according to Regulations regarding methods of applying microbiological analyses and superanalyses of food [19].
Internal laboratory method was used to determine the presence of coagulases in positive staphylococci, Proteus types and *Escherichia coli.* Fifty grams of analysed sample is blended with 450 ml of prepared sterilized nutritional broth in Erlenmeyer flask. Prepared sample is mildly homogenized and incubated for 24 hours at 37 ^{0}C. Isolation

and identification is carried out according to Regulation regarding methods for micro-biological analysis and superanalyses of foods [19].

RESULTS AND DISCUSSION

The quality of the nutrient (product), obtained by applying the process of excrusion (without steam) and hydro-thermal treatment of soybeans can be observed by analysing basic chemical and amino acid composition of soybeans before and after the treatment (Table 1). Results from Table 1 show that full-fat nutrients from soybean are qualitatively equal, although they were produced using different thermal treatments.
The content of the most important parameters that determine the quality of these nutrients, raw proteins and raw fats, are very similar, with a relatively similar destruction of individual amino acids that occurred during thermal treatments. Production of the above-mentioned nutrients resulted in the loss of content in amino acids where amino acid content of total proteins in extruded full-fat soy equaled 87.04%, and 86.81% in hydro-thermally treated soybeans. In soyabeans, the content of amino acids in total proteins is 95.88%.
Quality of the final product, the extruded full-fat soybean and hydro-thermally treated soybean, can also be viewed from authoritative data in determining the adequacy of applied thermal treatments, shown in Table 2.

Table 1. Parameters for soybean quality, full-fat soya and hydro-thermically treated soybean

Quality parameters	**Soybean**		**Full-fat soybean**		**Hydro-thermally treated soybean**	
Basic chemical composition	% in sample	% in DM*	% in sample	% in DM	% in sample	% in DM
Moisture	10,06	-	4,67	-	10,66	-
Raw proteins	37,48	41,67	39,40	41,33	36,92	41,32
Raw fat	19,27	21,26	20,26	21,25	19,28	21,58
Raw cellulose	4,39	4,88	4,08	4,28	4,55	5,09
Minerals	4,63	5,15	4,81	5,05	4,68	5,24

*DM – dry matter

Table 2. Quality parameters for determining adequacy of applied thermal treatments

Parameter of quality	Soybean	Full-fat soyabean	Hydro-thermally treated soybean
Trypsin inhibitor (mg/g)	61,66	3,27	3,91
Activity of urease (mgN/g·min na 30 ºC)	10,95	0,26	0,28
NSI (%)	65,82	25,64	23,79

Thermolable trypsin inhibitor, which is a dominant anti-nutrient in soyabean, is significantly thermally inactivated by the process of extrusion (94.70%), and to a lesser extent using hydro-thermal process (93.66%). Van der Poel [27] states that the treatment using steam (100 ºC > 15 min) reduces the content of trypsin inhibitors in soyabeans by 65-97%, and by 78-98% in the case of extrusion (145 ºC, 16 s). Gundel i Matrai [9] allow even lower levels where the content of trypsin inhibitors dimishes (97-99% for extrusion, and 88-94% when steam treatment is used). Results shown in Table 2 are in accordance with the data presented by Aurmor and associates [3], who have determined that hydro-thermal treatment (100 ºC, 10 min) almost completely eliminates the effect of trypsin and himotrypsin inhibitors and lectins.
Nitrogen Solubility Index (NSI), as one of the parameters of quality that is used in optimization of thermal treatment and quality control, amounts to 65-75% in soybean [7, 21], which is lower than in thermally treated products. Although the literature citations for optimal values of NSI for thermally treated soys differ from one another, citations made by Holmes [12], which we use the most for evaluating treatments and products, can serve as a good guideline – the level of 12.5% is considered to be a result of progressive treatment, while 25.1% the optimal level is considers a result of optimal treatment. By comparing these values with NSI values in extruded full-fat soya (25.64%) and hydro-thermically treated soybean (23.79%) (Table 2), it can be concluded that thermal treatments were optimal that were responsible for producing nutrients of similar quality.

Table 3. Parameters for soybean quality before and after thermal processing

Parameters	Soybean	Thermally processed soybean
Moisture content, %	9,01	8,22
Raw ash content, %	4,30	4,38
Raw celluloze, %	8,36	8,23
Raw fat content, %	16,06	16,00
Raw protein content, %	38,26	38,37
Activity of urease, mgN/g/min	7,83	0,08
Micro-biological analyses	Not found	Not found
Salmonella spp., in 50g	Not found	Not found
Coagulase of positive staphylococcus, in 50gr	Not found	Not found
sulfido-reducing clostridia, in 1gr	Not found	Not found
Proteus types, in 50gr	Not found	Not found
Escherichia coli, in 50gr	Not found	Not found
Total number of yeasts, in 1gr	Not found	Not found
Total number of molds, in 1gr	100	55
Total number of micro-organisms, in 1gr	800000	100

Soybean is thermically treated in a device for hydro-thermal processing without adding steam, which is toasted by using temperature generated by heaters that are installed inside of device sheathing. Pressure in the device was 0.98 bars, temperature of the first heater was 127 ^{0}C, second one 138 ^{0}C and third one 250 ^{0}C with intensive mixing using vertical screw. Thermally processed soybean, compared to soybean which had not been thermally processed, has a slightly lower moisture content (8.22%) and significantly lower urease activity (0,08 mgN/g/min). The total number of micro-organisms was reduced Substantially sighnificantly. It is certain that this system of thermal processing without steam must be technologically examined.

CONCLUSION

Thermal processing of soya beans in products intended for human and animal consumption require implemetation of technological extrusion processes, hydro-thermic treatments, thermal treatments without steam through application of optimal technological parameters in order to ensure that the final product is of high quality.
It is vital to monitor soybeans, which has been thermally-processed by implementing above-mentioned processes, prior to and during technological processing in order to

ensure that the product is of high quality and is in accordance with Regulations regarding the quality and other requirements for animal feed [18].

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POSSIBILITY TO MODIFY THE CONTENT OF CONJUGATED LINOLEIC ACID IN MILK FAT FROM COWS' MILK BY NUTRITION

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ABSTRACT

Modification of milk fat content by changing the regime of nutrition is not a completely new concept. The intensive development of the dairy industry, as well as the market trend increasingly targeted at using "health foods" are moving in the direction of finding ways to lower high contents of saturated fatty acids and cholesterol in milk, and increase the level of nutritionally valuable substances. Since variations milk quantity and composition are dominantly conditioned by hereditary factors (55%), the remaining variation (45%) is conditioned by paragenetic factors, among which nutrition is by all means the most important. Fat predominantly consists of triglycerides containing over 200 different fatty acids. Fatty acids can have short (C_4 – C_{10}), medium (C_{12} – C_{16}) and long chain fatty acids (C_{18}) on the carbon atom. Short and medium chain fatty acids account for 30% of milk fat. Long chain fatty acids originate from meals and depending on the type of meal, account for 40 to 60% of milk fat. Milk fat contains numerous anticancerogenic substances among which conjugated linoleic acid is the most important, and its content in milk can significantly be changed via nutrition, i.e. by utilizing certain feeds. In addition, the content of conjugated linoleic acid in milk fat depends to a large extent on seasonal variations, as well as on existing individual variations in animals fed the same rations, and the manner and system of cow nutrition, i.e. the share of individual feeds in the ration.
Key words: *nutrition, food supplements, milk fat, conjugated linoleic acid, fatty acids*

INTRODUCTION

In addition to a significant influence on quantity, nutrition can also influence milk quality, even though to a limited extent. Cows can not achieve their highest production or its stability during lactation, without daily adequate and balanced nutrition (29, 28, 24). Milk fat is the richest source of conjugated linoleic acid. Conjugated linoleic acid (CLA) is a common name for position and geometric isomers of octadecadienoic acid resulting as intermediary products in the process of hydrogenation of linoleic acid in the rumen of ruminants, and to date 9 different compounds were identified in commercial products (30,36). During the last 15 years, two CLA isomers, cis9, trans11 – isomer and trans 10, cis12 – isomer, attract considerable attention of scientists (30). There is data indicating that these isomeric fatty acids have numerous physiological effects, an anticancerogenic action, antiaterogenic, and immunomodulatory action on experimental

modules (36, 40), and reduce adipose tissue (4, 6). Milk and dairy products are the best natural sources of CLA and mainly contain a cis9, trans11-isomer originating in the rumen of ruminants during biological biohydrogenation of linoleic and linolenic acid originating from vegetable feeds (5).
Carbohydrates and fats ingested by animals via food are very important for forming fatty acids in milk (37, 40). In food, carbohydrates are found in the form of structural carbohydrates (cellulose, hemicellulose, pectin), sugars, and starch. Fodders such as hay, straw and silage contain relatively high quantities of structural carbohydrates, and their digestion in the digestive tract depends to a high extent on the fodder itself (hay is digested to a higher extent than straw), and this is important for the presence of milk fat in milk (37, 38, 29). Sugars and starch (nonstructural carbohydrates), which dominate in seeds, can also be converted to milk fat by disintegration to butyric acid (39). Fatty substances that are not degraded in the rumen (protected fats), and are absorbed in the small intestine of goats can also be used for this purpose.

DIETARY FACTORS AND THEIR INFLUENCE ON CLA CONTENT IN MILK FAT

Milk fat is the richest natural source of conjugated linoleic acid with a content ranging from 2.4 to 28.1 mg/g, however with a very pronounced seasonal variation, and with a summer concentration which is 2 to 3 times higher than in winter months (25).
The typical composition of milk fat, depending on the source of fat in the ration, as well as the content of fatty acids and CLA (conjugated linoleic acid) in various fat sources are presented in Table 1.

Table 1. Influence of fat in the diet on the composition of milk fat in cumulative cows' milk (27)

Fatty acid	Diet		
	No added fat	Whole soybeans	Fat [a]
4:0	[b]5.68	5.10	4.99
6:0	3.24	3.16	2.86
8:0	1.74	1.76	1.54
10:0	3.50	3.63	3.21
12:0	3.73	3.80	3.47
14:0	10.13	10.46	10.45
15:0	1.07	0.92	0.98
16:0	30.43	27.98	30.30
18:0	11.19	13.14	12.12
18:1 trans	1.76	1.74	1.95
18:1 cis	19.22	19.14	20.40
18:2	2.71	4.52	2.84
18:3	0.35	0.75	0.30
CLA [C]	0.45	0.50	0.65

[a] commercial protected fats, tallow and cottonseed
[b] as percentage relevant to total fatty acid content
[c] conjugated linoleic acid

Griinari and Bauman (31) have proposed that dietary factors which affect milk CLA content could be grouped into of two categories. The first would be factors that provide lipid substrates for formation of CLA or *trans* C18:1 oleinic acid in the rumen. The second would be factors that change the microbial activity associated with ruminal biohydrogenation (4). Literature data are presented in Table 2. according to the ranges of observed milk CLA content.

Table 2. Effects of dietary factors on milk fat CLA content in dairy cows (literature review)[a]

low values (0.2–0.8%)	**medium values (0.8–1.6%)**	**high values (> 1.6%)**
- corn silage - grass silage/hay/pasture - animal or vegetable saturated fats - raw soybeans - micronized soybeans - soybeans treated by heat processing - extruded soybeans or cottonseeds	- fresh pasture/young grass - low fiber diets - restricted feeding - extruded soybeans - peanut oil - rapeseed oil[b] - soybean oil[b] - linseed oil[b] - calcium salts of rapeseed oil	- rapeseed oil[c] - soybean oil[c] - sunflower oil[c] - linseed oil[c] - calcium salts of soybean and linseed oils - fish oils

a (1, 3, 5, 12, 17, 18, 21, 22, 33, 34, 35, 41, 43, 45).
b At low doses
c At medium doses

However, no definitive conclusions can be drawn from these data, because a large proportion of the data arose from indirect comparisons of experiments in different laboratories or experimental conditions, and also because a lot of the potential interactions in practical farm conditions have not yet been studied. It nevertheless appears that plant oils high in linoleic acid (e.g. sunflower, soya and rapeseed) are very efficient at increasing milk CLA content. Besides directly increasing the yield of CLA and *trans* C18:1, it is likely that linoleic acid inhibits the final reduction of *trans* C18:1, thus increasing its accumulation in the rumen (31). Calcium salts of rapeseed also increase milk CLA content (17), in agreement with the concept that calcium salts are not resistant to ruminal biohydrogenation. Limited research shows that the frequency of feeding cows fats and the physical form of oils (free or in raw seeds of oleaceous plants), as well as thermal processing of seeds of oleaceous plants, exert a relatively low influence on altering milk fat composition (7, 27). Furthermore, vegetable oils are more efficient than extruded seeds (which are themselves more efficient than raw seeds) at increasing milk CLA content (32) (Tab. 2). This potency could be inversely related to the protection of PUFA against biohydrogenation. On the other hand, supplementation with animal fats is not very efficient at increasing CLA content because of their low PUFA content. When soya oil was offered 24-times daily, instead of twice, the milk fat content increased, and the percentage of *trans* C18:1 decreased whereas that of C18:0

increased (2). This suggests that ruminal hydrogenation was more complete and that milk CLA synthesis was probably decreased.
Feeding 4% rapeseed oil to dairy goats (35) greatly increased milk fat CLA content (by 204%), and more efficiently than similar doses of rapeseed in dairy cow diets. It should be stressed that the milk yield and composition responses to dietary fat differ notably between goats and cows. Feeding vegetable oils or seeds increases milk fat content in goats (review by Chilliard and Bocquier (9)), whereas it generally decreases it in cows (8). This peculiarity of goats (31, 34) could be related to some differences in the metabolism of *trans* FA in the rumen or in the mammary gland.
Feeding linseed oil (a C18:3-rich oil) greatly increases milk fat CLA content (17, 22) and is at least as efficient as C18:2-rich vegetable oils. Since C18:3 is not a precursor of CLA in the rumen, this suggests that feeding linseed oil results in a large increase in the production of ruminal *trans*-11 C18:1, which can be used by the mammary gland for CLA synthesis.
Dietary fish oil is more efficient at increasing milk CLA content than an equal amount of plant oils. However, fish oil increases ruminal and milk (10, 11 and 12) *trans*-11 C18:1. So, CLA proportions increased from 0.2–0.6% with the control diet to 1.5–2.7% with diets supplemented with fish oil (200–300 g·d–1, (14, 15).
There is a linear relationship between milk fat CLA and *trans* C18:1 content across a variety of feeding conditions. However, the CLA: *trans* C18:1 ratio is much lower with fish oil.
However, the effects of forage: concentrate ratio were variable for different studies, as discussed by Griinari and Bauman (31). Pasture feeding increases milk CLA (see above), especially with grass at an early growth stage (25). The high C18:3 content of young grass (see above) and its low fiber content probably interact to increase the production of CLA or its *trans* C18:1 precursors. Animals on pasture have a considerably higher CLA content in milk fat, relevant to animals fed concentrates. In fact, dairy products from animals on pasture may contain 300-500% more CLA compared to cattle fed a ration consisting of 50% hay and silage and 50% concentrates. Generally speaking, fodders contain a higher concentration of linoleic acid (C 18:3), while linolenic acid (C 18:2) is mostly contained in cereals and seeds.
The influence of cow breed on milk CLA is either not significant or of limited extent, with milk from Montbeliardes showing slightly higher values (34). However, relevant to the phase of lactation, high variability (14) of CLA content was established (9.9 to 51.7 mg/g fat) in cows in the same phase of lactation, and fed the same ration.
The proportions of *trans*-18:1 and CLA in cow's milk produced from maize silage based diets (more than 60% of the ration) are small (1.1 to 2.2% and 0.4 to 0.6%, respectively) (14). CLA concentration in the milk of dairy cows switching from winter diet to young, natural meadow grass increases sharply (25, 26).
Nevertheless, the milk CLA proportions measured in cows at pasture are variable (0.5 to 1.7%) (Tab. 3). Milk CLA concentration increases with green grass availability (19, 25) and is further increased by lipid supplements (20). In other respects, the observed concentrations are higher in the Spring and in the Autumn than in Summer.

Table 3. Effects of pasture with or without lipid supplements on milk fat CLA in dairy cows (13)

Milk fat CLA (% of total FA)			Treatment duration
Winter diet	**Pasture**	**Pasture + Lipid supplement**	
0.3	1.3	-	-
0.3	0.6	-	4 months
0.4	1.2	-	3 weeks
0.5	1.1	-	4 weeks
0.4	0.7	-	4 months
0.4	1.1/1.4	-	3 months
-	0.5	0.5/0.8	8 weeks
-	1.7	2.5/2.2	3 weeks
-	0.8	1.3/1.8	6 weeks
0.3	-	1.3	4 weeks
0.6	1.7	-	3 weeks
0.6	0.8	-	6 weeks

Young grass high C18:3 concentration and low fiber content probably combine to increase CLA and *trans*-18:1 production. Also, the particular botanical composition of natural highland meadows seems to promote high milk CLA concentrations (up to 2.4%, (44)), whereas a botanical composition effect of cultivated swards appears to be low.

CONCLUSION

Conjugated isomers of omega-6 linoleic acid (CLA) are naturally occurring in food in small quantities, especially in milk. Concentration of CLA can vary in food and the levels of CLA in milk are mainly dependant on animal diet. Feeding factors make it possible to vary milk FA composition in many ways. Recent advances in the knowledge of FA synthesis mechanisms (digestion and metabolism) and their putative physiological effects in human consumers have significantly boosted on going research and potential applications. Research by numerous authors has established that by using different feeds as well as supplements in cow nutrition, increases milk fat content and milk yield, as well as the fatty acid profile in milk fat, and can lead to a considerable increase of the content of unsaturated fatty acids and CLA which demonstrate efficacy in reducing cholesterol in plasma, and the ingestion of which is very important from the aspect of human health. As regards ruminant nutrition, the aim is to better understand the effects of using grass-based diets, new combinations of feedstuffs in concentrates, and oil seed technology and processing. However, very few direct comparisons have been made between the main types of basal diets (different types of forages, starchy concentrates, etc.) combined with various lipid supplements (oils, seeds, technological processing and lipid dose added to the basal diet). However, it is clear that the plasticity of milk fat composition is very large, according to numerous interactions between forage-concentrates-oils-minerals-vitamins, time after dietary changes, as well as ruminant

species, on almost all major and minor FAs, including several *trans* isomers of C18:1, C18:2 and C18:3.

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ALTERNATIVE POSSIBILITY TO SUBSTITUTE MAIZE WITH SUDAN GRASS FOR PREPARING SILAGE FOR DAIRY COWS

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ABSTRACT

Under our conditions, there are numerous factors that can considerably reduce maize yield. The most important factor is certainly drought, which frequently appears during maize vegetation. In addition to adequate implementation of required agritechnical measures, one of the ways to alleviate consequences of draught, on terrains not adequate for irrigation, is also the introduction of cultures more resistant to draught than the present most frequent cultures. One such culture is sudan grass. This paper makes an attempt to answer the question to what extent growing sudan grass can replace growing maize for producing silage for cattle feed.
Research was carried out in the region of the village of Cantavir, where, maize and sudan grass silage were prepared in 2003 and 2004 for the needs of a cattle farm with 200 dairy cows. To prepare sudan grass silage, both in 2003 and 2004, the strain NS Srem was planted. Results of chemical analyses show that whole maize plants are without a doubt a better quality feed than sudan grass silage. First, whole maize plant silage contains considerably less NDF and ADF fibers, and considerably more nonstructural carbohydrates than sudan grass silage. The result of this different chemical composition of analyzed silage is that maize silage is much more digestible and richer in energy. In addition, maize silage had higher yield only of the easily digestible fraction, which is by all means the most important, and this yield was almost 50% higher than for sudan grass. A comparison of results of this research permits the conclusion that owing to its ability to regenerate, sudan grass can be used freshly cut as green feed, and when used in this manner it offers three cuttings annually without any problems. Sudan grass is resistant to draught and has higher yields of green mass, however sudan grass silage has lower quality than whole maize plant silage. For this reason, sudan grass silage should be used exclusively for feeding those categories of cattle that do not require high quantities of energy in the ration (dry cows, pregnant heifers, growing heifers, and heifers in insemination).
***Key words**: maize, sudan grass, ADF, NDF*

INTRODUCTION

It is a known fact that agricultural production and production of animal feed as its integral part, are facing numerous problems, very often coinciding in time and space. Very frequently, the solving of one problem can result in another problem, and for

this reason the goal of organizing plant and animal production is not to bring all factors of production to an optimum, because this is not possible, but to mutually harmonize all factors of production at an optimum, resulting in the best possible results of production under given conditions.
During the last decade, production of maize for silage and maize for kernel is facing a very dangerous pest – *Diabrotica virgifera*, able to cause much damage in maize production. The main measure to fight this pest is to avoid growing maize in repeat planting, i.e. by growing maize in a planting sequence (4). One factor that very frequently appears in maize production in our country, and results in a huge decrease of yield, is also draught. Under draught conditions maize had considerably lower yields, both of kernel and of the whole plant. As a consequence of this yield decrease, very often in draught years many farms are not able to produce sufficient quantities of silage to feed cattle.
In addition to adequate implementation of required agritechnical measures, one of the ways to mitigate the consequences of draught on terrains not suitable for irrigation, is also the introduction in plant production of cultures more resistant to draught than the presently most used cultures in production. One such culture is sudan grass. Sudan grass can have very high yield of green mass, even above 120 t/ha (2), which is much higher than the yield of maize for silage, and this difference is especially pronounced in draught years (5).
From the aspect of plant production, sudan grass has many advantages recommending it for growing, and increasing its planting would not be disputable if it were not for the fact that maize has certain characteristics placing it above other cultures from the aspect of animal feed production, and making it irreplaceable for silage production. Above all, silage prepared from maize is much more easily digested than sudan grass silage, i.e. the share of the fraction insoluble in neutral detergent (NDF – hemicellulose, cellulose and lignin) is much lower in maize silage than in sudan grass silage. From the aspect of cow nutrition, above data means that whole maize plant silage tastes good, and that cows more readily consume maize silage, i.e. that they are able to consume more dry matter and energy from maize silage than from sudan grass silage. The goal of this paper was to investigate the possibility to replace maize silage with sudan grass silage under conditions of intensive production in arid plant production. To answer this question, yield of digestible matter per surface unit was analyzed.

MATERIALS AND METHODS

Maize silage and sudan grass silage were produced for the needs of "Jadran" farm in Cantavir, in 2003 and 2004. Both in 2003 and 2004, the strain NS Srem was planted to prepare sudan grass silage. In production of sudan grass silage all technological operations were implemented. Cutting and preparing sudan grass silage was done twice a year, in the phase of ear formation. In 2003, plants were cut between 21 – 23 July and 14 – 16 September, while in 2004 this was between 26 – 27 July and 11 – 13 September.

Both in 2003 and 2004, to prepare whole plant maize silage, hybrids NS SC 663 and ZP SC 677 were planted. In addition, in production of whole plant maize silage, all technological operations were implemented.
In 2003, maize was harvested and silage prepared from 25 August to 1 September, and in 2004 between 30 August and 6 September. Silage was prepared in the milky-waxy phase of maize ripening.
A chemical analysis for the content of moisture, raw proteins, raw fat and ash was done using the Weende method. The chemical analysis for the content of neutral (NDF) and acid (ADF) detergent fibers the Van Soest method was used.
The chemical analysis for total content of lactic, acetic and butyric acid was done using the Wigner Magasanic method.

RESULTS AND DISCUSSION

Having in mind that in both years virtually identical agritechnical measures were applied, it can be concluded that the large differences in yield in 2003 and 2004, for both cultures, were caused by extreme differenced of precipitation in the monitored period. In the exceptionally dry 2003, yields were drastically decreased, and in the rainy 2004, yields were up to two times higher than in the preceding year (Table 1).
Indexes in Table 1 show that yield decrease in the dry 2003 was less pronounced for sudan grass than for maize, i.e. that sudan grass proved to be more resistant to the draught than maize.

Table 1. Achieved yield of fresh green mass (t/ha)

Species	**Year**		**Index**
	2003	2004	
Maize	15,2	32,4	
Sudan grass	41,8	66,7	

Realized yields are one of the two most important indicators of suitability of a culture for producing animal feed. Table 1 shows that, according to this indicator, sudan grass, especially in unfavorable years, has significantly higher yields, recommending an increase of its share in the planting structure in order to increase production of animal feed (silage). The second most important indicator of suitability of a culture for producing animal feed is its chemical composition, which directly influences the quality of feed (in this case silage) prepared from that culture. The quality of prepared silage directly influences both the level of consumption of dry matter per animal, and the level of utilization of consumed dry matter. Table 2 presents the quality of prepared whole plant maize silage and sudan grass silage in 2004.

