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Content

EFFECT OF FEEDING MIXTURE ADJUSTED BY SUNFLOWER AND LINSEED OIL ON SELECTED FATTY ACIDS IN MEAT OF LAMBS FROM ARTIFICIAL REARING

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ABSTRACT

The aim of this study was to analyze the effect of sunflower and linseed oil supplementation on selected fatty acid content in meat of light lambs. Forty Lacaune artificially reared lambs were divided into four groups (4x10) and were fed a control diet (C group) and three experimental diets (5% sunflower oil, 5% linseed oil, and 5% linseed + sunflower oil supplement; SO, LO and SLO groups). Lambs were slaughtered at an age of 49 days and a mean live weight of 16.26 ± 2.15 kg. Fatty acid composition were analysed in intramuscular fat of *Musculus longissimus lumborum et thoracis* by gas chromatography. Differences between dietary groups were calculated using General Linear Model of SAS 9.2. Linseed oil supplementation increased C18:3n-3 (0.22 in C vs. 0.37 g/100 g FAME in LO group, $P<0.001$) but decreased a total amount of CLA (0.45 in C group vs. 0.33 g/100 g FAME in LO group, $P<0.05$). The supplementation of sunflower oil had no significant influence on selected fatty acid content but in combination with linseed oil increased C18:3n-3 (0.44 vs. 0.22 g/100 g FAME, $P<0.001$) and decreased content of C18:3n-6 (0.10 vs. 0.15 g/100 g FAME, $P<0.05$) and LA : ALA ratio (35.22 vs. 55.80, $P<0.01$). There were no significant effects of

sunflower and linseed supplementation on C20:5n-3 (EPA), C22:5n-3 (DPA) and C22:6n-3 (DHA) contents or n-6 : n-3 ratio of intramuscular fat of light lambs.

Keywords: light lamb, artificial rearing, fatty acid composition, linseed oil, sunflower oil

INTRODUCTION

The amount of fat and especially a composition of fatty acids (FAs) in consummated meals are very important in the human diet. A proportion of saturated (SFA) and polyunsaturated (PUFA) fatty acids in the diet play an important role in human nutrition. Meat of ruminants may be a good dietary source of some FAs with health benefits such as long chain PUFA (LC-PUFA) and different isomers of conjugated linoleic acid (CLA). Some isomers of CLA have been associated with inhibition of carcinogenesis, reduction atherosclerosis, modification of the immune response and body fat repartitioning. The increase of health beneficial FAs and the decrease of SFA has been an important topic of ruminant meat research. Production systems, mostly nutrition of lambs may have considerable effect at carcass quality (Bas & Morand-Fehr, 2000; Díaz et al., 2005; Arsenos et al., 2006; Vasta et al., 2008) and fatty acid profile of meat (Wood et al. 2003; Lanza et al., 2006; Nuernberg et al., 2008; Jerónimo et al., 2009).

The production of suckling lambs prevails in Slovakia due to higher demand for light lambs at Easter and Christmas time in Mediterranean countries. Two basic rearing systems for production of light lambs can be found. A common production system is traditional rearing (TR), which is used for less productive dairy breeds and is based on mother´s milk. This system is usually applied with using nurseries until the end of weaning (Margetín, 2007; Margetín et al., 2009). The second one is artificial rearing (AR), which is usually applied in highly productive dairy breeds. This system is based on feeding a commercial milk replacer. There is a close relationship between meat quality and feed composition. The lamb meat from AR system is worse source of health beneficial *n-3* LC-PUFA and CLA than lamb meat from TR system and also AR lambs had fourfold higher n-6/n-3 ratio than TR lambs (Margetín et al., 2009; Margetín et al., 2013; Margetín et al., 2014; Ľuptáková, 2016; Janíček-Margetín, 2017). Mother milk

intake may have significant influence on *n-3* LC-PUFA level in intramuscular fat and weaning decrease the level of these FA (Osorio et al., 2007; Cividini et al., 2014).

