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BIOTECHNOLOGY IN ANIMAL HUSBANDRY

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Original scientific paper

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DETERMINATION METABOLIC AND NUTRITIONAL STATUS IN DAIRY COWS DURING EARLY AND MID LACTATION

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Original scientific paper

Abstract: The objective of the present study was to investigate nutritional and metabolic status in Simmental cows during early and mid-lactation. Fifteen early lactating cows and 15 mid lactating cows were chosen for the investigation. Blood samples were collected to measure beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), triglycerides (TG), glucose and the activity of aspartate transaminase (AST). Early lactation as compared to mid lactating cows were found to have significantly higher ($P<0.05$) blood serum concentrations of NEFA, BHB and AST and lower blood serum concentrations of glucose ($P<0.05$) and TG ($P>0.05$). Significantly negative correlations were observed between BHB and glucose ($P<0.01$), BHB and TG ($P<0.05$), NEFA and glucose ($P<0.05$). Significantly positive correlations were observed between NEFA and BHB ($P<0.05$), NEFA and AST ($P<0.05$), glucose and TG ($P<0.01$). The results suggest that these parameters can serve as useful indicators of the nutritional and metabolic status of dairy cows during lactation.

Key words: blood metabolites, dairy cows, early lactation, mid lactation

Introduction

Production diseases, those associated with improper nutrition or management are common in dairy cows. Dairy cows suffer from negative energy balance (NEB) during the first weeks of lactation due to energy expenditure associated with milk production and limited feed intake, resulting high mobilization of lipids from body fat reserves, and hypoglycaemia (*Veenhuizen et al., 1993; Drackley, 1999; Oetzel, 2004*). Nutrition, age, heredity, body condition score (BCS), management and energy imbalance are various risk factors which possibly play a role in NEB, periparturient fatty liver and ketosis (*Morrow et al.,*

1990; Pechova et al., 1997; Duffield et al., 1997). Clinical ketosis in dairy cows usually occurs between the second and seventh week of lactation. Nevertheless, most of cows in this stage of lactation may suffer a subclinical form of ketosis defined as increased blood ketone bodies without any other symptoms but accompanied by considerable decrease in milk yield and susceptibility other diseases (*Duffield et al., 1997*). Consequently, stressors and poor nutritional management causing reduction in dry matter intake will result in large increases in NEFA around calving (*Drackley, 1999*). NEFA are preferentially and greatly accumulated as TG in the liver, primarily because of a decrease in the very low density lipoproteins (VLDL) synthesis by hepatocytes (*Herdt et al., 1982; Sevinc et al., 2003*). However, when steatosis occurs, endogenous liver synthesis decreases, leading to a reduction in blood glucose, total proteins, albumins and globulins, cholesterol, TG and urea. (*Veenhuizen et al., 1993; Drackley, 1999; Sevinc et al., 2003; Djokovic et al., 2007; Djokovic et al., 2011*). Fatty liver infiltration and hepatocyte degeneration involve cell membrane damage and hepatocyte destruction (15) coupled with the release of cytoplasmic enzymes (aspartate aminotransferase (AST), gamma-glutyml transferase (GGT), lactate dehydrogenase (LDH)) and marked increases in their circulating activities (*Lubojacka et al., 2005; Pechova et al., 1997*).

The objective of the present study was to investigate nutritional and metabolic status in Simmental cows during early and mid lactation.

Materials and methods

Animals, diets and milk production

This experiment was carried out in a dairy herd (166 Simmental cows) suffering from several metabolic and reproductive disorders (Farm: Miličić-Ćurćić, Mrsać, Kraljevo). Two groups (n=15 cows) of clinically healthy cows were chosen from the herd. Group 1 consisted of early lactation cows, in the first month of lactation (16.1±9 days), and Group 2 included mid lactation cows between 3 to 5 months of lactation (124.8±27 days). The cows were mid-yielding with a preceding lactation of about 6500 l. The body condition scores (BCS) of the test cows were 3.42 ± 0.55 (early lactation) and 3.27 ± 0.74 (mid lactation) *(Ferguson et al., 1994).* The experimental cows were kept in tie-stall barns. Diet and the housing facilities were adapted to research purposes, with diet suited to the energy requirement of early and mid lactation cows. Early lactating cows were fed a diet consisting of 7 kg lucerne hay, 20 kg maize silage (30% Dry Matter, DM), 5 kg concentrate (18% crude proteins, CP). Mid lactating cows received a diet consisting of 5 kg meadow hay, 7 kg lucerne hay, 30 kg maize silage (30% DM), 8 kg concentrate (18% CP).). Dietary nutrient contents for dairy cows in early and

mid lactation are given in Table 1. The chemical analysis of the feed was performed by Weende methodology (*Givens et al., 2000*).

Table 1. Nutrient contents in daily ration for early lactation and mid lactation dairy cows

	Early lactating cows	**Mid lactating cows**
Dry Matter (DM) (kg)	16.05	24.82
Net Energy of Lactation (NEL) (MJ)	87.15	130.23
Crude Protein (CP) (% of DM)	13.58	13.38
Rumen undegradable protein (RUP) (% of CP)	35.91	28.33
Fat (% of DM)	3.09	3.14
Fiber (% of DM)	23.26	24.33

Biochemical analysis of blood

Blood samples were collected at 10:00 h or 4 to 6 hours after milking and feeding, by puncture of the jugular vein into sterile disposable test tubes, without anticoagulant. After clotting for 3 hours at 4°C and centrifugation (1500g, 10 minutes, 4°C), sera were carefully harvested and stored at -20°C until analysis. Blood samples collected on fluoride were immediately centrifuged in the same manner and plasmas were assessed for glucose concentrations. The following biochemical blood components were measured by different colorimetric techniques using spectrophotometers (Cobas Mira, Roche, Belgium and Gilford Stasar III, Gilford, USA): BHB and NEFA levels were measured by Randox (United Kingdom) kit, AST and glucose by Human (Germany) kit, and TG by Elitech (France) kit.

Statistical analysis

Difference between metabolic adaptation in early and mid lactation was confirmed by difference in concentration of metabolic parameters, by t-test. Pearson's test was performed to evaluate significant correlations between biochemical metabolites in pooled sample including cows in early and mid lactation. For this purpose was used statistic software Statgraphic Centurion (Statpoint Technologies Inc.Warrenton, Va, Virginia, USA).

Results and Disscusion

Blood biochemical metabolites in early lactation and mid-lactation cows were compared in this study. Homeostasis induces intense lipid mobilization and ketogenesis, and the liver has been adapted to metabolic changes in dairy cows (*Drackley, 1999*). Intensive postpartum lipid mobilization and ketogenesis are

sufficient for a series of compensatory metabolic processes with changes in blood metabolic profile during early lactation in healthy cows (*Drackley, 1999; Cincovic et al., 2012*). Results of blood biochemical metabolites, for both groups of cows are shown in Table 2.

Table 2. Blood metabolites in early and mid-lactating dairy cows (n=15 in each group). Results are expressed as mean ± standard deviation (SD). NS: non-significant

	Early lactating cows	Mid-lactating cows	P
Glucose (mmol/l)	2.29±0.48	2.76±0.43	< 0.05
BHB (mmol/l)	1.59±0.25	0.91±0.16	< 0.05
NEFA (mmol/l)	0.38±0.29	0.13±0.04	< 0.05
TG (mmol/l)	0.12±0.02	0.15±0.04	NS
AST (U/l)	69.46±27.54	39.31±18.90	< 0.05

The correlation coefficients among the biochemical parameters calculated for all cows in this experiment are summarized in Table 3.

Table 3. Correlation coefficients for the biochemical metabolites calculated for all cows in the present study. Significant correlations are marked with asterix (* P<0.05; ** P<0.01).

	NEFA	BHB	TG	AST
Glucose	r= -0.35*	r=-0.47**	r=0.65**	r=-0.23
NEFA		r=0.39*	r=-0.21	r= 0.34*
BHB			r=-0.36*	r=0.15
TG				r=-0.04

In early lactating cows, NEFA and BHB values were significantly higher (P<0.05) than in mid-lactating cows. NEFA concentrations > 0.40 mmol/l indicate problems with energy balance and subsequent intensive lipomobilization *(Oetzel, 2004)*. According to this report, in early lactating cows, NEFA values in blood were 0.38 ± 0.29 mmol/l, showing evidence of high lipomobilization in the present study. Given the fact that serum NEFA concentrations > 0.70 mmol/l are associated with ketosis *(Oetzel, 2004)*. These are the result of some early lactating cows in the present study having NEFA concentrations above the values indicative of subclinical ketosis. Subclinical ketosis also may be diagnosed when serum BHB concentrations are above 1.2 mmol/l, while clinical ketosis is associated with BHB concentrations above 2.6 mmol/l *(Oetzel, 2004; Duffield, 2000)*. The results of early lactating cows in the present study showed BHB concentrations above the value indicative of subclinical ketosis (1.59±0.25 mmol/l). The data presented show that serum NEFA may be used for detecting high lipomobilization, but not subclinical ketosis. This is in agreement with *(Duffield, 2000)*, who stated that the

use of NEFA is a better indicator of energy imbalance in prepartum animals than BHB, but BHB is more useful at postpartum. In the present study, a significant positive correlation was established between NEFA and BHB ($P<0.05$) in the sera, suggesting that both parameters are helpful indicators of EB during lactation.

Blood glucose values in mid-lactation cows were within the physiological range 2.5 - 4.2 mmol/l *(Radostis et al., 2000*), whereas hypoglycemia (2.29 ± 0.48 mmol/l) was detected in early lactating cows. Taking this criterion into account, early lactating cows had indicative values, but did not display any clinical signs, suggesting that they had a typical subclinical condition. In fact, a significant correlation was observed between NEFA values and glucose ($P<0.05$) and BHB and glucose ($P<0.01$). Similar correlations were observed by other authors (*Bobe et al., 2004; Djokovic et al., 2011).*

Fat infiltration into the liver may also affect the concentration of some blood components. *(Morrow et al., 1990; Lubojacka et al., 2005).* Serum level of TG, is an indicator of hepatic functionality, and decreases in their concentration may suggest fat infiltration in the liver *(Lubojacka et al., 2005; Djokovic et al., 2007).* The concentration of serum TG was significantly lower ($P<0.05$) in ketotic cows compared to healthy cows *(Djokovic et al., 2007).* These results may show that TG accumulate in the liver cells of ketotic cows and causes blood TG to decrease. In the present study, TG in the blood was lower (0.12 ± 0.02 mmol/l vs 0.15 ± 0.04 mmol/l) in both groups of cows, but without significant difference. This study has shown a possibility of the development a fat infiltration of the liver in early lactation cows which was confirmed by a significant correlation between TG and glucose, ($P<0.01$) and TG and BHB ($P<0.05$). When fat infiltrates the liver, a hepatocyte degeneration involve cell membrane damage and hepatocyte destruction, and the levels of enzymes that indicate liver injury (AST, GGT, and LDH) are generally augmented (*Pechova et al., 1997; Lubojacka et al., 2005; Djokovic et al., 2011).* AST values in the present study were statistically higher ($P<0.05$) in early lactation cows than in mid-lactating cows. AST activity higher than 100 U/l is indicative of hepatic disorders (*González et al., 2011).* These are result, early lactation cows in our study showed a changes in the morphological and functional state of liver cells, probably due to mild fat infiltration. Also, a positive correlation ($P<0.05$) was observed between AST activity and NEFA values. Mild fatty infiltration of liver in dairy cows during transition and maximum lactation is considered to be almost physiological (*Bobe et al., 2004).* In the present study, all data concerning serum AST activities suggested that the process of lipomobilization was sufficient to cause mild fat infiltration of liver cells in of the early lactating cows.

Conclusion

In conclusion**,** on the basis of changes of blood biochemical metabolites, this study suggests that early lactation cows showed physiological adaptive changes, which were associated with subclinical ketosis and mild fat infiltration of liver cells. They can serve as useful indicators of the nutritional and metabolic status of dairy cows during lactation.

Acknowledgment

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Određivanje metaboličkog i hranidbenog statusa kod mlečnih krava tokom početka i sredine laktacije

Radojica Djoković, Zoran Ilić, Vladimir Kurćubić, Milan P. Petrović, Violeta Caro Petrović, Božidar Milošević, Izeta Omerović

Rezime

Cilj ovog rada je bio da se ispita matabolički i hranidbeni status mlečnih krava simentalske rase za vreme rane laktacije i tokom sredine laktacije. 15 mlečnih krava na početku laktacije i 15 tokom sredine laktacije je odabrano za ispitivanje. Uzorci krvi su uzeti i merene su vrednosti za beta-hidroksi-buternu kiselinu (BHB), slobodne masne kiseline (NEFA), trigliceride (TG), glukozu i aktivnosti aspartat-amino transaminaze (AST). Krave na početku laktacije su imale statistički značajno veće koncentracije ($P<0.05$) NEFA, BHB i AST u krvi i značajno niže vrednosti za glukozu i trigliceride ($P<0.05$). Statistički značajne negativne korelacije su utvrđene između vrednosti BHB i glukoze ($P<0.01$), BHB i TG ($P<0.05$), NEFA i glukoze ($P<0.05$). Statistički značajne korelacije su utvrđene između NEFA i BHB ($P<0.05$), NEFA i AST ($P<0.05$), glukoze i TG ($P<0.01$). Rezultati ukazuju da ovi parametri krvi mogu biti korisni pokazatelji metaboličkog i hranidbenog statusa kod mlečnih krava za vreme laktacije.

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DETERMINATION OF THE ACTIVITY OF SPECIFIC ENZYMES OF BLOOD IN THE PERIPARTUM PERIOD AND DURING THE FULL LACTATIONS

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Original scientific paper

Abstract: This study examined the activities of aspartate-amino-transferase (AST), gamma-glutamyl-transferase (GGT) and lactate-dehydrogenase (LDH**)** in the blood serum of 45 dairy Simmental cows divided into three groups according to production period. The first group (n=15) consisted of late pregnant dairy cows, the second group (n=15) cows in the early lactation, and the third group (n=15) cow in mid lactation. The significant higher activity ($P<0.05$) of AST, GGT and LDH were determined in the early lactation period than in dry period and during full lactation. Research results showed possibility of mild degree of hepatic lesions, probably due to fat infiltration in early lactation cows. Serum AST enzyme activities were significant correlated ($P<0.05$) with GGT and LDH activities and may be most sensitive indicator.

Keys words: dairy cows, serum enzymes activities

Introduction

Priorities intensive dairy production is the prevention of the generation of metabolic and other diseases. Metabolic diseases cows are closely associated with poor diet and management. The most important disease of dairy cows are: fatty liver, ketosis, lameness, mastitis, puerperal paresis, retained placenta and endometritis. Determination of metabolic profile in dairy cows is considered as a routine diagnostic method in detecting metabolic diseases. This involves taking blood samples from at least 8 to 12 cows, 4 times a year, in the dry period, during early, full and mid-lactation (*Gross et al., 2001; Oetzel, 2004; Stengårde et al.*

2008). A purpose of these tests is to determine a blood serum enzyme activity, such as aspartate amino-transferase (AST), alanine amino-transferase (ALT), γ-glutamyl transferase (GGT) and lactate dehydrogenase (LDH). The activities of these enzymes in the blood are very significant when assessing the degree of damage to liver cells. Fatty infiltration and degeneration of liver cells in dairy cows in early lactation, usually leads to destruction of cell membrane of liver cells (hepatocytes), which releases enzymes (AST, ALT, GGT, LDH) and a significant increase in their activity in the circulation (*Pechova et al. 1997; Lubojacka et al. 2005; Stojević et al. 2005*). Increased activity of AST in blood serum is a very sensitive indicator in assessing damage liver cells, especially in the infiltration mass and degeneration of hepatocytes (fatty liver) (*Kauppinen, 1984; Pechova et al., 1997; Meyer and Harvey, 1998; Lubojacka et al. 2005*). GGT is a microsomal membrane and the enzyme-linked highest in cells of the liver, kidneys and small intestine. Increased activity of this enzyme in the blood can indicate damage to the cellular structure of hepatocytes (*Kupczyński et al. 2002; Lubojacka et al. 2005). Tainturier et al. (1984*) find that the activities of AST and GGT enzymes showed irregular changes during pregnancy and early lactation, while activity. LDH is not for organ-specific enzyme, since it is in large concentrations in the muscles, heart, kidneys and liver and is released during acute inflammation of these organs. Also, the activities of LDH in the blood are closely correlated with the degree of fatty liver cells infiltration (*Pechova et al. 1997*).The aim of this study was to evaluate the functional state of liver cells, through changes in AST, GGT and LDH in the blood serum with the Simmental dairy cows during the peripartum period and full lactation.

Materials and Methods

The experiment was conducted on a farm of Simmental cows with frequent occurrence of metabolic and reproductive disorders (Farma Farmad, Vrdila-Kraljevo). The cows had an average lactation of about 6,200 l. Three groups of clinically healthy cows were chosen for the tests as follows: Group of cows in the dry period (n = 15), or the time of 15 ± 2 days before calving, another group of cows (n = 15), in early lactation, 14 ± 5 days of lactation and the third group (n = 15) in full lactation, 112 ± 25 days of lactation. Cows were kept in loose housing in an open barn. Feeding cows has been adapted to the energy needs in late pregnancy and lactation. Blood samples were taken at around 10:00 in the morning, or 4 to 6 hours after milking and feeding, puncture of the jugular vein. Was allowed to clot for 3 hours at 4 °C and centrifugation (1500g, 10 minutes, 4 ° C), the serum was frozen at -20 ° C until analysis. Serum AST, GGT and LDH were determined using a variety of methods spectrophotometer (Cobas Mira plus) and commercial kits. Statistical analysis was performed ANOVA procedure (Statgraphic Centurion, StatPoint Technologies Inc. Warrenton, Va, Virginia, USA).

Results and Discussion

High milk production often poses a major risk for the development of metabolic disorders. For the purpose of diagnosis of fatty liver, it is important to determine the value of the important liver enzyme levels (*Stojević et al. 2005*). Average AST, GGT and LDH in the blood serum are shown in Table 1.

Table 1. Mean values (x±SD) of AST, GGT and LDH activities in dairy cows during transition period and full lactation.

	Late pregnancy	Early lactation	Full lactation
AST (IJ/l)	43.78±15.18 [a]	64.41±18.08[b]	39.47±17.36 [a]
GGT (IJ/l)	9.37±4.16 [a]	14.64±4.24 [b]	8.45±1.86 [a]
LDH (IJ/l)	1250.73±482.54 [a]	1850.33±586.78 [b]	1167.40±336,12.95 [a]

Legend: Mean values within a row with no common superscript differ significantly (P<0.05)

Table 2. Correlation coefficients for testing the activity of enzymes in the blood of dairy cows in peripartum and during full lactation

	GGT	LDH
AST	0.32*	0.43*
GGT		0.18

Legend: Significant correlations (P<0.05) are marked with *.

AST is considered as the most sensitive indicator in the diagnosis of fatty liver in cows (*Pechova et al. 1997; Kupczyński et al. 2002; Lubojacka et al. 2005)* AST is located in the cytoplasm and mitochondria of different tissues and organs, but the maximum activity determined in skeletal muscle, heart and liver in cows (*Lubojacka et al. 2005*) Accordingly, changes in activity of this enzyme in the blood may be due to damage to the cellular structure of the body (primarily the liver). In this study, serum AST were significantly higher (P<0.05) in cows in early lactation in relation to activities in pregnant cows and cows during a full lactation, which may indicate the development of fatty infiltration of the liver cells, damage to hepatocytes and release of the intracellular enzymes into the circulation. GGT is microsomal and membrane-bound enzyme *(Lubojacka et al. 2005*). Increased activity of this enzyme is the result of liver cell destruction (*Kupczyński et al. 2002; Lubojacka et al. 2005*). GGT activity also depends on the observed period. In this study, a statistically significantly higher activity in blood serum were found in cows in early lactation compared to the peak of high pregnancy and lactation (P<0.05). Similar results have been found by *Bobe et al. (2004).* LDH is not organ specific enzyme, as it has a high concentration in muscle, heart, kidneys and liver *(Pechova et al. 1997; Lubojacka et al. 2005*). Its activity is increased in acute

damage to these organs (*Lubojacka et al., 2005*). In this research, the activity of LDH was significantly higher (P<0.05) in cows in early lactation, in relation to the gestating cows and cows in full lactation. The results suggest that cows in early lactation have disrupted morphological and physiological condition of the liver, probably as a result of a mild degree of fatty liver cells infiltration. In this paper, AST, GGT, and LDH levels were mostly within the normal range (AST: 78-132 IU / l; GGT: 10-25 IU / l; LDH: 692-1445 IU / l) *(Stojić, 1996*), and were significantly higher after calving, which may indicate the development of a mild degree of fatty infiltration of cows' liver cells in early lactation. *Pechova et al. (1997*) showed that serum liver enzymes, AST in particular, are closely correlated with the degree of fatty infiltration and degeneration of liver cells. According to this paper, only the serum activities of AST significantly correlated (P<0.05) with the activities of GGT and LDH. Based on the results AST can be considered as the most sensitive indicator in the assessment of the functional state of the liver in dairy cows.

Conclusion

Biochemical tests of blood serum showed a statistically significantly greater activity for AST (P <0.05), GGT (P <0.05) and LDH (P <0.05) of cows in early lactation, compared to values blood in the other two groups. Serum AST activity was significantly correlated with GGT and LDH and may be considered as the most sensitive indicator to evaluate the morphological and functional state of the liver in dairy cows. Results indicate that early lactating cows have reduced functional capacities of hepatocytes, which is probably associated with a mild degree fatty infiltration of the liver cells.