Table 2. The quality of maize silage and sudan grass silage in 2004

Index	Maize silage yield 32.4 t/ha		Sudan grass silage yield 66.7 t/ha	
	% DM	Yield, t/ha	% DM	Yield, t/ha
Dry matter	(37,00)** 100	11,99	(9,75)** 100	19,84
Crude protein	8,67	1,04	9,80	1,94
Crude fat	2,71	0,32	2,37	0,47
Ash	4,09	0,49	4,27	0,85
Neutral detergent fiber (NDF)	39,38	4,72	72,34	14,35
Acid detergent fiber (ADF)	21,60	2,59	43,92	8,71
Hemicellulose	17,78	2,13	28,42	5,64
Unstructured carbohydrates *	45,15	5,41	11,22	2,23
	% on natural moisture content			
Fully lactic acid	1,60		0,90	
Fully acetate acid	0,93		0,96	
Fully butyric acid	0,00		0,21	
pH	3,78		4,92	

- 100 - % crude protein - % crude fat - % ash - % NDF (37.00)
- ** % dry matter in full mass with natural moisture content

Lower values for individual carbohydrate fractions (ADF and NDF) in sudan grass silage were reported in research by Petkova and Zhelev, (3), who used rations with maize silage and sudan grass silage to feed Holstein cows.

Results presented in Table 2 show that whole plant maize silage is a higher quality feed than sudan grass silage. First, whole plant maize silage contains considerably less NDF and ADF fibers, and considerably more unstructural carbohydrates than sudan grass silage. The result of this different chemical composition of analyzed silages is that corn silage is much more digestible and richer in energy. Lower fiber content, i.e. better digestibility, mean that whole plant maize silage is a tastier feed, more readily consumed by cattle, and that therefore ingestion of dry matter and energy from the fodder in the ration are higher, which in fact should be the goal for cattle production (meat, milk, offspring).

The claim about the undoubtedly better quality of maize silage is substantiated also by the much more favorable structure of organic acids created in maize silage during fermentation (Table 2). Sudan grass has a much less favorable ratio between lactic and acetic acid, and above all between lactic and butyric acid, which has a very unfavorable effect on its taste, and therefore on its level of consumption. Under production conditions on farm "Jadran" in Cantavir, maximum consumption of whole plant maize silage was up to 25 kg daily per animal, while consumption of sudan grass silage was rarely above 15 kg daily per animal. Translated into dry matter intake, the potential for dry matter intake for cows is twice higher for maize silage, than for sudan grass silage.

The content of butyric acid could be reduced by wilting the cut mass of sudan grass to 35% dry matter content, which would improve competitiveness of lactic acid bacteria and increase production of lactic at the expense of butyric acid. This would considerably improve consumption by cattle.
Having in mind indicators of silage quality presented in Table 2, it would be important to review realized yields per hectare for individual fractions with various digestibility for both cultures. The light digestible fraction would contain raw protein, raw fat, and nonstructural carbohydrate. The middle digestible fraction would contain hemicellulose, while the hardly digestible fraction would include ADF fibers.

Table 3. Yield of individual fractions with different digestibility

Fraction / Species	Yield of maize silage 32,4 t/ha		Yield of sudan grass silage 66,7 t/ha	
	Yield of fraction, t/ha	Cumulative, t/ha	Yield of fraction, t/ha	Cumulative, t/ha
Light digestible fraction	6,77	6,77	4,64	4,64
Middle digestible fraction	2,13	8,9	5,64	10,28
Hardly digestible fraction	2,59	11,49	8,71	18,99

A careful analysis of results in Table 3, shows that for maize there is a higher yield only for the light digestible fraction, which is by all means the most important, and that this yield is almost 50% higher than for sudan grass. However, the cumulative yield of the light and the middle digestible fraction, that can still be fairly well utilized by cattle, was considerably higher for sudan grass than for maize. This difference in yield is especially in favor of sudan grass if the calculation includes all three fractions. Although the hardly digestible fraction is a hardly accessible source of energy and body building substances, still a considerable part of this fraction (cellulose) is also utilized by cattle, owing to the presence of symbiotic cellulytic bacteria in their pregasters (1).
When analyzing results from Table 3, one should keep in mind that these results pertain to 2004, which had high above average precipitation. There is cause to presume that the relative yield increase of light and middle digestible fractions of sudan grass compared to maize was even more pronounced in unfavorable years such as 2003 (draught).
Owing to its ability to regenerate, sudan grass can be used freshly cut as green fodder, and in such use it offers three cuts a year without any problem (5). Used thus (at earlier phases of development) its digestibility is much higher, and cattle readily consumes it in large quantities in this form, therefore it often happens that during periods of feeding freshly cut sudan grass, the average milk yield on the farm grows by as much as two liters per animal, with a certain decrease of dry matter in milk.

Naturally, this type of nutrition requires more human labor (daily cutting and driving the cut mass to the site), and implementation depends on climate conditions to a high degree.

CONCLUSION

The above permits the conclusion that reasons for partial substitution of maize and introduction of sudan grass in the so-called "farm" plant sequence are:

- Heightened risk of growing maize as a monoculture
- Better resistance of sudan grass than maize to draught, diseases, weeds, and pests
- Sudan grass had higher yields of dry matter per surface unit
- The possibility to use sudan grass for feeding cows as hay and green mass

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EFFECT OF UTILIZATION AND COMPOSITION OF CONCENTRATES IN GOAT NUTRITION ON QUANTITY AND QUALITY OF PRODUCED MILK

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ABSTRACT

In order to determine the quantity of concentrate mixture to feed goats in a ration, the level of milk yield, quantity and quality of ingested fodder, appetite and status of the constitution should first be determined. In general, if milk yield is not at any higher level, goats are able to produce sufficient quantities of milk with very low concentrate consumption. Two basic periods can be differentiated in the nutrition of goats and their offspring: the summer or pasture period, and the winter or stable period. Depending on this, the nutritional balance is composed, both for feed requirements while on pasture, and for preserved feed (hay, haylage, silage, etc.) in the winter period. Goats are very good at utilizing fodder: pasture, hay, and haylage.
Therefore the yield of a goat will to the largest extent depend on the quantity and quality of such fodder. In high producing goats, fodder quality is very important. In such cases, certain quantities of concentrates are also included in the ration, since fodder is not sufficient to fulfill all nutritional requirements of the animal. Concentrate mixtures are differed to increase the share of energy and protein in the ration, but also to supplement the ration with individual mineral elements. Since goats can excrete as much as 1.5 times more dry matter than dairy cows by unit of body weight, their nutritional needs are high. For high milk production, realized by very valuable animals, fodder quality becomes even more important. In this process, utilization of certain quantities of concentrates, as well as their composition, must also be considered. Concentrate mixtures used for this purpose are similar to those given to dairy cows, with a protein share of 12 to 18%. Also, the share of concentrates in the total ration is 40 – 60%.
Key words: *feeding, goat, concentrate, production of milk*

INTRODUCTION

In order to enable adequate growth, development, and high milk production, as well as adequate balancing of rations for goats, it is very important to know the changes of their metabolic cycle the whole year round. Also, all these changes are primarily conditioned by the geographic latitude, i.e. the effect of climatic factors (6). Similar to cattle and sheep, during the yearly production cycle of goats, there is an alternation of periods during which they form body reserves of nutritious substances, and periods when they utilize such reserves (7, 8).

Two basic periods can be differentiated in the nutrition of goats and their offspring: summer or pasture period, and winter or stable period. Depending on composition, nutrition is balanced, both for nutritional requirements on pasture, and for preserved feed (hay, haylage, silage, etc.) during the winter period. Goats are very good at utilizing fodder: pasture, hay, and silage.
Therefore, production of goats will mostly depend on the quantity and quality of these feeds (10). In high producing goats, fodder quality is very important. In such cases, a certain quantity of concentrate is also included in the ration, since fodder is not sufficient to fulfill all needs of the animal for nutritional substances. Concentrate mixtures are offered to increase the share of energy and protein in the ration (12, 13), and also to supplement the ration with certain mineral elements (9, 11). Special attention should be paid to nutrition of goats during lactation.
In the case of goats producing higher quantities of milk, rations with higher energy concentration should be provided during lactation, where milk production is in high correlation with the quantity of energy consumed from food, especially in mid-lactation.

NUTRITIONAL REQUIREMENTS OF GOATS

Energy and proteins are the most important factors for calculating nutritional requirements of dairy goats. The lack of energy in the ration limits productivity, while protein is indispensable for growth, gestation and milk production. There often is a deficit of energy in goat nutrition, and not only in animals with high milk production (13). Abortions can result, especially from 90 to 110 days of gestation when insufficient nutrition causes stress in the goat, due to hypoglycemia. Insufficient energy will reduce weight gain and milk production, and will also change the fatty acid composition of milk fat, i.e. will lead to a decrease of medium chain fatty acids which the are the most desirable in human nutrition (4). In table 1 are presents data about daily nutritional requirement of adult goat. Total intake of feed dry matter/hd/day ranges from 2,5 to 5.5% of body weight with 3,5% being typical. Protein requirements for maintenance are 20-30 g on 50 kg of body weight or 60-70 g/kg milk in lactation.

Table 1. Daily nutritional requirement of an adult goat (2)

Item of diet	**Daily requirement**
Dry matter	
	Intake of dry matter / 100 kg body weight
	2.5 – 3.0 kg / Maintenance
	3.5 – 4.0 kg / Growth
	3.0 – 3.5 kg / Gestation
	3.5 – 5.5 kg / Lactation
	2.5 – 3.5 kg / Meat and hair
Energy	
	562 SE[1]/50 kg BW / Maintenance
	3 SE/g BW / For live weight gain
	350 SE/kg BW / Lactation
Protein	
	20-30 g /50 kg BW / Maintenance
	60 – 70 g/kg milk / Lactation
Water	450-700 gm/day for a goat weighing 19-20 kg

[1] SE – Starch equivalent

Frequently, in rations for goats with high milk yields, there is the need to increase energy density, because the volume of the ingested ration is limited, especially during early lactation. Adding fats to the concentrate ration is an efficient way to increase energy density, as long at it goats not hinder normal rumen flora. Addition of fats not accessible in the rumen or protected, at a level of 5% of the dry matter of the ration, is very efficient, increasing milk yield, fat and protein content in milk, however, the milk fat composition changes (14). Another means of increasing energy density of the ration is pelleting, which increases weight gain and milk production by increasing feed intake, but frequently decreases milk fat content if the effective length of cellulose fibers in the ration is not sufficient.

PROGRAM FOR GOAT NUTRITION DURING LACTATION

In order to establish the quantity of a concentrate mixture to be given to goats in the ration, first the level of milk production should be established, as well as the quantity and quality of ingested fodder, appetite and constitution status. Skinny goats, with high milk production, should be provided *ad libitum* access to hay and grain according to their appetite. Goats in mid-lactation, and well fattened goats, should be provided sufficient quantities of hay, i.e. *ad libitum*, as well as a supplement of 450g of concentrate mixture per 1.5 kg of milk produced (the concentrate : milk production ratio is 1:3). In goats in late lactation, this ratio should not be over 1:5. The following table presents the recommended quantity of concentrate per animal, depending on the animal's category.

Table 2. Feeding schedule of goats with concentrate, green and dry fodder (2)

Category	Concentrate	Green and dry fodder
Highly productive animals	400 g/for each liter milk and 150 g for maintenance	*ad libitum*
Yearling goats	250 g	*ad libitum*
Pregnant goats	300 – 500 g	*ad libitum*
Lactating goats	300-400 g for each liter of milk	*ad libitum*

CONCENTRATE COMPOSITION

When goats browse, an abundance of fodder should be available to enable them to be very selective and to ingest high quality rations that will fulfill their nutritional requirements. When fodder or browse are limited or of poor quality (< 10% protein), lactating goats (during the last 30 days of gestation) should be fed 450 g/day of a 16% protein mixture (77 : 20 : 2.5 : 0.5 coarsely ground maize : soy meal: mineral mixture for goats : calcium carbonate). As an alternative, for lactating goats, coarsely ground maize and soy meal can be substituted with cotton whole grain (15). Low to medium quality fodder (> 10% protein) will satisfy the needs of dry goats and goats not in service. When fodder or browse are poor quality (< 10% protein), yearling goats should be fed 450 g/day of a mixture containing 16% protein (15).
Table 3 presents certain examples of rations that can be prepared for goat nutrition.

Table 3. Utilization of seed rations for goats (1)

Component	Protein level (DCP[1]) in the ready mixture (%)			
	14%	16%	18%	20%
Broken or rolled maize	38	33	27	22
Crumpled oats	20	20	20	20
Soya oil meal (44%)	19	24	30	35
Beet or citrus pulp	10	10	10	10
Molasses	10	10	10	10
Salt with oligominerals	1.0	1.0	1.0	1.0
Dicalcium phosphate	1.8	1.8	1.8	1.8
Magnesium oxide	0.2	0.2	0.2	0.2

[1] DCP – Digestible crude protein;

Add a vitamin premix to provide 1000 units of vitamin A, 500 units of vitamin D and 3 units of vitamin E per 0.5 kg grain.

LEVEL OF CONCENTRATE IN THE RATION

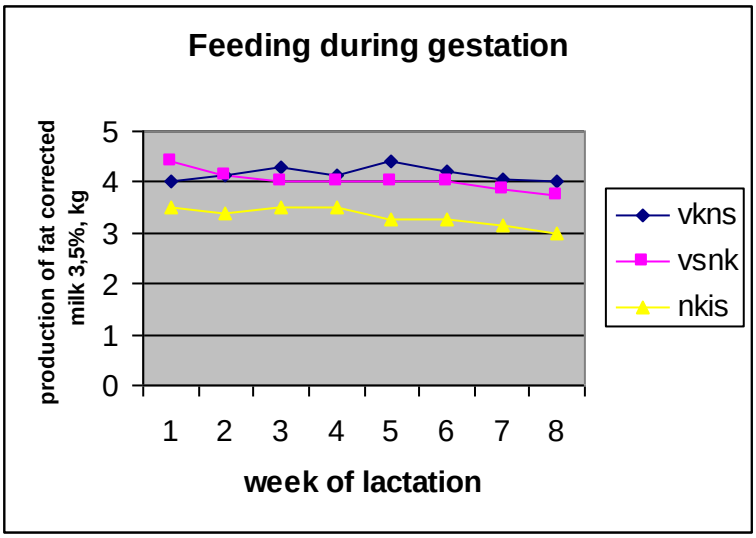
Based on abundant research dealing with the problem of the effect of added concentrate in goat nutrition during lactation, it is evident that high milk production as well as preservation of health during lactation, require the adding of higher quantities of concentrates to rations for goats (7,8,12). Adding concentrates during the final period of gestation influences milk production and composition during early lactation. The quantity of concentrate added during the second part of the drying-off period should be adapted according to the quality of fodder, in order to provide a sufficient quantity of energy and protein for production during early lactation. Insufficient nutrition during the drying-off period (final period) directly influences milk production during early lactation. When food intake covers only 60 to 70% of requirements, goats are more prone to metabolic disorders caused by inadequate nutrition. The level of concentrate in the ration can not influence doe performance in the later period of lactation very much, compared to adequate levels of nutrition during the drying-off period and the early lactation, both in adult goats-mothers, and in young yearling goats undergoing significant growth (15).
Addition of concentrate during the final period of gestation has an effect on milk composition during mid-lactation. In research by Sauvant and Morand Fehr (16) nutrition of goats with alfalfa hay (*ad libitum*) in 32 Alpino goats, with an additional 600g of concentrate, compared to 150g (fed to Group 2), during the last 6 weeks of gestation, led to an increase of milk production during mid-lactation (the test period was from week 9 to week 27 of lactation) (Table 4). Higher milk production during mid-lactation was achieved based on higher body reserves accumulated during late gestation that the animal had access to during lactation, i.e. the easier overcoming of the lack of energy balance at the beginning of lactation.

Table 4. Effect of supply of concentrates before parturition on milk production of goats in early lactation (16)

Items	**Concentrate supply**		**Significance**
	Low (150 g)	High (600 g)	
Gestation			
Alfalfa hay intake g/day	1652	1395	
Energy balance (MJ/day)	+ 6.50	+ 8.93	
Lactation			
Fat corrected milk production (3.5%) (kg/day)	2.79	2.93	P<0.01

In an investigation on 8 Alpino goats fed rations with various concentrate : hay ratios during gestation (1. high level of concentrate – low level of hay; 2. high level of hay – low level of concentrate, and 3. low level of both concentrate and hay in rations for goats), and milk production during the first 8 weeks of lactation, by Sauvant and Morand

Fehr (16), it was established that the highest milk production was present in the system of nutrition when the ration contained a higher quantity of concentrate compared to hay. At the same time, when compared to the other two systems of nutrition, milk yield remained at approximately the same level during the 8 weeks of lactation.



Graph 1. The effect of various concentrate: fodder ratios during gestation on the quantity of milk produced in early lactation (16)

RELATION BETWEEN CONCENTRATE AND FODDER IN THE RATION

However, the fodder : concentrate ratio in goat nutrition, in addition to raising the level of milk yield, also has an effect on changing milk fat and protein content in goat milk. Changing the relationship between concentrate and fodder can significantly influence milk fat content, and even reduce it by 20%. If the share of concentrate in dry matter of the ration is raised by 50-60%, milk fat content can drop significantly due to the production of propionate and the lower quantity of the milk fat precursor acetate (3).
However, the effect of highly concentrated rations is reduced if mixed rations are used, or if concentrate is fed in smaller portions, i.e. three or more times a day. Under such circumstances, conditions in the rumen remain relatively stable, and the ratio between the end products of fermentation – acetate and propionate – remains relatively constant. Thus, in an experiment by *Kawasa et al.* (5), a reduction of the fodder : concentrate ratio to 45:55 lowered the milk fat percent, increased protein content, milk yield and weight gain, while the period goats spent feeding and ruminating decreased (Table 5). However, the potential to alter protein content in milk via nutrition is considerably lower when compared to milk fat, for several reasons, the most significant being that their natural variation is low, and that all nutritional factors have not been fully studied. In addition,

the basic factors influencing protein synthesis and concentration are still not sufficiently known.

Table 5. Effects of Forage to Concentrate Ratios on Milk Yield and Composition of Saanen-Marota goats (5)

Items	Forage : Concentrate			S.E.
	75:25	60:40	45:55	
Milk yield, g/d	469	480	582	157
FCM yield, g/d	459	431	491	126
Fat, %	3.62	3.29	2.92*	0.48
Protein, %	3.4	3.90	3.73	0.42
Weight change, g/d	-50	-10	120	13
Rumination, min/d	363	339	299	40
Eating, min/d	207	214	188	30
Chewing, min/g NDF/MW	21.2	18.7	16.3*	1.8

CONCLUSION

Based on presented data from numerous investigations dealing with the problem of the effect of adding concentrate to goat nutrition during lactation on production, it is evident that for high milk production as well as for maintaining health during the lactation period, the addition of larger quantities of concentrate to rations for goats becomes indispensable. During the initial period of lactation, lower quantities of concentrate in the ration have no unfavorable effect on milk production if goats have sufficient body reserves accumulated during late gestation. Namely, to maintain the persistence of lactation, it is recommended to fortify nutrition from the fifth month, until the end of lactation, to enable goats to accumulate body reserves. It is deemed that the program of nutrition for dairy goats should be regarded an annual cycle during which proper goat nutrition is very important to enable formation of body reserves. Thus, during the production cycle, goats can mitigate variations that may appear relevant to their needs for nutritional substances for production, and at the same time be able to adapt to various conditions of nutrition.

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DETERMINATION OF CONSTITUENTS OF ANIMAL ORIGIN IN FEED

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ABSTRACT

Soon after the outbreak of BSE (Bovine spongiform encephalopathy) world crisis a theory of feed-born contamination through infected ruminant protein in meat and bone meal (MBM) has been set up. Eradication has started immediately and legislation has been established throughout the world to avoid MBM entering the feed chain. Total MBM ban for all farm animals, except fish meal for non-ruminants, is applied in EU regulation. Legislation in our country comprises ban on MBM in feed for ruminants, while presence of these components is allowed in mixes for other animal species.
With the aim to enforce the legislation and to detect cross-contamination, which means presence of unacceptable traces, the most appropriate method is optical microscopy. This is the only official method in EU and it has been applied in Institute of Veterinary Medicine of Serbia in Belgrade since 2006.
During three years period (2006, 2007 and 2008) feed for ruminants, pigs and poultry, from the feed mills in central Serbia, were sent by veterinary inspectors to the Institute of Veterinary Medicine in Belgrade to be examined on presence of MBM. During the first year among total of 118 samples, 49 samples of feed for cattle were analysed and in 6.12% constituents of animal origin were found, as well as in 25% of ovine feed, 25% swine and 32.14% of poultry mixes.
Significantly more samples were analysed in year 2007. There were 579 samples in total, mostly bovine feed - 344, with similar result to that in previous year - 6.69% with presence of MBM. But, percentages of positive samples of ovine feed (15.79%), swine (16%) and poultry feed (15.19%) were much lower than in year 2006, what is a clear indicator of good results and success of the program for feed control and education of manufacturers.
During 2008 microscopic analysis on the presence of animal contaminants in 509 samples of different mixtures was done. MBM was recorded in about 8% of samples of feed for cattle, although general trend of replacement of proteins of animal origin by other sources of proteins was more clearly observed.
Although improvement in facilities for feed production is evident, yet upgrading criteria for their work is still needed, as well as implementation of GMP and HACCP concept. Only multilevel monitoring of raw materials and final products is the way to ensure good manufacturing quality and consumer's safety.
Key words: *feed, microscopy, BSE*

INTRODUCTION

Soon after the outbreak of BSE (Bovine spongiform encephalopathy) world crisis a theory of feed-born contamination through infected ruminant protein in meat and bone

meal (MBM) has been set up (3). Therefore the first measure in preventing the spread of the disease was to establish a legislation which avoids the entry of MBM in the food chain. Today in the European Union a complete ban on the use of MBM for all farm animals, except fish meal for non-ruminants is applied. In our country is in force the Directive on the quality and other requirements for animal feed (Sl. list SRJ, 38/2001), which prohibits the use of MBM in feed for all ruminants (1), while the presence of these nutrients is still allowed in mixtures for other animal species.
In order to control the implementation of existing regulations and detection of cross-contamination, which represents the presence of traces of undesirable nutrients, it is necessary to practice the appropriate analytical methods. Proved to be the most adequate, classical microscopy, which is the only official method that is used for these purposes in the European Union (2), has been applied as accredited method in the Institute of Veterinary Medicine of Serbia in Belgrade since 2006.
The paper presents the results of feed monitoring on the presence of ingredients of animal origin using microscopy during the 2006th, 2007th and 2008th year, in order to perform data analysis for risk assessment, and also to stress the importance of this control as part of surveillance and prevention of BSE.

MATERIAL AND METHODS

During the three-year period Veterinary Inspection has sent for analysis on the presence of meat and bone meal feed for ruminants, pigs and poultry from the factories and feed mills from the territory of central Serbia to the Institute of Veterinary Medicine of Serbia in Belgrade . As part of regular check-ups in 2006 year it was examined a total of 118, in 2007 year 579, and in 2008 year 509 samples of feed mixtures for ruminants, pigs and poultry.
Tests for the presence of ingredients of animal origin were carried by accredited classical microscopy method used in accordance with EU regulations (2), and the interpretation and evaluation of results were carried out under the Directive on the quality and other requirements for animal feed (Sl. list SRJ, 38/2001) (1).

RESULTS AND DISCUSSION

In 2006 among 118 samples examined by classical microscopy mostly cattle feed was tested and 6.12% were positive on the presence of elements of animal origin, which made them unsuitable for feeding these animals. For the same reason 25% of samples of feed for sheep were eliminated, while only one goats feed sample was insufficient for any conclusion. Regarding mixtures for feeding pigs and poultry, although actual regulations in Serbia allow the presence of components of animal origin, it is an interesting fact that many manufacturers decided to avoid the risk of their usage, and it is found in only 25% of feed samples for pigs and 32.14% of feed samples poultry (Table 1). This reduces the risk of crossing the undesirable ingredients into feed for ruminants and prevents the possibility of cross contamination in feed mills where the same lines, preparing feed for different animal species, are used.

Table 1. Results of feed analyses on presence of igredients of animal origin in 2006.

	cattle	**sheep**	**goat**	**pigs**	**poultry**
TOTAL	49	4	1	36	28
negative	46	3	1	27	19
positive	3	1	-	9	9
% of positive	6.12	25.00	0.00	25.00	32.14

Significantly larger number of feed samples was examined in 2007. From a total of 579, 344 samples of cattle mixtures were analysed, with a similar percentage of positive results to these in the previous year - 6.69% (Table 2). The percentage of positive samples of feed for sheep, pigs and poultry, however, was far lower than in 2006, which clearly points to the good results and success of feed control programs and informing producers.

Table 2. Results of feed analyses on presence of igredients of animal origin in 2007.

	cattle	**sheep**	**pigs**	**poultry**	**Mixed: pigs + poultry**
TOTAL	344	19	75	79	62
negative	321	16	63	67	48
positive	23	3	12	12	14
% of positive	6.69	15.79	16.00	15.19	22.58

During 2008 microscopic analysis on the presence of animal contaminants of 509 samples of different mixtures was done (Table 3). MBM was recorded in about 8% of samples of feed for cattle, although general trend of replacement of proteins of animal origin by other sources of proteins, i.e. necessary amino acids was more clearly observed. Obvious indicator was further decrease of positive samples of feed for non-ruminants as a result of reduction of MBM usage.