The linseed oil and sunflower oil have a high proportion of essential FAs, especially C18:2n-6 (linoleic) and C18:3n-3 (*α*-linolenic). Feed which contents sunflower and linseed oil may increase *n-3* PUFA and CLA in intramuscular fat of lambs (Bessa et al., 2007; Díaz et al., 2011). Feeding by diet with higher content of C18:2n-6 and C18:3n-3 increase a proportion of C18:1*trans*11 (*trans*-vaccenic acid), which is precursor of CLA isomers, but feeding with supplement of linseed oil has less effect on increasing CLA in intramuscular fat than supplement of sunflower oil (Noci et al.,2007; Bessa et al., 2007). Ivan et al. (2001) reported increase of CLA level in lambs´ rib muscles after adding of sunflower oil (6 % in dry matter of feed). Ebrahimi et al. (2013) founded the increase a content of *n-3* PUFA, mostly C18:3n-3 in subcutaneous fat of kid goats after supplementation linseed oil to palm silage, however the level of C20:5n-3 (eicosapentaenoic - EPA), C22:5n-3 (docosapentaenoic - DPA) and C22:6n-3 (docosahexaenoic - DHA) showed a low values. According to Kitessa et al. (2009), the strongest increase of C18:2n-6 and C18:3n-3 in lamb meat is in first weeks after supplementation of linseed oil to lambs´ feed, while contents of C20:5n-3, C22:5n-3 and C22:6n-3 are increased only after minimally six weeks of linseed oil supplementation to the feed. These fatty acids are important for proper human fetal development and also they may affect many aspect of cardiovascular function (Swanson et al., 2012). Urrutia et al. (2014) observed the increase of *n-3* PUFA more than twofold after linseed supplementation in the diet. Increase of PUFA and PUFA/SFA ratio after adding sunflower oil to lamb feed (6 % in feed) was reported by Mir et al. (2000) and Noci et al. (2005). The aim of this experiment was to evaluate an impact of sunflower and linseed oil on composition of fatty acids in intramuscular fat of light lambs from artificial rearing.

MATERIAL AND METHODS

The experiment was carried out with forty Lacaune light lambs, which were artificially reared in common environmental condition on farm. After colostral nourishment, lambs were randomly assigned to four groups of ten animals each and given one of the

following feeding treatments *ad libitum*: control group of lambs received control diet (commercial supplemental feed mixture OV-02 as presented in Table 1; C group), SO group (lambs received OV-02 enriched by 5 % of sunflower oil), LO group (lambs received OV-02 enriched by 5% of linseed oil) and SLO group (lambs received OV-02 enriched by 2.5 % of sunflower oil and 2.5 % of linseed oil). Every lamb had *ad libitum* access to grass hay and commercial milk replacer available from an automatic feeding machine.

Table 1. Ingredients and chemical composition in 1 kg of commercial supplemental feed mixture OV-02

Ingredients	**(%)**	**Chemical composition**	**(g)**
Barley	20.5	Dry matter	887.3
Corn	24.2	N-free substances	190.7
Soybean meal	16.0	Fat	35.0
Sunflower meal	13.5	Crude fiber	86.8
Alfalfa meal	12.0	Ash	52.3
Barley malt flower	10.0		
Lamivit	3.0		
Calcium carbonate	0.8		

Lambs were slaughtered in the Department of Animal Husbandry at SUA Nitra. Twenty-four hours after slaughtering and chilling were taken 100 g meat samples from *Musculus longissimus lumborum et thoracis* (*MLLT*) between 9th and 13rd thoracic vertebra of each lamb to determine the fatty acid profiles in intramuscular fat (IMF). Samples were minced, vacuum packed and stored at -25 °C until lipid analysis. For analysis of content almost 70 fatty acids, gas chromatographic analyse (GC) was used, which was realized by gas chromatograph Agilent Technologies 6890N with flame ionization detector (Agilent, Waldbronn, Germany) and 5973 Network mass-selective detector. Fatty acids methyl esters (FAME) were separated in a capillary column 100 m x 0.25 mm i.d. x 0.2 µm film thickness of HP-88 stationary phase (J&W Scientific, Agilent Technologies, California, USA). Separated fatty acids were identified by reference materials (Supelco 37 Component FAME mix, PUFA No. 3 from menhaden oil, Sigma, Aldrich, Germany), published retention data and mass spectrometric measurements. The chromatograms were published response factors of flame ionization detector for FAME (Ackman, 2000). The fatty acid composition of IMF was expressed