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Određivanje aktivnosti specifičnih enzima krvi u peripartalnom periodu i tokom pune laktacije

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Rezime

U ovom radu je određivana aktivnost aspartat-aminotransferaze (AST), gama-glutamat-transferaze (GGT) i laktat dehidrogenaze (LDH) u krvnom serumu kod 45 simentalskih mlečnih krava, podeljenih u tri grupe u zavisnosti od produktivnog perioda. Prvu grupu (n=15) su činile visoko gravidne krave, drugu grupu (n=15) krave u ranoj laktaciji, a treću grupu (n=15) krave tokom pune laktacije. Statistički značajno veće aktivnosti AST ($P<0.05$),GGT ($P<0.05$) i LDH ($P<0.05$) u krvnom serumu su utvrđene kod krava u ranoj laktaciji u odnosu na aktivnosti ovih enzima u serumu kod zasušenih krava i krava u punoj laktaciji. Dobijeni rezultati ukazuju na mogućnost blagog stepena oštećenja ćelija jetre, odnosno masnu infiltraciju hepatocita kod krava na početku laktacije. Serumske aktivnosti AST su bile u značajnoj korelaciji ($P<0.05$) sa aktivnostima GGT i LDH u krvnom serumu pa se AST može smatrati pouzdanim indikatorom u tvrđivanju funkcionalnog stanja jetre kod mlečnih krava tokom peripartalnog perioda i pune laktacije.

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PLASMA HORMONES AND MILK PRODUCTION PERFORMANCES IN EARLY LACTATION BUFFALOES SUPPLEMENTED WITH A MIXTURE OF PRILLED FAT, SWEETENER AND TOXIN BINDER

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Abstract: The effect of combined feed supplements (prilled fat, sweetener and toxin binder) was studied on 24 apparently healthy early lactating rural and urban maintained Murrah buffaloes. The feeding of combined feed supplement was carried out for a period of 90 days. DMI, BCS, body weight were recorded at fortnightly intervals and milk composition was analyzed at weekly intervals. Blood samples were analyzed for hormones, plasma metabolites and lipid profile. The supplementation increased ($p<0.01$) milk yield by 13.6 and 17.0% in urban and rural Murrah buffaloes with respective increases of 20.14 and14.98% in milk fat ($p<0.01$). BCS and DMI varied non-significantly ($P>0.05$) between the groups. Body weight increased in rural buffaloes in comparison to urban buffaloes. Plasma GH was higher ($p<0.05$) before supplementation and fluctuated non-significantly ($P>0.05$) during supplementation period. Mean leptin levels decreased ($p<0.05$) while plasma estradiol and IgG level increased during the supplementation period. Plasma progesterone and ghrelin level varied non-significantly before and during supplementation. Plasma IGF-1 and glucose levels was more and NEFA level was lower ($p<0.05$) during the experiment. Mean HDL, triglyceride and cholesterol concentration increased ($P<0.05$) during supplementation than before supplementation. Blood urea nitrogen and plasma urea level was lower before feeding and increased during the experiment. The conception rate was more and service period was less ($P<0.05$) in urban buffaloes as compared to rural buffaloes. The complete feed supplementation was highly economical and generated an additional income of Rs. 114.45/day/buffalo with cost benefit ratio of 1:5. It was concluded that complete feed comprising of prilled fat, sweetener and toxin binder augment overall productive performance of rural and urban buffaloes.

Key words: Prilled fat, lipid profile, hormone, milk yield, milk composition, buffaloes

Introduction

Most of the animals in developing countries including India are being fed on agriculture by-products and low quality crop residues, which have inherent low nutritive value and digestibility. The requirement of energy is very high during early lactation but increased nutrient demand and limited feed intake due to physiological stage affects production potential of animal (*Sirohi et al., 2010*). Due to this reason animals are often forced to draw on body reserves to satisfy energy requirements (negative energy balance) leading to substantial loss in body weight and lower milk yield (*Kim et al.,* 2003). Cereal grains and fats play an important role as source of energy in the ration of high yielding dairy animals for optimum productivity. However use of cereals for human consumption and monogastric animals, the alternate source of energy in the form of bypass fat in dairy ration has been reported (*Saijpaul et al., 2010; Singh et al., 2015*). Inclusion of unprotected fat in dairy ration up to 3% of dry matter (DM) intake, reduces digestibility of fibre (NRC,2001) and depresses rumen cellulolytic microbial activity (*Ranjan et al., 2012*). Supplementation of bypass fat increases energy intake and unsaturated fatty acid content of buffalo milk and provide more economic returns to dairy farmers (*Parnerkar et al., 2010*). The experiment on effect of prilled fat containing vegetable palm oil have shown significant impact on milk yield, fat percent and reproductive performance in cows and buffaloes of organized herd (*Rajesh et al., 2014; Yadav et al., 2015; Singh et al., 2015a*). Since prilled fat feeding does not affect digestibility of feed and improves the reproductive performance, its effect on milk production and reproductive performance needs to be investigated in buffaloes maintained in tropical condition. In present investigation the effect of a supplementation mixture containing prilled fat, sweetener, toxin binder on milk production, composition, lipid profile and hormones was studied in rural and urban Murrah buffaloes. Sweetener was added to enhance the palatability of feed. Further, to determine its economic impact the cost benefit ratio of feeding was also determined.

Materials and Methods

Experimental design, diets and procedures

Lactating Murrah buffaloes (24) on day 38 postpartum in 2^{nd}- 3^{rd} parity were selected from Gohargarh rural village (group I) and progressive urban dairy farm (group II). Buffaloes of both the group were supplemented with a mixture of prilled fat, sweetener and toxin binder, 100gm, 30gm and 20 gm/d respectively for

aa period of 90 days. The experiment included two weeks observations prior to supplementation (before) followed by feeding period of twelve weeks. The withdrawal effect of the supplementation on milk yield was recorded for one week. DMI was recorded daily based on the amount of green fodder, wheat straw and concentrate offered and the residue left (Table 1). Body condition score was recorded based on fat cover in the brisket, on the ribs, back, hooks, pins and around the tail head adopting a 5-point scale method. Body weight was recorded at fortnightly intervals. Milk yield of individual buffalo was recorded daily during morning (6am) and evening (6pm) milking. Milk composition viz., fat, protein, lactose and SNF were estimated by Lactoscan machine. Blood samples were collected from rural buffaloes before feeding and at monthly interval on day 30, 60 and 90 of experiment. Plasma growth hormone (GH), insulin like growth factors (IGF-1), leptin, ghrelin, progesterone and estrogen levels were determined by enzyme specific enzyme immunoassay kits. Plasma glucose was determined by analytical kit and plasma NEFA was estimated by *Shipe et al. (1980)* method. Plasma triglyceride, total cholesterol and HDL were estimated by the commercially available analytical kits. Statistical analysis of data i.e. two ways ANOVA was carried out by sigma stat-3 programme. Effects were considered to be significant at probabilities ≤ 0.05 while a trend was assumed for probabilities between 0.05 and 0.1. The mean and standard errors are being presented in table and figure.

Table 1. Chemical Composition (% DM basis) of feeds

Parameter	**Feed**			
	Concentrate mixture	**Maize**	**Berseem**	**Wheat Straw**
DM	89.25	20.91	16.75	89.10
CP	20.95	8.75	16.92	3.60
EE	4.28	1.62	2.32	0.95
Total ash	3.75	11.02	9.88	10.11
NDF	30.60	63.91	50.71	75.27
ADF	18.14	44.52	35.95	52.30

Results

Supplementation of the mixture significantly increased ($p<0.01$) milk yield by 13.6% (1.25kg/d) and fat by 20.14% in urban buffaloes. In rural buffaloes the milk production increased ($p<0.05$) by 17.00% (1.63 kg/d) and fat by 14.98% (Fig 1, 2).

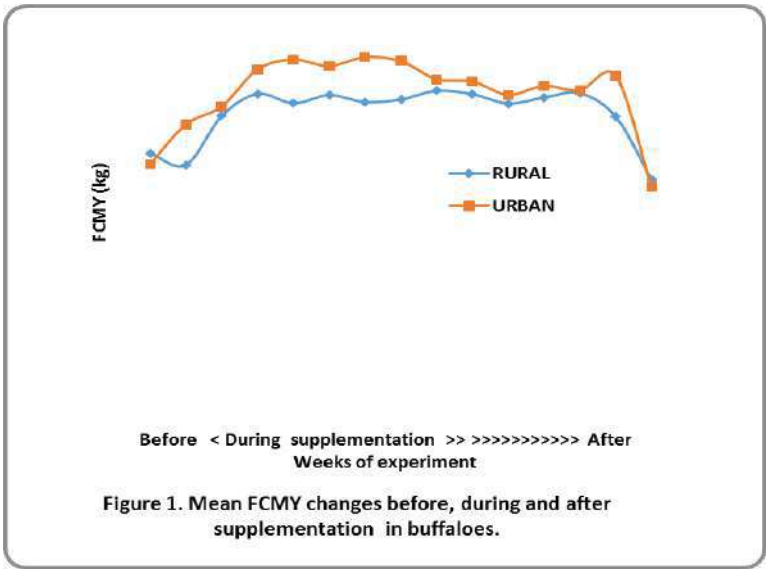

Figure 1. Mean FCMY changes before, during and after supplementation in buffaloes.

However, milk protein and lactose content was not influenced ($p>0.05$) by supplementation in both the groups. Solid not fat (SNF) was higher ($p<0.01$) in urban buffaloes than the rural buffaloes. Supplementation did not influence body condition score (BCS) of both the groups. The dry matter intake (DMI) increased ($P<0.05$) during the supplementation in rural and urban buffaloes. Body weight varied ($P<0.05$) between the groups. The body weight was not influenced by supplementation in rural buffaloes however a significant increase ($p>0.05$) in body weight of urban was observed (Table 2).

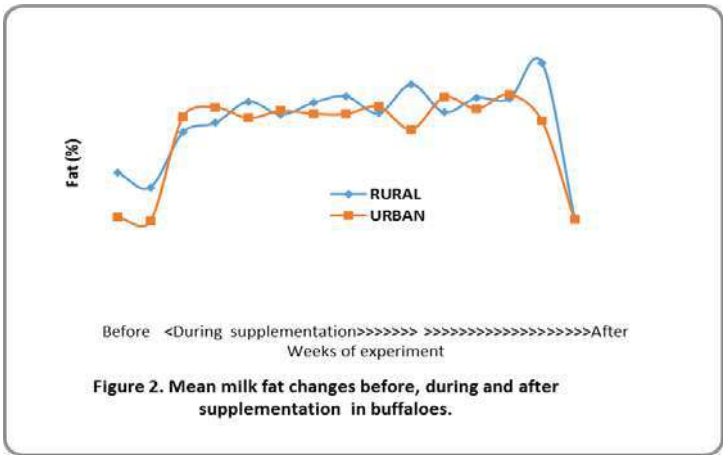

Figure 2. Mean milk fat changes before, during and after supplementation in buffaloes.

Table 2. Mean body condition score, body weight changes and dry matter intake before, during and after supplementation

Parameters		Before	Days during supplementation			After
			30	60	90	
BCS	Urban	3.58±0.12	3.58±0.12	3.54±0.11	3.50±0.08	3.72±0.09
	Rural	3.38±0.13	3.38±0.13	3.47±0.14	3.72±0.14	3.75±0.16
Body weight (kg)	Urban	480.0^{a}±24.59	524.21^{b}±17.23	519.3^{b} ±17.29	516.37^{b} ±16.38	523.42^{b} ±15.21
	Rural	493.9±7.94	515.11±16.68	510.92±15.73	509.03±15.08	508.66±13.77
DMI (kg)	Urban	14.78^{ax} ±0.52	16.62^{bx} ±0.87	17.59^{bx} ±1.04	17.35^{bx} ±0.80	17.02^{bx} ±0.99
	Rural	18.31^{ay}±0.96^{y}	19.89^{ay}±1.97	19.78^{ay} ±1.07	19.74^{ay}±1.97	19.79^{ay}±1.84

Values bearing different superscripts ^{a,b,c,d} differ ($p<0.05$) in a row.

Plasma GH level was numerically higher ($p>0.05$) before supplementation and was not influenced during the experimental period. Plasma leptin level was higher ($p>0.05$) before supplementation and a gradual decrease was observed during supplementation period (Table 3). However such decline in plasma ghrelin level was not observed. Plasma estrogen concentration was lower ($p>0.05$) before supplementation and increased upon supplementation during the experiment. Plasma progesterone level did not indicate any set pattern of change before and during supplementation periods. Plasma leptin, ghrelin and estradiol levels varied ($p>0.05$) between animal. Plasma IgG level was lower ($p<0.05$) before supplementation and increased during the supplementation period. Plasma IGF-1 level declined ($p<0.01$) during supplementation in comparison to before supplementation.

Table 3. Mean plasma hormones during different months of experiment in rural Murrah buffaloes

	Days of experiment				
	Before	**30**	**60**	**90**	**Av.**
GH (ng/ml)	$4.91^{a}\pm0.96$	$4.77^{a}\pm0.39$	$4.81^{a}\pm1.27$	$4.93^{a}\pm1.47$	4.84^{a}
Leptin (ng/ml)	$2.22^{a}\pm0.79$	$1.44^{b}\pm0.54$	$0.97^{bc}\pm0.12$	$1.14^{bd}\pm0.15$	1.18^{b}
Ghrelin (ng/ml)	$3.11^{a}\pm0.60$	$2.99^{a}\pm0.60$	$2.53^{a}\pm0.18$	$3.23^{a}\pm0.39$	2.92^{a}
Estradiol (pg/ml)	$146.03^{a}\pm44.37$	$185.32^{b}\pm47.90$	$264.03^{c}\pm21.24$	$228.69^{d}\pm24.73$	226.31^{d}
IGF-1(ng/ml)	$680.17^{a}\pm18.85$	$594.42^{b}\pm65.57$	$617.67^{c}\pm21.60$	$602.38^{c}\pm23.95$	604.82^{c}
Progesterone(ng/ml)	$0.66^{a}\pm0.13$	$0.46^{b}\pm0.07$	$0.67^{a}\pm0.11$	$0.76^{c}\pm0.10$	0.63^{a}

Values bearing different superscripts ^{a,b,c,d} differ ($p<0.05$) in a row.

Plasma glucose level increased ($p<0.05$) gradually during the supplementation period concomitant to increase in milk yield, however plasma NEFA levels declined gradually. Plasma NEFA varied ($p>0.05$) during different days of sampling. Further, plasma glucose and NEFA varied between animal ($p>0.05$). Plasma cholesterol and HDL increased during the supplementation period in comparison to control (Table 4). Plasma triglyceride also increased ($p>0.05$) steadily during the supplementation. Plasma urea and blood urea nitrogen levels were lower before supplementation and an increase ($P<0.05$) was observed during supplementation.

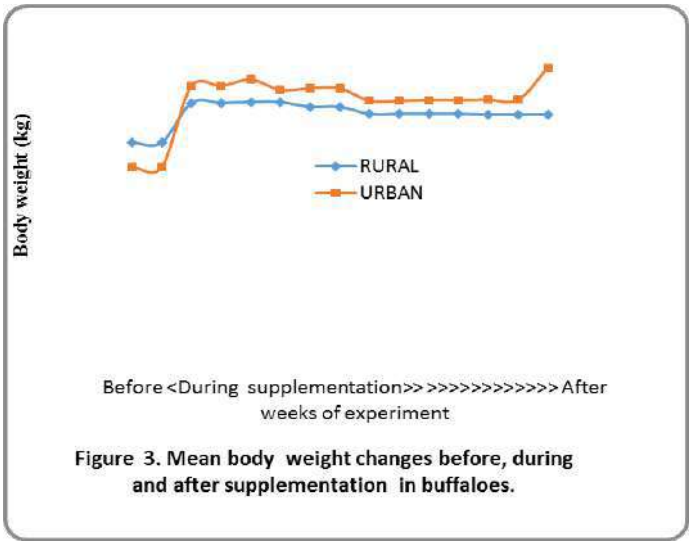

Figure 3. Mean body weight changes before, during and after supplementation in buffaloes.

Table 4. Mean plasma metabolite levels during different months of experiment in rural Murrah buffaloes

	Days of experiment				
	Before	**1**	**2**	**3**	**Av.**
Glucose(mg/dl)	51.07^{a}±2.40	57.83^{b}±2.71	59.94^{bc}±3.57	73.45^{d}±2.15	63.74^{b}
NEFA (µm/l)	71.44^{a}±5.82	55.07^{b}±1.92	57.68^{b}±2.36	54.00^{b}±1.93	56.25^{b}
Triglycerides (mg/dl)	25.77^{a}±1.76	26.77^{a}±2.90	29.81^{b}±2.94	31.84^{bc}±2.36	29.47^{b}
Cholesterol (mg/dl)	103.88^{a}±23.00	128.71^{b}±17.22	113.17^{ab}±6.09	113.84^{ab}±4.73	118.57^{b}
HDL (mg/dl)	62.80^{a}±11.17	86.32^{b}±9.02	85.96^{b}±7.20	86.01^{b}±5.43	86.10^{b}
Urea mg/dl	23.10^{a}±2.71	26.18^{ab}±2.58	28.98^{b}±2.85	32.76^{c}±2.43	29.31^{b}
BUN (mg/dl)	10.79^{a}±1.26	12.23^{a}±1.21	13.54^{b}±1.33	15.25^{c}±1.12	13.67^{b}
IgG (mg/ml)	12.17^{a}±5.87	34.34^{b}±5.04	22.78^{b}±7.48	22.88^{b}±5.34	23.31^{b}

Values bearing different superscripts ^{a,b,c,d} differ ($p<0.05$) in a row.

The urban buffaloes exhibited first post-partum heat earlier (65 day) in comparison to rural Murrah buffaloes (87day) in spite of similar number of artificial inseminations. Conception rate was higher ($p<0.05$) in urban than the rural Murrah buffaloes. The supplementation resulted in lesser service period in urban buffaloes by 51 days in comparison to rural buffaloes. The overall conception rate was 46.87 % in buffaloes**.** Supplementation generated additional income of Rs 115/day, however withdrawal of feeding declined ($p<0.05$) milk yield leading to loss of Rs 274.5/ buffalo/day. The cost: benefit ratio during the experiment was 1:5.

Discussion

The increases in milk production in both the groups of buffaloes suggest that the mixture of prilled fat was galactopoitic and sustained the milk production during the experiment. This was also evident from the significant decline in milk yield after the withdrawal of feeding. However inclusion of sweetener did not increased DMI though it was supposed to enhance the palatability of feed and increase in DMI. The greater response in milk yield and fat content in rural Murrah buffaloes indicated that energy was limiting the milk production. Such effects on milk yield have been reported earlier in mid-lactation cows fed with prilled fat 75g/d (*Singh et al., 2014*) and in buffaloes maintained in organized farm of the institute (*Singh et al., 2015*). The increase in milk fat content resulted probably due to elevated saturated fatty acids level in blood which is taken up by mammary gland for milk fat synthesis. The expected change in BCS was not observed in this study as increase in milk yield was persistent. Body condition score is used for

accurate determination of energy reserves as energy balance during entire lactation period (*Coffey et al., 2003*). Due to this reason BCS have strongest genetic association with cow fertility (*Banos and Coffey, 2010*). The decline in plasma NEFA concentration which is an important energy marker and transporters of fatty acids in blood remain unaffected by prilled fat supplementation (*Quiroz-Rocha et al., 2009*). However in this study decline in plasma NEFA reflected less adipose tissue triacylglycerol mobilization (*Pullen et al., 1989*). Stressors and poor nutritional management during peripartum results in large increases in NEFA immediately after calving due to decrease in voluntary dry matter intake (*Drackley, 1999*). The enhanced milk production therefore could be attributed to prilled fat. The significant increase in DMI could be attributed to bypassing of the prilled fat and not affecting the rumen microflora. Cows in NEB have lower leptin concentrations, higher NEFA and produce more milk with lighter live weight compared to cows in positive EB (*Liefers et al., 2003*). Moreover plasma leptin concentration is positively correlated with glucose and insulin concentrations and negatively with plasma NEFA concentrations (*Block et al., 2001*). However such type of correlation and pattern was not evident as buffaloes were producing less milk yield than the cows. But in medium producer cows similar effect of prilled fat supplementation on plasma leptin concentration has been reported (*Singh et al., 2014*). The non-significant decline in plasma ghrelin was probably due to increasing stage of lactation and maintenance of milk yield in buffaloes. Ghrelin, a 28-amino acid octanoylated peptide is secreted primarily by cells in the abomasum in ruminants (*Huang et al., 2006*) and is the "ultimate anabolic hormone" because it causes the body to consume and store energy (*Litwack et al., 2008*). *Singh et al. (2014)* report no effect of prilled fat feeding on plasma ghrelin concentration in cows as observed in this study. Plasma GH did not vary significantly though its role as galactopoitic hormone in buffaloes have been established (*Prasad and Singh 2010; Singh et al., 2014*). GH partitions nutrient towards the mammary gland at the expense of other tissues (*Aschenbach et al., 2011*). An increase in plasma cholesterol, triglycerides was observed as expected but lower HDL level during supplementation suggest that animal health remains unaffected by prilled fat supplementation. The improved reproductive performance could be attributed to higher plasma cholesterol and higher IGF-I levels as both play important role in reproduction (*Wadhwa et al., 2012*). Plasma insulin, leptin and insulin-like growth factor- I control ovarian follicular development and serve as mediators of energy balance on cow's fertility (*Diskin et al., 2003; Webb et al., 2004*). The feeding supplement was highly economical for dairy farmers in urban and rural conditions and corroborates the similar findings on additional income generation in prilled fat fed buffaloes (*Singh et al., 2015*) and with calcium salts of fatty acids in cows (*Naik et al., 2009; Parnerkar et al., 2011*).

Conclusion

Supplementation of a mixture of prilled fat along with sweetener and toxin binder significantly improves milk yield and fat percent without affecting milk protein, lactose and body condition score. The supplementation increased plasma glucose, decreased NEFA and improves the reproductive performance. Further being cost effective it could be used successfully to augment the productive performance of rural and urban reared buffaloes.