Table 3. Results of feed analyses on presence of igredients of animal origin in 2008.

	cattle	**sheep**	**pigs**	**poultry**	**Mixed: pigs + poultry**
TOTAL	299	23	51	36	100
negative	275	20	45	34	71
positive	24	3	6	2	29
% of positive	8.03	13.04	11.76	5.56	29.00

On the other hand, the constant presence of traces of undesirable substances in feed for ruminants can not be deemed to have reached a satisfactory level. Therefore, it is

important to educate all participants in the production chain "from farm to fork", it is necessary to separate the lines for feed for ruminants, or exclude the feed of animal origin from use. Also, the implementation of the principles of good manufacturing practice and HACCP systems are important postulates for good quality and safety of products. The motive for the application of high standards and harmonization with European regulations should be based on the need to produce safe food, as well as creation of vital conditions for export and integration into the EU.

CONCLUSION

Although improvement in facilities for feed production is evident, yet upgrading criteria for their work is still needed, as well as implementation of GMP and HACCP concept. Only multilevel monitoring of raw materials and final products is the way to ensure good manufacturing quality and consumer's safety.

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THE EFFECT OF MINERAL ADSORBENTS ADDED TO DIETS, ON THE PRODUCTION PARAMETARS OF BROILERS

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ABSTRACT

This study reveals the original results of an application of mineral adsorbent in feeding of broiler chickens. The goal of research was to determine if mineral adsorbents "Min-a-zel" and "Min-a-zel Plus" have an influence on the dressing of slaughtered chicken carcasses when added to broiler feed.
The research included 400 broilers, breedCobb 500, divided into 4 groups, taking into consideration the level of added mineral adsorbent: control group K (100 chickens, without added mineral adsorbent, 0,0%); experimental group O-I (100 chickens, with 0,5% Min-a-zel); experimental group O-II (100 chickens with 0,2% Min-a-zel Plus) and experimental group O-III (100 chickens, with 0,3% Min-a-zel Plus). The feeding experiment lasted 42 days.
As yield of carcases is an important quality parameter, the influence of feed treatment on this characteristic was followed. The research results indicate that chickens from O-I group had the best production results of chilled carcasses (%), O-III %, O-II % and K group % (P) the worst. Differences in values between the groups that were analyzed are also statistically significant.
We can conclude that that the addition of mineral adsorbents into feed in broiler feeding resulted in better production results.
Key words: *broiler feeding, mineral adsorbents, yield, carcasses*

INTRODUCTION

Modern poultry production is the fastest method of obtaining quality products of animal origin for human consumption. Optimal and healthy food is essential in order to reach full genetic potential, improvement of health and chicken productivity. Mycotoxins present a distinct problem in animal nutrition. There are various methods of decontamination of animal feed, and the use of mineral adsorbents as an inactivator of mycotoxins has been mentioned more frequently in recent times.
Mycotoxin adsorbents of mineral origin, based on natural zeolites with a high content of clinoptilite can be effective agents for absorption of most of the toxic matter in animal feed (Adamović *et al.*, 2003).
According to statements made by numerous authors, the application of zeolites as a means of preventing mycotoxicosis in poultry, produces good results in experimental as well as practical conditions (Radović and Bogosavljević-Bošković 2006).

Addition of zeolites in poultry feed contaminated with different mycotoxins effectively reduces and prevents pathomorphologic changes to target tissues. Also, there is a significant reduction of residue in edible tissues of meat and eggs (Radović 2003, Sinovec *et al.*, 2003). Furthermore, the application of zeolytes in the poultry feed has positive effects on production results, diminishing the mortality, good health conditions and improving the quality of meat and eggs (Radović *et al.* 2001).
Zeolite minerals in inorganic form adsorb more polar mycotoxins such as aflatoxins and ergot alkaloids and do not absorb vitamins, microelements and amino-acids, thus not leaving any residues in meat and eggs. Min-a-Zel is one of the products based on natural zeolite with a well balanced relationship of interchangeable cations Ca / Na / K. Min-a-Zel Plus is another product developed by modifying the surface of zeolite using organic cations (Daković *et al*., 2001, Lemke *et al*., 1998, Tomašević-Čanović *et al*., 2003).
The goal of this research was to compare the influence of different types and levels of mineral adsorbents on the production and slaughtering characteristics of broiler feeding, and the criteria for determining this influence included: increase in body mass, health state and mortality of chickens, production index, dressing.

MATERIAL AND METHODS

One day old chickens of breed COBB 500 were sampled as experimental material for the analysis of production characteristics of chickens and the effect of mineral adsorbents-zeolites on them. In the experiment, chickens were held inside of the floor system and fed in groups, with a nutritional mixture that had the same raw material and chemical composition (Table 1). The only difference was the type and level of added zeolite in the feed.

Table 1. Formulation of diet

Feed (%)	**Group**			
	K	O-I	O-II	O-III
1.Corn	49.023	48.777	48.924	48.875
2.Corn gluten	2.500	2.500	2.500	2.500
3.Soybean meal (44%)	21.894	21.894	21.894	21.894
4.Sunflower meal (33%)	10.000	10.000	10.000	10.000
5.Yeast 50%	5.000	5.000	5.000	5.000
6.Fish flour	3.000	3.000	3.000	3.000
7.Limestone	1.604	1.604	1.604	1.604
8.Monosodium phophate	1.003	1.003	1.003	1.003
9.Salt	0.149	0.149	0.149	0.149
10.Soy oil	5.328	5.328	5.328	5.328
11.Premix broil	0.500	0.500	0.500	0.500
12.Min-a-zel		**0,5%**		
13.Min-a-zel Plus			**0,2%**	**0,3%**

In this experiment chickens were divided according to the group-control system, in accordance with the scheme with a completely random distribution (100 chickens in the group): control (K), experimental I (O-I), experimental II (O-II) and experimental III (O-III). Different levels and types of zeolites that had the same raw material composition were added: K-0,0%; O-I=0.5% Min-a-zel; O-II=0.2% Min-a-zel Plus; O-III=0.3% Min-a-zel Plus. During the experiment, chickens were not seperated into halves until the moment of slaughter.
Zeoilte products (clinoptilites) under trade names "Min-a-zel" and "Min-a-zel Plus" were used in the experiment which are produced at the Institute for technology of nuclear and other mineral raw materials in Belgrade.
During feeding the following parameters were followed: health state and mortality. Broiler feeding lasted for 42 days.
When the feeding of broilers was completed, the mass of 8 live chickens from each group as well as the mass of chilled carcasses after slaughtering were measured. Measurements were carried out using electrical scale with the accuracy of ± 0.1 g. The yield was calculated based on the results that were produced.
Analyses were performed at the Institute for food technologies in Novi Sad.
In order to ensure the results are interpreted correctly, the given data were statistically analyzed by calculating: arithmetic mean ($\overline{X}$ **)** and standard deviation (Sd) (Hadživuković, 1991).

RESULTS AND DISCUSSION

Average body mass of broilers breed Cobb 500 in groups, mortality of chickens during feeding, average mass of chilled carcasses (yield) are shown in Table 2.

Table 2. Yields of chickens carcases form control and experimentale group

Group	**Mortality, pcs.**	**Sex**	**Weight of alive chicken, g**	**Mass of chiled carecass, g**	**Yield, %**
K	7	M	1754.5 ± 42.75	1265.0 ± 31.36	72.1 ± 1.27
		F	1649.0 ± 58.35	1176.3 ± 34.49	71.3 ± 0.72
O-I	3	M	2179.8 ± 57.48	1570.0 ± 41.23	72.0 ± 0.78
		F	2086.3 ± 21.76	1510.0 ± 33.17	72.4 ± 1.90
O-II	3	M	1874.8 ± 7.63	1358.8 ± 34.49	72.5 ± 2.12
		F	1836.0 ± 20.54	1303.8 ± 10.31	71.0 ± 0.81
O-III	2	M	1976.5 ± 38.80	1428.8 ± 56.48	72.3 ± 1.77
		F	1899.3 ± 9.07	1371.3 ± 25.94	72.2 ± 1.26

From the results shown in Table 2. it can be concluded that mortality was the highest in chicken groups in which mycotoxin adsorbents were not added to chicken feed (7 chickens). and lowest in experimental group with added 0.3% of Min-a-zel plus O-III (2 chickens). while 3 chickens died in each of experimental O-I (0.5% Min-a-zel) and O-II (0.2% Min-a-zel plus) groups.

Upon completion of the feeding period. that is 42 days. the chickens from experimental group O-I (M-2179.8 g and F-2086.3 g) which were given 0.5% of Min-a-zel reached the largest average body mass. The mass of chickens from experimental O-III group (0.3% Min-a-zel plus) varied from 1976.5 g in males to 1899.3 g in females. The mean value of the mass in chickens from O-II group (0.2% Min-a-zel plus) amounted to 1874.8 g for males and 1836.0 g for females. Chickens from the control group weighed the least. where the mass of males and females equaled 1754.5 g and 1649.0 g respectively.

From the results shown in Table 2. it can be concluded that chickens from the control group (without added mycotoxin adsorbents). which received feed without added mycotoxin adsorbents had the lowest body mass before slaughtering.

The same Table also shows that male chickens in control and experimental groups had higher mass values than female chickens.

During the experiment. the average total mass of chilled carcasses was also established. considering that it is an essential piece of data when determining the value of the chilled carcasses.

The largest total mass of chilled carcasses was confirmed in chickens from experimental group O-I (M-1570.0 g and F-1510.0 g). followed by: O-III (M-1428.8 g and F-1371.30 g); O-II (M-1358.8 g and F-1303.8 g) and K (M-1265.0 g and F - 1176.3 g). Also. chickens in the control group had the lowest mass value of chilled carcasses

O-II (F) group had the lowest carcass yield value while the highest value of this parameter of 72.50 was identified from the same group in males. Yields which were determined in control and experimental groups I and III had aproximate values in the above-mentioned interval. From the aforesaid results it can be concluded that deviations in values of the yields from the groups are not high. despite differences in mass of live chickens and value of this indicator in chilled chicken carcasses.

Taking into consideration the research results and the data from Table 2 that were presented it can be concluded that the yield from chilled carcasses largely depends on the body mass of live chickens. This conclusion is in agreement with the data found by Radović *et al*.. 2003. Sinovec *et al*.. 2003.

CONCLUSION

Based on data obtained during feeding of broilers which were given feed with added mineral adsorbents. the following conclusions can be drawn:

1. Addition of mineral adsorbents in feed during broiler feeding resulted in better production results.
2. The highest mortality rate was recorded in the group in which mycotoxin adsorbent was not added.

3. Chickens from the group in which adsorbent (K) was not added during broiler feeding had the lowest values in terms of body mass and the mass of chilled carcasses.
4. The highest mass value in live chickens. as well as the those in chilled carcasses were identified in the group in which 0.5% of Min-a-zel (O-I) was added during broiler feeding.
5. Irrespective of the differences in diets and different values in mass of live chickens and chilled carcasses. the yields in control and experimental group were almost equal.

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COMPARATIVE DETERMINATION OF SALINOMYCINE CONTENT IN POULTRY FEED BY SPECTROPHOTOMETRIC AND HPLC METHOD

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ABSTRACT

The paper presents the results of the efficiency of spectrophotometric methods compared with liquid chromatographic method (HPLC) for determination coccdiostatic salynomicin in premixes and poultry feed. Salinomycine was extracted from formulations and feed by our original method – column chromatography on the column of silica gel G and elution with methanol. The content of salinomycin in eluates was determined by spectrophotometric method at 520 nm with the vanilin reagens. The developed spectrophotometric method enables to determine the quantities of salinomycin in samples with detection limit of 0,5 mg/kg of feed. The recovery of spectrophotometric determination of salinomycin content in eluates was 83,8 and 103,5%, respectively. The HPLC determination of salinomycine residues in prepared samples was accomplished by post-column derivatisation with vanillin reagent and detection with VIS detector at 520 nm. TSK ODS-120 T, 10µm (7,8 x 300mm) column and methanol-water-acetic acid (94:6:0.1, v/v/v) as mobile phase were used to determine SL. The recovery test was carried out by adding standard solutions in concentrations from 10 to 60 ppm of feed samples. The recovery rates for the HPLC method was over 96,3 and over 112,7% respectively. Detection limit of HPLC method was 15 µg/kg of feed.
Key words: *Salinomycine, spetrophotometry, liquid chromatography, poultry feed*

INTRODUCTION

Coccidiosis is an infective disease of the digestive tract which is most frequent with poultry, causing a decrease in daily increment, prolonged fattening, poorer skin pigmentation, slower feed conversion and increased mortality. The disease is caused by Protozoas from the genera of *Eimeria, Isospora and Cryptospora,* and it is manifested by damaging the intestine epithelial cells, less frequently the bile duct and renal tubuli [3, 10].

Worldwide intensive study of new products which would be added to poultry feed to prevented the coccidiosis, methods for their determination in premixes, finished mixtures, as well as methods for the determination of their residues in tissues of treated broilers. Development of methods of determining and mastering existing analytical procedures are of great importance for producers of broilers, pharmaceuticals and feed for poultry producers. Manufacturers of broilers can get testing type and content of coccidiostatic in poultry feed. The existence of reliable methods of determining the drug during manufacturing, premixes or solutions, and stability of the product. Coccidiostatic application is rarely applied with the most important form of containment vaccination coccidiosis especially in raising broilers for meat production. Since a large number of anticoccidial preparations used in the prevention of coccidiosis we chose ionophore coccidiostatic – salinomycin. Salinomycin, except for prophylaxis, as anticoccidial preparations is applied as a growth promoter [4, 5]. To determine the content of salinomycin in samples of poultry feed before they primarily used microbiological methods [9]. Spectrophotometric methods for determination salinomycin and other ionophore coccidiostatics include spectrophotometric measurement characteristics in the visible and UV region. The visible area (colorimetry) [12] consists in the reaction of salinomycin solution and methanolic solution of vanilin with measuring the absorbance of mixture at 520 nm. Thin layer chromatography is one of the most applied technique for separation and identification of substances due to their simplicity and selectivity in determining [1, 7, 14]. High performance liquid chromatography is the method of choice for determining salynomicin and other ionophore coccidiostatics in biological samples, allows for quantification with the increasing sensitivity and reducing the lower detection limit [2, 6, 15]. Direct performance liquid chromatography is possible only in case of lasalocid with the application of fluorescent detectors [13]. Lasalocid, alone among the carboxylic acid ionophores, has a fluorescent chromophore. All of the HPLC-based assays that have been developed to determine the other carboxylic acid ionophores in tissue require derivatisation to introduce a suitable chromophore. Salynomicin and other ionophore coccidiostatics not have chromophore or luminophore groups, which allow UV or fluorescent detection, and due to their thermal degradability, impossible the use of gas chromatography, resorting to post-column derivatisation (NSD-method) in order to prepare derivatives with dansylhidrazin [6] and pyridinium dichromat derivatising reagens [8]. Frequently described is still NSD-method with vanillin reagent [2]. Since other methods for determination salynomicin and other ionophores shoud be noted enzyme-immuno (ELISA) method [16]. In general, the ELISA procedures developed to detect ionophore residues were significantly more sensitive compared with the earlier TLC-bioatography based sreening assays. Detection limit of this method is 0,2 ng/g sample.

MATERIAL AND METHODS

Preparation of samples

The extraction efficiency and recovery of applied spectrophotometric and liquid chromatography method for the determination of salinomycin content were studied by

the standard addition method. Original extraction procedure and determination salinomycin content in premixes and poultry feed was developed [11]. Three grams of Silica-gel G (for column chromatography, 70-230 mesh) was puted into the glass chromatography column. On the top of the chromatographyc column was one gram of broiler feed or premix with salinomycine and salinomycine was eluated with methanol in five fractions of the 1 cm^3. Eluents were evaporated under a stream of nitrogen and the remainder was reconstituted in cm^3 of methanol. For one gram saple of feed (which is not contained salinomycin) was added 10, 20, 40 and 60 µl primary standard solution of salinomycin in methanol mass concentration of 1 mg/cm^3. In this way were obtained feed samples with standard addition of 10, 20, 40 and 60 µg salinomycin per 1 g of feed. Then, extraction was performed on the previously described manner.

Spectrophotometric determination of salinomycine content

We developed a spectrophotometric method for the determination of salinomycine in premixes and broilers feed on a spectrophotometer Spekol 21 at wavelength of 520 nm. For the preparation of standard curves were prepared standard solutions of salinomycine in mass concentrations from 1 to 10 $\mu g/cm^3$. The 1 cm^3 of each standard solution was added per 1 cm^3 vanilline reagent solution. Mixture is heated 25 minutes in the bath water at 60ºC and after 10 minutes of cooling is done, colorimetric measuring at 520 nm. Identical manner done is to determine the content of salinomycin in samples of premixes and poultry feed from commercial use. After extraction, summary eluents were evaporated under a stream of nitrogen and the remainder was reconstituted in cm^3 of methanol and continue to comply with the previously described analytical procedure of spectrophotometric determination. Salinomycine concentration in investigation samples read with a calibration curve by calculating the dilution factor.

HPLC determination of salinomycine content

Since salinomycin and other ionophore coccidiostatics don't contains chromophore groups, don't absorbed in the UV region, and has therefore been necessary to develop a post-column derivatisation method. The investigations were conducted on the Bio Rad HPLC system with spectrophotometric detection with tungsten lamp at 520 nm. Eluates with salinomycin were separated on a TSK ODS-120 T, 10 µm (7,8 x 300 mm) column with a mobile phase consisting of methanol-water-acetic acid (94:6:0.1) at a flow rate of 1cm^3/min. Post-column derivatisation was performed with vanillin-reagent solutions which was prepared as follows: methanol sulphate acid-vanillin (95:2:3, v/vw), protected from UV light. To post-column derivatisation in the shortest possible time came to develop color, which will alow detection salinomycin the tested samples, it is necessary reaction chamber heated to a temperature of 90ºC. Before use, the mobile phase is degase holding in the ultrasonic bath.

RESULTS AND DISCUSSION

Results of spectrophotometric determination salinomycin in broilers feed with standard addition method are presented in Table 1. The results show that the applied extraction method, based on the separation salinomycin from samples of broilers feed, on silica-gel

column chromatography, provides high efficiency of spectrophotometric extraction and determination of 83,8 ± 5,4% do 103,5 ± 6,8%. Also, determination of efficiency is even greater if the content salinomycin, or in addition to the standard broilers feed, which explains the smaller slower elution larger concentration of salinomycin applied to silica gel column, and is more efficient elution samples with high content of salinomycin application required greater volume of methanol.

Table 1. Results of the determination of contents of salinomycin in premixes and broiler feed by spectrophotometric method

Added [µg/g feed]	**Found ± SD [µg/g feed]**	**Efficacy of extraction (%) ± SD**
10	10,4±0,7	103,5±6,8
20	18,6±0,5	92,8±5,4
30	26,9±0,9	89,5±8,6
40	34,3±0,8	85,7±7,5
60	50,3±0,7	83,8±5,4

Based on the results we can conclude that our original extraction method is suitable to extract salinomycine from premixes and poultry feed samples., and that the spectrophotometric method can be successfully applied in monitoring the content of salinomycin in eluates, with detection limit of 0,5 mg/kg poultry feed. This means that the described method allows the determination of 120 times lower concentrations of preventive doses of salinomycine in broiler feed, which is 60 mg/kg feed.
For the quantitative determination of salinomycin content in the poultry feed samples comparative method was elaborated liquid chromatography with post-column derivatisation with vanillin reagent. Salinomycin from samples extracted by column-chromatography and eluated with methanol. Eluents was centrifuged and filtered. Separation was carried out with TSK ODS-120T, 10µm column with a mobile phase consisting of methanol- water – acetic acid (94:6:0,1, v/v/v) and than using post-column derivatisation with vanillin reagent. Detection was performed with a spectrophotometric detector with tungsten lamp at 520 nm. Retention time for salinomycine, under these conditions of the determination, is 12,0 min. Results of the determination of contents of salinomycin in premixes and broiler feed by high performance liquid chromatography are shown in table 2. Based on the results shown in table 2, we notice that the application of the described HPLC procedure is possible to determine the salinomyin content in the poultry feed samples with high reproducibility rates, ranging from 96,3±2,6 % to 112,7±2,7% and detection limit of 15 µg/kg of examined poultry feed.

Table 2. Results of the determination of contents of salinomycin in premixes and broiler feed by liquid chromatography

Added [µg/g feed]	Found ± SD [µg/g feed]	Efficacy of extraction (%) ± SD
10	11,3±0,3	112,7±2,7
20	20,8±0,2	103,8±1,8
30	29,6±0,5	98,5±4,9
40	33,2±0,6	98,0±5,4
60	57,8±0,3	96,3±2,6

CONCLUSION

Developed extraction method by column-chromatography and method of spectrophotometric determination in the visible area and the method of liquid chromatography can be applied successfully to determination of salinomycin content in premixes and final fodder mixtures for fattening broilers. The obtained detection limits are far below the doses used for coccidiostatic salinomycin in broilers feed (60 mg/kg of feed). Recovery of both procedures studied using the standard addition method was over 80%. Applied spectrophotometric method is faster, simpler and cheaper method of liquid chromatographic method, in term of recovery, accuracy and detection limit are not behind precise, but more expensive and durable liquid chromatographic method. Therefore, spectrophotometry should be a routine method of choice in determining the salinomycin content in the poultry feed samples.

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QUALITY OF FEED INGREDIENTS – CHEMICAL, MICROBIOLOGICAL AND MYCOTOXICOLOGICAL PARAMETERS

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ABSTRACT

Chemical, microbiological and mycotoxicological analysis included 11 different types of components of feed mixtures, 62 samples respectively, that were analyzed in the accredited laboratory in "Bio-Ecological Center" Ltd. in Zrenjanin from the beginning of 2009. Content of proteins was not adequate in 4.1% of tested samples, as well as cellulose content. Higher fat content was found in 6.1 % of feed ingredients, and higher protease activity in 2.0% of the samples. The most of samples (82.6%) matched the Serbian normatives for feed. The maximum number of bacteria (6.3 x 10^6/g), as well as yeasts and molds (9,267/g) was detected ino wheat grains harvested in 2009. Microbiological not adequate quality of the 4 analyzed samples was a consequence of the presence of *Proteus* species. Mycological analysis established the presence of 18 types of molds, and 10 genera respectively. The dominance of representatives of the genus Fusarium (56.52%) was reported with *F. subglutinans* and *F. verticillioides* as the most widespread species. Despite the findings of a number of potentially toxigenic molds mycotoxicological analysis in most cases gave negative results. From six analyzed mycotoxins (aflatoxin B1, ochratoxin A, zearalenone - ZON, deoxinivalenol, T-2 toxin and diacetoxyscirpenol) only two were identified. ZON, in the amount of 0.294 mg/kg was contaminant of one sample of wheat grains, and in the amount of 1.472 mg/kg one sample of crushed oil sunflower seed. Trichotecen of type A - T-2 toxin was determined only in herd pea in the concentration of 0.500 mg / kg.

Key words: *feed ingredients, chemical, microbiological and mycotoxicological parameters*

INTRODUCTION

It is well known that one of the priorities of every nutritionist is the improvement of the production potential of domestic animals as well as getting the product of high-quality. Balanced diet that matches the needs of certain categories of animals provides a good health and good reproductive state and thus reaching the desired production goals [6]. Quality of animal feed does not only means the optimal nutrient value and health safety (microbiological quality) but also the absence of harmful substance. Components of feed mixtures may be contaminated with toxigenic molds and their toxic metabolites (mycotoxins) that can cause health disturbances of animals, and sometimes even their death. Seriousness of their impact depends not only on the amount of toxic substance that enters the body, but also upon the frequency of the uptake. Small dose of

mycotoxins continuously getting into animal organism, especially young animals or those with weakened immune system, can cause severe health disorders [8]. Inadequate quality of feed components leads to inadequate quality of feed mixture because, beside using the determined fodder recipes strictly, sometimes the satisfactory quality of the mixture can not be achieved.
Accordingly, the goal of the present investigation was to evaluate the nutritious (chemical), microbiological and mycotoxicological quality of feed mixture ingredients originating from the region of Vojvodina.

MATERIAL AND METHODS

Samples. Total number of 62 samples of feed raw materials was analysed in the accredited laboratory in "Bio-Ecological Center" Ltd. in Zrenjanin from the beginning of 2009. Samples of herd pea, barley, triticale and wheat grains were freshly harvested or in the initial phase of drying (crop 2009). All tested samples originated from the region of Vojvodina (Serbia).
Chemical investigations were performed according to *Regulations on the quality and other requirements of fodder* [16], and *Regulations on changes and supplements of regulations on the quality and other requirements of fodder* [17].
Determination of moisture, fat, fiber, sodium, potassium, protein, phosphorus and sulfur were made according to accredited methods of " Center for Bio-Ecology"having in mind *Regulations on sampling methods and methods of physical, chemical and microbiological analysis of fodder* [14]. Calcium and magnesium were analyzed by atomic absorption spectrophotometry technique according to AOAC method 968.08 [1], amino acids by HPLC technique with UV-VIS detection according to ISO 13903:2005 [7] and urease activity by SRPS ISO 5506:2001 [18]. Manganese, iron, copper and zinc were determined by atomic absorption spectrophotometry technique using accredited methods of the house.
Microbiological investigations were performed according to *Regulations on maximal quantity of harmful materials and ingredients in fodder* [15]. 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 *Regulations on methods of microbiological analysis and superanalysis of foodstuffs* [13]. Identifications of fungi were performed according to Domsh et al. [5], and Samson and van Reenen-Hoekstra [11].
Mycotoxicological investigations.
The presence of aflatoxin B1 (AFL B1), ochratoxin A (OTA) and zearalenone (ZON) was determined according to standard method [14], while deoxynivalenol (DON), diacetoxyscirpenol (DAS) and T-2 toxin were analyzed by applying the method of Pepeljnjak and Babić [10].