in grams of each individual FAME per 100 g of sum detected FAME. The average relative standard deviation of analysed FAME with content > 0.5 g.100 g^{-1} was 1.1%, for the whole analytical procedure and 5 replicated samples.

The experimental data were analysed by ANOVA. General Linear Model procedure as implemented in programme SAS (2009) was applied (SAS Institute, 2009). The influence of treatment group (C, SO, LO and SLO groups), lamb sex (males and females) and interaction considered between treatment group and lamb sex on the fatty acid profiles was studied. Estimated least squares means were compared using Scheffe's tests.

RESULTS AND DISCUSSION

Lambs were slaughtered at mean live weight 16.26 ± 2.15 kg on 49th day of life in average. From 69 isomers of fatty acids diagnosed by GC only 10 FAs were significantly affected by treatment group (C12:1; *iso*C14:0; *iso*C16:0; 15cC18:1; C18:3*n-6*; C18:2+C19:1; C18:3*n-3*; tcCLA; ttCLA; C20:1*n-9*) - P<0.05 to P<0.001.

Table 2. Proportion of chosen fatty acids and groups of FAs in intramuscular fat of the *MLLT* (g/100 g FAME)

Trait	Group of lambs (LSM)				SE	P
	C	SO	LO	SLO		
C14:0 (MA)	2.16	1.96	2.17	2.02	0.71	ns
C16:0 (PA)	20.83	19.65	20.63	20.22	2.21	ns
C18:1*cis*-9 (OA)	36.14	35.74	35.38	32.25	5.55	ns
C18:1*trans*-11 (VA)	0.23	0.30	0.31	0.28	0.09	ns
C18:2n-6 (LA)	12.51	13.00	13.34	14.88	3.37	ns
C18:2*cis*-9, *trans*-11 (RA)	0.16	0.18	0.17	0.14	0.05	ns
C18:3n-6 (GLA)	0.15^{b}	0.15^{b}	$0.12^{a,b}$	0.10^{a}	0.04	*
C18:3n-3 (ALA)	0.22^{a}	0.25^{a}	0.37^{b}	0.44^{b}	0.09	***
C20:4n-6 (AA)	5.56	6.12	5.64	6.92	3.25	ns
C20:5n-3 (EPA)	0.01	0.01	<0.01	0.01	0.01	ns
C22:5n-3 (DPA)	0.79	0.74	0.58	0.60	0.33	ns
C22:6n-3 (DHA)	0.64	0.81	0.77	1.07	0.47	ns
SFA	36.13	35.02	35.92	35.70	2.58	ns
MUFA	42.23	42.03	41.34	38.02	6.07	ns
PUFA	21.69	22.95	22.74	26.29	7.81	ns
n-6 PUFA	18.48	19.53	19.38	22.29	6.65	ns
n-3 PUFA	1.87	2.04	2.01	2.59	0.96	ns
CLA	0.45^{b}	$0.42^{b,c}$	$0.33^{a,c}$	0.33^{a}	0.10	*

n-6 PUFA : n-3 PUFA	10.91	10.09	9.80	9.03	1.53	ns
LA : ALA	55.80[a]	52.38[a]	38.13[b]	35.22[b]	13.21	**