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Hormoni u plazmi i osobine mlečnosti u ranoj laktaciji bivola hranjenih dodatkom mešavine zaštićenih masti, zaslađivača i sredstva za vezivanje toksina

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Rezime

Uticaj kombinovanih dodataka hrani (zaštićena mast, zaslađivač i sredstvo za vezivanje toksina) je ispitan na 24 grla murah bivola, zdravih i na početku laktacije, u ruralnim i urbanim uslovima držanja. Kombinovani dodatak obroku je korišćen u periodu od 90 dana. DMI (konzumiranje suve materije), BCS (ocena telesne kondicije), telesna masa su evidentirani u dvonedeljnim intervalima, a sastav mleka je analiziran u nedeljnim intervalima. Uzorci krvi su analizirani na hormone, metabolite u plazmi i lipidni profil. Dopuna hrani je uticala na povećanje ($p<0,01$) prinosa mleka za 13,6 i 17,0% u urbanim odnosno ruralnim uslovima držanja murrah bivola sa odgovarajućim povećanjem od 20,14 i 14,98% sadržaja mlečne masti ($p<0,01$). BCS i DMI nisu značajno varirali ($P>0,05$) između grupa. Telesna masa povećana je kod ruralnih bivola u odnosu na one u urbanim sredinama. Plazma GH je bila veća ($p<0,05$) pre suplementacije i varirala je nesignifikantno ($P>0,05$) tokom perioda suplementacije. Srednji nivo leptina se

smanjio ($p<0,05$), dok su estradiol plazme i IgG nivo povećani u periodu suplementacije. Progesteron plazme i nivo grelina pokazali su nesignifikantne razlike pre i tokom suplementacije. Plazma IGF-1 i nivo glukoze bili su viši a nivo NEFA niži ($p<0,05$) tokom eksperimenta. Srednja vrednost HDL, koncentracija triglicerida i holesterola je povećana ($P<0,05$) tokom suplementacije u odnosu na period pre suplementacije. Nivo uree u krvi i plazmi je bio niži pre ishrane sa dodatkom i povećan je tokom eksperimenta. Koncepcija je bila viša i servis period kraći ($p<0.05$) kod bivola u urbanim u odnosu na bivole u seoskim uslovima držanja. Suplementacija kompletnog obroka je bila veoma ekonomična i generiše dodatni prihod od Rs. 114.45/dan/ bivo sa odnosom isplativosti 1: 5. Zaključeno je da je potpuna hrana koja se sastoji od zaštićene masti, zaslađivača i sredstva za vezivanje toksina povećava ukupni produktivni učinak bivola u seoskim i gradskim uslovima držanja.

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MORPHOLOGICAL CHARACTERISTICS OF BREAST AND THIGH MUSCLES OF SLOW- AND MEDIUM-GROWING STRAINS OF CHICKENS

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Original scientific paper

Abstract: Morphological characteristics of skeletal muscles of slow- and medium-growing strains of chickens are very important for meat quality and comparison with fast-growing strains. The aim of this paper was to evaluate morphological parameters of breast and thigh muscles of slow- and medium-growing strains in a free-range system. The slow-growing strains used in the experiment were autochthonous breeds Sombor crested and Banat naked neck, and the medium-growing strain was Red-bro. The tissue samples were taken from the thigh muscle and muscles of the breast of 10 chickens of each breed. Samples were stained with hematoxylin – eosin and enzyme succinate - dehydrogenase (SDH). The following morphological parameters were observed: diameter of muscle cells, nucleo-cytoplasmic ratio of muscle cells, volume density of connective tissue within the muscle and the presence of red, white and intermediate muscle cell types. Between strains, the type of muscle or genotype didn't have significant effects on diameters of muscle cells and nucleo-cytoplasmic ratio in the muscle cells. Results indicated that genotype had significant effect on volume density of the connective tissue in breast muscles. Red muscle cells were, in all strains, significantly more represented in *m. biceps femoris* than *m. pectoralis superficialis.* Genotype had significant effect on ratio between connective tissue and muscle cells and no significant effects on other morphological parameters.

Keywords: slow-growing, medium-growing, chickens, morphological parameters, muscle

Introduction

The interest of consumers in products from alternative systems (organic, free-range) is increasing mainly because these systems can be environmentally

friendly, sustaining animals in good health with high welfare standards and resulting in higher quality products (*Sundrum, 2001*).

Alternative systems for poultry meat production could be organized by using commercial fast-growing broiler hybrids or middle and slow-growing genotypes. *Bogosavljevic-Boskovic et al. (2007)* showed that a difference exists in production parameters of commercial broiler hybrids rearing in poultry house and free-range. The appropriate choice of genotype for meat production in free-range system is very important because final body weights are different based on genotype (*Blagojević et al., 2009*). Also, different broiler genetic potential for growth has significant influence on production results as well as on carcass quality (*Škrbić et al., 2013*). *Mobini (2015*) pointed out the correlation between intramuscular connective tissues and meat tenderness. For changing the commercial rearing system with a traditional, autochthonous breeds play a very important role for poultry meat production (*Pavlovski et al., 2009*). *Franco et al. (2013)* found out that meat quality was significantly different between slow-growing strain (autochthonous breed) and commercial (fast-growing) broiler hybrid.

The aim of this study was to evaluate morphological parameters of breast and thigh muscles of slow- and medium-growing strains of chickens in a free-range system.

Materials and methods

Morphological characteristics of breast and thigh muscles of slow-growing strains of chickens were examined on 10 chickens a both sexes of Banat naked neck and 10 chickens of Sombor crested. Ten chickens of Red-bro, as medium-growing strain of chickens were used. All chickens were rearing in free-range system. Chickens of slow-growing strains are sacrificed at age of 12 week and medium-growing strain at age of 9 weeks. Samples of tissue were removed from the *m. biceps femoris* of the thigh and the *m. pectoralis superficialis* of the breast. The muscle tissues were initially fixed in a 10% buffered formalin solution, followed by a sequence of dehydration and clearing. The samples were then embedded in Histowax (Histolab Product Ab, Göteborg, Sweden) then cut into serial 5 μm thick sections using a microtome. Histological preparations for determining diameter and nucleocytoplasmic ratio of muscle cells were stained with hematoxylin-eosin (H&E), while the Mallory method was used for showing connective tissue (*Disbrey and Rack, 1970*). For hystochemical analysis samples of muscles were taken from each bird with a size of 1 cm^3. The samples were frozen by liquid nitrogen at a temperature of -196˚C. In the laboratory they were cut on Cryo-cut (-20˚C, sections of 10μm). These sections were fixed on microscopic

plates and stained with standard methods for succinodehydrogenase (SDH) (*Gerebtzoff, 1970*).

The following parameters were observed in the analysis of histological preparations of the muscle samples: the diameter of muscle cells, the volume density of connective tissue of muscle, nucleocytoplasmic ratio of muscle cells and identification of different muscle cell types (red, white and intermediate). Microscopy analysis was performed using a light microscope Leica DMLS with a Leica DC 300 digital camera, and the software package IM 1000 (Leica Imaging Systems Ltd, Cambridge, UK). The diameter of muscle cells was measured as the average of the longest lines drawn across the length and width of their cross-sections. For stereological analyses of the volume density of connective tissue, the nucleocytoplasmic ratio of muscle cells and percentage of certain types of muscle fibers, measurements were performed using the M42 testing system *(Weibel, 1979).*

The statistical significance of differences obtained in measurements was determined using factorial ANOVA and the *post hoc* Tukey test for each of the parameters measured. The statistical significance of differences was expressed as significant at $P \leq 0.05$. Statistical processing of data was carried out using the software package Statistica for Windows ver. 12.0 (Statsoft, 2012).

Results

The results show that the diameter of the muscle cells in the thigh ranged from 51.10μm to 53.21μm (Table 1).

Table 1. Effect of genotype, muscle and interaction GxM on the diameter of muscle cells (μm), means ± SEM.

	Banat naked neck	Sombor crested	Red-bro
Breast muscle	51.41±1.64	55.14±1.53	50.62±1.28
Thigh muscle	51.10±1.69	53.21±1.91	52.66±0.93
Source		*P* value	
Genotype (G)		n.s.	
Muscle (M)		n.s	
GxM		n.s	

Diameters of muscle cells in the breast were from 50.62μm to 55.14μm. Genotype and muscle type showed no statistically significant difference on the diameter of muscle cells.

In order to determine the dynamics of development and activity of skeletal muscle cells of different strains, the nucleo-cytoplasmic ratio of muscle cells were compared in the experiment (Table 2).

Table 2. Effect of genotype, muscle and interaction GxM on the nucleocytoplasmatic ratio of muscle cells, means ± SEM.

	Banat naked neck	Sombor crested	Red-bro
Breast muscle	0.0155±0.0008	0.0143±0.0011	0.0177±0.0008
Thigh muscle	0.0163±0.0009	0.0172±0.0007	0.0166±0.0011
Source		*P* value	
Genotype (G)		n.s.	
Muscle (M)		n.s	
GxM		n.s	

The results indicate that nucleocytoplasmatic ratio ranged from 0.014 to 0.018. No significant difference was found between the three chicken strains, muscle type and interaction GxM.

The results of volume density of the connective tissue in the muscle directly indicate the degree and the speed of development of the skeletal muscle tissue as well as the organization and arrangement of muscle cells in muscle bundles. The genotype had significant influence of percentage of connective tissue in muscles (Table 3).

Table 3. Effect of genotype, muscle and interaction GxM on the volume density of connective tissue of muscle (%), means ± SEM.

	Banat naked neck	Sombor crested	Red-bro
Breast muscle	23.72±1.62^{a}	26.59±2.34^{a}	16.19±1.80^{b}
Thigh muscle	24.14±1.78	21.19±1.81	20.40±3.03
Source		*P* value	
Genotype (G)		p<0.05.	
Muscle (M)		n.s	
GxM		n.s	

A significant difference was observed in breast muscle, where Red-bro stains had significantly smaller volume density of connective tissue compared with other strains. A significant difference in thigh muscle was not found.

The examination of the percentage of the different types of muscle cells being determined based on the activity of the enzyme succinate dehydrogenase (SDH), suggest that the type of muscle significantly affects the observed parameter (Table 4).

Table 4. Effect of genotype, muscle and interaction GxM on the muscle fiber type percentage (%), means ± SEM.

Fiber type	Banat naked neck		Sombor crested		Red-bro	
	Breast muscle	Thigh muscle	Breast muscle	Thigh muscle	Breast muscle	Thigh muscle
Red	0.99±0.29[a]	22.29±1.02[b]	0.88±0.23[a]	24.43±1.00[b]	1.04±0.14[a]	23.32±0.39[b]
White	27.99±1.04[a]	16.60±2.23[b]	29.82±0.87[a]	16.04±1.35[b]	28.82±0.28[a]	16.07±0.74[b]
Intermed.	71.01±1.14[a]	61.11±2.41[b]	69.29±0.89[a]	59.53±1.20[b]	70.14±0.32[a]	60.61±0.72[b]
Source	*P* value					
Genotype (G)	n.s					
Muscle (M)	<0.01					
GxM	n.s					

Means in a row without a common superscript letter differ significantly, P<0.01.

The results show that in the thigh muscle of both genotypes intermediate muscle cells are the dominant type, followed by red and white muscle cells, whereas in the breast muscle all groups the intermediate are dominant, followed by white muscle cells, whereas the red- muscle cells are extremely minor.

Discussion

Our results indicated no significant effects of genotype and muscle on diameter of muscle cells. According to our results, *Khoshoii et al. (2013)* also reported that a statistically significant difference in diameter of muscle fibers in pectoral muscle between native chickens and broiler strains does not exist. Results are presented as the mean value for both sexes because our previous paper (*Žikić et al., 2014)* as papers of other authors (*Mobini and Khoshoii, 2013; Mobini, 2013a*) suggest that sex did not significantly affect the difference in diameter of muscle fibers. The comparison of our and *Mobini (2013b)* results show the difference in diameter of muscle cells. Comparing the results of these two studies could not be fully done because in the paper of *Mobini (2013b)* it could not be determined age, genotype and poultry breeding system, as the factors which have significant influence on muscle fiber characteristics. *Branciari et al. (2009)* investigated the effect of different genotypes and poultry rearing systems on characteristics of muscle cells. They observed three genotypes, slow-growing chickens (Leghorn), medium-growing (Kabir) and fast-growing chickens (Ross) and found that genotype significantly affects the surface of the muscle fibers in *m. pectoralis superficialis*, *m. ileotibialis lateralis* and *m. semimembranosus*. Furthermore, they found that the rearing system (with respect to the conventional organic) significantly affects characteristics of muscle cells only in the Leghorn strain which is adapted for growing in organic rearing systems.

Our results suggest no significant effects of genotype and muscle on the nucleo-cytoplasmic ratio where values were between 0.015 and 0.017. But *Stojanović et al. (2013)* shows that the value of the nucleo-cytoplasmic ratio in

broiler chickens Ross hybrids, at the age of 42 days was 0.15. In the same study, it was shown that age has significant influence on this parameter. Comparing age of birds in our experiment, we can conclude that the difference is a consequence of age, but we should not ignore the possibility that the difference is a consequence of hybrids relative to strains used in our study.

The results of our study indicate that the genotype affect the volume density of the connective tissue within the muscle. Precisely, volume density of connective tissue in breast muscle was significantly smaller in medium-growing strain then in slow-growing strains. *Mobini (2013c)* comparing the amount of intramuscular connective tissue of *m. pectoralis profundus* did not find the differences between domestic poultry and Ross hybrid broilers. Also, the same author points out that sex did not affect the percentage of intramuscular connective tissue. However, *Mobini (2013c)* observed significant histological differences in epimysium. The number of collagen connective fibers was higher in epimysium in broiler chickens compared to domestic poultry. The softness of the meat depends on the amount of collagen found in the epimysium and perimysium. These results are in accordance with authors who conclude that the autochthonous breeds have better meat quality then commercial hybrids (*Pavlovski et al., 2012*). In order to compare the volume density of connective tissue within the muscle of broiler (fast growing strains), Red-bro as medium growing strain and autochthonous breeds as slow-growing strains, we compared the results of our study and the results from earlier research done with broiler chickens (*Stojanović et al., 2013*). The results of our study showed that the volume density of connective tissue in pectoral muscle was from 16.19% at Red-bro to 26.59% at Sombor crested, while in broiler chickens the average value was 17.97 % in the pectoral muscle broiler chickens Ross 308 at 42 days (*Stojanović et al., 2013*). The same paper points out that the increase of age of broilers causes a reduction in volume density of the connective tissue. Due to the facts that we measured the volume density in slow- and medium-growing strains at the 12th week of life and we could not determine dynamics changes depending on the age, a complete comparison is not possible.

Our results of the activities of enzymes succinate dehydrogenase are consistent with the results obtained in the experiments of other authors. *Šijački et al. (1986)* examined the percentage of red, white and intermediate muscle cells in two breeds of poultry in the post-natal period. He also concluded that the skeletal muscles have all three types of cells and that the red muscle cells are much more present in the leg muscle than in the breast. *Uśćebrka et al. (1999)* examined the percentage in the red, white and intermediate muscle cells in the skeletal muscle of partridges. It has been shown that there exists of three types of muscle cells in the muscles of the breast and the legs. It is noted that intermediate muscle cells dominate both in the breast and leg muscles, while red muscle cells were significantly more present in the leg muscles compared with breast muscle. *Dahmane Gosnak et al. (2010)* investigated the effect of selection (slow-growing

line and fast-growing line) on the metabolic activity of muscle fiber in the muscles of chickens and found that genotype significantly affects the metabolic activity of muscle fiber. Our results indicate that the only muscle (thigh or breast) has significant influence on ratio between muscle fiber types, but it has be noticed that in contrary to *Dahmane Gosnak et al. (2010)*, in our study slow-growing and medium-growing strains were compared.

Conclusion

The results from this study indicate that genotype have a significant effect on volume density of connective tissue in the breast muscle. Within the other morphological parameters there are no significant differences between strains and muscles. Because the morphometric characteristics significantly affect the quality of meat, researches like this are important to provide answers about usage of different strains in alternative rearing systems.

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Morfološke karakteristike mišića grudi i bataka sporo-rastućih i srednje-rastućih pilića

Dragan Žikić, Slobodan Stojanović, Mirjana Đukić-Stojčić, Zdenko Kanački, Verica Milošević, Gordana Ušćebrka

Rezime

Izučavanje morfoloških karakteristika skeletnih mišića sporo- i srednje-rastućih pilića je veoma važno za kvalitet mesa kao i mogućnost poređenja sa brzo-rastućim hibridima. Cilj ovog rada je bio da se ispitaju morfološke karakteristike mišića grudi i bataka sporo- i srednje-rastućih pilića gajenih u *"free-range"* sistemu. Sporo-rastući pilići korišćeni u ovom ispitivanju su bile dve autohtone rase Somborska kaporka i Banatski gološijan, dok su srednje-rastući pilići bili hibrida Red-bro. Uzorci tkiva su uzimani od mišića grudi i bataka od po 10 pilića iz svake grupe u ispitivanju. Uzorci su bojeni hematoksilin-eozinom i metodom za detekciju sukcinat-dehidrogenaze (SDH). Od parametara su određivani: dijametar mišićnih vlakana, nukleo-citoplazmatični odnos, volumenska gustina vezivnog tkiva i prisustvo crvenih, belih i intermedijarnih mišićnih vlakana. Između grupa

pilića, korišćenih u ovom ispitivanju, nije uočena statistički značajna razlika u pogledu dijametra mišićnih vlakana i nukleo-citplazmatičnog odnosa. Rezultati ovog ispitivanja ukazuju da je genotip značajno uticao na volumensku gustinu vezivnog tkiva u mišićima grudi. Crvene mišićne ćelije su, kod svih grupa pilića, bile značajno prisutnije u *m. biceps femoris* u odnosu na *m. pectoralis superficialis*. Genotip je imao značajan uticaj na odnos između vezivnog tkiva i mišićnih ćelija i nije imao statistički značajan efekat na druge ispitivane parametre.

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THE EFFECT OF RAW SOYBEAN IN THE FINAL MIXTURES FOR BROILERS ON THE CONFORMATION MEASURES AND SHARE OF MAJOR CARCASS PARTS

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Original scientific paper

Abstract: The experiment of the supstitution of soybean meal with raw soybean in the final diet was carried out on Hubbard F15 chickens at the age of 35-42 days. The effect of different levels and varieties of raw soybeans in diets on carcass conformation and share of major carcass parts was determined in a two-factorial experiment 2 x 5 (2 soybean varieties x 5 levels of raw grains in the mixture), ie a total of 10 dietery treatments. At the end of the trial, by a random sample method, 12 chickens (6 males and 6 females) from each group were sacrificed and examined. The results showed that the index of drumstick girth and share of drumstick were under significant ($p<0.05$) influence of the soybean varieties. The level of raw soybeans in diets had significant effect ($p <0.05$) on the index of drumstick girth and on the absolute value of the breast depth and breast angle. Shares of breast and thighs of broiler chickens of both sexes were not significantly influenced by the studied factors. It was concluded that the share of raw soybean of 10, 15 and 20% in the final mixtures for broilers hinders the utilization of protein in the ration, resulting in poorer quality of chicken carcasses.

Key words: diet, broilers, raw soybean, conformation measures, major carcass parts

Introduction

An important goal of broiler production, resulting directly from preferences of processors and consumers of chicken meat, is good quality chicken carcasses with the preferred conformation and with as large a share of muscle tissue in the breasts, thighs and drumsticks. Nutrition, in addition to genetics, is a key factor that may affect the achievement of this goal. By composing mixtures that are fully tuned to the nutritional needs of a specific genotype of broiler

chickens, the carcass yield and the yield of major carcass parts can be influenced *(Sinovec and Ševković, 1995).* High-quality protein feeds in the ration are essential for maximum development of muscle tissue, but also the most expensive component of a diet. In the broiler nutrition, soybean is the main component and it is used in the form of soy meal or other heat processed soy products. However, in broiler farms with their own crop production there is a continuing interest in the use of raw soybeans in animal feeding as a cheaper and easier option.

It is known that trypsin inhibitors (TI) present in the raw soybean that reduce utilization of soy protein and are the main anti-nutritional factors that hinder the utilization of nutrients which are contained in this feed (*Zhang et al., 1993*). In addition to TI, lectins are present in soybean which are also important anti-nutritional substances (*Douglas et al., 1999*). The negative effects of increased concentrations of TI in mixtures for broilers on their performance have been confirmed by many authors, *Palacios et al. (2004), Beuković et al. (2010)* and *Petričević et al. (2013)*. The problem can be solved by thermal denaturation of anti-nutritional factors in soybeans before use, or genetic selection/breeding of new TI free varieties. The positive effects of both methods, as well as their possible combination in the diet for non-ruminants, especially in the application of new soybean varieties, are subject to constant research.

The aim of this research was to investigate the effects of partial replacement of heat processed soybean of two local varieties ("Lana" and "Lydia") with raw grain, in the final mixtures for broilers on conformation measures and shares of major carcass parts.

Materials and Methods

The research was conducted at the experimental farm of the Institute for Animal Husbandry in Zemun using Hubbard F15 heavy line hybrid broilers. In the final mixtures for broilers two local varieties were used, variety "Lana" with reduced TI by 50% and variety "Lydia" with standard TI level, extruded and raw (Table 1).

Table 1. Level of tripsin inhibitor in soybean

Treatment	Raw soybean		Heat-treated (extruded) soybean	
Variety	Lana	Lydia	Lana	Lydia
TI (mg/g)	17.71	36.74	4.38	14.03

A total of 2000 one-day broilers were distributed in 40 equal boxes reared on deep litter (50 chickens per box, 4 boxes replicates per treatment diet). Chickens in all groups had uniform requirements in terms of population density, food area, temperature and light. Until the age of 35 days all birds in the experiment were fed

the same diets. Finisher as the final mixture contained 17.5% crude protein and 13.2 MJ/kg metabolic energy in all experimental groups, it was available to broiler chickens from 35 to 42 days and differed for all the tested treatments in regard to soybean varieties and the participation of the heat-treated and the raw soybean. (Table 2).

Table 2. Trial design/plan and extruded and raw soybean ratio in the final mixtures

Treatment	Broilers	% of soybean in the diet (Extruded : Raw)	Soy bean ratio % (Extruded : Raw)
Lana - 0% (K)	200	20% (20% : 0%)	100/0
Lana - 5% (I)	200	20% (15% : 5%)	75/25
Lana - 10% (II)	200	20% (10% : 10%)	50/50
Lana - 15% (III)	200	20% (5% : 15%)	25/75
Lana - 20% (IV)	200	20% (0% : 20%)	0/100
Lydia - 0% (K)	200	20% (20% : 0%)	100/0
Lydia - 5% (I)	200	20% (15% : 5%)	75/25
Lydia - 10% (II)	200	20% (10% : 10%)	50/50
Lydia - 15% (III)	200	20% (5% : 15%)	25/75
Lydia - 20% (IV)	200	20% (0% : 20%)	0/100

Effect of different levels of raw soybeans in diets on conformation measures and shares of major carcass parts was determined in a two-factorial trial 2 x 5 (2 varieties of soybean x 5 levels of participation of raw grains in the mixture) with 10 dietary treatments.