RESULTS AND DISSCUSION

Obtained results of the present investigation are shown in Tables 1-4.

Table 1. Chemical quality of feed ingredients of oleaceous plants origin

Parameter	**Soybean grits**	**Soybean cake**	**Soybean pellet**	**Sunflower pellet**
Proteins (%)	33.81-38.53	37.31-45.81	41.06-45.27	31.12-35.29
Moisture (%)	5.21-8.30	4.88-9.11	9.92-11.52	6.76-12.93
Fats * (%)	19.14-21.41	7.18-12.18	2.38-3.05	0.71-4.56
Celullose (%)	3.46-6.51	3.12-6.23	2.97-7.44	18.05-24.24
Ash (%)	4.49-5.22	4.91-6.49	5.62-5.75	5.48-6.65
Urease activity (mgN/g/min)	0.08-0.52	0.06-0.44	0.07-0.23	-
No. of samples	9	19	5	16
Adequate quality (%)	**67**	**100**	**80**	**75**

*Fat content in the sunflower pellets is not defined by *Regulations on the quality and other requirements of fodder* [16], and *Regulations on changes and supplements of regulations on the quality and other requirements of fodder* [17], but is given in the manufacturer's declaration.

During the investigation of chemical quality of animal feed ingredients of oleaceous plants origin inadequate quality was found in 16.33% of samples, which is significantly smaller percentage compared with invalid samples of feed ingredients and fodder mixtures (41.97%) originating from surroundings of Niš and South Morava region [6].
Based on the results shown in Table 1 can be noticed that the protein content did not match the quality of the *Regulations on the quality and other requirements of fodder* [16], and *Regulations on changes and supplements of regulations on the quality and other requirements of fodder* [17] in 4.08% of oleaceous plants samples. In one sample of soybean pellet and one sample of soybean grit detected protein contents were below the value of this parameter permitted by mentioned *Regulations* (44% or 36%). In the case of cellulose content inadequate quality of 2 samples was a consequence of higher values of this parameter than the maximum values issued by the current Serbian *Regulations*: 4.5% for soybean grit, and 21% for sunflower pellets. Three samples of sunflower pellet (18% of tested samples) were incorrect with respect to the content of fat above 3% (max value listed in the manufacturer's declaration). Urease activity did not match the value given in the *Regulations on the quality and other requirements of fodder* [16], and *Regulations on changes and supplements of regulations on the quality and other requirements of fodder* [17] in one soybean grit sample because it was higher higher than 0.40 mgN/g/ min.
Correctness of feed components has beneficial impact on the quality of forage mixtures in which content the individual nutrients take part. Our previous investigations of feed mixtures from Banat region of Vojvodina in the period from 2006-2008 [8] also revealed the small percentage of unaccurate samples - only 3.12% of them had inadequate protein content, 6.26% calcium content and 3.12% phosphorous content.

Table 2. Nutritive value of cereal plant and herd pea feed ingredients

Parameters	**Barley**	**Triticale**	**Herd pea**	**Wheat**
Proteins (%)	9.78-12.28	11.96	22.12	12.23-13.00
Moisture (%)	5.31-8.63	13.22	10.31	10.27-10.97
Celullose (%)	3.64-4.72	2.21	3.31	1.60-2.05
Ash (%)	1.85-2.19	1.44	2.67	1.23-1.37
Fats (%)	1.41-1.61	1.34	0.88	1.06-1.35
Phosphorous (%)	0.24-0.30	0.24	0.36	0.21-0.26
Sodium (%)	0.014-0.03	0.04	0.01	<0.01
Potassium (%)	0.30-0.35	0.39	0.95	0.27-0.37
Sulfur (%)	0.10-0.15	0.17	0.44	0.10-0.24
Calcium (%)	0.08	0.06	0.07	0.04
Magnesium (%)	0.11-0.12	0.11	0.12	0.10-0.11
Mannan (mg/kg)	13.4-14.7	33.4	11.3	30.1-36.6
Iron (mg/kg)	21.7-25.0	27.2	54.2	27.9-33.6
Cuprum (mg/kg)	4.3-5.7	11.1	6.2	4.6-7.1
Zink (mg/kg)	14.3-17.7	17.9	25.4	16.5-21.8
Glycine (%)	0.361-0.466	0.461	0.980	0.443-0.454
Histidine (%)	0.138-0.233	0.263	0.451	0.226-0.270
Methionine (%)	0.150-0.200	0.173	0.239	0.183-0.225
Threonine (%)	0.333-0.413	0.370	0.837	0.346-0.351
Arginine (%)	0.475-0.574	0.581	0.201	0.544-0.578
Valine (%)	0.455-0.561	0.538	0.978	0.503-0.530
Phenylalanine (%)	0.477-0.653	0.596	1.110	0.561-0.609
Isoleucine (%)	0.349-0.776	0.426	0.850	0.400-0.436
Leucine (%)	0.668-0.851	0.819	1.650	0.803-0.846
Lysine (%)	0.282-0.382	0.750	1.560	0.223-0.241
Tryptophan (%)	0.102-0.136	0.200	0.214	0.127-0.154
No of samples	4	1	1	3

In all analyzed samples shown in Table 2 moisture content matched with the values issued by the *Regulations on the quality and other requirements of fodder* [16], and

Regulations on changes and supplements of regulations on the quality and other requirements of fodder [17]. Other parameters given in Table 2 were analyzed at the request of users, in order to obtain information necessary for the preparation of feed mixtures for different categories of domestic animals according to the nutritious recommendations given by Sinovec and Ševković [12].

Table 3. Microbiological quality of fodder ingredients analyzed in 2009

Sample type	No. of samples	BCFU/g (aver. value)	MYCFU/g (aver. value)	Adequate quality (%)
Wheat grain	3	6303000	9267	100
Wheat brain	1	1070000	920	100
Barley grain	4	4312500	3530	100
Triticale	1	2350000	3400	100
Herd pea	1	655000	330	100
Protilac	1	1700	150	100
Soybean cake	4	278000	345	75
Soybean grits	1	720000	300	0
Sunflower pellet	5	3386200	5790	60
Flax seed	1	148000	1600	100
Corn silage	1	565000	1900	100
TOTAL	**23**	**1700 – 6303000**	**150 – 9267**	**82.6**

Legend: BCFU/g – bacterial colony forming units per gram,
MYCFU/g – colony forming units of molds and yeasts per gram

The majority of tested samples (82.6%) had satisfactory microbiological quality according to the *Regulations on maximal quantity of harmful materials and ingredients in fodder* [15]. The maximum numbers of bacteria (6.3×10^6/g), as well as yeasts and molds (9,267/g) were detected in wheat grain samples harvested in 2009, while the lowest number of microorganisms (1700 BCFU/g and 150 MYCFU/g) was recorded in Protilac additive.

Microbiologicaly anadequate quality of 1 sample of soybean cake, and 2 samples of sunflower pellets was a consequence of the presence of *Proteus* species. From other pathogenic bacteria only *Clostridium* spp. were found in one sample of soybean cake, but in much smaller number compared to the max allowed number according to Serbian regulations (20/g compared to 1000/g).

Table 4. Fungal species identified in feed ingredients

No.	Species	Feed component (%)							
		a	**b**	**c**	**d**	**e**	**f**	**g**	**Total**
1.	*Absydia corimbifera*					20			**4.35**
2.	*Acremonium cerealis*	11							**4.35**
3.	*Acremonium fusidioides*				20	20			**8.70**
4.	*Acremonium rutilum*					20			**4.35**
5.	*Alternaria* spp.	78	100	100	20				**42.48**
6.	*Aspergillus clavatus*					60			**13.04**
7.	*Aspergillus flavus*	11			40	60			**26.09**
8.	*Aspergillus fumigatus*				20	20			**8.70**
9.	*Aspergillus ochraceus*				20	20			**8.70**
10.	*Aspergillus ustus*					20			**4.35**
11.	*Aspergillus* sp.	11	100	100	20	80			**34.78**
12.	*Fusarium oxysporum*		100	100		20	100		**17.39**
13.	*Fusarium poae*	33							**13.04**
14.	*Fusarium subglutinans*	11		100	20	20			**17.39**
15.	*Fusarium verticillioides*	11		100	40				**17.39**
16.	*Fusarium* spp.	33				20			**17.39**
17.	*Geotrichum candidum*					20			**4.35**
18.	*Mucor* spp.	22			60	60			**34.78**
19.	*Penicillium aurantiogriseum*	22			20	40		100	**26.09**
20.	*Penicillium* spp.	33	100	100	20	40		100	**39.13**
21.	*Rhizopus nigricans*				20				**4.35**
22.	*Scopulariopsis brevicaulis*			100		20			**8.70**
23.	*Scopulariopsis* sp.			100	20	20			**13.04**
	Total No. of species	**11**	**4**	**8**	**16**	**18**	**1**	**2**	

Legend: **a –** cereals, **b -** herd pea, **c –** protilac, **d -** soybean cake and grits,
e - sunflower pellets, **f -** flax seed, **g –** corn silage

Mycological analysis established the presence of 18 specieses of molds and 10 genera, respectively (Table 4). The dominance of representatives of the genus *Fusarium*

(56.52%) was reported with *F. subglutinans*, *F. verticillioides* and *F. oxysporum* as the most widespread species. By the frequency followed the typical storage mycobiota - the genus of *Penicillium* (47.83%) with *P. aurantiogriseum* as the dominant species and *Aspergillus* (43.48%) with most common species *A. flavus.* Beside *Fusarium* spp., significantly were represented and other field mycobiota - the genera *Alternaria* and *Mucor* (30.43%). The dominance of species *A. flavus, F. oxysporum* and *P. aurantiogriseum* in fodder mixtures, as well as in food in Serbia was also found by Lević et al. [9]. The greatest number of fungal species was identified in the samples of sunflower pellet (18) and soybean cake and grit (16) (Table 4), while the least one in the crn silage (2). Presented results are in accordance with the results of our previous many years lasting researches on feed ingredients and fodder mixtures on the territory of Vojvodina [5]. Despite the findings of a number of potentially toxigenic fungal species (*A. flavus, A. ochraceus, F. oxysporum, F. poae, F. subglutinans, P. aurantiogriseum* etc.) in most cases mycotoxicological analysis gave negative results. From six analyzed mycotoxins (aflatoxin B1, ochratoxin A, zearalenone - ZON, deoxynivalenol, T-2 toxin and diacetoxiscirpenol - DAS) only two fusariotoxins were identified during the present study. In the amount of 0.294 mg/kg ZON was the contaminant of one sample of wheat grain, and in the amount of 1.472 mg/kg was found in one sample sunflower pellet. The possibility of the presence of mycotoxins in sunflower pellets is considered in our previous investigations [2]. Unlike our current results the samples of sunflower pellet (2004 harvest) contained low concentrations of AFL B1 (0.0003 mg/kg), and more significant quantities of T-2 toxin (0.75 - 1.00 mg/kg). This trichotecene of type A (T-2 toxin) was determined in the present study only in one sample of herd pea in the concentration of 0.500 mg/kg. The importance of fusariotoxins as contaminants of feed mixtures and their components point out and our previous research [3]. We found that the main cause of mycotoxicological inaccuracy of feed samples in 2004 and 2005 in Banat region of Vojvodina was the presence of ZON and T-2 toxin in quantities higher than the maximum one allowed by the current *Regulation* in the Republic of Serbia [15].

CONCLUSION

On the basis of the presented results it can be concluded that it is necessary to perform regular quality control of feed components and fodder mixtures on the market in Serbia. It is not enough to track only nutritional value of these samples ie. chemical quality, but also microbiological and mycotoxicological parameters must be taken into account due to the fact that adequate nutrition is a prerogative of good health and the desired production performance of domestic animals.

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THE EFFECT OF EXTRUDED CORN IN BROILER FEEDING

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ABSTRACT

Signigicant physico-chemical changes occure in corn grain structure, due to extrusion, thus positively contributing to its nutritive value, i.e. nutritive components became easily digested by enzymes. Also, corn extrusion is beneficial concerning hygiene and sensor characteristics (sweet taste is becoming more apparent).
The objective of this research is to point at the efficiency of feed meal extrusion in growing chicken diet. Experiment was carried on 3000 chickens, hybrid ROSS. Chickens were devided in two groups, experimental and control. Growing period was 49 days. The diet was the same for both groups of chickens, except in the experimental group corn was replaced with extruded corn.
In growing period up to 42 days, chickens fed with diet containing extruded corn grew more rapidly, had higher weight gain (1985 g), with less cunsumed feed (2644 g) in comparison to control group (1940 g; 2685g). Mortality also decreased (20:96).
Concerning this data, the use of extruded corn in growing chicken diet is beneficial.
Key words: *chicken growing, extrusion, corn*

INTRODUCTION

Increased production of human food and animal feed can be attained by applying new technologies in the biotechnology, i.e. biotechnology, (Lazarević, et al., 2005). Basic orientation is either toward new technological processes witch contribute to increased food or feed nutritive value or giving extra value to by-products from food industry and from prinary agricultural production. Today in the world a variety of thermic processes is applied for treating oilseeds and cereals: toasting, extrusion, hydrothermic treatment, micronization, micro wave treatment or dielectic thermic treatment (Sakač at al., 1996, Marssman et al., 1998) but in Serbia extrusion and hydrothermic processes are in comom practice (Sakač at al., 2001, Filipović et al., 2007).
In domestic feed production, in comparison to other cereals, corn is having a leading position due to high energy (16,2MJ/kg), starch and fat but low cellulose level. It is considered that corn, beside the best digestibility, in comparison to other cereals, also has the best taste (Bekrić, 1999).
Appropriate temperature in extrusion process can reduce the content of thermically nonstable antinutrients at acceptable level and improve the digestibility of some nutrients (protein, fat, carbohydrates), also sensor characteristics and microbiology of the product (Kormanjoš, 2007) Along with thhe reduction of antinutrient content, it is neccessary to

preserve thermolabile nutritive components, therefore process need to compromise these two demands (Jansen, 1991).
Thermic treatment of cereals is commonly practiced for the improvement of their nutritive value, hygiene, physico-chemical and other characteristics, that way positively contributing to increasing nutritive value of certain nutrients, sensor characteristics (increasing corn sweet taste due to extrusion) and inactivation of thermically unstable anti-nutrients if present.
Extrusion of corn, which is a basic raw material in feed production, as well as, extrusion of corn dry milling by products is contributing to better feed utilization in animal fattening (Filipović et al., 2008).
Due to extrusion, carbohydrates from corn meal are undergoing to certain changes resulting in starch content decrease and therefore in dextrin content increase, also to the inactivation of amylase inhibitors. Concerning the fact that gelatinized starch is easily digested by enzyme these changes are in favor of either *in vitro* or *in vivo* starch digestibility (Douglas et al., 1992; Filipović et al., 2003).
The objective of this research is to determine the efficiency of corn extrusion in growing chicken diet.

MATERIAL AND METHODS

Chicken feeding
The experiment was carried on 3000 chickens, hybrid ROSS. Chickens were devided in two groups, (experiment 0) and control group (Control K) and fed under the same conditions in the period of 49 days. The diet was the same for both groups of chickens, except in the experimental group corn was replaced with extruded corn. Up to 21 day chickens in both groups were fed with starter diet than followed finisher formulation.

Table 1. Composition of the diet

Feedstuff (%)	Starter	Finisher I	Finisher II
Corn	50	56	52
Soyabean grits	20	20	17
Soyabean meal	22	10	5
Sunflower meal	-	5	10
Short	-	-	8
Yeast	3,5	3,5	3,0
Fat	-	3,5	3,0
Limestone	1,7	1,5	1,5
Monocalciumphosphate	1,45	1,10	1,00
Salt	0,35	0,40	0,50
Premix	1,0	1,0	-
Lysine	0,1	0,1	-
Methyonine+cistine	0,15	0,15	

During whole chicken growing period water and feed were fed *ad libitum*. Every 7 days body weight was tested. After growing and 12h starving period, chickens were slaughtered, body mass was weighted and data statistically interpreted according to computer program Origin.

Corn extrusion

Corn with moisture content of 12% was grinded on a hammmer mill having Ø 5 mm sieve openings, than tempered to 18% moisture.

Corn was extruded in extruder with following characteristics: capacity, 900 kg/h; installed power of extruder, 100 kW; power of worm dosing device, 1,1 kW; extrusion temperature 90 and 95˚C; and die diameter 7,5 mm.

Chemical procedures

Basic chemical composition (moisture, crude proteins, crude cellulose, crude fat and mineral matters) of chicken feed was determined according to official A.O.A.C. methods (1984). Starch and total reducing sugars were determined according to the Regulations for methods for quality control of cereals, milling and bakery products, pasta and frozen doughs (1988). Content of following elements: calcium, phosphorus, are determined according to Regulations for methods for sampling and methods for feed physical, chemical and microbiological analyses (1987).

Nitrogen solubility index was determined according to official A.O.C.S. method (1987).

Microbiological procedures

Total number of microorganisms, yeast and mold count, were done in accordance with the Regulation for methods for microbiology analyses and superanalyses of food (1980).

RESULTS AND DISCUSSION

Results for basic chemical composition of chicken feed are shown in the Table 2.

Table 2. Chemical composition of the diet

	Starter (%)	**Finisher I (%)**	**Finisher II (%)**
Moisture	12,52	12,24	12,37
Crude protein	22,55	19,93	19,14
Crude fat	5,85	7,57	7,56
Crude cellulose	2,35	3,11	4,09
Mineral matters	5,30	5,20	5,50
Calcium	0,95	0,81	0,74
Phosphorus	0,72	0,65	0,64

Microbiology, total number of microorganisms, yeast and mold count of corn before and after extrusion is presented in table 3.
In the tested diet, molds, yeasts and other microorganisms were present. Prior to extrusion 63.000 molds were present in corn and due to extrusion the count was only 55 per 1g. Total number of microorganisms also significantly decreased after corn extrusion.

Table 3. Content of microorganisms in corn and extruded corn

Microorganism	**Number**	**Non-treated corn**	**Extruded corn**
Total number of molds	In 1 g	63.000	55
Total number of yeasts	In 1 g	45.000	0
Total number of microorganisms	In 1 g	1.200.000	310

Though extrusion temperature and duration are relatively low 90-125°C and 6-10 s, respectively, but a significant decrease in total number of microorganisms is evident, probably due to very high presure, 30-40 barr, table 3.
Nurtitive and chemical characteristics of non-treated and extruded corn is presented in table 4. After tempering moisture of 17,6% of ground corn is favorable for corn extrusion process under so called controled temperature conditions. Moisture content round 20% is also recommended by Venou et al., (2003) as an optimum concerning extrusion of wheat and corn.
Chemical characteristics of corn extruded at temperatures of 90 and 95°C are presented in table 4. The decrease of moisture (table 4) after corn extrusion is statistically significant ($p<0,05$) therefore extrudat is characterized by a long shelf-life, i.e. it is suitable for storing.
In comparison to nontreated corn, dry extrusion contributed to statistically significant changes ($p<0,05$) in crude fat i.e. crude fat decreased 57 and 45% at temeratures of 90 and 95°C, respectively, (table 4). Similar fat reduction round 60% after corn extrusion at 115-125°C, was also reported by Venou et al., (2003). Though extrusion is contributing to fat content decrease, extrudates may undergo lipid oxidation due to increased area that is in contact with the air (Namiki, 1990).
In comparison to non-treated corn, starch content in extruded corn is according to statistic data, ($p<0,5$), significantly lower and, as a consequence, there is an increase of reducing sugars, (table 4) which contributes to the change of sensor characteristics i.e. slightly sweet taste is registered in extrudate.

Table 4. Chemical composition of nontreated and corn extruded at 90 and 95°C

Quality characteristics	**Non-treated corn**		**Corn extruded at 90°C**		**Corn extruded at 95°C**	
Moisture (%)	17,60	D.M. basis	9,07	D.M. basis	5,25	D.M. basis
Crude protein (%)	7,62	9,25[b]	8,25	9,07[c]	8,50	8,97[a]
Crude ash (%)	1,51	1,83[b]	1,42	1,56[a]	1,500	1,58[a]
Crude cellulose (%)	2,84	3,45[c]	2,25	2,47[a]	2,65	2,80[b]
Crude fat (%)	3,96	4,80[c]	1,89	2,08[a]	2,52	2,66[b]
NSI	13,11	15,91[b]	6,06	6,66[a]	5,88	6,21[a]
Starch (%)	58,42	70,90[c]	60,98	67,06[b]	61,55	64,98[a]
Total sugars (%)	0,82	1,00[a]	3,63	3,99[a]	3,90	4,12b
Reducing sugars (%)	0,33	0.40[a]	0,38	[0,42]	0,43	0,45[a]

D.M. – dry matter
Mean values of quality characteristics expressed on dry matter basis with the same exponent in the row are not statistically different ($p<0,05$).

Basic data concerning chicken gowing with diets containing extruded or nontreated corn are presented in table 5.

Table 5. Basic data of chicken growing

Period (days)	O					K				
	Body weight	Total feed usage	Mortality	Chicken number	Feed conversion	Body weight	Total feed usage	Mortality	Chicken number	Feed conversion
	(gr)	(gr)	(kom.)	(kom)	(kg/kg)	(gr)	(gr)	(kom.)	(kom)	(kg/kg)
0	44,2			1.500		44,2			1.500	
0-7	127,5	200	6	1.494		111,5	215	10	1.490	
0-21	565,0	1.250	8	1.486		519,0	1.265	53	1.437	
0-28	966,0	2.250	2	1.484		907,0	2.255	23	1.414	
0-35	1.490,0	3.650	1	1.483		1.420,0	3.645	3	1.411	
0-42	1.985,0	5.250	2	1.481		1.940,0	5.210	3	1.408	
0-49	2.760,0	8.350	1	1.480	2,04	2.780,0	8.330	4	1.404	2,13

If extruded corn was included in the diet, chickens grew faster, had better body weight, utilized less feed per kg of body gain in comparison to chickens in control group.
In chicken growing, the most evident positive result of the diet with extruded corn, low mortality should be emphasized. In control and experimantal group total number of dead chickens was 96 and 20, respectively, so concerning better chicken health, the great advantage of extruded corn in the diet is obvious. This result is particularly characteristic within first four weeks of chicken growing.
The other fact, significant for total production effects and realised economic data is the feed conversion. Calculation of realised feed conversion point at the better results in experimental group, i.e. in the diet with extruded corn. Feed conversion of total experimental and control group was 2,04 kg and 2,13 kg of feed per one kg of body gain, respectively. Better insight in feed conversion can give the change of feed conversion during growing period.
From table 5. it is evident that diet containing extruded corn is beneficial at the beginning of chicken growing. That is one more proof of extruded corn positive performance concerning younger animals. Related to the data from table 5 it is possible to conclude that the feed consumption is nearly the same in both groups. I should be stressed that experimental group, due to lower mortality, had greater number of chickens at the end of growing period, thus on the whole, better production results were experienced if extruded corn was included in the diet.

CONCLUSION

Based on presented data it can be concluded that, in comparison to chickens in control group, extruded corn in the diet contributed to:

- faster growth,
- better body weight
- better feed utilization per kg of body gain and
- lower mortality.