C=control diet, SO=sunflower oil diet, LO= linseed oil diet, SLO=sunflower + linseed oil diet, SE= standard error, MA=myristic acid, PA=palmitic acid, VA=vaccenic acid, LA= linoleic acid, RA=rumenic acid, GLA= γ-linolenic acid, ALA= α-linolenic acid, AA=arachidonic acid, EPA= eicosapentaenoic acid, DPA=docosapentaenoic acid, DHA=docosahexaenoic acid, SFA=sum of saturated fatty acids, MUFA=sum of monounsaturated fatty acids, PUFA= sum of polyunsaturated fatty acids, n-6 PUFA= sum of n-6 polyunstaturated fatty acids, n-3 PUFA= sum of n-3 polyunstaturated fatty acids, CLA=Conjugated linoleic acids, ns: non-significant (P > 0.05); *P< 0.05; **P < 0.01; ***P < 0.001

The proportion of chosen fatty acids and groups of FAs in intramuscular fat of *Musculus longissimus lumborum et thoracis* is shown in Table 2. There was no significant effect on the proportion of main fatty acid groups such as SFA, MUFA, PUFA, *n-6* PUFA and *n-3* PUFA. The treatment group had significant effect on the γ-linolenic acid (C18:3n-6), which is a Δ6-desaturation product of linoleic acid (C18:2n-6). Proportion of these FA was lower in SLO group than in the control group (0.10 vs. 0.15 g/100g FAME, P<0.05). It is probably related with higher proportion of C18:3n-3 (*α-l*inolenic acid) in SLO lambs meat (0.44 vs. 0.22 g/100 g FAME, P<0.001). The α-linolenic (ALA) acid is important *n-3* fatty acid and it is converted into EPA, DPA and DHA. We observed also significant difference in proportion of *α*-linolenic acid between the LO and control group (0.37 vs. 0.22 g/100 g FAME, P<0.001). There was no significant difference between treatment groups in content of health beneficial FAs: C20:5n-3 (EPA), C22:5n-3 (DPA) and C22:6n-3 (DHA). These results are in agreement with Kitessa et al. (2009). They reported significant increase in proportion of EPA, DPA and DHA only after 6 weeks of feeding of lambs by linseed supplements. Total content of CLA was significantly higher in C group than in SLO group (0.45 vs. 0.33 g/100 g FAME, P<0.05). The higher proportion of *α*-linolenic acid in LO and SLO group had also effect on LA : ALA ratio. These ratio was 38.13 in LO group and 35.22 in SLO group, whereas in control group these ratio was 55.80 (P<0.01).

CONCLUSION

In this study the effect of feed mixture enriched by linseed and sunflower oil on chosen FAs and groups of FAs was compared with standard lamb feed mixture for artificially reared lambs. Supplementation of oils which are rich source of *n-3* PUFA to light lambs increased mainly essential *n-3* PUFA - α-linolenic acid. Supplementation of SO and LO

oils did not affect proportion of long chain *n-3* fatty acids, probably because of short time of lamb fattening. Also important fatty acid groups and ratios of FAs groups were not significantly affected by treatment group.

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REFERENCES

Arsenos, G., Kufidis, D., Zygoyiannis, D., Katsaounis, N., Stamataris, C. (2006): Fatty acid composition of lambs of indigenous dairy Greek breeds of sheep as affected by post-weaning nutritional management and weight at slaughter. Meat Science, 73, 55-65.

Bas, P., Morand-Fehr, P. (2000): Effect of nutritional factors on fatty acid composition of lamb fat deposits. Livestock Production Science, 64, 61-79.

Bessa, R.J.B., Alves, S.P., Jerónimo, E., Alfaia, C.M., Prates, J.A.M., Santos-Silva, J. (2007): Effect of lipid supplements on ruminal biohydrogenation intermediates and muscle fatty acids in lambs. European Journal of Lipid Science and Technology, 109, 868-878.

Cividini, A., Levart, A., Žgur, S., Kompan, D. (2014): Fatty acid composition of lamb meat from the autochthonous Jezersko-Solčava breed reared in different production systems. Meat Science, 97, 480-485.