At the end of the trial, by method of random sample, 6 broilers for each test treatment and sex (gender), a total of 120 chickens, were slaughtered. The conformation measures werere determined according to the method *Pavlovski and Mašić (1983)*:

- Breast depth (which indicates the roundness of the breast and carcass) was measured with a caliper placed between the cranial part of the keel and the dorsal surface above the first thoracic vertebrae.
- Drumstick girth, as an indicator of development of the limbs was measured by a measuring tape at the widest part of the drumstick.
- Breast angle, which is considered the most significant measure of conformation and which is an indicator of the development of breast muscle and its roundness is measured using a protractor vertically relative to the back line.

In addition to absolute values of conformation measures that are result of pre-slaughter body mass of chickens, indices were calculated that represent the ratio of pre-slaughter live weight and the observed measure (g / mm).

In order to determine the share of major carcass parts and the share of muscle tissue in them, carcasses were cut according to the *Regulations on the*

quality of poultry meat (1981). Muscle tissue was obtained by dissecting the breast, thighs and drumsticks.

For statistical analysis software package STATISTICA, version 6 (StatSoftInc.) was used. The level of statistical significance of differences between groups was determined by Tukey test.

Results and Discussion

Absolute and relative carcass conformation measures of broiler chickens are presented in Table 3.

Table 3. Absolute and relative carcass conformation measures

Treatment	Breast depth (BD), mm	Body mass/BD, g/mm	Breast angle, degrees	Drumstick girth (DG), mm	Body mass/DG, g/mm
Soybean variety					
Lana	92.08	23.05	124.32	138.95	15.30[a]
Lydia	91.18	22.58	122.83	139.43	14.76[b]
Level of raw soybean. %					
0 (K)	93.04[a]	23.46	126.38[a]	139.79	15.64[a]
5 (I)	93.25[a]	22.90	123.96[ab]	140.29	15.20[ab]
10 (II)	90.46[ab]	22.97	122.83[ab]	139.25	14.92[ab]
15 (III)	91.79[ab]	22.89	123.21[ab]	140.21	15.00[ab]
20 (IV)	89.63[b]	21.85	121.50[b]	136.42	14.39[b]
p value					
Soybean variety	0.252	0.197	0.130	0.740	**0.022**
Level of raw soybean	**0.014**	0.080	**0.032**	0.423	**0.019**
Variety x Level	0.796	0.236	0.127	0.222	0.249

* a-b Average values in each column without a common designation are significantly different at the level of 5%

Significantly higher ($p<0.05$) index of drumstick girth was determined on carcasses of chickens that consumed a mixture containing soybean variety Lana. Other carcass conformation measures were not influenced by the different soybean varieties. The analysis of aggregate data for absolute and relative measures of carcass conformation showed that the level of raw soybeans in diets affected the significant differences ($p<0.05$) in the index of drumstick girth as well as the absolute value of the breast depth and angle. The differences were not statistically significant under the influence of the interaction of investigated factors.

Data on the share of major carcass parts and share of muscle tissue in broiler chickens of both sexes can be found in Table 4.

Analysis of data on share of breast showed that the use of different soybean varieties and with different levels of raw soybean in chicken diet had no statistically significant influence on this trait. Statistically significant differences ($p<0.05$) occurred under the influence of soybean varieties for the share of drumsticks. Chicken diet which included soybean with the standard TI level has affected significantly higher shares of drumsticks compared to soybean variety Lana.. The investigated factors and their interaction caused no significant differences in share of drumsticks.

Using different soybean varieties in the final mixtures for broilers had no significant impact on the share of breast muscle tissue, also thigh and drumstick muscle tissue in chickens of both sexes. The level of raw soybeans in diets affected the statistically significant differences ($p<0.05$) in the share of breast muscle tissue. The highest average share of breast muscle tissue was determined in groups, without the inclusion of raw soybean (K). The shares of drumstick and thigh muscle tissue did not differ significantly under the influence of the level of raw soybeans, nor the interaction of investigated factors had an impact on these properties.

Table 4. Share of major carcass parts and share of muscle tissue, %

Treatment	Breast	Breast meat	Drumstick	Drumstick meat	Thigh	Thigh meat
Soybean variety						
Lana	19.88	15.65	10.22^{b}	6.45	12.34	8.78
Lydia	19.91	15.66	10.74^{a}	6.55	12.45	8.95
Level of raw soybean. %						
0 (K)	20.93	16.86^{a}	10.21	6.46	12.35	8.67
5 (I)	19.96	15.75^{ab}	10.36	6.57	12.38	8.48
10 (II)	19.60	15.30^{b}	11.07	6.52	12.48	9.87
15 (III)	19.42	15.21^{b}	10.41	6.56	12.47	8.67
20 (IV)	19.55	15.13^{b}	10.35	6.41	12.30	8.87
p value						
Soybean variety	0.929	0.983	**0.042**	0.257	0.451	0.954
Level of raw soybean	0.059	**0.019**	0.234	0.727	0.926	0.332
Variety x Level	0.647	0.487	0.113	0.672	0.891	0.607

* a-b Average values in each column without a common designation are significantly different at the level of 5%

The term carcass conformation means the physical shape which is directly related to the amount and distribution of body muscles, especially the breasts, thighs and drumsticks, ie. the slaughter value and leanness (*Pavlovski et al., 2006*).

The carcass conformation can largely be influenced by genotype and sex (gender) *(Bhardway and Mohapatra, 1996).* The share of main carcass parts, in addition to diet, depends on other factors: genotype, sex, age and housing system. In the literature, there are few results that are directly related to the impact of increased concentrations of TI on conformation and shares of chickens' major carcass parts. Similar to the results obtained in our study *Sardary (2009),* using 20% of raw soybeans in diets for chickens at the age of 42 days has established by 1% lower share of breasts compared to chickens fed a diet containing 20% of cooked soybeans. The differences were not statistically significant. *Petričević et al. (2015)* have found significantly lower values of carcass yield with an increase in the share of raw soybean in the final mixtures for chickens. Comparing raw and heat-treated soy in the chicken diet, *Beuković et al. (2012)* have found statistically significantly higher yield in case of conventional carcass dressing, carcass „ready to roast“ and „ready to grill“ and share of breast in the carcass of chickens fed heat-treated soybeans compared to raw soybeans.

Conclusions

Based on the research results of the individual impact of the variety and level of participation of raw soybeans and interactive influence of both factors in the diet of broilers aged 35 to 42 days, the following can be concluded:

- The analysis of the impact of soybean varieties on carcass conformation measures showed significantly lower ($p<0.05$) index value of drumstick girts in chickens fed diets which included soybean variety Lydia. For other conformation measures as well as shares of major carcass parts, it was determined that the use of Lana variety soybean did not affect the higher values of these parameters in relation to soybean with a standard level of TI.
- the share of 20% raw soybean in diets had negative effect ($p<0.05$) on the values of the breast depth, breast angle and drumstick grith index compared to the group without the raw soybean in the mixture.
- In general it can be concluded that the level of raw soybean, 10, 15 and 20% in the final mixtures for broiler chickens hinders the utilization of protein in the ration resulting in weaker development of the most valuable parts of the carcass, primarily the breast.

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Efekat upotrebe sirove soje u završnim smešama za ishranu brojlerskih pilića na mere konformacije i udele vrednijih delova trupa

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Rezime

Ogled je izveden na pilićima hibrida Hubbard F15 u uzrastu od 35-42. dana. Efekat upotrebe različitog nivoa sirove soje u smešama na konformaciju trupa i udeo vrednijih delova trupa utvrđen je dvofaktorijalnim eksperimentom 2 x 5 (2 sorte soje x 5 nivoa učešća sirovog zrna u smeši) sa 10 tretmana ishrane. Na kraju ogleda metodom slučajnog uzorka iz svake grupe žrtvovano je po 12 pilića (6 muških i 6 ženskih) u cilju utvrđivanja mera konformacije i udela vrednijih delova trupa.

Dobijeni rezultati su pokazali da je indeks obima bataka bio pod značajnim ($p<0,05$) uticajem sorte soje. Nivo sirove soje u smešama imao je značajnog uticaja ($p<0,05$) na indeks obima bataka kao i na apsolutnu vrednost dubine grudi i grudnog ugla. Udeo bataka je bio pod značajnim ($p<0,05$) uticajem sorte soje. Udeli grudi i karabataka brojlerskih pilića oba pola nisu bili pod značajnim uticajem ispitivanih faktora.

Zaključeno je da učešće sirove soje od 10, 15 i 20% u zavrsnim smešama za ishranu brojlerskih pilića otežava iskorišćavanje proteinskog dela obroka. što rezultira slabijim kvalitetom pilećih trupova.

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THE INFLUENCE OF PROTEIN SOURCE AND CROSSING SYSTEM OF LAMBS ON WOOL QUALITY PARAMETERS

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Original scientific paper

Abstract: The experiment included 30 lambs-crosses F1 generation: Pirot Pramenka (50%) x Württemberg (50%) and 30 crossbred F1 generations: Pirot Pramenka (12.5%) x Württemberg(37.5) x Ille de France (50%), weaned at 60 days of age, the average body weight of 18.0 kg. The mixtures varied in protein source: I - sunflower meal, II - soybean meal and III - fish meal. The share of undegradable protein was 43 : 51 : 58 %. The average diameter of the fibres in lambs on treatments I:II:III was 26.14 : 24.96 : 25.20 μm, and of two-breed (PxW) and three-breed (PxWxIDF) crosses: 25.38 and 25.49 μm. The average height of the wool fibre in lambs on treatments I:II:III was: 2.97 : 3.06 : 3.17 cm, and in two-breed (PxW) and three-breed (PxWxIDF) crosses 2.98 : 3.15 cm. The average length of the fibre in lambs on protein sources I:II:III was 4.62 : 5.08 : 5.11 cm and in two-breed (PXW) and three-breed (PxWxIDF) crosses 4.77 : 5.11 cm. Protein source in feed mixtures, and genotype of lambs significantly influenced the quality of wool expressed through diameter, height and length of the fibres.

Key words: lamb, protein source, wool, diameter, height length

Introduction

Wool is not a uniform biological product because its physical characteristics vary depending on sheep genetics, environment and management strategies (*Warn et al., 2006*; *Poppi and McLenan, 2010*).
Wool value is intrinsically linked to its characteristics and the ability to meet commercially pre-determined parameters (*Wood, 2003; Jones et al., 2004; Purvis and Franklin, 2005; Bidinost et al., 2008*).
The quality of wool has determined by the physical and mechanical properties: diameter (fineness), height, length, tortuosity, strength and ductility of the wool fibres (*Ružić-Muslić, 2006*). In addition, these properties have ascertained by

factors of genetic and paragenetic nature. The most important characteristic of wool is definitely diameter (fineness) fibres, which implies an average thickness or diameter of the cross section of fibre expressed in micrometres (μm). Fibre diameter (FD) refers to the average width of a single cross section of wool fibre (*Gillespie and Flanders, 2010*). It is measured in microns (μm) which equates to one thousandth of a millimetre (*Cottle, 1991; Cottle, 2010; Poppi and McLenan, 2010; Rowe, 2010*). FD is widely acknowledged as the most important wool characteristics when assessing wool quality and value (*Edriss et al., 2007; Kelly et al., 2007; Rowe, 2010*) accounting for approximately 75% of the total price of raw wool (*Jones et al., 2004; Mortimer et al., 2010*).

Growth of lamb wool fibre is a continuous process influenced by: a genetic basis, nutrition, general physiological status and different environmental factors. The potential of sheep for wool production was determined during their embryonic development. During intrauterine development of lambs, begins the formation of the hair, to the extent of which depends on the genetic potential of the animal. The number and size of wool fibres produced by follicles (structural units in the skin of sheep) determine the quantity of wool produced. Primary follicles occur in the skin of the foetus on the ninetieth day after fertilization, while the secondary follicles develop from that moment on until the birth of lambs (*Jovanović et al., 2001*). The volume of maturation of follicles and production of wool fibres have closely related to nutrition and intensity of lamb growth.

Because the wool fibre is a protein matter whose main ingredient is keratin, the presence and source of protein in the diet affect the yield and quality of fibre (*Zeremski et al., 1989*). According to the research results obtained by *Slen (1969)* increase of protein levels from 7 to 10 % in dry matter of isoenergy diet used for feeding sheep, has resulted in an increase in production of unwashed wool by 16 %. At the same time, influenced by the above nutrition treatment, in terms of length and thickness of wool fibre, improvement of 8-12 % was established. In order to investigate the optimal protein content in the diet for maximal growth of high-quality wool fibre, the author carried out a trial on Romney Marsh breed lambs fed diets to suit their basic requirements and rations for fattening with a high proportion of protein in dry matter. It was established that during the period of 6 months of the experiment the lambs fed fattening diets with a high proportion of protein realized by 343 % more of unwashed wool, superior tortuosity of fibre, by 172 % higher fibre, by 206 % stronger and slightly coarser fibre.

Researches by *Urbaniak (1994)* indicated that with the increase in protein levels of 9.3 to 15.9 % in the diets for sheep, a linear increase in the production of wool has recorded. In studies by *Pajak et al. (1992)* had found that the decrease in protein content in the diet for nutrition of lambs (17, 14 and 11 %) resulted in a decrease in wool production. Profile analysis of amino acids present in the wool showed that the protein of wool is significantly richer in cystine and serine and poor in lysine and methionine. *Jovanović et al. (2001)* point out that the amount of

available amino acids containing sulphur is one of the most important nutritional factors that affect the production and quality of wool. Degradation of feed proteins in the rumen prevents supply the sheep organism with large quantities of mentioned amino acids. Proteins that avoid bacterial hydrolysis in the rumen (undegradable protein), increase the wool production through increase in supply of the organism with amino acids, especially cystine, which is a limiting factor for the production of wool. According to the same author, the infusion of cystine into abomasum or blood can double the growth of wool, while the infusion of methionine increases the wool growth by providing sulphur for the synthesis of cystine. Another method to protect proteins from degradation in the rumen is treatment with formaldehyde. *Zeremski et al. (1989)* showed that lambs fed diets supplemented with casein (previously treated with formaldehyde) realized by 70% more wool than those who received untreated casein.

Chalupa (1975) studied the impact of application of formaldehyde treated feeds on growth of wool. Comparing the effects of soybean meal (untreated and treated) as the protein source, the author found that the increase of wool in the use treated soybean meal of 117 % compared to untreated (100 %). The use of untreated meat meal as a source of undegradable protein in the sheep diet had a greater effect on the growth of wool (100 %) compared to treated meal (96%). A similar relationship has noted in the use of flax meal (100:92 %). *Kiljpa and Kravcov (1989)* studied the effect of different protein supplements on the productivity of four (4) groups of sheep. As a source of protein, the Group I used sunflower meal, Group II used peas, Group III soybean meal and Group IV cottonseed meal. Respectively, wool yield in animals at the age of two years was 4.75, 4.78, 5.20, 4.73 kg. Effect of different concentrations of dehydrated alfalfa (0, 5, 10, 15 and 20 %) as source of undegradable protein in the diets for feeding lambs from 17.0 to 36.0 kg on wool production, *Urbaniak (1994)* found that the greatest accumulation of proteins in wool fibres (4.11 g day^{-1}) was achieved by lambs fed concentrate mixture that contained 10 % of dehydrated alfalfa.

The aim of the present study was to investigate the effect of different sources of protein in feed mixtures used in feeding of two populations of crosses: Pirot Pramenka x Württemberg (PXW) and Pirot Pramenka x Württemberg x Ille de France (PxWxIDF), on some physical and mechanical properties of wool.

Material and methods

The experiment included 30 lambs -crosses F1 generation: Pirot Pramenka (50%) x Württemberg (50%) and 30 crosses F1 generation: Pirot Pramenka (12.5%) x Württemberg(37.5%) x Ille de France (50%), weaned at 60 days of age, the average body weight of 18.0 kg. Animals fed with feed mixtures and alfalfa hay, in- group and ad libitum. The structure and nutritive value of mixtures have

presented in Table 1and Table 2, respectively. The mixtures varied concerning protein source: I-sunflower meal, II- soybean meal and III- fish meal and hence the share of undegradable protein was 43 : 51 : 58%, respectively.

Table 1. Structure of concentrate mixtures for fattening of weaned lambs, %

Feeds	Concentrate		
	I	II	III
Corn	73	79	82
Sunflower meal	23	5	7
Soybean meal	0	12	0
Fish meal	0	0	7
Livestock lime	2	2	2
Salt	1	1	1
Premix	1	1	1

Protein source: I - sunflower meal, II - soybean meal and III - fish meal

Table 2. Nutritional value of mixtures

Nutritional indices	Concentrate mixtures		
	I	II	III
*Dry matter, g kg^{-1}	870	860.5	860.8
*OFU	1.2	1.2	1.2
*NEM,MJ	7.51	7.98	7.91
**UFV	0.99	1.05	1.04
*Total protein,g kg^{-1}	142	137	141
RUP	43	51	58
**PDIN g $animal^{-1}$ day^{-1}	102	103	107
**PDIE g $animal^{-1}$ day^{-1}	102	112	118
*Ashes, g kg^{-1}	25	23	27
*Ca,g kg^{-1}	8.4	8.2	10.6
*P,g kg^{-1}	4.6	3.7	5.0

Protein source: I - sunflower meal, II - soybean meal and III - fish meal; RUP- rumen undegradable protein; PDIN - protein digested in small intestine depending on the fermenting nitrogen; PDIE - protein digested in small intestine depending on the fermenting organic matter ******INRA (1988) *Obračevic (1990)

The experiment lasted 75 days. The average body weight of animals at the end of the experiment was about 35.0 kg. To test the quality of wool, samples have taken from all animals in the experiment from three different locations: the left shoulder, the last rib and rump. For each sample, 10 fibres from the said locations had measured, i.e. 30 per head, or 1800 measured fibred. Samples were taken/cut using shear, along the skin, 2-3 cm in thickness and put in a form with the entered data for the animal and the place where the sample had taken.

As an indicator of the quality of wool, the following characteristics were analysed: height, length and diameter of wool fibre. In this study, the results of the analysis of wool related to the first three traits have presented, as seen from the point of impact of the sources of protein and lamb population. Fibre height has measured from the bottom to the top in a natural position and the length from the base to the top in the corrected position. These measurements had made according to JUS.F.B1O11 and JUS.F.B1O12. Evaluation of the thickness or fineness of wool fibres was performed by the method of short segments taken from the base, middle and top of fibre according to the Reichert lanometer, with a coefficient of 2 (magnification 500 times). Samples of wool used for testing of wool fibre diameter have pre-washed in hot water and detergent, and then rinsed in ethyl ether in order to remove all impurities.

Statistical analysis of the obtained data has done by analysis of variance (according to plan 3x2 factorial experiment, where a source of protein is one, and lamb population, the other observed factor) and assessment of the significance of the obtained differences, using the adequate tests (Tukey honest significant difference test, *Statistica 6 (2003).*

Results and Discussion

The results of measurements of wool fibre diameter in lambs (two-breed and three-breed crosses) fed different sources of protein have displayed in Table 3. The average diameter of the fibres in lambs on treatments I : II : III was 26.14 : 24.96 : 25.20 μm. Established difference of 2.45 μm in the diameter of rump wool fibres taken from three-breed crosses on the third and first treatment, and to the benefit of treatment III, was highly significant (P= 0.000172). Moreover, three-breed crossbreds of treatment II had smaller fibre diameter (measured at the rump) by 2.16 μm, compared with the same population on treatment I, which was statistically highly significant (P= 0.000484). Analysing the observed characteristic in terms of genotypes, we can conclude that the average fineness of the fibre of two-breed and three-breed crosses was 25.38 and 25.49 μm. The difference in fibre diameter (measured at the rump) between the two-breed and three-breed crosses fed the diet I was 2.08 μm and was highly statistically significant (P= 0.000729). At the same time, this population of crosses differed in terms of fibre diameter by 1.51 μm, in favour of the three-breed crosses, in treatment III, which was statistically confirmed (P=0.02). The difference in the fineness of fibres (rump) between the two-breed crosses fed diet with protein source II and three-breed crosses fed diet with source of protein I, was 2.06 μm and was highly statistically significant (P = 0.000875) as well as the difference of 1.62 μm, in diameter of back fibres.

Table 3. Average diameter of wool fibre, µm.

Protein			Indicators	
source	Crosses	Location	Average	CV
		Shoulder	25.61±1.56	6.08
		Back	26.11±1.73	6.62
	2	Rump	25.41±1.08***	4.24
		Average	25.71	
I		Shoulder	25.87±0.53	2.07
		Back	26.37±1.64*	6.24
	3	Rump	27.49±1.08***	3.94
		Average	26.57	
Average I			26.14	
		Shoulder	24.66±1.39	5.64
		Back	24.70±1.49*	6.05
	2	Rump	25.43±1.68*	6.60
		Average	24.93	
II		Shoulder	24.72±0.38	1.55
	3	Back	24.97±0.42	1.69
		Rump	25.33±0.53***	2.11
		Average	25.00	
Average II			24.96	
		Shoulder	24.82±2.34	9.43
	2	Back	25.12±0.47	1.88
		Rump	26.55±0.90*	3.38
		Average	25.50	
III		Shoulder	24.54±1.16	4.74
		Back	25.12±0.66	2.65
	3	Rump	25.04±0.60***	2.41
		Average	25.20	
Average III			26.14	

Protein source: I - sunflower meal, II - soybean meal and III - fish meal; 2-two-breed crosses (PxW); 3-three-breed crosses (PxWxIDF); *(P<0,05); ** (P<0,01); ***(P<0,001)

The results of the measurements of this property in experimental lambs have shown in Table 4. The highest average height of fibre has found in treatment III and it was 3.17 cm and the lowest in treatment I (2.97cm), while the animals on food type II had height of fibres of 3.06 cm. The value of the studied trait in two-breed and three-breed crosses was 2.98 and 3.15 cm. Difference between crosses, fed the source of protein II, in regard to the height of fibres measured at the shoulder was 0.37 cm in favour of the three-breed crosses and was statistically highly significant (P = 0.005) as well as difference in the above said trait measured at the rump (P = 0.003). Also, the established difference in the height of wool fibre between two-breed crosses on the type of diet I and three-breed crosses on II

treatment, measured at the shoulder or rump was also confirmed at the level of statistical significance (P = 0.04) and (P = 0 .01), respectively.