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POSSIBILITIES OF THE IMPROVEMENT OF STILLAGE OBTAINED FROM BIOETHANOL PRODUCTION ON STARCH BASED FEEDSTOCKS

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ABSTRACT

Today, the production of ethanol for biofuel is in expansion and an intensive research and investments in the technology development has been done to improve the process economy and its ecological impact. The most pronounced is a trend of the process integration and byproduct valorization in order to achieve higher process productivity with minimum negative environmental impact.
In the process of bioethanol production from corn, 293 kg of ethanol is produced from 1000 kg corn (with 12% of moisture and 65% of starch on dry matter). In this process 229 kg of stillage with 90% of dry matter is obtained. In the majority of industrial facilities in Serbia, the bioethanol byproducts have not been utilized posing therefore a hardly solvable environmental problem. The complex composition of stillage causes high BOD_5 values which range from 15-340 g/L.
The stillage content encompasses all the components initially present in the feedstock except the carbohydrates fermented to alcohol; the yeast biomass, as well as the products obtained by the feedstock saccharification and fermentation which cannot be metabolized by yeast. Many factors have an impact on the stillage composition: a type of raw material; fermentation conditions employed for ethanol fermentation; conditions employed for drying of stillage etc. Primarily due to its high protein content, the stillage obtained from bioethanol production on cereals (corn and triticale) could be an excellent basis for the production of a high quality feed. For this purpose, the possibilities of the enrichment of the stillage obtained from ethanol fermentation with various yeasts strains was studied in this paper. A chemical composition of the obtained mixtures was analyzed from the point of nutritive quality of animal feed.
Key words: *bioethanol, corn and triticale, stillage, animal feed*

INTRODUCTION

Production of bioethanol and protein co-products has been conducted in world for last few decades.
Fermentation derived ethanol can be produced from sugar and starch based feedstocks (corn, triticale, wheat) by yeast or bacteria. Concerning EU, in November 2001 a new directive is accepted, that requires of members states to establish legislative about utilization of fuels from renewable resources. In 2005 this utilization should cover 2% of

the total fuel consumption. This quota should increase to 5.75% in 2010 and furthermore. Thereby, it can be calculated that the world production of bioethanol from corn in 2010 will be about 32%.
The stillage obtained as a byproduct from bioethanol production on cereals is a high value feed, rich in protein content and could decrease a need for the addition of other protein rich components in feed mixes. Advantages of using stillage as animal feed are: better consumption of nutritive ingredients from the feed, better flavor of feed, presence of plant's proteins, it contains an adequate amount of energy for animals in fatling, a lot of minerals and vitamins, presence of crude fiber which enhance the state of ruminant's abdomen [1].
Besides, utilization of stillage could enhance bioethanol production in techno-economical aspect. In this way around 40% of the investment can be recovered [2].
In addition of stillage use for animal feed, researchers at The Faculty of Technology, Novi Sad have investigated recirculation of a thin stillage (a liquid portion obtained by the separation from a whole stillage) in the mashing process. By this method of stillage recycling a lower consumption of water in the process and higher ethanol production was achieved [3].
Nowadays, an integrated model of bioethanol production makes possible utilization of the enriched sillage for animal feeding and the manure from animal feeding for the production of biogas. A stabilized sludge remained in the process can be used as a fertilizer. Besides, by direct combustion of lignocelluloses part of the plants a considerably energy for heating can be released [4].
For the last few years, a use of probiotic microorganisms as animals` food ingredients has attracted a lot of interest. Some data indicate that the supplementation with probiotics may improve the health of animals. The supplementation with the yeast culture may improve food intake, digestion and increase the number of anaerobic and cellulolytic bacteria. Yeast cells consist of some immunomodulant components, such as β-glucan, nucleic acids, oligosaccharides, which stimulate the resistance to viruses and bacteria and the immune system of animals [5, 6].
The aim of this study was to perform a chemical and nutritive analysis of the stillage obtained as a byproduct from bioethanol production on corn and triticale, as well as of the stillage obtained after bioethanol production on corn which was enriched with 1% w/w of the *Saccharomyces cerevisiae* yeast. The chemical analysis encompassed the detection of components such as moisture, fat, cellulose, phosporus, proteins, and mineral materials – Cu, Zn, Mn, Fe, Ca and Na. The chemical composition of the obtained mixtures was assessed from the point of nutritive quality required for animal feed.

MATERIALS AND METHODS

Three samples of stillage were analyzed: 1) Stillage of triticale 2) Stillage of corn 3) Stillage of corn enriched with *S.cerevisiae* (1%w/w stillage).
By the chemical analysis determinated: moisture, protein content, cellulose, minerals, microelements (P, Cu, Zn, Mn, Fe, Ca i Na) [7].

Preparation of starch hydrolyzates from corn meal and triticale [8,9]
Starch hydrolyzates were obtained by a two step hydrolysis (liquefaction and saccharification). A 100 g of corn meal was mixed with water at the weight ratio 1:3, and 60 ppm of Ca^{2+} (as $CaCl_2$) ions was added. The liquefaction was carried out at 85ºC and pH=6.0 for 1h by adding 0.026% (v/w starch) enzyme Termamyl SC. The liquefied mash was saccharified at 55ºC and pH=5.0 for 4h with 0.156% (v/w starch) enzyme SAN Extra L. The hydrolysis was performed in flasks in a thermostated water bath with shaking at 150 rpm.
Grinded and mashed triticale samples were mixed with water warmed at 50 C in metallic jars, keeping the sample to water ratio at 1:3. After mixing of the samples with water Term amyl 120 L was added. The samples were held in a water bath with mixing (150 rpm) for 30 min at 50 C. After that triticale samples were heated to 60 C. At this temperature the samples were held for 60 min with mixing. Temperature was then lowered to 55 C and SAN Super 240 L was added. Samples were held on this temperature for 30 min after which the temperature was lowered to 30 C.

Laboratory preparation of cell culture [8]
Saccharomyces cerevisiae was used for the fermentation of the hydrolyzed corn meal and triticale samples. The culture originated from the collection of BIB-TMF, Belgrade, and was maintained on a malt agar slant. Before use as an inoculum for the fermentation, the culture was aerobically propagated in 500 ml flasks in a shaking bath at 30ºC for 24 h and then separated by centrifugation.

Ethanol fermentation of starch hydrolyzates
Starch hydrolyzates were subjected to ethanol fermentation by *Saccharomyces cerevisiae* under anaerobic conditions (pH=5.0; 30ºC). The initial yeast concentration was 2%. Fermentation time was 36 hours.

Separation of stillage after distillation
After ethanol distillation, the stillages of corn and triticale were removed by centrifugation. The solid samples were dried at 60^0C and used for chemical analysis.

Enrichment of corn stillage with yeast
Dried corn stillage was enriched with spray dried *Saccharomyces cerevisiae (*1%w/w stillage) [10].

RESULTS AND DISCUSSION

The results of the chemical composition of corn stillage, the stillage of triticale and the corn stillage enriched with yeast are presented in Table 1.

Table 1. Chemical composition of the samples of stillages

	Triticale stillage	Corn stillage	Corn stillage enriched with yeast
Moisture, %	10,39	3,91	6,24
Proteins, %	30,51	39,96	42,90
Fat, %	5,80	13,14	14,28
Sugar, %	15,37	7,27	3,84
Cellulose, %	6,74	2,95	4,33
Phosphorus, %	0,534	1,12	1,49
Cu [mg/kg]	398,34	17,72	22,26
Zn [mg/kg]	146,05	49,57	47,90
Fe [mg/kg]	84,42	74,67	60,46
Se [mg/kg]	<2	<2	<2
Mg [mg/kg]	6339,12	4615,10	1971,18
Ca, %	0,079	0,195	0,031
Na, %	0,432	0,112	0,055

The protein content, which is the most important part of the cattle food, is extremely high. The triticale stillage contains 30.51% of proteins, while the corn stillage contains 31% proteins more and its content is 39.96%. The protein content of the sample of corn stillage enriched with yeast is higher due to the contribution of the yeast biomass and its value is 42.90%.
This protein content is satisfactory considering the values officially prescribed by the regulations for animal feed mixes. According to the regulations, maximal protein content of 50 % is recommended only for full feed mixes for young trout, while the common

values of proteins in animal feed mixes are in the range from 15- 40% [11]. The changes in the protein content are shown in Figure 2.

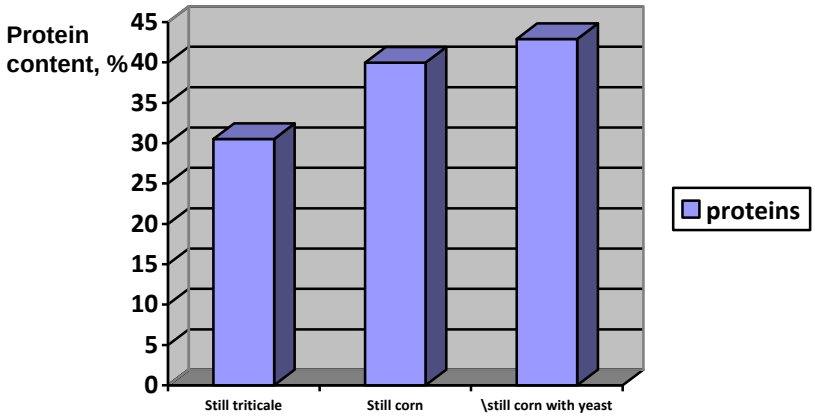

Figure 2. Protein content (%) of the tested samples

The cellulose content of the complete animal feed mixes is limited to 10% due to its low digestibility. In the stillage samples tested its content was in limits which are officially allowed. The cellulose content reaches the value of 6.74% in the triticale stillage, while the corn stillage has a lower content of cellulose for around 55%.

The fat content of the stillage samples was in the range from 5.8-14.3%. The corn stillage has a higher fat content compared to the triticale stillage. The fat content is very important for fatlings` feed and according to the official food regulations it shouldn't be below 5% [11].

The phosphor content of the samples is in the range from 0.534% to 1.49% with the greatest value detected in the sample of corn stillage enriched with yeast. The value of phosphor content of 1.49% is higher from the majority of upper phosphor limits prescribed by the national feed regulations (average recommended values are from 0.6-0.8%), with an exception for complete animal feed mixes for the fatlings, where the upper phosphor value limit is 1.5%

For a good animal feed it is important that it contains particular minerals in a proper amount [12]. The content of minerals contributes to a higher quality of animal feed, especially in the case of mixes based on corn stillage. The content of copper with values ranging from 17.72 mg/kg (corn stillage enriched with yeast) to 398.34 mg/kg (triticale stillage) is far above of the officially recommended value (5 mg/kg). A high increase in the content can be noticed for the zinc, which is ranging from 47.90 mg/kg (corn stillage enriched with yeast) to 146.05 mg/kg (triticale stillage).

In the sample of corn stillage enriched with yeast, a low sodium content of 0.055% may be noticed. This is acceptable only for a few animal feed mixes officially prescribed by food regulations. The sodium content of the other two samples is 0.112% (corn stillage)

and 0.432% (triticale stillage), which satisfy quality requirements for the majority of animal feed mixes prescribed by our national feed regulations [11].
The content of calcium increased from 0.31% (stillage enriched with yeast) up to 0.195% (corn stillage), but still remained of poor quality compared to the calcium content prescribed for animal feed mixes by animal feed regulations [11].

CONCLUSSION

Chemical analysis of the samples of triticale stillage, corn stillage and corn stillage enriched with 1% of *Saccharomyces cerevisiae* yeast has shown that all stillage samples contained a high percentage of proteins (above 30%). Maximum protein content (42.90%) contains the corn stillage enriched with 1% of *Saccharomyces cerevisiae* yeast. The addition of the yeast contributed to an increase in protein content, fat content and the content of particular minerals - P, Ca and Cu. The triticale stillage contains more minerals such as Zn, Fe, Mg and Ca, compared to the corn stillage.
The chemical composition of the stillage indicated that it can be a complete feed mix for several animal categories. The enrichment of the corn stillage with *Saccharomyces cerevisiae* yeast can contribute to develop a new animal feed of a high quality. Its valorization can significantly improve the economy of the bioethanol production on corn.

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THE EFFECT OF MICRO AND MACROELEMENT CONCENTRATONS IN FEED AND WATER ON THEIR DISTRIBUTION IN BEEF CATTLE TISSUES

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ABSTRACT

A six-month study was conducted to determine the concentrations of micro- and macroelements (iron, manganese, potassium, sodium and calcium) in the water, hay and complete feed mixtures (CFM) used for beef cattle. The water, hay and CFM samples were collected for analysis at fifteen-day intervals, totalling ten samples per each test material.

The test beef cattle (n=10) were raised on a mini-farm in Kraljevo. Samples of muscular tissue, liver and kidney were obtained from each test animal immediately after slaughter to determine the content of the above elements. All samples used for the analysis, excepting water, were prepared by ash process and the mineral concentrations (contents) were read by atomic absorption spectrophotometry (AAS).

The average iron content was 1.92 mg/l in water, 185.52 mg/kg in hay, 137.43 mg/kg in CFM, 38.87 mg/kg in the muscular tissue of the beef cattle, 67.60 mg/kg in the liver and 78.83 mg/kg in the kidney. The average manganese content was 1.57×10^{-2} mg/l in water, 76.01 mg/kg in hay, 12.64 mg/kg in CFM, 0.34 mg/kg in the muscular tissue, 2.37 mg/kg in the liver and 1.12 mg/kg in the kidney. The average sodium content was 2.81 mg/l in water; 1.34 g/kg in CFM; 464.05 mg/kg in the muscular tissue; 547.58 mg/kg in the liver and 774.24 mg/kg in the kidney. The average potassium content was 2.37 mg/l in water; 11.28 g/kg in CFM; 5.71 g/kg in the muscular tissue, 5.59 g/kg in the liver and 5.69 g/kg in the kidney. The average calcium content was 12.98 mg/l in water; 9.62 g/kg in CFM; 729.07 mg/kg in the muscular tissue; 534.04 mg/kg in the liver and 252.14 mg/kg in the kidney.

The obtained results suggested that the iron intake by the tested beef cattle from the water supply was substantially above the maximum tolerable concentrations (MTC), whereas the manganese content was considerably lower. The detected iron content of CFM largely exceeded the minimum requirements for cattle, as opposed to the manganese content which was below the limit values prescribed by the Regulation. The highest content of iron was detected in the kidney and that of manganese in the liver of the test animals. Potassium was uniformly distributed within the tissues, whereas sodium and calcium accumulated in the kidneys and muscles, respectively.

Key words: *iron, manganese, potassium, sodium, calcium, water, hay, CFM, beef cattle, muscular tissue, liver, kidney*

INTRODUCTION

Meat is a very rich and suitable source of nutrients, including microelements, among others. The chemical composition of meat is dependent on the livestock feeding methods and plane of nutrition. Mineral requirements of cattle depend on their age, physiological state and feed intake as well as on the raising conditions employed [14]. Minerals account for almost 1% and they have specific functions in terms of metabolism and breeding technology [30]. Meat contains significant amounts of phosphorus, calcium, sodium and iron salts and low amounts of copper, zinc, aluminium and manganese.

Iron is an essential component of a number of proteins involved in oxygen transport or utilization (hemoglobin, myoglobin, cytochromes and iron-sulphur proteins participating in the electron transport chain). There are several mammalian enzymes that either contain iron or are activated by iron [16]. More than 50% of the iron in the body is present in hemoglobin. Since three quarters of the iron in the organism is contained in hemoglobin and myoglobin, it is quite natural that the highest iron concentrations are found in the blood and organs having hematopoietic, hemolytic and storage functions.

The iron requirement is about 50 mg/kg diet in beef cattle, due to the reports on young calves fed milk diets indicating that 40-50 mg Fe/kg is adequate to support growth and prevent anemia [4, 2]. Minimum requirements for complete feed mixtures (CFM) in beef cattle are estimated at 20 mg/kg. The iron content in adult animals is variable across organs and tissues. Total iron levels differ among individual animals.

Iron occurs in meat as either heme iron or free iron and is stored in ferritin in ionic form.

Iron resorption takes place through the intestinal mucous membrane providing and regulating maintenance of iron equilibrium in the body. Iron resorption in the digestive tract is dependent on the iron reserves in the body and therefore an equilibrium is maintained between the course of iron resorption and size of iron reserves. The course of resorption may be affected by diet components: it decreases substantially at low calcium and high phosphorus and phytic acid levels. Iron excretion from the organism is minimal (through urine and faeces).

The first signs of iron deficiency in animal diet include anemia (hypochromic microcytic), languidness, reduced feed intake, weight loss, pale mucous membranes and atrophy of the papillae of the tongue [3, 4]. Anemia can develop in calves fed exclusively milk diets for extended periods of time lacking sufficient amounts of other nutrient supplements.

Given the fact that anemia treatment in humans is also associated with iron intakes through beef meat and viscera, we deemed it important to determine the iron content of the muscular tissue, liver and kidneys following slaughter of the tested beef calves. Both meat and fish meat are good sources of iron in human diet (400-500 mg Fe/kg).

Manganese functions as a component of the enzymes pyruvate carboxylase, arginase and superoxide dismutase and as an activator for a number of enzymes, including hydrolase, kinase, transferase and decarboxylase [13, 1]. Only glycosyltransferases are specifically activated by manganese. The mitochondrial concentrations of manganese imply that manganese is involved in partial regulation of oxidative phosphorylation.

Manganese participates in oxidation and reduction processes, as well as in the conversion of fat, sugars and proteins. It influences growth, reproduction and the function of endocrine organs. High levels of manganese can reduce those of hemoglobin.

The absorbed manganese is readily transported by blood to the liver and bones. Manganese is not concentrated in any particular organ or tissue. The concentrations in the bones, liver, kidneys and pancreas (1-3 mg/kg fresh tissue) are higher than those in the skeletal muscles (0.1-0.2 mg/kg).
The manganese concentration in livestock feed is variable, depending on the fodder plants used, soil pH and soil drainage [17]. Therefore, complete feeds for beef cattle must be supplemented with adequate forms of manganese, most commonly manganese oxide and manganese sulfate. If the relative biological availability of manganese from manganese sulfate is 100%, the availability from manganese oxide and manganese carbonate is 60-80 % and 25-40%, respectively [16]. As compared to manganese sulfate, the relative availability of manganese from manganese methionine is approximately 120 % [12].
The manganese requirements of animals depend on the animal species and dietary levels of calcium and phosphorus –high levels of calcium and phosphorus have been proved to increase the need for manganese [11, 7, 15]. The evaluation of manganese requirements is hampered by inadequate research data on manganese availability (manganese availability in poultry nutrition is estimated at 5%). The manganese requirement for beef cattle is 20 mg Mn /kg diet.
Manganese deficiency in young animals leads to skeletal abnormalities (including stiffness, twisted legs, swollen joints and reduced bone strength). In older cattle, it causes poor or irregular oestrus response, low conception rate, abortion, stillbirths and low birth weight of offspring.
No limits are prescribed by the Codex Alimentorum, for iron and manganese.
The highest amounts of potassium and sodium in animal tissues are stored in muscles, and those of calcium in the bones. Potassium is the major cation in the intracellular fluid involved in the acid-base balance, regulation of osmotic pressure, water balance, smooth, skeletal and heart muscle contractility, nerve impulse transmission and certain enzymatic reactions. The potassium requirement of beef cattle is around 0.6%. Potassium is absorbed from the rumen, omasum and the intestines and its absorption is very high. Feedstuffs are excellent sources of potassium, containing between 1 and 4% potassium. Cattle diets can be supplemented with potassium as potassium chloride, potassium bicarbonate, potassium sulfate or potassium carbonate (all forms are readily available).
Sodium is the major cation in the extracellular fluid, the function thereof being to maintain osmotic pressure, control water balance and regulate the acid-base balance. Another important function of sodium is its involvement in muscle contractions, nerve impulse transmission, and glucose and amino acid transport.
The sodium content of feedstuffs shows high variations. Animal-derived products generally contain higher sodium and chlorine amounts than plant-derived products [19].Calcium is the most abundant mineral in the body. As much as 98 % of calcium functions as a structural component of bones and teeth and the remaining 2% is distributed in extracellular fluids and soft tissues where it is involved in vital functions (blood coagulation, membrane permeability, muscle contraction, nerve impulse transmission, cardiac regulation, secretion of certain hormones and activation and stabilization of some enzymes).
The calcium requirements have been calculated by adding the available calcium required for maintenance, growth, pregnancy and lactation and correcting for the percentage of

dietary calcium absorbed. The Agricultural and Food Research Council (AFRC) has recently used a value of 68% absorption to calculate calcium requirements of beef cattle [27].
Calcium is absorbed primarily from the duodenum and jejunum by active transport and passive diffusion [16]. Vitamin D is required for active absorption of calcium [6]. In natural feedstuffs, calcium occurs in oxalate or phytate form. In alfalfa hay, 20-33 % is present as insoluble calcium oxalate which is apparently unavailable to the animal [36].
Calcium deficiency in young animals prevents normal bone development and retards growth and development, resulting in bone softening and fragility leading to fructures. In adult animals, the deficiency induces osteomalacia. Blood calcium concentration is not a good indicator of calcium status as plasma calcium is maintained at 9-11 mg /dL by homeostatic mechanisms. Parathyroid hormone is released in response to a reduction in plasma calcium levels. It stimulates the production of 1,25-dihydroxy cholecalciferol (vitamin D_3), which enhances calcium resorption from the intestines and, in conjunction with parathyroid hormone, increases calcium resorption from the bones. Elevated plasma calcium concentrations induce the production of caltinonin that inhibits the production of parathyroid hormone, leading to decreased calcium absorption and bone resorption [20].
The calcium content of feedstuffs is affected by plant species, portion of plant consumed, maturity, amount of exchangeable calcium in the soil and climate [17]. A higher content of calcium is found in legumes than in grasses.
Given the similar role that sodium and potassium play in regulating the cation balance and osmotic pressure in intra- and extracellular fluids, their relative dietary amounts are considered highly important. Determination of the potassium, sodium and calcium contents of water and feedstuffs can help adequately define their amounts through complete feeds consumed. The mineral intake affects the mineral content in the tissues of the animal and, hence, beef cattle. In view of the importance of beef and viscera (liver and kidney) diets in human nutrition, it is necessary to determine the content of the above micro- and macro elements (minerals) in them.

MATERIAL AND METHODS

This study was conducted to determine the contents of iron (Fe), manganese (Mn), potassium (K), sodium (Na) and calcium (Ca) in water, hay and complete feed mixtures (CFMs) for beef cattle as well as their concentrations in the muscular tissue, liver and kidney samples obtained immediately after slaughter of the beef calves. The analysis included 10 samples of each test material.
The samples of water, hay and CFM were collected for analysis at fifteen-day intervals over a six-month period and those of tissue and organs were secured from the slaughtered feed cattle.
The water samples collected for analysis were prepared by nitrate acid preservation at the experimental farm and were subsequently concentrated in the laboratory. The hay samples were homogenized, chopped and heated on a hot place until burnt. Then, they were incinerated in an incinerating furnace at 550^0C. Following the incineration process, the residue was dissolved in 1:1 HCL and redistilled water and decanted into 50 mL weighing vessels.

The complete feed samples were homogenized in a "Cyclotec" sample mill, burnt on a hot plate and incinerated in an incinerating furnace at 550^0C. The obtained ash was dissolved in HCl 1:1, and iron and manganese contents of the test materials were read by atomic absorption spectrophotometry (AAS) using a Perkin Elmer 3300 device.
The content of calcium was determined by air-acetylene flame atomic absorption spectrophotometry and that of sodium and potassium by flame emission spectrophotometry. To prevent flame ionization, potassium chloride was added to all standard dilutions and samples to obtain the final potassium chloride concentration in the solution of at least 0.1%.

RESULTS AND DISCUSSION

The results of the present study on the contents of iron (Fe), manganese (Mn), potassium (K), sodium (Na) and calcium (Ca) in water, hay, CFM and tissues of the test beef cattle are presented in tabular form (Tabs. 1 - 9).

Table 1. Average concentration of iron (Fe) in water (mg/l), hay and CFM (mg/kg)

	Water	**Hay**	**CFM**
$\bar{x}$	1.92	185.52	137.43
Sd	1.48	83.25	18.073
Cv	77.31	44.87	13.15
Iv	0.47 - 5.60	66.44 - 302.41	103.32 - 158.87

Table 2. Average concentration of manganese (Mn) in water (mg/l), hay and CFM (mg/kg)

	Water	**Hay**	**CFM**
$\bar{x}$	1.57×10^{-2}	76.01	12.64
Sd	1.97×10^{-2}	22.21	12.84
Cv	125.15	29.22	30.18
Iv	0.002 - 0.070	32.08 - 100.16	8.84 - 54.62

Table 3. Average concentration of potassium (K), sodium (Na) and calcium (Ca) in water (mg/l)

	Potassium (K)	**Sodium (Na)**	**Calcium (Ca)**
$\bar{x}$	2.37	2.81	12.98
Sd	1.06	1.57	4.92
Cv	44.68	55.93	37.92
Iv	1.22 - 3.84	1.78 - 5.56	6.22 - 17.56

Table 4. Average concentration of potassium (K), sodium (Na) and calcium (Ca) in CFM for beff cattle (mg/kg)

	Potassium (K)	**Sodium (Na)**	**Calcium (Ca)**
$\bar{X}$	11,283.07	1,344.00	9,623.14
Sd	556.06	41.63	218.87
Cv	4.93	3.10	2.27
Iv	10,787.99 – 12,101.90	1,299.30 – 1,410.40	9,310.80 – 9,840.60

The average iron intake from water by beef cattle was 1.92 mg/l, the iron content ranging from 0.47- 5.60 mg/l and that of manganese - 1.57 x 10^{-2} mg/l. The current Regulation on the Hygienic Safety of Drinking Water does not prescribe limits for iron, but the maximum tolerable concentration (MTC) for manganese is 0.050 mg/l. Water supplies for the cattle farm used in the experiment as well as for the Town of Kraljevo came from the Ibar alluvion. The listed results on the manganese content of the Ibar river water show that the content remained almost unchanged as compared to the 1987, 1988 and 1989 data reported by *Vukašinović Marija and Mihajlović R.* [35].

The present results on the iron concentration in the water used for the test animals show that the obtained values were considerably higher than those measured by *Vukašinović Marija and Mihajlović R.*. [35] who reported them to be within a range of 0.12 - 2.20 mg/l during 1987-1989, which suggested an apparent increase in iron concentration.