Table 4. Average height of wool fibre, cm

Protein source	Crosses		Indicators	
		Location	Average	CV
		Shoulder	3.03±0.22*	7.46
	2	Back	2.85±0.37	12.88
		Rump	2.96±0.19**	6.43
I		Average	2.95	
		Shoulder	3.02±0.22	7.24
	3	Back	3.01±0.19	6.49
		Rump	2.98±0.23	7.84
		Average	3.00	
Average I			2.97	
		Shoulder	2.91±0.24**	8.17
	2	Back	2.88±0.22	7.62
		Rump	2.88±0.19**	6.56
II		Average	2.89	
		Shoulder	3.28±0.22**	6.58
	3	Back	3.14±0.24	7.73
		Rump	3.26±0.30**	9.28
		Average	3.23	
Average II			3.06	
		Shoulder	3.20±0.20	6.16
	2	Back	3.03±0.09	3.01
		Rump	3.09±0.18	5.75
III		Average	3.11	
		Shoulder	3.45±0.10	2.90
	3	Back	3.15±0.11	3.49
		Rump	3.10±0.10	3.22
		Average	3.23	
Average III			3.17	

Protein source: I - sunflower meal, II - soybean meal and III - fish meal; 2-two-breed crosses (PxW); 3-three-breed crosses (PxWxIDF); *(P<0,05); ** (P<0,01); ***(P<0,001)

Length of wool fibre is the distance between the ends of straightened fibre (without extension). Wool fibre staple length is becoming an increasingly important determinant of wool quality and value (*Edris et al., 2007; Valera et al., 2009; Gillespie and Flanders, 2010*), and is expressed in millimetre (mm) (*Thompson et al. 1988*). In Table 5, the results of measurements of this trait in experimental lambs are exposed.

The average length of the fibre in lambs on protein sources I : II : III was 4.62 : 5.08 : 5.11 cm, respectively. Established difference in fibre length, measured

at the shoulder and rump, between two-breed crosses on source of protein III and I was a statistically significant (P = 0.02) and (P = 0.01).

Also, the difference between the two-breed crosses on treatments II and I in the length of the fibres measured on the back, was statistically confirmed (P = 0.03). Established fibre length of the three-breed crosses was 5.11 cm, and it was by 0.34 cm higher than in the two breed crosses.

Table 5. Average length of wool fibre, cm

Protein source	Crosses		Indicators	
		Location	Average	CV
		Shoulder	4.37±1.09**	28.61
	2	Back	4.10±1.27*	41.5
		Rump	4.27±1.07*	25.20
I		Average	4.25	
		Shoulder	5.02±0.25	26.88
	3	Back	4.95±0.17*	27.88
		Rump	5.01±0.22*	4.38
		Average	4.99	
Average I			4.62	
		Shoulder	5.02±0.33**	21.57
	2	Back	4.95±0.20*	26.70
		Rump	4.90±0.18*	3.76
II		Average	4.96	
		Shoulder	5.27±0.20	18.97
	3	Back	5.08±0.29*	16.50
		Rump	5.24±0.29**	5.51
		Average	5.20	
Average II			5.08	
		Shoulder	5.17±0.18*	25.41
	2	Back	5.01±0.09*	30.86
		Rump	5.09±0.19*	3.74
III		Average	5.09	
		Shoulder	5.21±0.28	21.45
	3	Back	5.15±0.24	30.24
		Rump	5.03±0.025	0.49
		Average	5.13	
Average III			5.11	

Protein source: I - sunflower meal, II - soybean meal and III - fish meal; 2-two-breed crosses (PxW); 3-three-breed crosses (PxWxIDF); *(P<0,05); ** (P<0,01); ***(P<0,001)

The studied populations of crosses on treatment I and about this trait measured on the back differed by 0.85 cm, which was statistically significant at P = 0.03, and measured on the rump by 0.74cm, which was also statistically significant (P = 0.03).

Comparing the ratio of the length and height of wool fibres, we concluded that, on the protein sources I : II : III it was 155.55 : 166.01 : 161.20 %, and in two-breed and three-breed genotypes 160.07 and 162.22 %. This relationship is consistent with the already known fact that in animals with finer wool there is significant difference between the length and the height of fibre.

During intrauterine development of lambs begins the formation of wool follicle, the extent of which depends on the genetic potential of animals. The yield and quality wool have influenced by genetic factors and environmental conditions within which the most important are nutrition and seasonal influences (*Zeremski et al., 1994*).

Comparing our results with data obtained by *Mitić (1984)* who examined the effect of genotype on some qualitative wool indicators in Württemberg breed of sheep, and found fibre fineness of 24-26 microns, we can state a certain agreement, given that the values obtained ranged from 24.96 - 26.14 micrometres.

By studying the phenotypic variability of wool of Merinolandschaf population, *Petrović et al. (1995)* found that the average height of fibre was 9.13 cm, fibre fineness 28.38 microns, strength 12:51CN / tex and extensibility 25.62 %, which is not fully consistent with our results. Furthermore, in the study of sheep wool quality of Ile de France breed, *Mitić (1984)* found the diameter of the fibres of 23-27 microns, and the average length was about 8.0 cm *Mekić et al. (1998),* found that the diameter of wool fibres in animals of Ile de France breed was on average 25.27 microns (rams) and 24.43 microns (sheep). Height of fibres amounted to 6.85 and the length of 10.30 cm. *Ćeranić (1970)* studied the impact of two-breed and three-breed crosses of domestic Merino on improvement of some fibre properties. The experiment involved the following genotypes: F_2 (domestic Merino X Precose); F_2 (domestic Merino x Stavropol); F_1 (domestic Merino X Precose) x Caucasian and F_1 (domestic Merino x Stavropol) x Caucasian. The average thickness of the fibres was 23.02 : 21.85 : 19.73 : 20.66 micrometres, respectively.

In terms of the impact of nutrition on yield and quality of wool, *Jovanović et al. (2001)* point out that the amount of available amino acids containing sulphur is one of the important nutritional factors that affect the quality of the wool. Protein degradation in the rumen prevents food supply to the sheep organism with more of the above-mentioned amino acids. Proteins that avoid bacterial hydrolysis in the rumen (undegradable protein) increase the growth and quality of wool, by increasing the supply of amino acids to the organism, especially cystine, which is a limiting factor for the production of wool. This conclusion is fully consistent with our results, since the best results in terms of quality of lamb's wool were obtained on treatment III which included fish meal as a protein source and the highest share of undegradable protein (58 % of the total), and thus the optimal content of amino acids necessary for the production and quality of wool. In addition to this, the study results by *Jovanović et al. (2001)* show that lambs fed diets supplemented with

casein (previously treated with formaldehyde) reported a 70 % more wool than those who received untreated casein.

Also, *Chalupa (1975),* has studied the impact of the application of formaldehyde treated feeds on growth of wool. Comparing the effects of soybean meal (untreated and treated) as the protein source, the author found an increase in wool by 117% when used treated soybean meal compared to untreated (100 %). The use of untreated meat meal as a source of undegradable protein in the sheep diet had a greater effect on the growth of wool (100 %) compared to treated meal (96 %). A similar relationship noted in the use of flax meal (100 : 92 %).

In order to investigate the optimal protein content in the diet for maximal growth of high-quality wool fibre, *Slen (1969)* performed a trial on Romney Marsh breed lambs fed diets to suit their maintenance requirements and rations for fattening with a high proportion of protein in dry matter. It has found that during the period of 6 months of the experiment, the lambs fed fattening diets with a high proportion of protein had by 343 % more of unwashed wool, superior fibre tortuosity, by 172 % increase in the height of fibre and by 206 % stronger and slightly coarser fibre.

Generally feed protein containing a high level of sulphur-containing amino acids that is less degradable in the rumen would favour increased wool production. For example, canola (rapeseed) meal and lupin seed both contain similar and high levels of crude protein, but canola meal is less degraded in the rumen (*AFRC, 1993*). Merino lambs fed a diet containing canola meal grew 7-64 % more wool than sheep fed a lupin seed diet (*Masters and Mata, 1996; White et al., 2000*) and the response depends on the level of intake and the proportion of canola meal in the diet. When ruminal degradation of protein is avoided, substantial increase in wool growth rate can be obtained with protein, and only small responses are associated with energy *(Allden, 2001). Reis (2000)* showed that very high rates of wool growth could be obtained with moderate energy intakes when casein was given through the abomasum.

Conclusion

It is determined the quality of wool by the physical and mechanical properties: diameter (fineness), height, length, tortuosity, strength and ductility of wool fibres.

Diameter (fineness) of fibres implies an average thickness or diameter of the cross section of fibre expressed in micrometres (μm). The average diameter of the fibres in lambs on treatments I : II: III was 26.14 : 24.96 : 25.20 μm, and in two-breed (PxW) and three-breed crosses (PxWxIDF): 25.38 and 25.49 μm.

The average height of the wool fibre in lambs on treatments I: II: III was 2.97 : 3.06 : 3.17 cm, and in two-breed (PxW) and three-breed crosses (PxWxIDF) 2.98 : 3.15 cm.

Length of wool fibre is the distance between the ends of straightened fibre (without extension). The average length of the fibre in lambs on protein sources I : II: III was 4.62 : 5.08 : 5.11 cm, and in two-breed (PxW) and three-breed crosses (PxWxIDF) 4.77 : 5.13 cm.

Protein source in feed mixtures, and genotype of lambs significantly influenced the quality of wool expressed through diameter, height and length of the fibres, with the best results achieved in lambs on treatment with fish meal as a protein source, while the superior genotype were three-breed crosses (PxWxIDF).

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Uticaj izvor proteina i sistema ukrštanja jagnjadi na parametre kvaliteta vune

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Rezime

Eksperimentom je obuhvaćeno 30 jagnjadi-meleza F1 generacije pirotska pramenka (50%) x virtemberg (50%) i 30 meleza F1 generacije: pirotska pramenka (12,5%) x virtemberg (37.5) x Il de frans (50%), odbijenih na 60 dana starosti, prosečne telesne mase 18,0 kg. Smeše su se razlikovale u izvoru proteina: I – suncokretova sačma, II - sojina sačme i III – riblje brašno. Udeo nesvarljivih proteina je 43: 51: 58%. Prosečan prečnik vlakana u jagnjadi na tretmanima I: II: III je bio 26.14: 24.96: 25.20 μm, a kod dvorasnih (PxW) i trorasnih meleza (PxWxIDF): 25.38 i 25.49 μm. Prosečna visina vune u jagnjadi na tretmanima I: II: III je bila: 2.97: 3.06: 3.17 cm, a kod dvorasnih (PxW) i trorasnih (PxWxIDF) meleza: 2.98: 3.15 cm. Prosečna dužina vlakana u jagnjad na proteinskim izvorima I:II:III je bila 4.62: 5.08: 5.11 cm, a kod dvorasnih (PxW) i trorasnih (PxWxIDF) meleza 4.77: 5.11 cm. Izvor proteina u smešama hrane, kao i genotip jagnjadi, značajno su uticali na kvalitet vune izražen kroz prečnika, visinu i dužinu vlakana.

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WHEY AND ITS INHIBITION OF LIVER ENZYMES

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Abstract: Whey is lately used as functional food, and whey proteins and albumens are considered to have therapeutic influence on diseases associated with oxidative stress. Whey proteins contribute to the reduction of the level of transaminases in blood, especially of alanine transaminase. In this paper we examine some components (total proteins, albumens from whey proteins, minerals: potassium (K), iron (Fe), calcium (Ca) and phosphorus (P), from whey and their inhibition effects to transaminases (alanine aminotransferase (ALT), aspartate aminotransferase (AST), and ɣ glutamyl transferase (ɣ -GT)). The increased level of transaminases in serum is an indicator of an illness of the liver. Additionally, several samples of whey were examined by using a photometric and spectrophotometric method. The results from examination of ALT, AST and ɣ -GT *in vitro* show that whey performed inhibition on the activity of these enzymes: ALT 10.71%, AST 8.51% and ɣ -GT 18.16% in pathological serum, and in serum with normal values, whey performed inhibition on ALT 39.33%, AST 29.08% , ɣ -GT 39.59%. The examination of the whey composition shows that the proteins represented in sufficient quantity to make reduction of enzymes and of the mineral potassium (K) is the most common. From the obtained results can be concluded that whey impacts on the reduction of transaminases and performs inhibition of enzyme activity in the *in vitro* test.

Key words: whey, transaminase, whey proteins, alanine aminotransferase, liver enzyme

Introduction

Whey in its original form is a liquid comprising less than 1% protein and 93% water. Whey is a byproduct in the production of cheese, which was previously considered as waste material. Regardless of the type of treatment and duration of the processing, the whey must be previously removed from curd by filtration or

clarification procedures and then the fat is removed with centrifugal separation. The separated milk fat can be used in the cheese manufacture, other dairy desserts and the production of butter whey (*Presilski, 2004*). Separation of whey depends on the technology of producing the main product, and the quality of used milk.

Whey contains β-lactoglobulin, α-lactoalbumin, serum albumin, lactoferrin, immunoglobulins, lactoperoxidase, glycomacropeptides, lactose and minerals. The whey protein boosts the immune system, which helps the body to produce the antioxidant glutathione (*Marshall, 2004*). Because of the wide range of essential and non-essential amino acids, minerals, fats and biologically active protein, whey is often used in the treatment of various diseases.

Whey is rich in minerals: calcium/phosphorus and potassium/sodium ratios, also contains Cl, Cu, Zn, Fe, Mn and Mo in trace which are good for cell maintenance, prevention of high blood pressure, stroke and heart diseases. The healing power of whey is still being researched, but it is known that the main problem occurs when the human body reduces its ability to regenerate organs or the slowing of life processes, which normally takes years, but with intensive use of whey in the diet in all its original forms or derivatives leads to regeneration.

Enzymes are a special class of proteins that catalyze chemical reactions in biological systems, whereas in humans, animals and plants there are many metabolic processes of decomposition and synthesis (*Dzekova, 2006*). Liver enzymes which are examining liver function are: ALT-alanine aminotransferase, AST-aspartate aminotransferase, LDH-lactate dehydrogenase, γ-GT-gamma glutamyl transferase, alkaline phosphatase ALR, iron, copper, ceruloplasmin and others. In the liver, ALT catalyzes the transfer of α-amino nitrogen of the alanine to α- ketoglutarate to form pyruvate, which is used in gluconeogenesis (*Jerry Kaneko et al., 2008*).

Hepatic analyzes are indicators of liver disease, a viral liver disease; autoimmune liver disease; Toxic liver disease; hereditary liver diseases; oncological diseases of the liver (*Karlsson et al., 2009*).

The aim of this study is to prove the impact of whey of liver enzymes in human control serum with normal and abnormal values, and to present whey inhibitory activity.

Materials and Methods

Tests are made with protein-rich whey obtained in production of mixed cheese (cow's and sheep's milk) and other various types of cheese in "Ideal Shipka" Dairy – Bitola. Applied to photometric - colorimetric and nephelometric methods for proving the components of whey, minerals Ca, K, Fe and P, total protein, albums and spectrophotometric methods spectrophotometer (Screen Master) for enzymes. The inhibitory power of the whey liver enzymes ALT, AST and γ - GT was studied in vitro with HUMATROL P (serum pathological values) and

HUMATROL N (normal serum) control serum based on animal serum which was added a certain amount of whey.

Examination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) - spectrophotometric test, as recommended by the International Federation of Clinical Chemistry (IFCC), using the kinetic method (Schumann, G., et al, 2002), as recommended by the International Federation of Clinical Chemistry (IFCC). Reagent consisted of two parts, buffer / reagent and enzyme substrate are mixed in a 1: 4 ratio. The absorbance is measured at a wavelength of 340nm, optical path 1 cm at 37 ° C are placed 50 μl serum in 500 μl working reagents baked.

Examination of γ GT- gamma glutamyl transferase-colorimetric test, method is a colorimetric kinetic method standardized against the IFCC recommended method. The principle of the reaction is followed (*Schumann et al., 2010*) at a wavelength of 400-420nm.

Examination of iron with photometric colorimetric test for iron factor in clearing fat (LCF). Iron (III) is reacted with Chromeazurol B (CAB) and Cetyltrimethilammonium bromide (CTMA) and form a colored complex at absorbance maximum of 623nm. The intensity of the color is directly proportional to the concentration of iron in the sample (*Garcic, 1979*), work carefully to avoid contamination of the reagent, oily samples cause falsely high results, and distillate water must not contain iron.

Examination of calcium with photometric method. Calcium ions reacts with o-Cresolphthalein complexion in an alkaline medium and form a violet colored complex. The 570nm absorbance of this complex is proportional to the concentration of calcium in the sample (*Gitelman, 1967*)

Examination of phosphorus-UV photometric test. Phosphorus reacts with molybdate in an acidic environment and form a complex that absorbance is directly proportional to the concentration of phosphorus (*Gamst and Try, 1980*).

Examination of potassium-nephelometric method (endpoint). Potassium ions in an alkaline environment without proteins react with sodium Tetraphenylboron (TPB-Na) to form a dispersed colloidal suspension of potassium Tetraphenylboron. The resulting turbidity is proportional to the concentration of potassium in the sample (*Terri and Sesin, 1958).*

Examination of the albumen colorimetric photometric tests, BCG-method. Bromocresol green in citrate buffer with albumen forms a colored complex. The

apsorbance of this complex is proportional to the concentration of the albumen in the sample (*Rodkey, 1965; Doumas et al, 1971*).

Examination of total proteins photometric colorimetric method (method Biuret). Copper ions from proteins and peptides in an alkaline environment form violet colored complex, the 520-580nm absorbance of this complex is proportional to the concentration of protein in the sample.

Results and Discussion

I. Determination of the composition of whey

According to literature data, whey proteins and mineral substances have enormous impact on transaminases. The content of total protein and albumen from whey proteins in various types of whey obtained from different types of cheeses is shown in Table 1.

Table 1. Contents of the total protein and albumen in whey proteins

No. samples TP/ (g/L)	**W_1**	**W_2**	**W_3**	**W_4**	**W_5**
n	10	10	10	10	10
$\bar{x}$	17.6	13.7	11	9.5	12.4
SD	3.97	2.16	1.94	1.17	1.26
CV	22.60	15.78	17.66	12.40	10.20
Alb/ (g/L)					
$\bar{x}$	2.36	1.76	1.53	1.34	1.99
SD	0.95	0.47	0.33	0.27	0.15
CV	40.40	27.07	21.79	20.57	7.65

The average of total protein content in whey is 12.8 g / L, the average concentration of the albumen is 1.79 g / L, so the highest concentration of albumens is in 1 W_1=2.36 g / L, the lowest is in W_4=1.34 g / L, equivalent to the total protein content.

Minerals, along with whey proteins, give whey a high biological value. Macro and micro elements are of particular importance for the animal organism (Table 2). The content of calcium and phosphorus are different for different types of whey and have different proportional ratio. The average calcium content is 7.31 mmol / l, and it has the highest concentration in the W_3=9.47 mmol / l, while the average phosphorus content of 8.09 mmol / l, and the highest level in the W_2=9.39 mmol / l. Whey contains a certain amount of potassium with the highest concentration in W_4=26.6 mmol / l. Whey contains a very small amount of iron, or

the average amount is 10.19 μmol / l. From the data presented in Table 2, it can be seen that the highest iron content is in the W_1=11.74 μmol / l, which is also rich in protein and albumen.

The protein and albumen from whey protein, as well as relative proportions of various minerals seen from Figure 1, the units of measurement from all parameters are converted into mg / dL.

Statistical analysis of the results made in Microsoft Office Excel by using the test ANOVA for comparison of the multiple modalities of one factor (*Ott and Longnecker, 2001*).

The results obtained for the total protein and albumin from different whey is done comparing the exactly defined values of the normal and pathological serum expressed in percentages.

The elements: Ca, P, K, Fe, total protein and albumen in whey proteins have different values, and from that variable is found the composition of whey according to the composition of milk and the applied technological process of preparation of cheese.

Table 2. Ca, P, K, Fe contents of various whey types

No. of sample Ca/ (mmol/l)	**W_1**	**W_2**	**W_3**	**W_4**	**W_5**
n	10	10	10	10	10
$\bar{x}$	7.87	5.842	9.469	6.205	7.178
SD	0.34	0.55	1.12	0.08	0.06
CV	4.36	9.50	11.83	1.44	0.951
P/ (mmol/l)					
$\bar{x}$	7.056	9.395	8.065	7.769	8.186
SD	0.30	1.92	1.31	0.09	0.07
CV	4.32	20.52	16.32	1.20	0.85
K/ (mmol/l)					
$\bar{x}$	24.042	25.34	25.975	26.6	25.691
SD	1.54	1.33	1.30	0.85	0.44
CV	6.42	5.28	5.01	3.21	1,74
Fe/ (μmol/l)					
$\bar{x}$	11.74	9.202	9.99	10.05	9.999
SD	0.89	1.54	0.24	0.94	0.32
CV	7.59	16.83	2.42	9.39	3.29

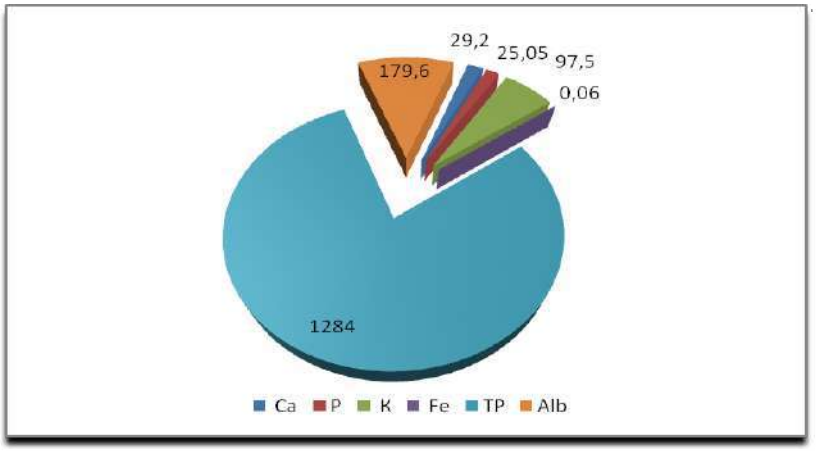

Figure 1. The proportions of surveyed substances in the whey (mg / dL)

II. *In vitro* tests for ALT, AST and ɣ-GT

In vitro test was followed by the effects of whey on the transaminase with adding whey into pathological and normal serum. Inhibition of whey on Pathological and Normal serum (HUMATROL P and N) are shown in Tables 3 and 4, where it can be concluded that whey inhibit the enzymatic activity of ALT, AST and ɣ-GT.

Table 3. Impact of whey on Pathological serum

Transaminases	**ALT/(U/L)**	**AST/(U/L)**	**γ - GT/(U/L)**
Pathological serum-1	203.5	145.181	129.991
Whey 1	181.56	143.69	102.34
Pathological serum-2	186.0	165.368	120.153
Whey 2	167.43	148.56	101.04
Pathological serum-3	179.4	151.151	118.443
Whey **3**	158.86	130.11	98.06

Table 4. Impact of whey on Normal serum

Transaminases	ALT/(U/L)	AST/(U/L)	γ - GT/(U/L)
Normal serum-1	50.4	46.7	48.3
Whey-1	31.46	39.2	29.0
Normal serum-2	48.3	45.4	45.4
Whey-2	28.42	26.1	27.6

Calculation of inhibitory activity of the whey on enzymes, the percentage of inhibition of the whey on transferases is calculated by bellow mathematical formula (*Kaiser et al., 2007*).

$$\% \text{ Inhibition} = [(\text{normal activity} - \text{inhibition}) / (\text{normal activity})] \cdot 100\ \%$$

The resulting values show that whey inhibits the pathological serum ALT 10.71%, of AST 8.51% and γ-GT 18.16%. In serum with normal values whey also inhibits ALT 39.33%, of AST 29.08% and γ-GT 39.59%, Figure 2.

Whey appears as an inhibitor of the activity of ALT, AST and γ-GT, it can be seen in Table 5, where their activity is expressed as a percentage, and notes that in both (pathological and normal serum) reduces activity around 0.2%.

Table 5. Enzyme activity (%)

Type of sample	ALT/ %	AST/ %	γ- GT/ %
P	1.89	1.53	1.22
P + W	1.69	1.41	1.0
N	0.49	0.46	0.47
N + W	0.29	0.33	0.28

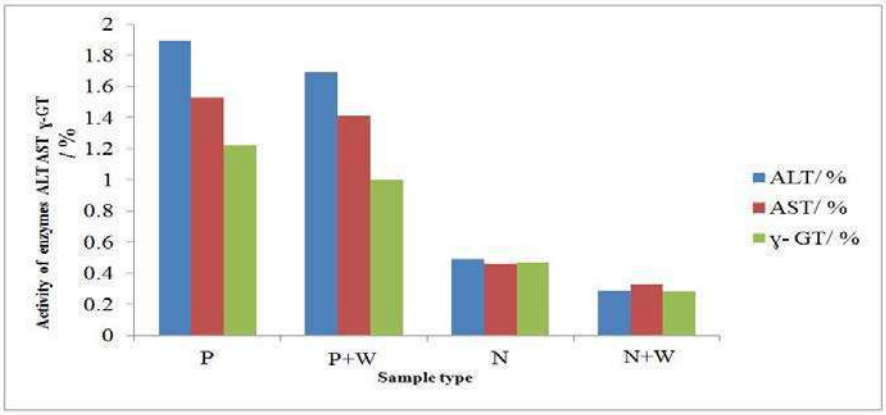

Figure 2. Chart of the enzyme activity of pathological and normal serum with and without of whey.

Most whey (92%) is obtained in the manufacture of various types of cheese. First the fat content of milk is standardized between 2.5% (40% fat cheese) and 3.5% (whole fat cheese). Starter culture provides the desired characteristics of the cheese, and rennet (or a replacement) produces gelatin of casein, including milk fat globules from the milk. This milky gel forms for about 30 minutes at 30° C and then cut into cubes. They will precipitate in curd, leaving the whey as the clear liquid lies above a sediment as precipitate i.e. supernatant (*de Wit, 2001*).

Whey consists of several proteins, including beta-lactoglobulin, alpha-lactalbumin, serum albumin (BSA) and Glukomakropeptides (GMP). Whey proteins contain all the essential amino acids in higher concentrations compared with some vegetables that are sources of protein such as soy, corn and wheat (*Walzem et al., 2002*). Leucine has been identified as the key amino acid in the metabolism of proteins (Anthony J, et al., 2001). Whey protein is not susceptible to the action of acids or enzymes, and during coagulation remains unchanged and after the removal of casein lump transferred in whey. Therefore, there's a similar amount of protein in sweet and sour whey (*Presilski, 2004*).

Whey acts as an antioxidant and detoxifies, due to its participation in the synthesis of glutathione (GSH) which is an intracellular antioxidant. Whey is rich in cysteine which is combined with glutamate and glycine to form glutathione. GSH containing thiol (sulfhydryl) group serves as an active reducing agent in preventing oxidation and tissue damage. Carried out by direct conjugation, it detoxifies endogenous and exogenous toxins, including toxic metals, petroleum distillates, lipid peroxides, bilirubin and prostaglandins. Riboflavin, niacinamid and glutathione reductase are essential cofactors in the reduction of glutathione (*Marz, 2010*). Whey lately is used as a supplement for lowering blood pressure, as

antihypertensive peptides are isolated from the primary sequence of the bovine lactoglobuline β- (*Michael et al., 2005, Tomovska et al., 2006)*.

From minerals, iron is present at least 10.19 μmol / l which in hemoglobin is as metaloenzymes, lactoperoxidaze, catalaze, and participates in the transmission of oxygen. Lactoferrin can prevent some unwanted bacteria to connect with iron, thus inhibiting their growth in the gut (*Fox, 2000*). Potassium, with the average amount of 25.5 mmol / l, is an important element for the optimal functioning of cells, tissues and organs. It regulates the activity of the heart, participate in the construction of proteins and metabolism of carbohydrates.

Hepatic analysis: ALT, AST and ɣ -GT *in vitro* show that the whey inhibits the activity of these enzymes of ALT 10.71%, AST 8,51% and ɣ -GT 18.16% in serum P, and ALT 39.33%, AST 29.08% and ɣ -GT 39.59% in serum N. By measuring the ALT, AST and ɣ -GT, it can be detected the disruption of liver cells and monitoring of the clinical progress (*Center, 2007*).

Conclusion

In the 21st century whey is still an enigma and is insufficiently used, it's interesting for researchers, production and marketing.

The use of whey in normal and pathological control serum values indicates that whey has reduction reaction of the transaminases meaning inhibit enzyme activity in *vitro test* of ALT, AST and γ -GT.

The content of total protein, albumen whey protein and minerals Fe, K, Ca and P was determined in samples of whey, and concluded that from minerals mostly present is potassium (K) then calcium (Ca), while iron (Fe) is present in a very small amounts.

Ingredients that actually impact on inhibitory activity of transaminases we can't prove, but we explored some components such as total protein, albumen of whey proteins and mineral elements (Ca, P, K, and Fe) present in whey and possible the impact of whey on enzymatic activity inhibition.

Whey proteins contribute to the reduction of the level of transaminases in blood, especially of alanine transaminases, and it's very important for an organism that increased level of transaminases in serum is as an indicator of illness of the liver.

Surutka i uticaj na inhibiciju enzima jetre

J. Tomovska, G. Dimitrovska, S. Presilski, K.Velkova

Rezime

Surutka se u poslednje vreme koristi kao funkcionalna hrana, a smatra se da proteini i belančevine surutke imaju terapeutski uticaj na bolesti povezane sa oksidativnim stresom. Proteini surutke doprinose smanjenju nivoa transaminaza u krvi, posebno alanin transaminaze. U ovom radu razmatraju se neke komponente (ukupni proteini, belančevine iz proteina surutke, minerali: Kalijum (K), gvožđe (Fe), kalcijum (Ca) i fosfor (P) iz surutke i njihov efekat inhibicije transaminaze (alanin aminotransferaza (ALT), aspartat aminotransferaze (AST) i ɣ glutamil transferaza (ɣ -GT)). Povećan nivo transaminaza u serumu je pokazatelj bolesti jetre. Osim toga, nekoliko uzoraka surutke su ispitivani korišćenjem fotometrijske i spektrofotometrijske metoda. Rezultati ispitivanja ALT, AST i ɣ -GT *in vitro* pokazuju da surutka inhibira aktivnost ovih enzima: ALT 10,71%, AST 8,51% i ɣ -GT 18,16% u patološkom serumu, a u serumu sa normalnim vrednostima, surutka inhibira ALT 39,33%, AST 29,08%, ɣ -GT 39,59%. Ispitivanje sastava surutke pokazuje da su proteini zastupljeni u dovoljnoj količini da dovedu do smanjenja enzima i kalijuma (K) koji je najčešći. Iz dobijenih rezultata može se zaključiti da postoji uticaj surutke na smanjenje transaminaza i inhibiciju aktivnosti enzima u *in vitro* testu.

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FUNGAL AND MYCOTOXIN CONTAMINATION OF MAIZE HYBRIDS IN DIFFERENT MATURITY GROUPS

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Original scientific paper

Abstract: In the present study, the frequency of toxigenic fungi and occurrence of aflatoxin B_1 (AFB_1), deoxynivalenol (DON) and total fumonisins (FBs) in the kernels of six maize hybrids from different FAO maturity groups (ZP 341, ZP 427, ZP 434, ZP 560, ZP 606, ZP 666) in three localities (Belosavci, Lađevci and Divci) in Serbia, during the harvest in 2013, was investigated. Using standard mycological tests of maize kernels, the presence of potentially toxigenic fungi species from the genera *Aspergillus*, *Fusarium* and *Penicillium* was found. In the studied localities, species *Fusarium verticillioides* was the most frequently isolated from the most hybrids, with a maximum frequency of 30%, while the presence of *Aspergillus* spp. ranged from 0 to 16%, and *Penicillium* spp. from 0 to 20%. By applying Immunoadsorbent enzymatic assay (ELISA) the concentrations of AFB_1, DON and FBs were determined in maize. The differences between tested hybrids in the level of mycotoxins in kernels were statistically significant ($P \leq 0.01$) for DON and FBs, but not for the content of AFB_1. Also, the interaction between the hybrid and location was significant ($P \leq 0.01$) for the level of DON and FBs, while there was no statistical significance for the level of AFB_1. Maximum values of AFB_1, DON and FBs level were 1.02 μg kg^{-1} (ZP 427), 12 μg kg^{-1} (ZP 341) and 1528.56 μg kg^{-1} (ZP 427). The concentrations of the tested mycotoxins in kernels did not exceed the maximum allowed limits stipulated by Serbian regulations *(Službeni glasnik RS, 2014)*. Given that agro-ecological conditions in Serbia are favourable for the occurrence of toxigenic fungi and their mycotoxins, it is necessary to exert the control of maize kernels annually, in harvest and postharvest periods.

Key words: toxigenic fungi, mycotoxins, maize hybrids

Introduction

Maize is one of the economically most important cultivated plants in Serbia and around the world and is the main energy source for animal feed. Toxigenic fungal species can develop in maize crops in the field and during storage and contaminate food and/or feed with mycotoxins that exhibit toxic effects in animals and humans *(Biagi, 2009)*. The most important fungal species and mycotoxins in maize are: *Aspergillus flavus* and aflatoxins, *Fusarium verticillioides* and *F. proliferatum* and fumonisins, *F. graminearum* and trichothecenes and zearalenone *(Chulze, 2010)*. Aflatoxin is a problem in many food stuffs, but it is the primary problem in maize. This is because the maize is infected yet in the field which is in relation to external environmental conditions. Contamination of maize kernels depends on the co-existence of sensitivity of hybrids and environmental conditions suitable for fungal infection, development and toxinogenesis *(Blandino et al., 2009)*.

The aflatoxins are a group of closely related, highly toxic, mutagenic and carcinogenic compounds produced primarily by *A. flavus* and *A. parasiticus*. Nearly all strains of *A. parasiticus* are toxigenic, and synthesis of aflatoxins in *A. flavus* varies considerably between strains *(Resanović, 2000)*. Aflatoxin B_1 was the first mycotoxin isolated in feed causing death of 100,000 turkeys in 1960 in England *(Kuhn and Ghannoum, 2003)*. Aflatoxins have been globally established in different types of animal feed, and their amount varies depending on numerous factors. There is a big difference in the frequency of contamination of animal feed in some years. The occurrence of aflatoxin B_1 (AFB_1) in feed for cattle may impair the safety of milk and dairy products, because AFB_1 in food for cattle is transmitted into milk as aflatoxin M_1 (AFM_1). Therefore, many countries have legally permissible limits for AFB_1 in feed and AFM_1 in milk *(Driehuis et al., 2008)*.

Fumonisins are the group of structurally related mycotoxins, which were first isolated from cultures of *F. verticillioides*, one of the most common type of fungus that contaminate maize. Fumonisins are grouped into four series: A, B, C and P. The most widespread and directly related to the pathogenic effects are fumonisins B (FB_1, FB_2, FB_3, FB_4) and A (FA, FA_1 i FA_2) series *(Meronuck and Concibido, 1996)*.

From the group of trichothecenes, deoxynivalenol (DON) is the most common in animal feed (between 20 and 100%) *(Driehuis et al., 2008)*.

In animals and humans mycotoxins cause diseases called mycotoxicoses. Consumption of food and/or feed contaminated with mycotoxins can cause acute and chronic effects that may be teratogenic, carcinogenic, neurotoxic, estrogenic or immunosuppressive in humans and/or animals *(Binder et al., 2007)*.

Because of the potential risk and the inevitable occurrence of toxigenic fungi and their secondary metabolites (mycotoxins) in maize, as one of the most important and the most cultivated cereal in Serbia, the paper presents the results of mycological and mycotoxicological analysis of six maize hybrids obtained from three different locations in Serbia.

Materials and Methods

The incidence of potentially toxigenic fungi species and the natural occurrence of mycotoxins were studied in the samples of maize kernels of six hybrids from different FAO maturity groups (ZP 341, ZP 427, ZP 434, ZP 560, ZP 606, ZP 666), which were collected during the harvest in 2013 originating from three locations in Serbia: Belosavci (near Topola), Lađevci (near Kraljevo) and Divci (near Valjevo). Samples of each hybrid, taken from each location, were homogenized, so that the final sample was about 1 kg. Total of 18 samples were tested, 6 samples per site, 1 sample per hybrid. The samples were stored in a refrigerator at 4°C until analysis. Moisture content of the samples was determined using a laboratory moisture meter (OHAUS MB35, USA).

According to standard mycological methods toxigenic fungal species were isolated. For each sample 25 kernels of maize were analyzed, with four replications. Obtained colonies were purified in the procedure where monospore cultures were obtained and subsequently used to identify *Fusarium* species. Monospore cultures were subcultured on potato dextrose agar (PDA), medium with fragments of sterile carnation leaf (CLA) and the synthetic substrate (SNA). The cultures were incubated in the dark at 25±1°C, and on the CLA and SNA 12 hours in the combined light (fluorescent and ultraviolet light) and at 25±1°C, and 12 hours in the dark at 25±1°C. The characterization of these species was performed according to *Nelson et al. (1983)* and *Burgess et al. (1994).*

The incidence (I) of potentially toxigenic fungi was calculated according to the formula of *Lević et al. (2012):* I (%) = [Number of kernel samples in which a species occurred/Total number of kernel samples] x 100.

To test the presence of aflatoxin B_1 (AFB_1), deoxynivalenol (DON) and total fumonisins (FBs) samples were pulverized in a mill (IKA A11, Germany) to a fine powder. To 5 g of each sample was added 1 g of NaCl and homogenized in 25 ml of 70% methanol for 3 minutes in an orbital shaker (GFL 3015, Germany). Samples were filtered over Whatman 1 filter paper and the resulting filtrate was further analyzed using the competitive ELISA method according to the manufacturer's instructions Celer Tecna® ELISA kits. The limits of detection for AFB_1, DON and FBs were 1 μg kg^{-1}, 40 μg kg^{-1} and 750 μg kg^{-1}, respectively. Each sample was analyzed in triplicate.

The results were analyzed using ANOVA with STATISTICA (StatSoft version 10). The level of significance was assessed at $P \leq 0.05$ and $P \leq 0.01$. The significance of the difference between the parameter mean values (means) was estimated using the F test at $P \leq 0.05$ level.

Results

In the analyzed samples the kernel moisture content was from 11.21% (ZP 434) to 12.30% (ZP 427), with the average for all hybrids of 11.83% in the locality Belosavci, from 12.25% (ZP 666) to 13.24% (ZP 427) with an average for all hybrids of 12.63% in the locality Lađevci, and from 12.36 % (ZP 666) to 13.60% (ZP 341), with the average for all hybrids of 13.07% in the locality Divci.

Table 1. Incidence (%) of potentially toxigenic fungal species from *Aspergillus*, *Fusarium* and *Penicillium* genera in six tested maize hybrids samples from three locations

Parameters	*Aspergillus* spp.	*Fusarium graminearum*	*Fusarium subglutinans*	*Fusarium verticillioides*	*Penicillium* spp.
Location Belosavci					
ZP 341	6	0	0	0	0
ZP 427	8	0	0	30	4
ZP 434	16	0	2	2	20
ZP 560	10	0	2	20	14
ZP 606	8	0	0	30	12
ZP 666	8	0	0	30	6
Location Lađevci					
ZP 341	0	0	2	6	4
ZP 427	2	0	0	10	0
ZP 434	6	0	0	22	4
ZP 560	0	0	0	0	8
ZP 606	0	0	0	14	0
ZP 666	4	0	6	8	14
Location Divci					
ZP 341	0	0	10	8	10
ZP 427	0	0	6	6	10
ZP 434	0	0	4	14	0
ZP 560	0	10	2	6	16
ZP 606	0	4	0	18	12
ZP 666	0	0	12	16	0

Potentially toxigenic species of the genera *Aspergillus*, *Fusarium* and *Penicillium* were identified in the most hybrids at all locations, with the exception of the genus *Aspergillus* in locality Divci. *Aspergillus* spp. were isolated from all the hybrids from the site Belosavci with the incidence from 6% (ZP 341) to 16% (ZP 434). In the genus *Fusarium* three species were identified: *F. graminearum*, *F. verticillioides* and *F. subglutinans*. Among them species *F. verticillioides* was the

most commonly isolated in majority of studied hybrids from all three localities with the highest incidence of 30% in three hybrids (ZP 427, ZP 606 and ZP 666) in the locality Belosavci. Species *F. subglutinans* was the most frequently isolated from the majority of hybrids in the locality Divci with the highest incidence of 12% (ZP 666), as well as the species *F. graminearum* with the highest incidence of 10% (ZP 560). *Penicillium* spp. were isolated with the highest incidence of 20% in ZP 434 in the site Belosavci (Table 1). Representatives of the fungal genera *Alternaria, Rhizopus* and *Nigrospora* were isolated with an average incidence of 15%, 47.56% and 4%, respectively, from all the hybrids in all three considered test sites.

Table 2. Effect of hybrids and locations on AFB_1, DON and FBs levels

Factor	AFB_1 (μg kg^{-1})	DON (μg kg^{-1})	FBs (μg kg^{-1})
Hybrid effects (A)			
ZP 341	0.95	12^{a}	1206^{b}
ZP 427	1.02	nd	1528.56^{a}
ZP 434	0.90	nd	853.22^{c}
ZP 560	0.90	nd	595.67^{d}
ZP 606	0.92	nd	657.56^{d}
ZP 666	0.96	nd	957.11^{c}
F test	ns	**	**
Locations effects (B)			
Belosavci	1.21^{a}	nd	930.61^{b}
Lađevci	0.85^{b}	nd	1094^{a}
Divci	0.77^{b}	6^{a}	874.44^{b}
F test	**	**	**
Interactions (F test)			
AB	ns	**	**

nd – not detected; means followed by the same letter within a column are not significantly different by F Test at P≤0.05 level, ** - significant at the 0.01 level of probability, * - significant at the 0.05 level of probability, ns – not statistically significant

In mycotoxicological analysis it was found that the tested hybrids had statistically significant effect (P≤0.01) on the level of FBs and DON mycotoxins, but not on AFB_1 level (Table 2). The impact of location was highly statistically significant (P≤0.01) for the levels of all tested mycotoxins. However, there was no statistically significant difference between the tested hybrids in the level of AFB_1, while the hybrid ZP 341 statistically significantly differed from other hybrids in the level of DON. In this hybrid, 12 μg kg^{-1} of DON was recorded, while in other maize hybrids DON was not at all detected. The ZP 341 and ZP 427 hybrids were mutually and relative to other tested hybrids significantly different in the level of FBs, while between the hybrids ZP 434 and ZP 666, and ZP 560 and ZP 606 no statistically significant differences in the level of FBs were determined. The highest

level of FBs was 1528.56 μg kg^{-1} (ZP 427) and the lowest 595.67 (ZP 560). In regard to the studied locations, the level of AFB_1 was statistically significantly higher in the locality Belosavci, while between the other two sites, no statistically significant differences in the level of AFB_1 was established. Likewise, the level of DON was statistically significantly greater in the locality Divci, while on the other two sites DON was not identified. FBs level was significantly higher in the locality Lađevci compared to other test sites, among which there were no statistically significant differences in the level of FBs. *F* values for hybrid x location interaction were statistically highly significant ($P \leq 0.01$) for the levels of DON and FBs, and not significant for the level of AFB_1 (Table 2).