The average iron content of the meadow hay collected from the region of Kraljevo was 185.52 mg/kg, the iron content ranging from 66.44 - 302.41 mg/kg air-dried hay. The iron content of the meadow hay in the region of Kraljevo was also evaluated by *Ševković et al.* [29] who reported the values of 203.00-269.66 mg/kg iron, whereas the content of the Pozega region hay was slightly lower, ranging from 188.82- 247.28 mg/kg (*Ševković et al.)* [28]. The manganese content of the Kraljevo hay ranged from 32.08 - 100.16 mg/kg, the average being 76.01 mg/kg air-dried hay. The measured values conformed to the results obtained by *Ševković et al.* [29] who reported the manganese content of the meadow hay from Kraljevo and that from Pozega regions to fall within a range of 44.54 - 87.22 mg/kg and 68.42 - 143.95 mg/kg, respectively [28].

The average iron concentration in the complete feeds used for beef cattle was 137.43 mg/kg, ranging from 103.32 - 158.87 mg/kg. The measured iron concentration was significantly above the limit of 20.00 mg/kg set in the Regulation on the Quality and Other Requirements for Feedstuffs [23].

The average manganese concentration of the complete feed mixtures (CFM) used for the beef cattle was 12.64 mg/kg, the measured values varying from 8.84 to 54.62 mg/kg. The obtained average concentration was below the minimum requirements of 20 mg/kg prescribed by the Regulation on the Quality and Other Requirements for Feedstuffs ("The Official Gazette of the Federal Republic of Yugoslavia" No. 20/2000) [23]. The manganese requirements of beef cattle are difficult to evaluate due to the lack of research data on manganese availability.

The average potassium concentration in the water used for cattle was 2.37 mg/l, its range being 1.22-3.84 mg/l i.e. slightly exceeding the level measured by *Vukašinović Marija et al.* [33], in the waters of the Ribnica, the Studenica and the tributaries, but considerably

below the levels for the waters in the region of Smederevska Palanka, Srbobran and Rekovac reported by *Plavšić, K.* [21].
Sodium concentration in the water ranged from 1.78 - 5.56 mg/l, the average value being 2.81 mg/l. Significantly higher sodium concentrations in the waters in the regions of Smederevska Palanka (34.16 - 69.72 mg/l), Rekovac (4.30 - 56.90 mg/l) and Srbobran (5.80 - 14.20 mg/l) were detected by *Plavšić, K.* [21]. The sodium content in the waters in the region of Kraljevo (the Ribnica and Studenica basins) was reported by *Vukašinović Marija et al.* [33, 34] to be considerably lower.
The calcium concentration in the water used for beef cattle ranged from 6.22-17.56 mg/l, with an average of 12.98% mg/l, which was significantly lower than the values obtained by *Vukašinović Marija et al.* [33] for the Ribnica and Studenica rivers and those measured by *Plavšić, K.* [21] for the waters in the regions of Smederevska Palanka, Rekovac and Srbobran.
The maximum tolerable levels of potassium, sodium and calcium, as set in the *Regulation on the Hygienic Safety of Drinking Water ("The Official Gazette of the FRY" 42/98)* [22] are 12.0, 150 and 200 mg/l water, respectively, being considerably above the levels measured in this study.
The obtained results on potassium, sodium and calcium concentrations in the CFM used for beef cattle are given in Table 4. The average contents of potassium, sodium and calcium were 11,283.07 mg/kg, 1,340.00 and 9,623.14 mg/kg, respectively. The *Regulation on the Quality and Other Requirements for Feedstuffs ("The Official Gazette of the FRY" 20/12) and the Amendments No. 38/2001* [23], prescribe maximum levels of calcium and sodium in complete feeds for beef cattle at 0.9-1.1% and 0.2-0.3%, respectively, whereas the potassium level is not prescribed. The measured sodium concentration in the CFM for beef cattle was below the required regulated amount, whereas the calcium content conformed to the quantity prescribed.

Table 5. Average contents of iron (Fe) in the muscular tissues, liver and kidney of beef cattle (mg/kg)

	Muscular tissue	**Liver**	**Kidney**
$\bar{x}$	38.87	67.60	78.83
Sd	16.42	13.10	20.63
Cv	42.23	19.38	26.17
Iv	22.67 - 78.96	45.27 - 88.37	57.43 - 121.14

Table 6. Average contents of manganese (Mn) in the muscular tissue, liver and kidney of beef cattle (mg/kg)

	Muscular tissue	**Liver**	**Kidney**
$\bar{x}$	0.34	2.37	1.12
Sd	9.70×10^{-2}	0.32	0.13
Cv	28.68	13.69	11.95
Iv	0.17 - 0.52	1.81 - 2.86	0.90 - 1.30

The measured contents of iron and manganese in beef cattle tissues are given in Tables 5 and 6, respectively. The iron content in the muscular tissue ranged from 22.67 - 78.96 mg/kg, with an average being 38.87 mg/kg. The content of manganese varied from 0.17 - 0.52 mg/kg, averaging 0.34 mg/kg. The iron content of the liver and kidney tissues ranged from 45.27 - 88.37 and 57.43 - 121.14 mg/kg, the average values being 67.60 mg/kg and 78.83 mg/kg, respectively. The manganese content of the liver ranged from 1.81-2.86 mg/kg, averaging 2.37 mg/kg, whereas the kidney content fell within a range of 0.90-1.30 mg/kg giving an average of 1.12 mg/kg. The obtained liver manganese values complied with those (0.26 mg/100 g) reported by *Rogovski, B.* [24].
The measured results on the iron content of muscular tissue showed higher values than those of 3.00 mg/100 g determined by *Rogovski, B.* [24], whereas the iron content of the liver reported by the cited author was higher than in this study (12.00 mg/100 g). The iron content of the kidneys was almost identical to the values obtained by *Rogovski, B.* [24].
The iron contents of the leg muscles (1.40 - 3.04 mg/kg), shoulder blade muscles (1.22 - 2.56 mg/kg) and head meat (1.42 - 3.57 mg/kg) of beef cattle reported by *Djujić Ivana et al.* [8] were significantly lower than our results.
Skalická, M. et al. [26] compared the results on iron and manganese contents of the muscles in beef cattle sampled from different farms in Eastern Slovakia. The average iron values for the muscles were 23.787 mg/kg^{-1} in farm A cattle and 15.788 mg/kg^{-1} in farm B cattle. The maximum values obtained were 48.600 mg/kg^{-1} and 33.550 mg/kg^{-1}, respectively. The comparison revealed lower iron concentrations in the farm B beef cattle. The average manganese values in the muscles of the farm A and farm B cattle were 0.242 mg/kg^{-1} and 0.566 mg/kg^{-1}, respectively.
The average muscle manganese concentration reported by *Bruggemann, J & Kumpulainen, J.* [5] was 1.2 mg/kg^{-1}. The obtained results conformed to those of *Gallo, M. et al.* [10], who suggested that the highest manganese concentrations in emission-exposed areas were deposited mainly in internal organs of beef cattle. Manganese concentrations were highest in the liver, bone and kidney and lowest in the skeletal muscles. Manganese concentrations were rather stable in most major tissues of adult animals.
Valenzuela Carolina et al. [31] determined the content of total iron (TFe) and heme iron (HeFe) in major cuts of meat and principal viscera of bovine origin. ^{55}Fe (30 mCi) was injected into two 4-month-old calves. Triplicate samples of the 12 basic American cuts of meat and major viscera were obtained from each specimen. The samples were acid digested and their iron content was read by AAS. Duplicate samples of the basic cuts of meat and major viscera were analyzed to determine the concentration of ^{55}Fe using a double isotopic technique. The mean and standard deviation of Tfe for all cuts was 1.4 ± 0.3 mg/100 g of meat. The mean TFe for organs was (mg/100 g): 0.9 ± 0.1 brain, 3.0 ± 0.05 kidney, 3.2 ± 0.04 heart, 5.7 ± 0.2 lung, 6.0 ± 0.1 liver, and 31.2 ± 0.4 spleen. HeFe was 64% of TFe in meat and 72.8% in spleen, 53.8% in lung, 35.7% in brain, 35.0% in kidney, 27.3% in heart and only 13.6% in liver. Blood contained 85.5% of the radioisotope and only 1.4% was found in muscle and 1.6% in viscera. The results suggest that bovine cuts of meat have a low variation in Tfe and that HeFe comprises more than 60% of Tfe.

The muscles of wild game species, beef and pork contained 3.4 mg, 2.4 mg and 1.9 mg Fe, respectively. *Falandysz, J. et al.* [9] determined higher manganese contents on average in meat samples of roe deer, wild boar, mallard and wild goose than in those of dairy cattle, rabbits, male turkeys and chickens. The manganese concentrations were 0.013 mg/kg^{-1} in game meat, 0.016 mg/kg^{-1} in beef and 0.02 mg/kg^{-1} in pork.
Miranda, M. et al. [18] conducted a study in a Spanish industrial region and reported mean values for iron in the muscle (56.0 mg/kg^{-1}) and liver (96.2 mg/kg^{-1}) of beef cattle as well as a mean manganese concentration in the liver of 3.11 mg/kg^{-1}.

Table 7. Average contents of potassium (K), sodium (Na) and calcium (Ca) in the muscular tissues of beef cattle (mg/kg)

	Potassium (K)	**Sodium (Na)**	**Calcium (Ca)**
$\bar{x}$	5,712.60	464.05	729.07
Sd	393.39	44.91	1,063.25
Cv	6.89	9.68	145.84
Iv	5,041.02 – 6,037.48	410.34 – 524.28	133.07 – 2,616.24

The average muscle potassium content (Table 7) was 5,712.60 mg/kg. The measured values showed uniformity across the samples (Cv = 6.89%).
The potassium amount was higher than the value (400mg/100g) for beef determined by *Rogovski, B.* [24]. The daily potassium requirements (of an adult man of average body weight) reported by *Simić B.* [25] are 2,500 mg.
The average sodium content of the muscular tissue was 464.05 mg/kg (the measured range of 410.34 – 524.28 mg/kg), which conformed to the results of *Rogovski, B.* [24] who reported the 40 mg/100 g sodium content of beef. *Vukićević, D.* [32] determined a significantly higher sodium content (110 mg/100g) in game (mallard and pheasant) meat.
The calcium content of the tested meat samples showed high variations (from 133.07-2,616.24 mg/kg), the average being 729.07 mg/kg. The measured calcium content substantially exceeded the content (10 mg/100g) in beef determined by *Rogovski, B.* [24].

Table 8. Average contents of potassium (K), sodium (Na) and calcium (Ca) in the liver of beef cattle (mg/kg)

	Potassium (K)	**Sodium (Na)**	**Calcium (Ca)**
$\bar{x}$	5,597.31	547.58	534.04
Sd	652.25	89.39	788.87
Cv	11.65	16.32	147.72
Iv	4,728.03-6,208.70	418.96-636.50	125.57-1,943.63

Table 9. Average contents of potassium (K), sodium (Na) and calcium (Ca) in the kidney of beef cattle (mg/kg)

	Potassium (K)	**Sodium (Na)**	**Calcium (Ca)**
$\overline{x}$	5688.09	774.24	252.44
Sd	431.41	100.83	41.17
Cv	7.58	13.02	16.31
Iv	5,292.32-6,249.70	663.90-905.54	189.44-296.22

The measured calcium content of the liver and kidney of beef cattle was low and averaged 5,597.31 and 5,688.09 mg/kg, respectively. The sodium content was considerably higher in the kidney, whereas a significantly higher content of calcium was found in the liver.

CONCLUSION

This study suggests the following:

- The measured iron concentration in the water used for beef cattle was substantially above the levels detected in previous studies. The manganese content did not exceed the maximum tolerable concentration (MTC).
- The beef cattle had a considerably higher iron intake from complete feed mixtures (CFM) than prescribed by the current Regulation, whereas the average manganese content was below the minimum requirements.
- The average iron and manganese contents were highest in the kidney (78.83 mg/kg) and liver (2.37 mg/kg) of the tested beef cattle, respectively.
- Potassium was uniformly distributed in the cattle tissues (muscle, liver and kidney). The highest content of sodium was found in the kidney and that of calcium in the muscular tissue.

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EFFECT OF A COMMERCIAL ESSENTIAL OIL AND PROBIOTIC ON GROWTH PROMOTION IN BROILER CHICKENS

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ABSTRACT

The concern about possible antibiotic residues and disease resistance has led to investigation of alternative feed additives in animal production. Today, among other alternative for antibiotics, as growth promoters, in animal feed are both essential oils, and probiotics.
An experiment was conducted to determine the responses of broiler chickens to commercial blend of essential oils, or probiotics in feed. Trial involved day-old chickens of Ross 308 hybrid line assigned to 3 groups, namely, C- control, O- trial group (fed diets supplemented with essential oil extracts) and P- trial group (fed diet supplemented with probiotics). The following production and performance parameters of broilers were studied: daily gain, feed intake and feed conversion efficiency.
Daily bodyweight gain was lowest for the control group. At the end of the trail the body weight of chickens fed supplemented diets was improved for 13% and 9% for the experimental group O and P, respectively. Birds from the experimental group O and P had lower feed intake for a kilo of weight gain in relation to control group.
Key words: *broiler meat, essential oil, probiotics, growth performances*

INTRODUCTION

Feeding strategy is the management factor which is most actively used as a quality control tool in the production of meat and in relation to improvement and/or control of performance, animal welfare, safety, nutritional value, and eating and technological quality [1]. A number of feed additives have been widely used in the poultry industry for several decades. The manipulation of gut functions and microbial habitat of domestic animals with feed additives has been recognized as an important tool for improving growth performance and feed efficiency [3].
Modern trend in poultry production are going toward expelling synthetic medicines from breeding. The ban on use of antibiotics as feed additives has led to investigation of alternative feed additives in animal production. The effective replacement for the antibiotics should have a significant and sustainable beneficial impact on animal production and health, be proven safe for both animal and human population, be easy to apply and store and provide a substantial return on investment [13]. Aromatic plants and their extracts have received increasing attention as potential alternatives to growth

promoters. Most of their properties are due to the essential oils they contain as products of secondary metabolism [2]. Essential oils extracted from herb and spices are a complex mixture of various compounds, which consists of aromatic and volatile substances which are generally recognized as safe, admitted by the Food and Drug Administration (FDA) [3]. A blend of essential oils has been developed for use as alternatives to antibiotics in the animal industry partly due to their biological properties such as antimicrobial and antiseptic activities. Essential oils inhibit microbial growth in the gut and in that way enhance nutrient digestibility [3]. Essential oils are already used as feed supplements to improve growth performance under intensive management systems.
Another antibiotic replacement in poultry production, used as a growth stimulant and for improvement of the feed conversion rate, in farm animals could be some probiotics microorganisms [9]. Probiotics may affect the permeability of the gut and increase uptake of nutrients. By definition, a probiotic contributes to an improvement of the intestinal microbial balance and consequential beneficial effects for the host animal [12]. Therefore, the present study was designed to evaluate the effects of dietary supplementation with essential oil extract, and probiotic on broiler meat production.

MATERIAL AND METHODS

The trial involved one-day-old fattening chicks of ROSS 308 hybrid line. Day-old chickens were weighed at the beginning of the trial and housed in special pens under controlled ambient conditions and had ad libitum access to feed and water. Chickens were assigned in 3 experimental groups and received iso-protein and iso-energy diets with no supplements (control group C), with essential oil (group O), and with probiotic (group P) supplementation. The trial lasted 42 days (two 21-day periods). The first three weeks chicks were fed starter diets and then (4-6 week) finisher diets until the end of the trial. The main difference between starter and finisher diets was in protein level and energy: protein ratio. Composition of trial diets is given in Table 1.
For determination of body weight, daily gain, feed intake and feed conversion efficiency birds were weighed every 7 days of experiment.

Table 1. Composition (%) of starter and finisher diets

	Broiler starter (0 – 3 weeks)	**Broiler finisher (4–6 weeks)**

Trial group	C	O	P	C	O	P
Supplement	-	Essential oil	Probiotic	-	Essential oil	Probiotic
Maize	48.1	48.1	48.1	57.12	57.12	57.12
Soybean meal	14	14	14	20	20	20
Extrudate soybean meal	15	15	15	15	15	15
Sunflower meal 44%	14	14	14	-	-	-
Oil	4.5	4.5	4.5	4	4	4
Monosodium phosphate	1.3	1.3	1.3	1.1	1.1	1.1
Limestone	1.4	1.4	1.4	1.2	1.2	1.2
Salt	0.4	0.4	0.4	0.4	0.4	0.4
Methyonine	0.12	0.12	0.12	-	-	-
Methyonine +cystine	-	-	-	0.18	0.18	0.18
Lysine HCl	0.18	0.18	0.18	-	-	-
Premix	1	1	1	1	1	1

RESULTS AND DISCUSION

Average results data on birds body weight, daily gain and feed conversion ratio during the experimental period are shown in Fig. 1, 2 and 3.

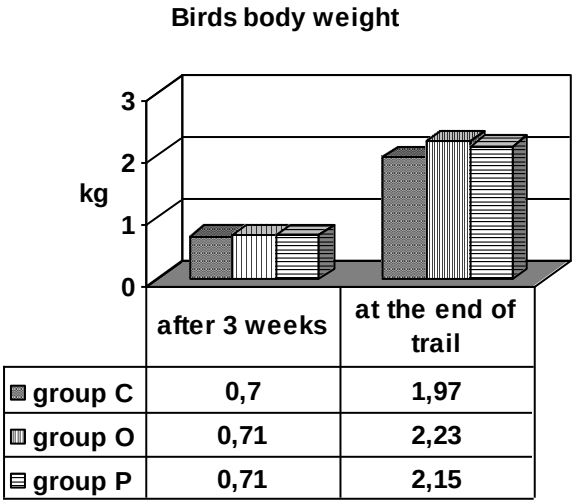

Fig. 1. Birds body weight after 3rd week (starter diet) and at the end of trail (finisher diet)

As it can be seen on Fig. 1. dietary supplementation of essential oil and probiotic did not influenced average bird body weight after starter diet. At the end of trail body weight of birds from control group was the lowest. Body weight of chickens fed diet supplemented

with essential oil improved for 13%, and with probiotic 9%, in relation to control group. Jang et al. [3] reported higher, but not statistically significant higher, final body weight of birds fed diet supplemented with essential oil for 2.5% comparing to control group (basal diet), and 5.5 % regarding the group fed with antibiotics. Higher body weight in birds fed with probiotic, comparing to the control, after 21 day (6.9%) and at the end of trail (16.35%) reported Savkovic et al. [7], and also increment of 11% in paper of Savkovic et al. [8]. Also, according to results of Savkovic [11] broilers fed diets fortified with probiotics (0,1%) had higher body weight (13,88%), than control group (diets without additives).

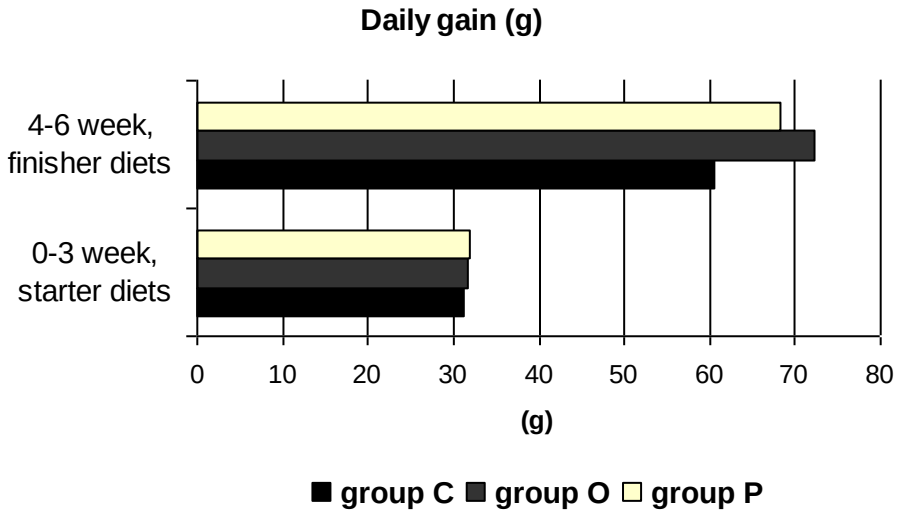

Fig. 2. Average daily gain (g) of chickens from experimental groups

According to the results shown in Fig. 2 there were only slit differences in average daily gain among experimental groups after 3rd week of experiment. Average daily gain, in this period, ranged between 31.22 and 31.85 g. With regard to the second feeding period during which chickens received finisher diets, the differences were more notable. Highest daily gain was for the chickens from the experimental group O (72.24 g), and lowest for control group (60.48 g). Jang et al. [3] reported not significantly different in total gain among basic and essential oil fortified dietary treatment groups. Positive effects diets supplemented with probiotics on body weight gain, were obtained in experiments of Milosevic [4], Savkovic et al., [5,8], and Savkovic and Tojagic [6]. Published data indicate that broiler feed supplemented with probiotics in concentration of 0,1% and 0,2% rise average daily weight gain for 22.02% and 24.32% respectively comparing to control group (diet without additives), and for 16.3% and 18.6% comparing to group fed diets with antibiotics [10].

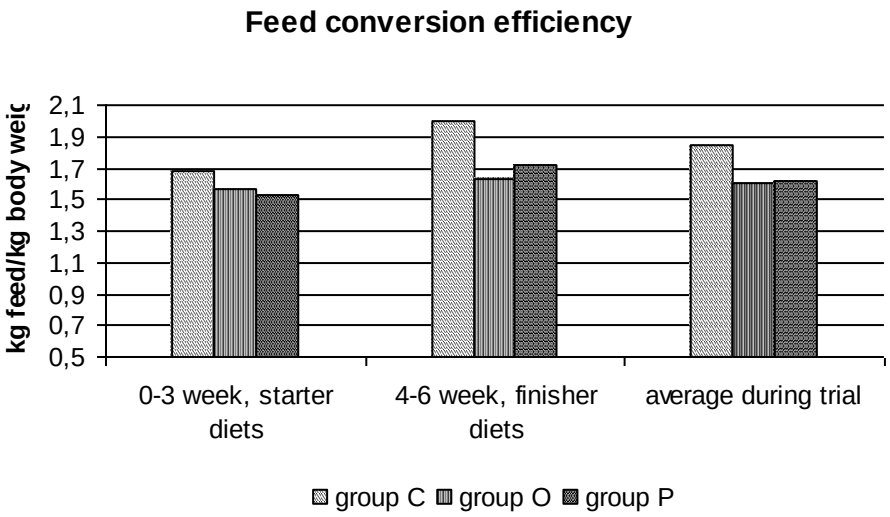

Fig. 3. Feed conversion efficiency of chickens from experimental groups

Differences in feed conversion efficiency among experimental groups can be seen on Fig. 3. Chickens from the experimental groups O and P had very similar feed intake (average during trial) for a kilo of weight gain, 1.6 and 1.62, respectively, what was approximately 14 % lower in relation to control group.

CONCLUSION

According to the results feed supplementation with commercial essential oil or probiotic had positive effects on broiler growth performances. At the end of the trail the body weight of chickens fed supplemented diets was improved for 13% and 9% for the experimental group O and P, respectively. Birds from the experimental group O and P had lower feed intake for a kilo of weight gain in relation to control group. The usage of essential oils and probiotics in feed supplementation, as antibiotics replacements, are also justified from the healthy food production and environmental standpoint.

ACKNOWLEDGEMENTS

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EFFECTS OF DIETARY GARLIC POWDER ON GROWTH PERFORMANCES AND QUALITY OF POULTRY MEAT

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ABSTRACT

The present study deals with growth performances, carcass and meat quality of broilers feed basal diet (control group) and broilers feed diet supplemented with garlic powder in content of 2% (experimental group I). Hubbard hybrids were used for the investigations. Fattening lasted 42 days, and during the whole trial chickens were fed ad libitum.
Positive effects of garlic supplementation on growth performance were noted after fourth week of trail, and at the end of the trail chickens of experimental group had significantly higher ($p<0.05$) body weight. During the trail experimental group had better feed conversion efficiency. Experimental group had higher ($p<0.05$) meat yield in chickens breast, but significant effect of garlic addition on nutritive quality of chicken breast meat were not noted.
Key words: *chickens, garlic powder, growth performance, meat quality*

INTRODUCTION

Term "meat quality" covers numbers of properties of meat decisive for the suitability of the meat for eating, further processing and storage including retail display. Meat quality depends on complex property of meat, influenced by multiple interacting factors including the conditions under which the meat is produced. Considering the already extensive knowledge of feed and meat quality, feeding seems to be the optimal tool in achievement of desired meat qualities [1].
Standard meals for chicken fattening are based on corm, soybean and fish meal. Beside nutrient necessary for chickens growth and development, feed very often contains some medications used therapeutically in animal feed to improve the health and well-being of animals and to improve the production results in poultry industries [2]. As alternatives for these synthetic growth promoters in animal feed, probiotics, prebiotics, organic acids and herbs, as well as essential oils have been investigated [12]. In recent years, aromatic plants and their extracts have received increased attention as potential alternatives to growth promoters [2].
Garlic (*Allium sativum*) has been used as spice and folk medicine since antiquity, mostly because of its antibacterial, antifungal and antioxidant activities. Bioactive components of garlic, including several sulphur-containing compounds such as alliin, diallylsulfides and allicin, may partly account for some of these effects of garlic [2].

Having in mind the mentioned above, the objective of this paper was to determine the effects of garlic powder addition in broiler feed on growth performances and carcasses and meat quality.