Maximum levels of studied mycotoxins did not exceed allowed limits stipulated by the Regulation on the amendments of the Regulation on maximum residue levels of plant protection products allowed in food and feedstuffs, and on food and feedstuffs for which the maximum residue levels of plant protection products are determined by *(Službeni glasnik RS, 2014).*

Discussion

Meteorological data of the Hydro meteorological Service of the Republic Serbia for studied sites in the vicinity of Topola (Belosavci), Kraljevo (Lađevci) and Valjevo (Divci), where the maize hybrids were cultivated, are shown in Table 3. Considering all of the investigated localities, the average daily temperature from 21.9 to 22.8 ºC, relatively low precipitation (23.2 - 50.6 mm) and relative humidity from 60 to 62% in July 2013, were favorable for intensive development of toxigenic *F. verticillioides* species compared to other *Fusarium* spp., and significant production of fumonisins. Minor development of *Aspergillus* and *Penicillium* species as well as mycotoxins AFB_1 and DON was observed. Between hybrids, there were no statistically significant differences in the level of AFB_1, DON was detected only in hybrid ZP341, and the highest level of FBs was detected in hybrid ZP427. Certain statistically significant differences in the level of mycotoxins between the tested sites were recorded; however, the impact of sites on the content of mycotoxins was not consistent. Thus, in the locality of Belosavci the highest level of AFB_1 was found, in the locality Divci the highest level of DON, and in Lađevci the highest level of FBs (Table 2).

Similar to our results, in Northern Italy, *Balconi et al. (2014)* have found a larger number of maize kernels infected with *F. verticillioides* in July 2009 when higher average daily temperatures were recorded (> 20°C) than in July 2010 (<20°C), while the levels of fumonisins (FBs) were similar in both years. Also, in the examination of the fungal and mycotoxin contamination in Bt maize and non-Bt maize grown in Argentina (during 2002-2003 and 2003-2004 harvest seasons), *Barros et al. (2009)* have identified toxigenic species of the genera *Fusarium*,

Penicillium and *Aspergillus* in both Bt and non-Bt maize in seven localities tested. *Fusarium* species were the most common with average values from 58% (Bt maize) to 82% (non-Bt maize). Between *Fusarium* species, *F. verticillioides* was the most frequently isolated species (70% of isolates). According to the same authors the effect of location was very important to the level of fumonisins, the average DON levels did not differ significantly between the sites, and the presence of aflatoxin was not detected in both genotypes in both examined growing seasons. In Poland in three-year studies (2007-2009), that considered the impact of sensitivity between different hybrids (flint and dent hybrids) to the *Fusarium* species, *Wit et al. (2011)* have found that the dent hybrids showed a significantly higher level of infection. Similarly, in Italy *Blandino and Reyner (2008)* have found in the hybrids with harder kernels had less damaged kernels by insects and thus disease development and production of mycotoxins were reduced. In that case contamination of maize with *F. verticillioides* and fumonisins can be related with greater damage incidence by insects, primarily by European corn borer (*Ostrinia nubilalis* Hubn.). Further, *Blandino et al. (2009)* have determined a statistically significant effect of the year ($P \leq 0.05$) and agricultural measures (time of sowing, plant density and nitrogen fertilization) ($P \leq 0.01$) on the level of FBs in the kernel, while the impact of hybrids from two different FAO maturity groups (400 and 600) was not statistically significant for the level of FBs. During the maize harvest in 2003 in Mexico, *Reyes-Velázquez et al. (2011)* have investigated natural phenomena of *Fusarium* species and their mycotoxins and found that in seven hybrids dominant species was *F. verticillioides* with an incidence from 44 to 80%, but the level of fumonisins (FB_1 and FB_2) (up to 606 and 277 μg kg^{-1}, respectively) was low in all samples of maize.

Table 3. Mean daily temperature (T), total monthly rainfall and mean relative humidity (RH) from June to October 2013 in investigated locations Belosavci (A), Lađevci (B) and Divci (C)

Year 2013	Location								
	A			B			C		
Month	T (ºC)	Rainfall (mm)	RH (%)	T (ºC)	Rainfall (mm)	RH (%)	T (ºC)	Rainfall (mm)	RH (%)
June	19.8	85.4	72	20	96.1	71	20.5	63.9	69
July	21.9	50.6	62	22.5	23.2	60	22.8	44.1	61
August	23.1	50.1	60	23.8	23	58	23.7	21	60
September	16.2	49.6	69	16.5	48.6	68	16.6	54.8	72
October	13.6	41.7	73	13.6	50.7	75	13.5	52	78
Mean temperature (June-October)	18.9			19.2			19.4		
Total rainfall (June-October)		277.4			241.6			235.8	
Mean RH (June-October)			67.2			66.4			68

Production of high quality maize kernels is of primary importance for livestock production because maize is the main component of animal feed. Summary of analysis of annual and multi-years results of the occurrence, the presence and frequency of potentially toxigenic fungi and their mycotoxins in feeds are important primarily due to the implementation of preventive measures, as well as increasing the awareness of the consequences of the harmful effects of these contaminants in the food chain. In order to prevent the biosynthesis of mycotoxins it is necessary to take measures that inhibit the development of fungi in the field. The most important preventive measure is cultivation of resistant genotypes. For this reason, continuous research of the sensitivity of domestic hybrids to toxigenic fungal species is required.

Conclusion

Potentially toxigenic species of fungi of the genera *Aspergillus, Fusarium* and *Penicillium* are identified in the present paper. However, in consideration of all of the test sites, *Fusarium* species were the most frequently isolated with *F. verticillioides* as the most common species in majority of tested hybrids. This points to the fact that environmental conditions in Serbia are very suitable for the development of fusariosis of maize ears and thus for production of fusariotoxins, in this case fumonisins. Low incidence of species *F. graminearum*, is reason why in most hybrids the presence of DON was not detected. In all hybrids and locations the presence of AFB_1 was detected, although the frequency of *Aspergillus* species was not high or was not observed in certain hybrids and not at all in the locality Divci. The interaction between genotype and location was not statistically significant for AFB_1 contamination, but it was statistically significant for DON and FBs contamination.

Although the detected concentrations of mycotoxins did not exceed the maximum residue levels determined by the Regulations of Republic of Serbia, it is necessary to carry out every year mycological and mycotoxicological analysis of maize in order to avoid potential adverse impacts from contaminants (fungi and mycotoxins) on human and animal health.

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Kontaminacija gljivama i mikotoksinima hibrida kukuruza različite grupe zrenja

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Rezime

U radu je ispitivana učestalost potencijalno toksigenih vrsta gljiva i prirodna pojava aflatoksina B_1 (AFB_1), deoksinivalenola (DON) i ukupnih fumonizina (FBs) u zrnu šest hibrida kukuruza iz različitih FAO grupa zrenja (ZP 341, ZP 427, ZP 434, ZP 560, ZP 606, ZP 666) u tri lokaliteta (Belosavci, Lađevci i Divci) u Srbiji, tokom berbe u 2013. godini.

Primenom standardnih mikoloških ispitivanja zrna kukuruza ustanovljeno je prisustvo potencijalno toksigenih vrsta gljiva iz tri roda: *Aspergillus*, *Fusarium* i *Penicillium*. U ispitivanim lokalitetima, vrsta *Fusarium verticillioides* je bila najčešće izolovana kod većine hibrida, sa maksimalnom učestalošću od 30%, dok je prisustvo *Aspergillus* spp. bilo od 0 do 16%, a *Penicillium* spp. od 0 do 20%.

Primenom imunoadsorpcione enzimske metode (ELISA) određen je sadržaj AFB_1, DON i FBs u zrnu kukuruza. Razlike između ispitivanih hibrida u sadržaju mikotoksina u zrnu bile su statistički značajne ($P \leq 0,01$) za DON i FBs, ali ne i za sadržaj AFB_1. Isto tako, interakcija između hibrida i lokaliteta je bila značajna ($P \leq 0,01$) za sadržaj DON i FBs, dok nije bilo statističke značajnosti za sadržaj AFB_1. Maksimalne koncentracije AFB_1, DON i FBs bile su 1,02 μg kg^{-1} (ZP 427), 12 μg kg^{-1} (ZP 341) i 1528,56 μg kg^{-1} (ZP 427).

U zrnu kukuruza sadržaj ispitivanih mikotoksina nije premašio maksimalno dozvoljene vrednosti propisane Pravilnikom o maksimalno dozvoljenim količinama ostataka sredstava za zaštitu bilja u hrani i hrani za životinje i o hrani i hrani za životinje za koju se utvrđuju maksimalno dozvoljene količine ostataka sredstava za zaštitu bilja *(Službeni glasnik RS, 2014)*. S obzirom da su agroekološki uslovi u Srbiji povoljni za pojavu toksigenih gljiva i njihovih mikotoksina, neophodno je svake godine vršiti kontrolu zrna kukuruza, kako u žetvenom, tako i u postžetvenom periodu.

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THE EFFECT OF CROP DENSITY ON MAIZE GRAIN YIELD

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Abstract: The aim of this investigation was to estimate the effects of crop density on the plant height (PH), ear height (EH), leaf number per plant (LN), ear length (EL), number of rows per ear (NRE), number of grain per row (NGR), number of grain per ear (NGE), grain weight per ear (GWE), cob weight (CW), 1000-grain weight (1000-GW), ear diameter (ED) and grain yield (GY) in staygreen maize hybrid Dijamant 6 (FAO maturity group 600). Three crop densities (51020 plants ha^{-1}, 59524 plants ha^{-1} and 71429 plants ha^{-1}) were tested. The field experiment was carried out during 2006 and 2007 at Srem region (Putinci: latitude 44° 59′ 19" N; longitude 19° 58′ 11" E). Plots were organized as completely randomized block system design in four replications. PH (284.3 cm), EH (119.9 cm), LN (13.9), EL (20.8 cm), GWE (232.5 g), CW (56.4 g), 1000-GW (378.4 g) and GY (13.56 t ha^{-1}) were significantly higher in 2006 (favorable climatic conditions) than in 2007 (258.5 cm, 112.8 cm, 13.2, 17.9 cm, 192.9 g, 46.9 g, 232.7 g and 11.50 t ha^{-1}, respectively). Increasing crop density significantly increased the PH, EH and GY, and significantly decreases the EL, NGR, NGE, GWE, CW and 100-GW. The crop density of 71429 plants ha^{-1} is the optimal for growing this hybrid in Srem region. On that crop density hybrid more efficiently used available resources and achieved the highest grain yield.

Key words: crop density, grain yield, maize, morphological traits

Introduction

In Serbia, maize (Zea mays L.) is very important agricultural plant which is grown on 1.2 million hectares of land. The total annual production of maize is about 6 million tons and an average grain yield of 4.9 t ha^{-1}. It is used for feeding livestock (80% of the total production), human food and as industrial raw material. Grain yield of maize depends upon variability of the rainfall and temperature

regimes during summer seasons *(Mandić et al., 2013)*. However, irregular schedule and an insufficient number of plants per unit area is a major problem in the production of maize and one of the most common reasons for poor production results *(Mandić, 2011)*. The number of plants per unit area is the most important component of yield because if there are not enough plants cannot be expected high number of ear per unit area and yield. In many regions in Serbia, the maize grain yield is reduced by 1.5 to 2.2 t ha^{-1} due to the loss about 30% of plants from sowing to harvest. Plants will produce smaller ears, fewer kernels per ear and/or less grain weight per year in densely sown crops, but greater number of ear will result in a higher yield. *Mandić (2011)* found that the ear length, number of grain per row, number of grain per ear, grain weight per ear, cob weight, 1000-grain weight decreases, grain yield increases and number of rows per ear and leaf number per plant did not change with the increase of plant density. *Sharifi et al. (2009)* reported that the highest grain yield obtained from plant density of 10 plants m^{-2}, number of grain per ear, number of grain per row, ear length, number of grain per ear at plant density of 8 plants m^{-2}, while plant height at plant density of 12 plants m^{-2}. *Amiri et al. (2014)* concluded that increasing crop density increases grain yield, decreases number of grain per ear, and not change ear length, number of rows per ear, 1000-grain weight and ear diameter. Plant height, ear height and grain yield is greater under high density and ear length and number of grain per row decreases with increase in plant density in maize *(Silva et al., 2014)*. However, maize grain yield rises with planting density to some maximum value and then declines because of water supply, plant nutrients and other available resources, become limiting. Maize grain yield declines due to a decline in the harvest index, total biomass per plant and increased stem barrenness *(Boomsma et al., 2009; Mandić, 2011)*.

The aim of this study was to determine the effects of crop density on morphological traits, yield components and grain yield of maize hybrid Dijamant 6.

Materials and Methods

The field experiment was carried out during 2006 and 2007 at Srem region (Putinci: latitude 44° 59′ 19" N; longitude 19° 58′ 11" E). The tests were carried out in dry land farming on calcareous chernozem soil type. Soil analysis in the layer of 0-30 cm showed: in 2006 - pH in water of 7.38; pH in n/1KCl of 7.16; $CaCO_3$ of 16.8%; humus of 3.61%;total N of 0.23%; P_2O_5 of 17.18 mg/100 g soil and K_2O of 28.20mg/100 g soil; in 2007 - pH in water of 7.67; pH in n/1KCl of 7.46; $CaCO_3$ of 10.08%; humus of 2.79%; total N of 0.12%; P_2O_5 of 21.86 mg/100 g soil, and K_2O of 22.2 mg/100 g soil.

Maize hybrid Dijamant 6 (FAO maturity group 600) was used as material. It is hybrid with stay green trait, recommended for grain and silage production.

Preceding crop was winter wheat in both seasons. An elementary plot was 16.8 m^2 (consisting of 4 rows of 6 m length) with plant densities of 51020 plants ha^{-1}, 59524 plants ha^{-1} and 71429 plants ha^{-1}. The field experiment was arranged in a randomized block system design with 4 replications. Sowing was carried out manually between the 16th and 18th of April, with 2 seeds in seed bed to spacing of 70 × 28, 70 × 24, and 70 × 20 cm. Land rolling was applied after sowing, and thinning seedlings after germination on planned number of plants. A standard cultivation practice was applied. Basic fertilization with N-P-K fertilizer 10:30:20 at the rate of 300 kg ha^{-1} in autumn and 90 kg ha^{-1} of KAN - 27% in two doses (1/2 at the stage of 3 leaves and 2/2 at the stage of 7-9 leaves) in spring were applied.

The amount of rainfall and monthly air temperature from April to September were 398.4 mm and 18.0°C in 2006, and 358.8 mm and 18.8°C in 2007, respectively (Table 1). In 2006, higher rainfall amount were in April for 63.9 mm, June for 7.1 mm and August for 93.8 mm than in 2007. In 2006, lower rainfall amount was in May for 47.6 mm than in 2007. In 2006, average month temperatures were lower than in 2007.

Table 1. Monthly meteorological data in 2006 and 2007

Year	Month							Σ /x̄
	X-III	IV	V	VI	VII	VIII	IX	(IV-IX)
	Rainfall (mm)							
2006	211.8	63.9	31.4	92.3	39.0	156.2	15.6	398.4
2007	254.8	0	79	85.2	38.7	62.5	93.4	358.8
	Relative humidity (%)							
2006	-	78	70	78	68	81	76	75.2
2007	-	56	67	69	66	73	80	68.2
	Teperature (°C)							
2006	-	12.5	16.4	19.6	22.8	19.1	17.5	18.0
2007	-	13.0	18.5	22.0	22.6	22.3	14.3	18.8

Maize harvest was performed manual on 15th October in 2006, and 16th in 2007. The central two rows from each plot were used to determine grain yield (GY). GY is calculated on a 14% moisture basis. Ten plants from each plot were taken for measuring plant height (PH), ear height (EH), leaf number $plant^{-1}$ (LN), ear length (EL), number of rows per ear (NRE), number of grain per row (NGR), number of grain per ear (NGE), grain weight per ear (GWE), cob weight (CW), 1000-grain weight (1000-GW) and ear diameter (ED).

Data were processed using analysis of variance (ANOVA). The statistical tests were carried out using STATISTICA (version 10; StatSoft, Tulsa, Oklahoma, USA). The significance level was set at $P \leq 0.05$ and $P \leq 0.01$. Differences between traits means were assessed using Duncan's Multiple Range Test at $P \leq 0.05$ level.

Results and Discussion

Results showed that the year had highly significant effect on PH, EH, LN, EL, GWE, CW, 1000-GW and GY (Table 2).

Table 2. Effects of year and crop density on morphological traits, yield components and grain yield of maize

Factor		PH	EH	LN	EL	NRE	NGR	NGE	GWE	CW	1000-GW	ED	GY
Year (A)	2006	284.3^{a}	119.9^{a}	13.9^{a}	20.8^{a}	15.1	39.2	594.0	232.5^{a}	56.4^{a}	378.4^{a}	4.8	13.56^{a}
	2007	258.5^{b}	112.8^{b}	13.2^{b}	17.9^{b}	14.8	38.5	570.9	192.9^{b}	46.9^{b}	232.7^{b}	4.6	11.50^{b}
Crop density (B)	51020	266.7^{b}	112.7^{b}	13.5	21.3^{a}	15.2	42.4^{a}	645.5^{a}	250.6^{a}	60.3^{a}	341.1^{a}	4.8	11.79^{b}
	59524	270.8^{b}	116.8^{ab}	13.6	19.4^{b}	15.0	38.6^{b}	579.7^{b}	210.8^{b}	51.2^{b}	300.7^{b}	4.8	12.51^{ab}
	71429	276.8^{a}	119.6^{a}	13.6	17.4^{c}	14.7	35.7^{b}	522.1^{b}	176.7^{c}	43.5^{c}	275.0^{c}	4.5	13.30^{a}
F test	A	**	**	**	**	ns	ns	ns	**	**	**	ns	**
	B	**	*	ns	**	ns	*	**	**	**	**	ns	*
	A × B	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
M		271.4	116.4	13.6	19.4	15.0	38.9	582.4		51.7	305.6	4.7	12.53

Note: PH, Plant height (cm); EH, Ear height (cm); LN, Leaf number per plant; EL, Ear length(cm); NRE, Number of rows per ear; NGR, Number of grain per row; NGE, Number of grain per ear; GWE, Grain weight per ear(g); CW, cob weight (g); GW, 1000-grain weight (g); ED, Ear diameter (cm); GY, Grain yield (t ha^{-1}); Means followed by the same letter within a column are not significantly different by Duncan's Multiple Range Test at the 5% level ($p \leq 0.05$); ** - significant at 1% level of probability, * - significant at 5% level of probability and ns - not significant

PH (284.3 cm), EH (119.9 cm), LN (13.9), EL (20.8 cm), GWE (232.5 g), CW (56.4 g), 1000-GW (378.4 g) and GY (13.56 t ha^{-1}) were significantly higher in 2006 than in 2007 (258.5 cm, 112.8 cm, 13.2, 17.9 cm, 192.9 g, 46.9 g, 232.7 g and 11.50 t ha^{-1}, respectively). However, in 2007 we have noticed slow initial growth of seedlings and uneven initial plant growth. The initial population of plants and adequate development affected maize grain yield *(Mandić, 2011)*. *Pommel et al. (2002)* concluded that difference in seedlings emergence affect canopy development of maize plant. *Egli and Rucker (2012)* have concluded that plants with a delay of emergence were responsible for reducing crop growth than plants with early emergence. In June maize is in stage of intensive growth of the stem. Although amount of rainfall in June in 2007 (Table 1) was higher than in 2006 the lagging of growth at the beginning of the growing season influenced that plants are lower. During the 2006, the amount of rainfall in August (156.2 mm) was above conditional-optimal for grain filling (95 mm). The grain filling stage of maize is in August. Thus, GWE and GW were higher in this year than in 2007 when amount of rainfall in August was lower (62.5 mm). *Mandić et al. (2013)* reported that drought stress in August reduced GWE and the share grain in ear was which leads to a reduction in the yield.

The crop density had significant effect on PH, EH, EL, NGR, NGE, GWE, CW, 1000-GW and GY. PH increased 266.7 to 276.8 cm, EH increased 112.7 to 119.6 cm, and GY increased 11.79 to 13.30 t ha^{-1} with increasing crop densities. EL decreased 21.3 to 17.4 cm, NGR decreased 42.4 to 35.7, NGE decreased 645.5 to 522.1, GWE decreased 250.6 to 176.7 g, CW decreased 60.3 to 43.5 g and 1000-GW decreased 341.1 to 275.0 g with increasing crop densities. The increased plant populations intensified interplant competition for light and stimulate apical dominance and lengthening internodes. Accordingly PH and EH is greater under high density. EL, NRE, NGR, NGE, GWE, CW and 1000-GW decreased linearly as crop density increased because the resources, light, water and nutrients are limited. Interplant competition for sunlight, soil nutrients and soil water increases with increasing crop density. Increasing crop density reduces the quantity of resources available for each individual, and intraspecific competition becomes more intense *(Mandić 2011)*. Also, *Sangakkara et al. (2004)* reported that the interspecific competition for nutrients uptake and sunlight interception increased with increasing plant population of maize. The interplant competition for light, water and nutrients induces barrenness, decreases the number of ears per plant, NKE and GY *(Sangoi and Salvador, 1998)*. Also, *Ottman and Welch (1989)* reported that high plant population increases barrenness and decreases kernel number per plant and kernel size. EL was reduced which resulted in fewer NGR and NGE. Higher planting densities increase plant sterility and the interval between pollen shed and silk emergence, reducing the NGE *(Sangoi et al., 2002)*. However, the lower values of the traits of the ear shall be compensated larger number of plants per unit area, and a large number of ears per unit area. Many research showed that increasing crop density increases PH and EH *(Shafi et al., 2012; Sharifi et al., 2009; Silva et al., 2014)*, decreases EL *(Gozubenli et al., 2004, Sharifi et al., 2009; Mandić, 2011; Khah et al., 2012; Silva et al., 2014; Ijaz et al., 2015; Imran et al., 2015)*, decreases NGR *(Sharifi et al., 2009; Abuzar et al., 2011; Silva et al., 2014; Ijaz et al., 2015)*, decreases NGE *(Abuzar et al., 2011; Mandić, 2011; Amiri et al, 2014)*, decreases GWE *(Gozubenli et al., 2004; Mandić, 2011; Ijaz et al., 2015)*, decreases CW *(Azam et al., 2007; Mandić, 2011)*, decreases 1000-GW *(Mandić, 2011; Zamir et al. 2011; Ijaz et al., 2015)*, increases GY *(Mandić, 2011; Shafi et al., 2012; Amiri et al, 2014; Silva et al., 2014; Mahdi and Ismail, 2015)*.