MATERIAL AND METHODS

Hubbard hybrids were used for the investigations. Chickens were assigned in two groups, of 40 birds each. Chickens were feed three basal diets: starter, finisher I and finisher II, each containing 23, 20 and 18% of protein, randomly. Diets were changed after every 14 days, and all the time chickens were fed *ad libitum.* Diets′ compositions are presented in Table 1. Chickens from control group (C) were fed with basal diet, and diet of chickens from experimental group I was supplemented with 2% of garlic powder. Body weight and feed intake were recorded every 7 days of the trail to determine growth performance and feed: gain ratio. Fattening lasted 42, after which broilers were hungered for 12 hours, slaughtered and processed by bloodletting, scalding, plucking and evisceration and chilled. Cutting and breast boneing were followed by measuring the meat yield and taking samples for determination of nutritive quality. Basic chemical composition of breast meat was established by determination of moisture [7], protein [10], free fat [8] and total ash [9] content.

Table 1. Formula (%) of experimental diets fed to broiler chickens

Diet	Starter	Finisher I	Finisher II
Soy oil	4	4	4
Maize	41.78	50.91	57.8
DL methionine	0.27	0.23	0.23
Soy grits	12.5	11.5	11
Soybean meal	37	29	23
Monosodium phosphate	1.4	1.31	1
Premix	1	1	1
Salt	0.25	0.3	0.4
Limestone	1.6	1.6	1.49
Lysine	0.2	0.15	0.08
Total	*100*	*100*	*100*
Crude protein	23.21	20.18	18.03
Metabolic energy	12.95	13.29	13.6

RESULTS AND DISCUSION

Results of chickens' body weight changes during the trial period are presented in Table 2.

Table 2. Body weight of chickens (g) of control and experimental group

Group	Control (C)	Experimental I
Start	43.3	43.4
I week	141.1	140.7
II week	357.4	345.7
III week	680.6	729.1
IV week	1048.9	1098.8
V week	1490.3	1570.0
VI week	1964.5[a]	2055.5 [b]

[ab]P<0.05

As it could be seen from the presented results, after the second week - the first period of the trail (starter diet), higher body weight was recorded for the control group (357.4 g). Reason for this could be lower feed intake because of the pungent smell that garlic has, and it is possible the chickens needed some time to adopt to this kind of feed [3, 6]. Though, in the second period of the trail garlic supplementation showed stimulant effects on growth performances, and after the fourth week (finisher diet I) experimental group I had 4.5% higher body weight, comparing to control. At the end of the trail chickens from experimental group I had significantly ($p<0.05$) higher body weight. Results of Freitas et al. [4] did not shown influence of diet supplementation with garlic on growth performances of broiler. Also, Horton et al. [6] reported that garlic addition in concentration of 1 and 10 g of garlic/ kg of feed, did not affect growth performances of pigs. Similar results reported Chen et al. [3].

Table 3. Feed conversion efficiency during trail

Group	Control (C)	Experimental I
1. - 2. week	1.45	1.22
Index (%)	100.00	84.48
3. - 4. week	1.77	1.53
Index (%)	100.00	86.31
5. - 6. week	2.19	2.04
Index (%)	100.00	93.36
1. - 6. week	1.92	1.73
Index (%)	100.00	89.81

Table 3 presents the results of feed intake needed to increase chicken body weight for one kg, feed conversion efficiency. Better conversion efficiency during the whole trial period was in chickens of experimental group I. Average feed intake per kg of gain was 1.73 kg experimental group, what was for 10% lower than for control group.

Table 4. Average values of chilled carcases weight, breast meat weight and basic components parts in chickens breast of the control (C) and experimental group I

Group	Chilled carcases weight ns		Breast meat weight ns		Meat part	Bon part ns	Skin and subcutaneo-us fat part ns
	(g)	(%)	(g)	(%)	(%)	(%)	(%)
C	1578.5	100	491.1	31.12	70.02 a	20.72	9.27
I	1539	100	476.9	31.01	73.36 b	19.95	7.48

ns ($p > 0.05$)
a,b($p<0.5$)

Investigation of carcasses quality (Table 4) of control and experimental group showed that higher weight of chilled carcasses (1578.5 g), and breast meat 491.1 g were in control group. Differences in weight of chilled carcasses and breast meat were not statistically significant ($p>0.05$). Although the breast meat weight in control group was higher, meat yield in chickens' breast was significantly higher ($p<0.05$) in experimental group (73.36%), comparing to the control. Bone, skin and subcutaneous fat shares were lower in experimental group.

Table 5. Basic chemical composition of chicken breast meat of control C and experimental group I

Group	Moisture ns	Protein ns	Free fat ns	Total ash ns
			(%)	
C	74.41 ± 0.53	21.82 ± 0.95	2.61 ± 1.43	1.15 ± 0.06
I	74.46 ± 0.51	22.86 ± 0.42	1.52 ± 0.59	1.15 ± 0.04

ns-($p > 0.05$);

Investigations of basic chemical composition of chicken breast meat are shown in Table 5.
Water content of both examined groups, as well as total ash content, were equal. Group I had higher protein content, comparing to the control group. Majewska et al. [11] reported that usage of free extract of raw garlic in a proportion of 0,5 g/cm^3 of water for turkeys fattening achieved a highly significant increase in protein content in breast meat. On contrary, Gardzielewska et al. [5] reported lower protein content in breast meat within the group that received feed supplemented with 0.3% crushed fresh garlic. The content of free fat was 42% lower in experimental group I, than in control group, what is in accordance with results of Gardzielewska et al. [5] who noted fat reduction of 43%.

CONCLUSION

Modification in chickens' diets, that is supplementation with garlic powder, significantly improved birds body weight at the end of trail, comparing to the control. Garlic addition

contributed better feed conversion efficiency in experimental group. Chilled carcasses of control group had higher weight, but the meat yield in chickens' breast was significantly higher in experimental group. Feed supplementation with garlic powder did not significantly effected nutritive quality of meat, although the protein content was a bit higher, and fat content lower in experimental group I.

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MYCOTOXINS ADSORBENTS IN PORK FEED – EFFECTS ON CARCASSES AND MEAT QUALITY

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ABSTRACT

This paper investigates the influence of adsorbents mycotoxins addition in pigs diet on the carcass and meat (*M. Semimembranosus*) quality. In basal mixture for pigs fattening three different adsorbents: Min-a-zel (inorganic adsorbent) and Mycosorb and Mycofix (organic adsorbent) were added.
Carcass quality (yield of meat in carcass) was determined by the method of two points, while the technological quality of meat was investigated determining: initial and final temperature, initial and final pH, water holding capacity (WHC), initial and final lightness (L^*), initial and final share of the red (a^*) and yellow (b^*) colour.
The nutritive quality of meat was investigated determining the content of moisture, protein, free fat, total ash and by determining the content of total pigments as colour parameters.
Results shown numerically but not significantly higher meat yield (P>0.05) in carcasses of pigs fed with the addition of adsorbents Mycosorb and Min-a-zel compared with the meat yield in carcasses of pigs fed standard mixtures. According to parameters and criteria for meat quality it was determined that the average technological quality of meat of control and experimental groups was normal RFN (reddish, firm, non-exudative).
The highest protein and the lowest free fat content had *M. Semimembranosus* of pigs fed with the addition of Min-a-zel, but these values were not statistically significant (P>0.05) comparing to the control group.
Results obtained during the investigations point to a positive opinion on the use of mycotoxins adsorbents (Mycosorb and Min-a-zel) in pigs feed.
***Key words**: mycotoxins adsorbents, pork, quality carcass and meat*

INTRODUCTION

Mycotoxins are toxic secondary metabolites of fungi (*Fusarium, Aspergillus, Penicillium*), and in the animals and human organisms reach via contaminated food [21]. Mycotoxins are harmful for animals and if they are present in feed, even in small quantities, they negatively affect the animal health and production results [20]. Disorders in animal production results are very common. Lower feed conversion and lower production parameters generally are associated with the use of food contaminated with mycotoxins [12].

Effective methods for decontamination of feed are very expensive and also impractical, especially if there is a large amount of feed. One of possibility is to add mycotoxins adsorbents to feed [3]. Adsorbents can bind mycotoxins during the feed passage through the digestive tract and in that way decrease resorption of mycotoxins that are in feed [18]. There are many types of adsorbents for binding mycotoxins, and they can be either organic or inorganic.
Commonly used inorganic adsorbents are: activated carbon, hydrated sodium calcium aluminosilicates and zeolite (Min-a-zel). Recently the possibility of organic adsorbents usage is investigated, and among these adsorbents is modified manan-oligosaharids isolated from cell wall of *S.cerevisaea* (Mycosorb) [2]. Experimental and practical experiences, indicate that the use of adsorbents can decrease and / or prevent negative effects of mycotoxins [13].
Quality of carcass and meat is influenced by a number of exogenic and endogenic factors. Feeding as the exogenic factor is responsible for more than 30% of halves and meat quality. That is why in regions with developed pork production, big attention is paid to feeding i.e. optimisation of animals′ meals [11].
However, literature data relating with the effects of feed supplementation with mycotoxins adsorbents on pork meat quality are lacking. Therefore, the objective of this study was to investigate the influence of feed supplementation with organic (Mycosorb and Mycofix) and inorganic (Min-a-zel) mycotoxins adsorbents on carcass and meat quality.

MATERIALS AND METHODS

In these investigations pigs of the same genetic origin and equal mass, of approximately 25 kg, were used. The control group (K) obtained standard mixture for pigs and the experimental groups were fed the same mixture with the addition of 2 g/kg of mycotoxin adsorbents: O_1 group - Mycosorb-organic adsorbent; O_2 group - Min-a-zel - inorganic adsorbent and O_3 group - Mycofix - organic adsorbent. Feeding of all groups of animals ended when the average mass of pigs was 97 – 110 kg. Transport, stunning, exsanguinating and carcass processing on the slaughter line were accomplished applying the standard technological procedure. At the end of slaughter line the carcass quality was examined.
Quality of carcass, that is, meat yield was determined by the method of two points on hot right carcass. The thickness of fat tissue was measured at two measuring points LF (between 3rd and 4th lumbar vertebrae), and RF (between 3rd and 4th ribs), and using the mathematical models the yield of meat was calculated [4] and determined quality classes of carcass (SEUROP) [1]. Technological and nutritional quality of meat was investigated on the caudo-cranial part leg, *M. Semimembranosus* (SM).
Temperature was measured on right carcass, in deep leg, 45 min ($T_{45'}$) and 24 h *post mortem* (T_{24h}), using the portable thermometer (Consort T651, Turnhout, Belgium). pH was measured in right carcass 45 min ($pH_{45'}$) and 24 h *post mortem* (pH_{24h}) with pH-meter ULTRA X, type US 390 (Gronert, Germany), with INGOLD combined penetrating electrode [10]. Colour measurements were performed with photo colorimeter MINOLTA CHROMA METER CR-400 (Minolta Co., Ltd., Osaka, Japan) on SM 45

min ($L^*_{45'}$, $a^*_{45'}$, $b^*_{45'}$) and 24 h *post mortem* (L^*_{24h}, a^*_{24h}, b^*_{24h}). Water holding capacity (WHC_{24h}) was determined by compression method and expressed as % of bound water [5].

From samples of SM external fat and connective tissue were removed and meat was homogenized and kept in polyethylene bags at temperature of -18°C until determination of the basic chemical composition.

Nutritional quality of meat was investigated determining: content of moisture [8], total ash [9], free fat [6], proteins [7] and the content of total pigments, as parameters of colour using Möhler's modification method.

All data are presented as ± standard deviation. The results were evaluated statistically using the analysis of variance and Duncan's multiple range test in the Statistical Analysis System [19].

RESULTS AND DISCUSSION

The results presented in Table 1 shows that the average yield of meat in carcass of pigs ranges from 48.71% (K) to 51.77% (O_2). Using the method of two points numerically but not significantly higher ($P>0.05$) meat yield in carcass of pigs fed with the addition of adsorbent mycotoxins was determined, comparing to the value found in carcass of pigs in the control group.

Carcass of pigs from experimental groups O_1 and O_2 belong to the quality class E, while carcass of control (K) and experimental group O_3 belong to the class U, based on the calculated percentage of meat yield (Table 1).

Table 1. Average values of LF, RF and meat yield in carcass of pigs from control and experimental groups

Parameters	LF (mm)	RF (mm)	Meat yield (%)	Class
K	$29.90^{ns} \pm 5.20$	$23.50^{ns} \pm 5.15$	$48.71^{ns} \pm 3.24$	U
O_1	$25.10^{ns} \pm 7.96$	$20.10^{ns} \pm 6.76$	$51.61^{ns} \pm 4.71$	E
O_2	$25.20^{ns} \pm 3.55$	$18.60^{ns} \pm 2.50$	$51.77^{ns} \pm 1.92$	E
O_3	$29.70^{ns} \pm 3.83$	$23.10^{ns} \pm 6.31$	$48.87^{ns} \pm 2.54$	U

^{ns} indicates not significant difference at $P > 0.05$

Average values of T_i (Table 2) of all examined groups of pigs carcasses exceed the maximum allowable temperature, i.e. all T_i values were higher than 40°C, what indicates that all four groups of pigs were under stress when exsanguinated, and that animals were not properly prepared for slaughter, and that welfare of animals was not observed [15].

Results of T_{24h} indicates that main demand in means of hygienic quality of processed meat of experimental O_3 and control K groups were not satisfied, while this demand were satisfied at experimental O_1 and O_2 groups, because average values of T_{24h} in deep leg of this groups were lower than 4°C [15]. Determined $pH_{45'}$ values were higher than 5.8 in SM of all examined groups. The highest $pH_{45'}$ value was for control group (K), with statistically significant differences with O_1, O_2 ($P<0.05$), and O_3 ($P<0.001$) group. The

pH_{24h} value measured in SM samples ranged from 5.72 (group O_1) to 5.90 (group O_3) but differences were not statistically significant (P>0.05). Lightness (L^*_{45}) of examined SM was between 41.25 and 42.55 and the differences were not statistically significant (P>0.05). Lightness (L^*_{24h}) of examined SM ranged between 42.79 and 46.98 for experimental groups O_3 and O_1, respectively. The L^*_{24h} value of the control group and experimental groups O_1 and O_2 were statistically significant higher compared to the values of the experimental groups O_3 (P<0.05).
The highest share of the red colour (a^*_{24h}) was found in SM experimental O_3 group, significantly higher than control group (P<0.05) and experimental group O_2 (P <0.01). The lowest share of the yellow colour (b^*_{24h}) was found in SM experimental O_2 group, statistically different than control group (P<0.01) and experimental group O_1 (P<0.05).

Table 2. Average values of technological quality parameters (M. semimembranosus) for control and experimental groups of pigs

Parameters	**K**	**O_1**	**O_2**	**O_3**
$T_{45'}$ (°C)	$41.65^{bcdAB} \pm 0.79$	$42.27^{aA} \pm 0.28$	$41.94^{abcAB} \pm 0.58$	$41.36^{dB} \pm 0.39$
T_{24h} (°C)	$6.23^{AX} \pm 0.99$	$3.70^{CZ} \pm 0.28$	$3.57^{CZ} \pm 0.47$	$5.46^{ABXY} \pm 0.33$
$pH_{45'}$	$6.25^{a,A} \pm 0.22$	$6.01^{b} \pm 0.16$	$6.04^{b} \pm 0.24$	$5.90^{B} \pm 0.28$
pH_{24h}	$5.78^{ns} \pm 0.27$	$5.72^{ns} \pm 0.21$	$5.75^{ns} \pm 0.27$	$5.90^{ns} \pm 0.27$
$L^*_{45'}$	$41.25^{ns} \pm 1.43$	$42.04^{ns} \pm 1.62$	$42.55^{ns} \pm 1.71$	$41.62^{ns} \pm 2.25$
L^*_{24h}	$46.27^{a} \pm 2.62$	$46.98^{a} \pm 3.56$	$46.49^{a} \pm 4.76$	$42.79^{b} \pm 1.32$
$a^*_{45'}$	$5.88^{ns} \pm 1.29$	$6.71^{ns} \pm 1.19$	$5.52^{ns} \pm 1.23$	$6.63^{ns} \pm 1.60$
a^*_{24h}	$7.96^{bcAB} \pm 0.84$	$8.34^{abAB} \pm 0.76$	$6.99^{cB} \pm 1.16$	$9.32^{aA} \pm 2.08$
$b^*_{45'}$	$3.12^{AB} \pm 0.72$	$3.59^{A} \pm 0.56$	$3.20^{AB} \pm 0.46$	$2.77^{B} \pm 0.35$
b^*_{24h}	$5.19^{aA} \pm 0.57$	$4.54^{aAB} \pm 0.96$	$3.61^{bB} \pm 1.07$	$5.18^{aA} \pm 1.15$
WHC_{24h} (%)	$81.32^{a,A} \pm 4.27$	77.38 ± 4.96	$75.10^{B} \pm 5.22$	$76.80^{b} \pm 3.27$

^{abc} indicates significant difference at P < 0.05;
^{ABC} indicates significant difference at P <0.01;
^{XYZ} indicates significant difference at P < 0.001

According to the defined parameters [16] and the criterions («normal meat» RFN: $pH_{45'}$>5.8; pH_{24h}<6.2; L^*=43–50; SVV (%)>50) for determination of technological quality of meat it was defined that the average technological quality of all examined SM (control and experimental groups) was RFN (reddish, firm, non-exudative).
Meat is very rich in nutrients and plays important role in human diet [17], thus it is very important to investigate if the addition of mycotoxins adsorbents have any influence on nutritional quality of pork meat.
Addition of mycotoxins adsorbents to feed mixture had no significant influence on average water content (Table 3) in the examined SM samples. The presented results indicate that adsorbents in the diet did not demonstrated a significant impact on the average water content in the SM. Content of total ash in meat is about 1 %, while in lean meat it can be higher, up to 1.5 % [17], as also confirmed in this investigation.

Fat content in muscles of control group was higher than in muscles in all experimental groups (O_1, O_2, O_3). The lowest fat content was found in experimental group O_2, however the differences were not statistically significant (P>0.05).
It is important to mention that the average free fat content in *M. semimembranosus*, both in control and experimental groups was higher than 2% - the upper limit for SM muscles of good quality, intended for processing highest quality meat product, especially cooked ham [14].
The highest average protein content (21.22%) was found in SM experimental O_2 group, significantly higher (P<0.05) than in group O_3. Which means that meat obtained from pigs fed with diets supplemented with inorganic adsorbent (Min-a-zel) had better quality, from nutritional point of view. Protein content higher than 21% had SM of experimental O_2 group. Vidovic [19] reported, that 21% of protein content is the bottom limit for quality muscles (*M. semimembranosus*) of pigs, what is the basic task in modern pig raising.

Table 3. Average values of nutritional quality parameters (M. semimembranosus) for control and experimental groups of pigs

Content of basic parameters	**K**	**O_1**	**O_2**	**O_3**
Moisture (%)	$74.47^{ns} \pm 0.75$	$74.54^{ns} \pm 0.70$	$74.55^{ns} \pm 0.64$	$74.64^{ns} \pm 0.60$
Total ash (%)	$1.13^{bcBXY} \pm 0.02$	$1.07^{cBY} \pm 0.12$	$1.17^{abABXY} \pm 0.08$	$1.24^{aAX} \pm 0.07$
Free fat (%)	$3.72^{ns} \pm 0.54$	$3.62^{ns} \pm 0.90$	$3.06^{ns} \pm 1.04$	$3.44^{ns} \pm 0.79$
Proteins (%)	$20.69^{a,b} \pm 0.46$	$20.77^{a,b} \pm 0.45$	$21.22^{bc} \pm 0.86$	$20.55^{d} \pm 0.46$
Total pigments (µg/g)	$59.02^{a} \pm 11.45$	$55.42^{b} \pm 9.94$	$48.96^{bc} \pm 7.92$	$54.54^{d} \pm 6.32$

^{abc} indicates significant difference at P < 0.05;
^{ABC} indicates significant difference at P <0.01;
^{XYZ} indicates significant difference at P < 0.001

The average content of total pigments (Table 3) was the highest in the SM of pigs′ carcasses from the control group (K) and amounted to 59.02 µg/g, while in the SM from carcasses of all experimental groups of pigs was lower. The lowest content (48.96 µg/g) was determined in the SM of group O_2. It is also observed that the SM of carcasses of pigs from the control group had significantly higher content of total pigments in comparison with the SM of all experimental groups (P<0.05). This indicates that the mycotoxins adsorbents affect the reduction of the total content of pigments, what is probably the result of slightly higher growth [4].

CONCLUSION

Analysis of the results indicated numerically but not statistically significant higher meat yield in carcasses of pigs fed with the addition of adsorbents Mycosorb and Min-a-zel compared with the meat yield control group, while the influence of adsorbent Mycofixa on meat yield had not been determined.

According to parameters and criteria for meat quality it was determined that the average technological quality of meat, control and all experimental groups, was normal RFN (reddish, firm, non-exudative).
Analysing the results of basic chemical composition, it can be given a positive opinion from nutritional point of view on the use of mycotoxin adsorbent Min-a-zel, because it was determined numerical but not significant higher protein content (P>0.05) in carcass of pigs fed with the addition of Min-a-zel.

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REGULATIONS AND ECOLOGICAL RISKS OF FOOD PROCESSING INDUSTRY OF SERBIA APPROACHING THE EUROPEAN UNION

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ABSTRACT

The inclusion in the international trends and approximation with the European Union brings also the necessity of harmonisation of national legislation with the European and world legislation. Adopting of regulations, harmonised with the international one, does not include only a legal aspect but it also makes the pre-condition for export of domestic products and possibility of financial support. Passing of a set of regulations in the field of environmental protection, which are harmonised with the EU legislation, makes a concrete contribution to these processes. The analysis of ecological risks and impacts on the environment, which needs to be carried out, represents a significant segment of these processes. The analyses of effluents (by-products) in food production have shown that those effluents also contain different quantities of toxic substances that pollute the environment as they are discharged or disposed. The largest polluters in our country include energy production, petrochemical industry, and chemical industry. Effluents and pollution caused by them have been processed and resolved as acute. Due to a circular flow of pollution through air, water, soil, flora, and fauna, it is transferred into the food chain and threatens health safety of food production, both in our country and in the world. The waste coming from food industry is a big problem but it can also make a rich source of raw materials in other bio-technologies, pharmaceutical industry, source of energy, compost etc. Through application of the best available techniques, obtaining of integrated permits for the operation of food-processing industry plants types of activities, supervision, and other issues of significance for prevention and control of environmental pollution are defined. "The best" technique refers to the most efficient performance in accomplishing of a high general level of environmental protection.
***Key words**: regulations, risk analysis, environmental protection, food industry*

INTRODUCTION

Understanding the significance of use of effluents in the chain of mass production of food, in accordance with scientific and technological capacities, the methods are searched for their practical, safe and economically justified resolving. An appropriate monitoring is set after the evaluation of the current status and analysis of environmental impact. The results of monitoring, best available techniques (BAT), national legislation that needs to be harmonised with the world standards are decisive for setting up of a model of environmental friendly disposal of effluents coming from food-processing industry. The environment is threatened by generating and piling up of waste materials.

More and more attention is paid to it and activities are in progress aimed at its protection and improvement.

Requirements and benefits of countries that are potential EU members from the aspect of environmental protection

Striving to produce all the larger quantities of material properties, which should satisfy the man's needs for all the higher standard of living and create the optimum conditions for maintaining of health status, modern civilisation also generates large quantities of waste materials that have negative impact on the environment degrading it up to such an extent that it becomes harmful to health of people and wildlife. [8]
Food industry is a large consumer of technical water. The extraction and separation of starch from proteins, which is isolated by means of flushing in the form of gluten in wheat flour in one of about twenty patented ways, requires water quantities that are up to 8 times larger on the average compared to quantities of the input raw materials. [6]
The presence of about 80% of organic waste in morphological composition of municipal waste is a large problem. [5]
The EU accession imposes a significant task in front of the potential member states, which also implies the transfer of the so-called EU "ecological" Directives into national legislation, their implementation, and enforcing. The European legislation in the field of environmental protection ("Environmental acquis") consists of about 300 Directives and regulations, including the "sister Directives" and amendments. The calculated necessary investments for reaching of standards range from 80 to 120 billion EUR for 13 countries of central and east Europe (which were the last to join the EU) in environmental protection sector alone. In other sectors, the investments necessary for harmonisation with the EU legislation are also significant. On the other hand, there is an emphasised limitation to necessary resources, both financial and administrative ones. The paper presents some of the aspects of benefits from the EU accession in environmental protection sector from the aspect of food-processing industry and key legislation that refer to it. Considering the obligations set in front of the potential EU member states by transferring and implementation of the EU legislation in environmental protection sector, the following requirements are also imposed:

- Improvement and extending of water supply network aimed at providing necessary quantity of drinking water for all inhabited areas in the country;
- Improvement and extending the sewerage and waste water disposal network, as well as construction of waste water treatment plants;
- Reduction of emissions of pollutants into air, in particular from large burning plants;
- Improvement of air quality, in particular in urban centres;
- Control and prevention of emissions of harmful substances from plants and minimising the risk from occurrence of accidents;
- Collection, treatment, and disposal of waste from households, industries and medical institutions;
- Cleaning of contaminated soil and polluted rivers where water quality is not satisfactory;

- Protection of eco-systems, habitats and protected species against economic and ecological pressure;
- Reduction of emissions from traffic (passengers' and freight);
- Reduction of emissions of pollutants from economic sector, such as large industrial plants and agriculture.

The above-mentioned requirements are not only imposed in front of the potential EU member states as all the EU Member States are faced with those problems. However, potential member states have to invest much larger efforts, taking into account the previous insufficient investments into environmental protection sector. On the other hand, they still have the examples of positive and negative experiences of countries that have already travelled along that road.
The benefits from implementation of the EU requirements that are visible in the first iteration include:

- Improvement of health status of population, reduced environmental pollution (the reduction of respiratory diseases and fatalities, in particular among infants occur as the results);
- Reduced damage to forests, built structures, agricultural surfaces, fish fund as the result of reduced acid rains and other forms of pollution. This leads to a wider economic benefit (increased profit) and reduction of maintenance costs (works on maintenance-building of facades);
- Reduction of the risk of deteriorating of quality of natural resources (ground waters);
- Better protection of natural eco-systems, endangered species;
- Promotion of tourism as the result of the clean environment (forests, water for bathing, natural resorts);
- Reduction of the risk of transmission of diseases caused by dirty waters and improvement of water taste as the result of requirements for better drinking water and quality of water for bathing;
- Increase of economic efficiency and productivity as the result of implementation of updated technology, which increases competitiveness of industry;
- Lower production and maintenance price through clean water availability. reduction of the need for pre-treatment of the supplied water;
- Lower consumption of primary raw materials as the result of implementation of an efficient system of a renewed use and recycling;
- Support to employment and benefits for local and regional development;
- Support to the development of companies and their benefit through raising of awareness on the risks for the environment, access to risk minimisation and response to the risk that has occurred.

When we talk about benefits of implementation of the EU legislation in the field of environmental protection the same can be shown in the form of the following Table:

Table 1. Benefits from transfer of the EU requirements in the field of environmental protection [4]

Type of benefit	**Air**	**Water**	**Waste**	**Nature**
Health	Prevention of respiratory diseases and mortality among infants	Clean drinking water in households and clean bathing water	Reduction of risk against Poisoning and accidents caused by emission of methane from landfills.	Preservation of nature, bio-diversity
Resources	Prevention of damage for structures and corporations	Clearer ground waters and surface waters for bathing	Reduction of use of primary materials and energy generating	Rational utilisation of resources
Eco-system	Prevention of global heating caused by CO_2 emission	Improvement of quality of river, lake, sea water	Prevention of global heating caused by emission of methane from landfills.	Protection of nature resorts and wildlife species
Social	Improvement of the status of cultural heritage of less damaged historical sites	Recreation on rivers and surface waters	Raising of awareness on personal responsibility and impact on the environment	Access to protected areas
Economic	Cultural tourism. Attractive investments, increase of employment rate through environmental protection	Development of tourism (clean beaches). Reduction of prices of pre-treatment and possibility of new investments at a local level.	Reduction of import of primary raw materials. Attractive investments contribute to quality of a site	Eco-tourism

Reduction of damage in agriculture is expected along with the implementation of EU Directives through reduction of pollution caused by excessive use of pesticides and fertilisers, which can contribute significantly to increase of yields and quality of

products, better protection of eco-systems that are under significant impact of pollution from the air and water.
The benefits that are the result of implementation of EU Directives on nature preservation are mainly connected with the establishment of Natura 2000 Network of special, protected areas in the candidate countries. Bio-diversity and eco-system shall also benefit from other Directives in the EU legislation on environmental protection, e.g. through better quality of water and air, which reduces the pressure on protected areas.
Finally, the implementation of Habitats Directive shall help to reduce the problems that arise with habitats due to the uncontrolled urbanisation, intensive forest cutting and intensive agricultural activities, as well as damages caused to protected areas, e.g. in the Danube delta. The implementation of the Directive on habitats and wild birds shall assist:
• In many cases through increase of surfaces within the protected area;
• Through raising of the level of protection within the protected area;
• Through identification of species that need to be protected;
• Through adopting of specific protection measures at each specific territory (e.g. prohibition of use of pesticides).
It is not possible to quantify, but social benefit shall be seen in better nature and protection of species. The civil society shall have significant benefits from the increase of communication and distribution of information on the environment, increase of consultation based approach and inclusion in the streams of implementation of the Directive (e.g. inclusion of consumers in the process shall assist in raising of awareness on their role and impact on the environment). Finally, the employment shall rise along with the investments in environmental protection, which shall support the stability of the society.

Outline of the adopted laws the field of ecology

Enactment of a set of laws in December 2004 made a concrete contribution to those processes. They include:

- Law on Environmental Impact Assessment
- Law on Strategic Environmental Impact Assessment
- Law on Integrated Environmental Pollution Prevention and Control; and
- Law on Environmental Protection [13]

Comparing it with the European Community Directive 96/ 61/ EC (1996) [1], the Law on Integrated Environmental Pollution Prevention and Control represents a national law that sets forth the same provisions. This Law regulates the conditions and procedures of issuing of integrated permits for plants and activities that can have negative effects on human health, environment, or material property, types of activities and plants/installations, supervision and other issues of significance for environmental pollution prevention and control. This Law implements certain new terms into practice, such as the term “best available technique” or “BAT” that implies the most effective and advanced stage in the development of activities and their methods of operation which enable more suitable implementation of particular techniques for compliance with the emission limit values prescribed to prevent and reduce emissions and impacts on the

environment as a whole. The technique implies the way in which the installation is designed, built, maintained, operated and decommissioned or closed, including the technology that is used. Available means techniques that have been developed on a scale which allows the implementation in the relevant industry sector, under economically and technically acceptable conditions, including costs and benefits, as long as they are reasonably accessible to the operator (physical or legal entity that operates the installation, controls it or that is authorised to make economic decisions in the field of technical functioning of the installation and in the name of which the integrated permit is issued). "The best" means the most effective in achieving a high general level of protection of the environment as a whole. The original European Directive (96/ 61/ EC (1996)) also lists the industrial branches that it refers to, which include the food-processing industry processing raw materials of plant origin with the daily capacity exceeding 300 tons of final products. [18]

The Council Directive 91/676/EEC on protection of waters against pollution with nitrites coming from agricultural sources requires the states to develop standards for healthy agriculture, which would also include training and information. [2]

The Directive 86/278/EEC on environmental protection, and soil in particular in thecae of use of secondary fertilisers in agriculture – use of mud in agriculture aimed at prevention of pollution of soil, vegetation, people and animals. The list of the main polluting substances for which it is necessary to set the emission values also includes the substances that pollute waters causing undesirable impact on the oxygen balance that can be measured through HPK, BPK5 and similar parameters and that can also encompass the oils of plant origin. [3]

The Guidelines of the Integrated Pollution Prevention and Control Bureau (IPPC) are laid down through BREF – BAT reference documents (2004) that refer to the best available techniques in certain industries – production of food, drinks and milk, production of plant oils etc. [14]

In May 2009, the Parliament of Serbia passed some additional laws in the field of ecology: Law on Protection Against Non-ionising Radiation and Law on Chemicals, with which the national legislation in that field is harmonised with the EU legislation. The Parliament also passed Law on Biocide Products, which should set a safer system of placing of those products on the market and their use with an adequate information for consumers on methods of their use [16], as well as the Law on changes and amendments of the Law on Environmental Impact Assessment, which should shorten the period of obtaining of permits for environmental impact assessment studies. The Law also provides the possibility for the competent authority to set the minimum environmental protection measures based on the decision on liberation from the obligation to elaborate the environmental impact assessment study. [24]

The Law on Protection Against Non-ionising Radiation anticipates decentralisation and more efficient implementation of measures and supervision in use of sources of non-ionising radiation. The enacted Law shall enable more efficient implementation of measures of protection of health of people and protection of the environment against detrimental effect of such radiation. It also defines special sensitive cases such as schools, nurseries, and health care institutions where it will not be possible to install sources of non-ionising radiation such as antenna systems of telecommunication devices. [21]

The Law on Chemicals prescribes that chemicals are manufactured, imported, and used in the way that is safe for human health and the environment. The main objective of this Law is safe placing of chemical products on the market with provision of a high level of protection of human health and the environment. A specific objective is improvement of safety in trade with chemicals with other countries. [17]
The Parliament also passed the Law on Air Protection that sets froth the measures of protection of quality of air and reduction of emission of substances with negative effect on health. [25]
They also enacted the Law on Nature Protection that prescribes the protection of natural resources, setting up of the system of monitoring of natural values and protected natural properties. It also defines protection of nature and landscapes in spatial plans and planning documentation, passing of programmes of management with natural resources and raising of awareness on the need for nature protection in the education process. [23]
The Parliament also enacted the Law on Protection Against Noise in the Environment that sets forth the measures for prevention and reduction of harmful effects of noise on human health and the environment. The elaboration of strategic noise maps at the level of Serbia, autonomous province and local self-government unit is the novelty contained in the Law, along with the elaboration of action plans that should define measures for reduction of exposure to noise. [20]
The Law on Fish Fund Protection and Sustainable Use has also been enacted and it regulates the preservation and protection, as well as catching, use and trade with fish. [22]
The Law on Waste Management should prevent inadequate waste handling, which is one of the largest environmental problems in Serbia. This Law, which is harmonised with the European legislation, sets up an integrated waste management aimed at creating the conditions for reduction of waste generation, and development of cleaner technologies. [19]
The Law on Packing Material Waste sets forth the establishment of environmental protection standards that packing material needs to satisfy, but it also creates conditions for an integrated management with packing material and waste originating from packing material. [15]
Through changes and amendments, the Parliament passed the Rotterdam Convention on permit issuing procedure based on previous information for certain hazardous chemicals and pesticides in international trade, as well as the amendments to the Annex B of the Kyoto Protocol accompanying the Framework Convention of Climate Change.

INSTEAD OF THE CONCLUSION

The evaluation of overall benefit from transposing and implementation of the Environmental Acquis is based on analyses of changes in pollution that could be attributed to harmonisation with the Directives and effects on recipients (e.g. health of the population, environmental amenities, and values and productivity of natural resources). The benefits from harmonisation with the EU Directives also contains the non-economic benefits, in particular the protection of sensitive eco-systems and biodiversity, as well as non-ecological benefits such as the increase in economic activities connected with the building and other works on infrastructure necessary in

order to preserve the environment. When it comes to protection of natural resources, full harmonisation with provision shall provide for protection of thousands of hectares of valuable habitats and hundreds of endangered species against threats from socio-economic activities. The contents of toxic metals and pesticides need to be monitored, micro-biological contamination at farms needs to be reduced, and hygiene of all those who handle food needs to be enhanced. The introduction of the HACCP System sets the implementation of analysis of threats and critical control points, which represents a prevention based approach and more efficient method compared to the control of finished products. This covers all the production stages – from the field to the table. In accordance with that, the animal feed production represents an exceptionally important segment due to its sensitivity to micro-biological correctness, viruses, and mycotoxins while the impact on the environment itself is brought down to noise, dust, and wastewater control and deodorisation in the case of organic waste use in production of ready-made mixtures or concentrates. Pollution of the environment with animal waste shows other negative sides as well. It is known that places where organic matter is deposited and degraded create ideal conditions for existence of other insects and rodents. They enable spreading of infections and contribute significantly to degradation of a visual impression of the area they inhabit. Irregular manipulation with dead animals and by-products from the farms and slaughter houses results with pollution of soil, running and ground water, food and different articles, which makes them unsuitable or less valuable for consumption. [10], [12], [7]

Aesthetic unacceptability of the environment that is threatened in such a way is one of the problems to which more and more time and space are devoted.

Pollution of the environment, in particular of air and water, can also occur in the process of harmless disposal of dead animals and indigestible by-products of slaughtered animals and their processing into animal feed products and products for chemical industry. Therefore, »the structures for animal waste processing« need to be analysed in a dual way – in the service of environmental protection, namely as production units and, in parallel, as polluters of the environment. [11]

The polluters from the process of harmless disposal include the bulk input raw materials, wastewater, waste gasses, organic dust and contaminated solid substances that are not suitable for processing.

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20. Zakon o zaštiti od buke u životnoj sredini
21. Zakon o zaštiti od nejonizujućih zračenja
22. Zakon o zaštiti i održivom korišćenju ribljeg fonda
23. Zakon o zaštiti prirode
24. Zakon o izmenama i dopunama Zakona o proceni uticaja na životnu sredinu
25. Zakon o zaštiti vazduha

FUTURE CHALLENGES FOR RESEARCH AND DEVELOPMENT IN FEED TECHNOLOGY

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ABSTRACT

The global demand for animal products is expected to grow dramatically over the coming decades, driven by an ever increasing population and better per capita income which translates into a higher demand for animal products. Also, the demand for feed is developing at a rapid pace and it is essential for the Fed Industry to be able to meet this demand in a sustainable manner. Solutions to these sustainability issues require a full production chain approach. A major technological jump will be required to achieve these big demands. For this technological jump to take place, the admittedly strong efforts of the feed industry on this front need to be complemented by public sector efforts, where leading academic, public research establishments, FAO, IFIF, FEFAC and similar organizations and inter-governmental institutions are to be involved. The Feed industry can and has to play a leading role in feed/livestock food technology development by supporting research in this area. Challenges for feed industry are also challenges for research and development in whole feed to food chain.
Key words: *feed, technology, challenges, development, research*

INTRODUCTION

Worldwide hunger has reached a historic high in 2009 with 1.02 billion people going hungry every day. A dangerous mix of the global economic slowdown combined with stubbornly high food prices in many countries, poor regulation of financial markets, food-feed-biofuel competition and failures in economic governance has pushed some 100 million more people since last year into chronic hunger and poverty. Many of the world's poor and hungry are smallholder farmers in developing countries. Yet they have the potential not only to meet their own needs but also to boost food security and catalyze broader economic growth. To tap this potential, however, governments, supported by the international community, need to ensure an enabling business environment that improves the efficiency at the production, processing, marketing and export stages. The public sector, *i.e.* governments at the national, regional and global level, have the responsibility to set up the normative, institutional, regulatory and legal frameworks which are necessary for securing the achievement of society's objectives. Such objectives are e.g. preservation of public health, social equity and justice and sustainability of natural resources and the environment.

The global demand for animal products is expected to grow dramatically over the coming decades, driven by an ever increasing population and better per capita income which translates into a higher demand for animal products. In a rapidly changing world where living standards are continually rising and consumerism is driving economic

progress we must not overlook the importance of keeping our food from animal sources safe and in sufficient quantities to meet growing demand. In 2050, 9.2 billion people will have to be fed, requiring much more animal source food than currently with little more than 6 billion people.When it is recognized that production will have to come from an agricultural surface which is shrinking and degrading in many areas, it will be required to increase resource use efficiency very drastically. FEFAC is involved in the debate on the future evolution of the Common Agriculture Policy (CAP) from a feed & food chain perspective. Beside challenges identified in the framework of the Health Check of the CAP, FEFAC as the challenges for the EU agriculture in 2013 and beyond underlined that EU agriculture must continue its basic mission of producing safe agricultural goods, offering a large choice of different types of products with different qualities, meeting the demands of the EU and global consumers at affordable prices, and **conclude that the EU agriculture must produce more, better, everywhere and at an affordable price.**

FEED INDUSTRY

A major technological jump will be required to achieve so big demands. For this technological jump to take place, the admittedly strong efforts of the feed industry on this front need to be complemented by public sector efforts, preferably in Public/Private Partnerships arrangements where leading academic, public research establishments and inter-governmental institutions are to be involved. According to FAO such type of synergistic action can be envisaged on most elements of FEFAC's mission statements of which noted:

- legislative framework and its implementation relative to the compound feed companies;
- safeguarding conditions of free access to raw materials, the functioning of their markets and the definition of their quality and
- development of professional rules and good manufacturing practices that ensure the quality and the safety of compound feed;

Collaboration between FAO, FEFAC, IFIF, and their members has resulted in a series of successful outcomes. Collaboration is particularly beneficial in areas such as the implementation of relevant international regulations and codes of practice – such as those of the Codex Alimentarius, Guide to good manufacturing practice (EFMC), Manual of Good Practices for the Feed Industry, but there is scope in other areas as well such as in environmental impact analysis and management of global feed supply and trade. The manual for the feed industry, prepared on the basis of cooperation between IFIF and FAO will be a powerful instrument for the achievement of the common goal of facilitating viable, safe, clean and sustainable animal production. It is also satisfying to note that the manual not only deals with the large scale feed industry requirements but also with those of smaller scale enterprises more frequent in developing, transition and emerging countries. Similarly synergistic effects are also available from the jointly organized meetings of feed regulators. FEFAC is also included in these activities.

SUSTAINABLE FEED PRODUCTION

The demand for feed is developing at a rapid pace and it is essential for the feed sector to be able to meet this demand in a sustainable manner. Solutions to these sustainability issues require a full chain approach. Sustainable development is strategy and development in sustainable feed technologies must involved different aspects like as:

- Feed production
- Animal feeding
- Fedd safe for animal, people and environment
- Feed control and evaluation
- Quality of food of animal origin
- Environmental impact

Animal feed production is not only milling and mixing of grains, nor is it just a feed manufacturing facility, but it denotes complete agricultural and food production throughout various processes of collecting, preserving, storing and processing of basic and by-products serving to improve quality and overall performance of food via animal feed. In that context, production of vitamins, mineral feeds and supplements, non-protein nitrogen compounds, amino acids, enzymes and other additives used in feedstuffs for improving it nutritional value and quality, feed conversion, improved shelf life, simplified technological procedures in feed preparation, coloring meat and eggs, and for many other purposes, is to be mentioned. All these factors together make up animal feed industry. Taking into consideration complexity and diversity of technological procedures involved, interaction and relationship with the agricultural, food and other industries and their place in the food chain, animal feed production cannot be considered separately.

Sustainability is a serious challenge for the whole feed and food chain including researches involved in various research areas within that chain. There is a direct impact of feed production on employments, emission into the environment (dust, noise, waste, gasses..), energy consumption, use of finite resources etc. and the indirect impact on the sustainability of the feed materials supply chain (outlet for by-products from food industry, soya production systems, fish meal production…), and on the sustainability of the livestock production with regard to food safety (salmonella, contaminants, etc), livestock effluents (N and P digestibility, use of feed additives, etc..), feed conversion rates, balanced diets etc.

FEFAC releases its first Environment report on industrial Compound feed as a contribution to the evaluation and the improvement of the sustainability of EU livestock production and published the “Credo”

The FEFAC "Credo"

- FEFAC believes that products of animal origin form an integral part of the European diet providing key nutritional benefits to the European population. Nutritionally optimized feed meeting the physiological requirements of animals and fish raised for food production purposes are essential to mitigate the environmental impact of production and consumption of animal products.
- The EU compound feed industry is willing to responsibly contribute to the sustainable development of livestock and aquaculture production systems. FEFAC believes that the key drivers for such improvements are:
  1. Promotion of ecologically intensive production systems for farm animals and fish, oriented towards maximization of resources efficiency and minimization of Green House Gas (GHG) emissions;
  2. Changes in diet patterns and composition for farm animals and fish to significantly reduce the GHG emissions attributed to livestock production systems (e.g. methane);
  3. Improvement of feed efficiency, i.e. the conversion of feed into animal products, in order to control the use of resources and to reduce the loss of nutrients;
  4. Further optimization of use of co-products from the food industry, biomass and non-organic raw materials to alleviate the pressure on natural resources.
- FEFAC and its Member Associations believe they have a role to play in providing feed companies with tools to measure and improve the environmental performance of their products. FEFAC is committed to play a role as spokesman of the EU compound feed industry to facilitate feed chain cross-sector initiatives to develop standardized methodologies to evaluate the carbon foot print and also to contribute to international agreements on sustainable criteria for feed production.

RESEARCH AND DEVELOPMENT IN FEED-TO-FOOD-CHAIN

The Feed industry can and has to play a leading role in feed/livestock food technology development by supporting research in this area. Challenges for feed production are also challenges for science and development in Feed industry. These challenges are changing during the time and were targeted to different aims:

- Best yield at least cost 1970-1980
- Quality of products (milk, meat, eggs) 1980-1995

- Feed & Food Safety 1996-2000
- Feed for Food 2000-2010

Feed producers became aware of the fact that the future of feed is in changing paradigm from "Feed research per se" to "Feed for food". Feed for Food Starts with the Consumers. Consumers have their food concerned priorities listed bellow in order of importance: raw material, quality, brand, and trust in the producer, official controls, certification, and known product, purity of product, right price, trade trust, origin and package information. In accordance with these demands for research and development in Feed industry are aimed at:

- Consumer Guarantees for safe, healthy, nutritious food
- Food chain collaboration
- Health & wellness:
    - functional foods
    - nutraceuticals
    - nutrigenomics

Research and development in Feed industry must be targeted on raw materials their availability, consistency and impact on animals people and environment. Many ingredients and nutrient variations within and between ingredients demand more research and knowledge in whole supply chain from agriculture to food. Knowledge about composition is necessary to assure consistent quality of products and as the starting point for future research and understanding the nutrient supply from ingredients to nutrients. Understanding ingredient and supplier variability allows formulation of consistent and accurate feeds.

For succesfull feed constitutent utilisation it is important to evaluate nutritient availability for the different animal species and categories on the right way. Contemporary metods include following characteristic as the main determinants of the nutritional value of a feed ingredient:

- Total nutrient content
- Nutrient availability
- Content of antinutritional factors
- Physico-chemical properties
- Ingredient-specific effects on the utilisation of absorbed nutrients
- Effects on voluntary feed intake and
- Effect on final animal product quality (meat, eggs, milk, manure, etc.)

Furtermore, additivity of these characteristics in mixtures of ingredients, variability in each of these characteristics between different batches of the same ingredient, changes in feeding value due to processing, and inexpencive, repeatable, rapid means to asses these characteristics should be considered.

It is important to take care about by-products from new industries especially from biofuel production. Kyoto Protocol ratification commits development countries to an 8% reduction in GHG emission by 2010 which will significantly increase the quantity of these products. Biofuel production pulls the carbohydrates & lipids (energy) out of the raw material leaving unused protein to be put back into the feed. Investigation

concerned on upgrading of biofuel by-products feeding value may have significant contribution on feed industry development.

One of the important research areas is the production of new components specifically designed to meet the nutritional requirements of the animal to produce desired results (fertility, growth, health or naturally enriched products in the food chain). Companies, producers of these new components keep as secret their investigation results, but for livestock and Feed Industry development it will be necessary to collaborate with other industries and share their technology, know how and expertise.

According to paradigm Feed for Food future research should be aimed at:

- Improving food safety
- Strengthening consumer confidence in traceability
- Creating unique foods through feed (ω3 – eggs, Se – milk)
- Collaboration with Human Nutritionists
- Nutrigenomics –Functional foods to prevent food related pathologies
    - Heart diseases
    - Cancer
    - Obesity
    - Osteoporosis
    - Diabetes

These all are the challenges for research and development in feed technology.

Feed safety: a prerequisite to food security and sustainability

Feed safety is nowadays increasingly recognized as an important topic in the feed-to-food value chain and subject to comprehensive EU legislation. The nutritional value, taste, texture and safety of food products such as meat, fish, dairy products and eggs are directly influenced by the nutrition of the animal concerned. Compound feed manufacturers have an important role to play in providing feeds that are nutritious and safe, thereby contributing to the supply to consumers of safe food of high and consistent quality. The challenge for the compound feed manufacturer is to make the best use of available resources without compromising on the high safety standards established in the EU.

Traceability

EU Directive 178/2002 lays down requirements for traceability of all materials used or processed in feed. Under EU law, "traceability" means the ability to track any food, feed, food-producing animal or substance that will be used for consumption, through all stages of production, processing and distribution. In that way the entire animal feed chain, including the primary production of feed, is put on the same level with food and included in quality assurance systems

Creating unique foods through feed

Feed directly contribute to the quality of meat, milk and eggs in possitive and negative direction. Throught feed diet content it is possible to manipulate the animal products quality and it is possible to achieved different nutritional, sensoric, physical and

chemical characteristics. Also, the different contaminants may be transmit to animal products through feed. This indicates the necessity of research related to determining the impact of feed on animal products quality and following the quality of animal products depending on the composition of diets consumed by animals.

Collaboration with Human Nutritionists
Because of the possibility that over feeding affects the composition of animal products for human consumption, cooperation between feed producers and human nutritionists is necessary . Research in this area must be focused on the welfare of animals and people.

Nutrigenomics –Functional foods to prevent food related pathologies
Research on health-promoting aspects of feeds and relatinship between bio-active components in feed and bio-active components in foods is necessary. Heart health, weight and energy management, bone & joint health, digestive health, glycemic control are major health issues that can be affected through feed. Health promotion through milk for people in different fisiological stages and enriched eggs is reality today. Cooperation between doctors and feed concerned researchers is necessary to reach new useful knowledge.

CONCLUSION

"The vision of the ETP Food for Life is to create greater synergy between economic growth, environmental protection and fair-minded social conditions in order to improve welfare and well-being of the European citizen". These also could be the future challenges for research and development in feed technology.

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