Different crop density had no significant effects on LN (ranging from 13.5 to 13.6), NRE (ranging from 14.7 to 15.2), and ED (ranging from 4.5 to 4.8). NRE is genetic traits that depend primarily on the genotype, rather than growing conditions *(Mandić 2011)*. Also, many researchers showed that crop density did not significantly affect NRE *(Sharifi et al. 2009; Ashrafi and Seiedi, 2011; Mandić, 2011; Amiri et al, 2014; Ijaz et al., 2015; Mahdi and Ismail, 2015)*, LN *(Mandić, 2011; Rahmani et al., 2015)* and ear diameter *(Amiri et al, 2014; Rahmani et al., 2015)*.

Year and plant density interactions were not observed for investigated traits.

Conclusions

Results of the study showed that the hybrid Dijamant 6 responded positively to high plant densities. In our study grain yields increased about 0.72-1.51 t ha^{-1} by increasing plant density. The maximum PH, EH, and GY were recorded at highest crop density (71429 plants ha^{-1}). Contrary, the maximum EL, NRE, NGR, NGE, GWE, CW and 1000-GW were recorded in the lowest crop density (51020 plants ha^{-1}). LN, NRE and ED did not change with increasing crop density. The crop density of 71429 plants ha^{-1} is the optimal for growing this hybrid in region Srem. This hybrid more efficiently uses available resources and achieves the highest grain yield with above mentioned crop density.

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Uticaj gustine useva na prinos zrna kukuruza

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Rezime

Cilj ovog istraživanja bio je da se ispita uticaj gustine biljaka na visinu biljke (VB), visinu klipa (VK), broj listova po biljci (BL), dužinu klipa (DK), broj redova zrna na klipu (BRZ), broj zrna u redu (BZR), broj zrna po klipu (BZK), prinos zrna po klipu (PZK), masu kočanke (MK), masu 1000 zrna (MHZ), prečnik klipa (PK) i prinos zrna (PZ) u hibrida Dijamant 6 (FAO 600 grupa zrenja). Tretmani su bili: 51020 biljaka ha^{-1}, 59524 biljaka ha^{-1} i 71429 biljaka ha^{-1}. Poljski ogledi izvedeni su 2006. i 2007. godine u regionu Srema (Putinci 44° 59′ 19" SGŠ, 19° 58′ 11" IGD). Ogledi su postavljeni po slučajnom blok sistemu u četiri ponavljanja. VB (284.3 cm), VK (119.9 cm), BL (13.9), DK (20.8 cm), PZK (232.5 g), MK (56.4 g), MHZ (378.4 g) i PZ (13.56 t ha^{-1}) bili su značajno veći u 2006 godini (povoljni klimatsku uslovi) nego u 2007. (258.5 cm, 112.8 cm, 13.2, 17.9 cm, 192.9 g, 46.9 g, 232.7 g i 11.50 t ha^{-1}). Povećanje gustine useva značajno je povećalo VB, VK i PZ, i značajno smanjilo DK, BZR, BZK, PZK, MK i MHZ.

Gustina useva 71429 biljaka ha^{-1} je optimalna za gajenje ovog hibrida u regionu Srema. Na toj gustini hibrid najefikasnije koristi raspoložive resurse i postiže najveći prinos zrna.

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IMPACT OF AGRO-ECOLOGICAL CONDITIONS ON PROTEIN SYNTHESIS IN HEXAPLOID WHEAT - SPELT (*Triticum Spelta*)

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Communication

Abstract: Technological quality of wheat is defined by physical and chemical indicators of quality and its baking properties. To make wheat a commodity, there are certain requirements to be met, defined by minimum values of trade quality indicators. As hexaploid wheat, spelt (*Triticum spelta* L.) belongs to a group of alternative cereal grains high in gluten, and its flour is therefore used for making most pastries. Due to its high nutritional value, spelt flour is used to enhance the quality or flavour of wheat bread and other bakery products. Two-year research was conducted during 2011 and 2012 to investigate protein content in crops grown on the Eutric Cambisol soil type. The research was conducted on two spelt cultivars: Hungarian *Ekö 10* and Serbian *NS Nirvana*. The results showed that *NS Nirvana* averaged a statistically significantly higher proteins content (16.76%) than Hungarian cultivar *Ekö 10* (15.65%). Climatic factors, temperatures, the intensity of light and duration of seed filling had an impact on the investigated parameter.

Key words: alternative cereal grain**,** spelt, climatic factors, protein content, correlation.

Introduction

Wheat is one of the most important crop cultures grown in Serbia, on approximately 500,000 ha; with an average yield of 3,700 kg/ha (*Statistical Yearbook of Serbia, 2012*). It has been used for thousands of years to provide food

for humans. For livestock feeding, wheat kernels can be used as concentrated livestock feed, whereas whole plant can be used as fodder *(Krnjaja et al., 2014*).
The group of alternative cereal grains comprises some old and nearly forgotten wheat cultivars, such as spelt, emmer (*Triticum dicoccum* Schrank), einkorn (*Triticum monococcum* L.), club wheat (*Triticum compactum* Host.) and khorasan wheat (*Triticum turanicum*). Spelt occupies an important place in this group, due to its biological, dietary and medicinal properties. Spelt (*Triticum spelta* L.) as hexaploid wheat belongs to a group of alternative cereal grains of the genus *Triticum*, with fragile spikes and chaffy kernels. The biological properties and chemical composition of spelt make it suitable for growing in our areas as well (*Pavićević, 1988; Glamočlija, 2004; Jankovic et al., 2013; 2015*). During harvest, spelt spikes fall apart into spikelets, which mostly contain two, and sometimes even three kernels (caryopsis). The kernels are closely covered with chaff, and in non-breed populations it is hard to separate the chaff from the kernels (*Pavicevic, 1988*; *Ugrenovic, 2013*). The chaff usually makes up 25%-35% of total kernel weight (*Medović, 2003*).

The nutritional value of kernels, as mentioned by *Ruibal-Mendueta et al. (2002)*, reflects in a high level of total protein (up to 19%) with increased essential amino acids. It comprises a lot of dietary fibres, vitamin B complex, and mucopolysaccharides that stimulates an immune response, and it also comprises increased levels of oil and mineral salts. Spelt kernels are high in gluten, and its flour is used for making most of pastries (*Pržulj et al., 2012*). Due to a high nutritional value, spelt flour is used as a quality and flavour enhancer for wheat bread and other bread and bakery products (*Galova and Knodlochova, 2000*).

Spelt is an alternative to wheat in making bread, to barley and oats in animal feed and to barley in beer brewing. It is mostly used as a substitute for wheat flour to make bread, pasta, biscuits, crackers, muesli for breakfast, puff pastry, pancakes, and waffles; partially de-husked spelt can be used for brewing and making gin and vodka, but also as a substitute for unglazed rice (*Pržulj et al., 2012*). Kernels of this cereal grain are easy to digest and therefore recommended to the sick and convalescent, but also to children and the elderly. Besides high dietary and nutritional value, one should also mention medicinal properties of kernels and whole plants of spelt (*Ikanović, 2013*; *Ugrenović, 2013*).

To make wheat a commodity, there are certain requirements to be met, defined by minimum values of trade quality indicators. These requirements are differently regulated worldwide. Indirect indicators comprise physical, chemical and rheological indicators of quality, namely all the indicators that indirectly imply technological quality of wheat. Direct indicators define what kind of end-product can be made from such wheat, thus including testing of its baking properties (*Živančev, 2014*).

The composition of wheat comprises the following chemical compounds: starch, proteins, dietary fibres and fat (*Goesaert et al., 2005*). Protein levels in

kernels are chemical indicators of spelt quality. Proteins make the second most abundant group of compounds in wheat kernels, right after starch. The share of proteins in kernels usually ranges from 8 to 15% (*Shewry, 2009*). The protein level is one of the most important chemical indicators according to which wheat is classified into quality classes (SRPS E.B1 200). Milling of hard wheat with high protein levels (from 11 to 14%) gives a suitable ingredient for the baking industry (*Halverson and Lawrence, 1988*).

The goal of this research was to investigate the impact of years on protein synthesis in spelt kernels (*Triticum spelta* L.) on the brown forest-type soil. Furthermore, the goal was also to point out the ecological potential of this alternative cereal grain as a key protein source of nutrients and the most versatile ingredients of life. Spelt is also an element of biodiversity; it is tolerant to adverse climatic factors and successfully uses favourable elements from the environment.

Material and Methods

The research on the impact of the year on protein synthesis in kernels of two spelt wheat cultivars (*Triticum spelta* L.): Hungarian (*Ekö 10*) and Serbian Novi Sad cultivar (*Nirvana)* were conducted on an experimental field of the Faculty of Agriculture in Banja Luka (Serb Republic) during 2010/2011 and 2011/2012, in early March (in the both years of research), and set up in a form in a randomized block design with three repetitions.

This spelt wheat was produced by using cropping practices commonly applied in wheat (*Triticum vulgare* L.) production. Sowing was done in the optimal period – in the first decade of November in both years of the research, on a basic plot size of 10 m^2 with potato as the preceding crop. Plant density for spelt varieties was 500,000 plants ha^{-1}. Harvest was carried out at full maturity of the crops. The harvest was conducted manually.

For analysing technological quality of the spelt kernels, the samples were taken after de-husking of the kernels, by sowing periods. The percentage of protein was calculated on a dry matter basis, according to the Kjedahl method at the Laboratory of the Faculty of Agriculture in Banja Luka.

The field trial was set on brown forest soil (eutric cambisol, according to FAO classification of soil). The soil had low levels of humus (1.38%) and total nitrogen (0.114%), and was poor in easily available phosphorus (5 mg in 100 g soil) and potassium (11.8 mg in 100 g soil). Due to a pronounced acid reaction (pH 6.1 in H_2O), it had high levels of available Fe, Cu, Mn and Zn as well as Pb derived from the rock it is formed on (*Glamočlija et al., 2015; Jankovic et al., 2015*).

The domestic NS cultivar *Nirvana* was created at the Institute of Field and Vegetable Crops in Novi Sad, by re-selecting a local population. This cultivar has been on the National List of Varieties in the Republic of Serbia since 2004. *Nirvana* belongs to a group of late cultivars, tolerant to winter. It is characterised

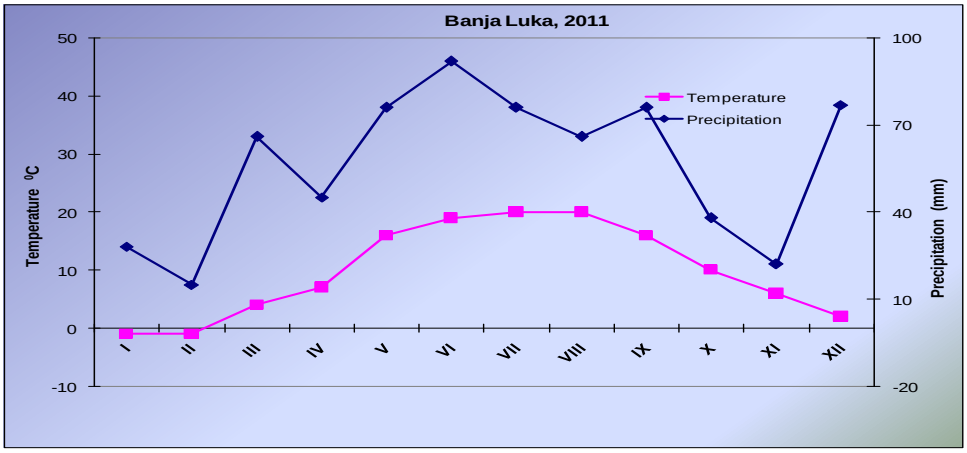
by high adaptability and tolerance to different soil and agro-ecological conditions. Its requirements for nitrogen are not so high, so it achieves the best results on soil of moderate fertility. However, its high stem makes spelt prone to lodging when soil is too fertile, as well as in intensive nitrogen use. The genetic cropping potential of this cultivar is over 4,000 kg ha^{-1} (*Mladenović and Denčić, 2010*). According to the research of *Bodroža-Solarov et al.* (*2010a*), its chaff averages 22.7% of total weight. High levels of protein, mineral salts and gluten, and a quite specific ratio between gliadin and glutenin make its flour be used for making special bakery products of high nutritional value.

Field data were analysed by using descriptive and analytical statistics, with the help of *STATISTICA 12 for Windows* software package. All estimations of significance were based on the LSD test (significance levels 0.5% and 0.1%). Relative dependence was determined with a correlation analysis and the obtained coefficients for the significance levels of 0.5% and 0.1%. The results are shown in tables and graphs.

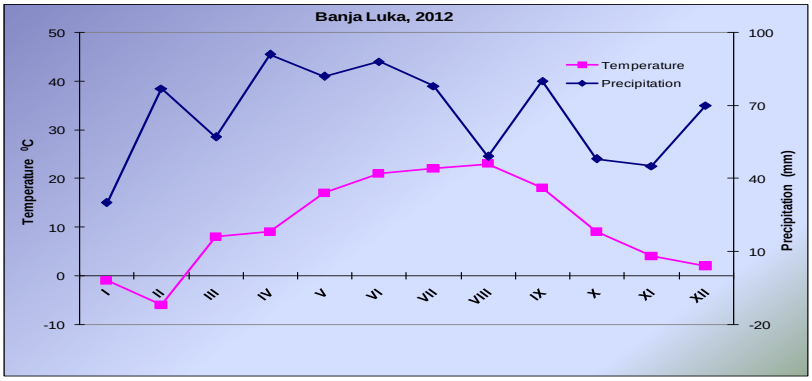
Results and Discussion

Meteorological conditions

In this research, meteorological data were retrieved from a weather station in Banja Luka, Graph 1 and 2.



Graph 1. Monthly precipitation (mm) and average temperatures (^{0}C), Banja Luka, 2010/2011



Graph 2. Monthly precipitation (mm) and average temperatures (^{0}C), Banja Luka, 2011/2012

Meteorological conditions are changeable and unpredictable (*Popovic*, 2010), having a major impact on plant growth (*Popović et al.*, 2013a; *Ikanović et al.*, *2014; Mandic et al., 2015*). In the years of the research, the average monthly temperature of air was 10.17^{o}C, being higher in 2012 (10.50^{o}C) than in 2011 (9.83^{o}C). The average monthly precipitation in the years of the research was 736 mm, varied from 677 mm in 2011 to 795 mm in 2012 (Graph 1). Climatic factors had a significant impact on wheat quality, Table 1, Graph 3.

Protein content in spelt seed

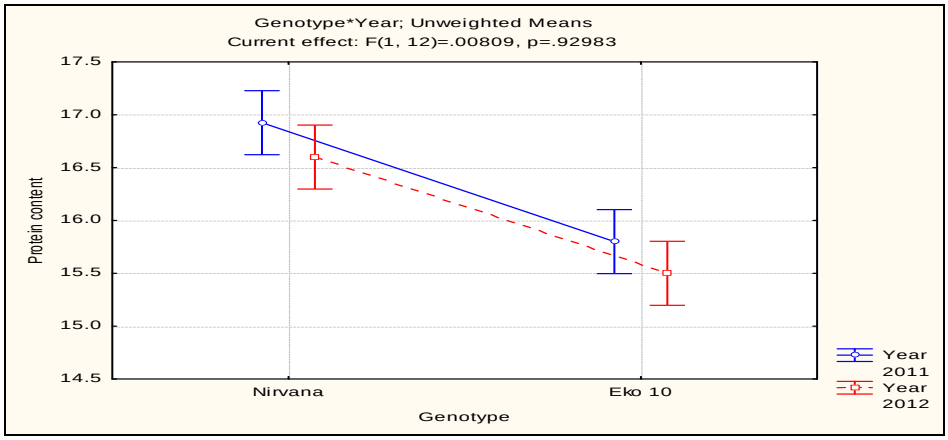
The results show that NS cultivar *Nirvana* had, on average, a statistically highly significantly higher protein content (16.76%) than *Ekö 10* (15.65%). The genotype and year had a statistically significant impact on the investigated property, Table 1.

Observed by year, the NS cultivar *Nirvana* had statistically highly significantly higher protein content than the Hungarian cultivar *Ekö 10*. *Nirvana* had 1.13% higher protein content (16.93%) than *Ekö 10* in 2011, and 1.10% higher (16.60%) in 2012, Table 1, Graph 3 and 4.

Table 1. Protein content (%) in spelt grain

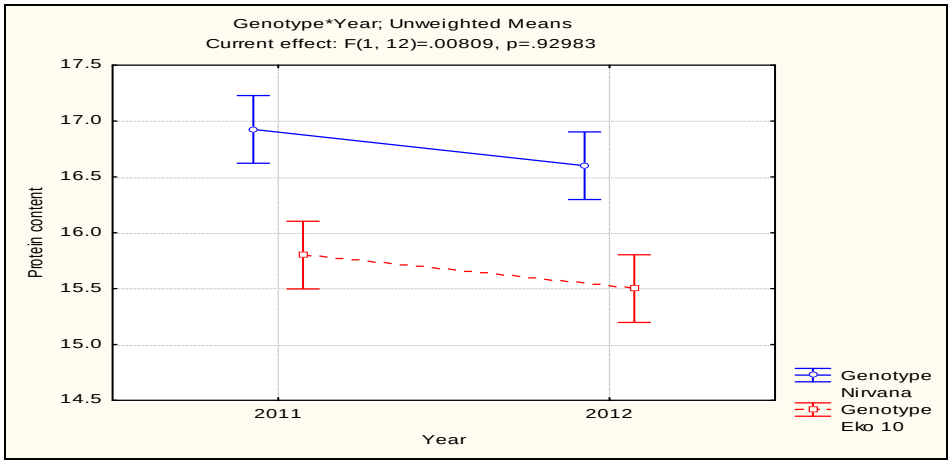
Year (A)	Genotype (B)		Average (B)	Std. Dev.	Std. Error	No. repl.
	Nirvana	*Ekö* 10				
Protein content, %						
2011	16.93	15.80	16.36	0.66	0.23	8
2012	16.60	15.50	16.05	0.64	0.23	8
Average, A	16.76	15.65	16.21	0.65	0.16	16

Indicator	LSD-test	G	Y	G x Y
Protein Content	0.05 0.01	0.31 0.43	0.30 0.43	0.43 0.61



Graph 3. Impact of genotypes on protein content in spelt seed, *Nirvana* and *Ekö 10* (%)

The years of the research had a significant impact on protein content in the spelt grain. In 2012, both cultivars had on average statistically significantly lower values of this property, 16.05% lower than in 2011 (16.36%). There difference of 0.31% between the years was recorded. The standard deviation for protein content averaged 0.65, Table 1 and 2, Graph 4.



Graph 4. Impact of years on protein content in spelt seed, *Nirvana* and *Ekö 10* (%), 2011-2012

Climatic factors, such as precipitation, temperatures, light intensity and duration of seed filling had an impact on protein content.

Protein content was negative non significant correlation with temperature and precipitation, table 2.

Table 2. Correlations in tested parameter

Parameter	Protein content	Temperature	Precipitation
Protein content	1,00	-0,25^{ns}	-0,25 ^{ns}
^{ns} – non significant			

According to *Malešević et al. (2008),* climatic conditions prolong the filling, resulting in well-filled seed low in protein. In contrast to this research, *Zhao et al.* (*2009*) determined that artificially caused draught did not cause an increase in wheat protein. In hot and arid areas, such as plains in the USA and regions of the Mediterranean, wheat is normally high in protein (*Živančev*, *2014*). Technological quality of wheat is defined by physical and chemical indicators of quality and its baking properties (*Malešević et al. 2008*). To make wheat a commodity, there are certain requirements to be met, defined by minimum values of trade quality indicators.

Conclusions

Spelt (*Triticum spelta* L.) is an alternative cereal grain, a key source of nutrients and the most versatile ingredients of life. Spelt is also an important element of biodiversity and it is tolerant to adverse climatic conditions.

The results of this research show that NS *Nirvana* averaged statistically significantly higher protein content (16.76%) than Hungarian cultivar *Ekö 10* (15.65%). The years of research also had a statistically significant impact on protein content in spelt seed. In 2012, the tested cultivars averaged statistically significantly lower protein content (16.05%) than in 2011. The difference between the years was 0.31%.

Climatic conditions, such as: precipitation, temperatures, the intensity of light and duration of seed filling had an impact on the investigated parameter, namely protein synthesis. Protein content was negative non-significant correlation with temperature and precipitation.

Acknowledgement

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Uticaj agroekoloških uslova na sintezu proteina hehaploidne pšenice krupnik - *Triticum Spelta L.*

Jela Ikanović, Vera M. Popović, Snežana Janković, Gordana Dražić, Slobodanka Pavlović, Mladen Tatić, Ljubiša Kolarić, Vladimir Sikora, Ljubiša Živanović

Rezime

Tehnološki kvalitet pšenice definisan je fizičkim i hemijskim pokazateljima kvaliteta i pecivnim osobinama. Da bi pšenica bila predmet trgovine potrebno je da zadovoljava određene uslove koji su definisani minimalnim vrednostima pokazatelja prometnog kvaliteta. Heksaploidna pšenica krupnik (*Triticum spelta* L.) pripada grupi alternativnih žita koja ima visok sadržaj glutena, te se od njegovog brašna uspešno pravi većina peciva. Zahvaljujući visokoj hranljivoj vrednosti brašno krupnika koristi se kao poboljšavač kvaliteta i ukusa pšeničnog hleba i drugih hlebno-pekarskih proizvoda.

Dvogodišnja istraživanja izvedena su tokom 2011. i 2012. godine u cilju ispitivanja sadržaja proteina na zemljištu tipa gajnjača. Ispitivane su dve sotre krupnika: mađarske sorta *Ekö 10* i srpska NS sorta *Nirvana.* Rezultati su pokazali da je NS sorta *Nirvana* imala u proseku statistički značajno veći sadržaj proteina (16.76 %) u odnosu na mađarsku sortu *Ekö 10* (15.65 %). Klimatski faktori temperatura, intenzitet svetlosti i dužina trajanja faze nalivanja zrna imali su uticaj na ovaj ispitivani parametar.

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Corresponding author: Milan M.Petrović, milpet99@gmail.com
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Example 2

EFFECTS OF REARING SYSTEM AND BODY WEIGHT OF REDBRO BROILERS ON THE FREQUENCY AND SEVERITY OF FOOTPAD DERMATITIS

Zdenka Škrbić, Zlatica Pavlovski, Miloš Lukić, Veselin Petričević

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