Díaz, M.T., Álvarez, I., De la Fuente, J., Sanudo, C., Campo, M.M., Oliver, M.A., Font i Furnols, M., Montossi, F., San Julián, R., Nute, G.R., Caneque, V. (2005): Fatty acid composition of meat from typical lamb production systems in Spain, United Kingdom, Ger many and Uruguay. Meat Science, 71, 256-263.

Díaz, M.T., Caneque, V., Sánchez, C.I., Lauzurica, S., Pérez, C., Fernández, C., Álvarez, I., De la Fuente, J. (2011): Nutritional and sensory aspects of light lamb meat enriched in n – 3 fatty acids during refrigerated storage. Food Chemistry, 124, 147-155.

Ebrahimi, M., Rajion, M.A., Goh, Y.M., Sazili, A.Q., Schonewille, J.T. (2013): Effect of linseed oil dietary supplementation on fatty acid composieiton and gene expression in adipose tissue of growing goats. BioMed Research International, Article ID 194625, 11 pages.

Ivan, M., Mir, P.S., Koening, L.M., Neil, L., Entz, T., Mir, Z. (2001): Effect of dietary sunflower seed oil on rumen protozoa population and tissue concentration of conjugated linoleic acid in sheep. Small Ruminant Research, 41, 215-227.

Janíček, M., Margetín, M. (2017): Health beneficial omega-3 fatty acids levels in meat of lambs from artificial and traditional rearing. In: Animal breeding 2017, Brno, Czech Republic, 55-64.

Jerónimo, E., Alves, S.P., Prates, J.A.M., Santos-Silva, J., Bessa, R.J.B. (2009): Effect of dietary replacement of sunflower oil with linseed oil on intramuscular fatty acids of lamb meat. Meat Science, 83, 499-505.

Kitessa, S.M., Williams, A., Gulatia, S., Boghossianc, V., Reynolds, J., Paercea, K.L. (2009): Influence of duration of supplementation with ruminally protected linseed oil on the fatty acid composition of feedlot lambs. Animal Feed Science and Technology, 151, 228-239.

Lanza, M., Bella, M., Priolo, A., Barbagallo, D., Galofaro, V., Landi, C., Pennisi, P. (2006): Lamb meat quality as affected by a natural or artificial milk feeding regime. Meat Science, 73, 313-318.

Ľuptáková, L. (2016): Kvalita ľahkých jatočných jahniat z tradičného a umelého chovu. PhD Diss. Nitra: SPU v Nitre. 113 p., FAPZ-17352-33311.

Margetín, M. (2007): Spôsoby odchovu dojných oviec. Chov oviec a kôz, 27, 8-10.

Margetín, M., Margetínová, J., Gálisová, M., Čapistrák, A., Špánik, J. (2009): Intenzita rastu jahniat odchovaných pomocou dôsledného škôlkovania a jahniat z umelého odchovu. Slovenský chov, 14, 27-29.

Margetín, M., Margetínová, J., Oravcová, M., Luptáková, L. Horečná, Z. (2013): Carcass quality and physico-chemical characteristics of meat of light lambs. Acta fytotechnica et zootechnica, 16, 83-89.

Margetín, M., Apolen, D., Oravcová, M., Vavrišínová, K., Peškovičová, D., Luptáková, L., Krupová, Z., Bučko, O., Blaško, J. (2014): Fatty acids profile of intramuscular fat in light lambs traditionally and artificially reared. Journal of Central European Agriculture, 15, 117-129.

Mir, Z., Rushfeldt, M.L., Mir, P.S., Paterson, L.J., Weselake, R.J. (2000): Effect of dietary supplementation with either conjugated linoleic acid (CLA) or linoleic acid rich oil on the CLA content of lamb tissues. Small Ruminant Research, 36, 25-31.

Noci, F., French, P., Monahan, F.J., Moloney, A.P. (2007): The fatty acid composition of muscle fat and subcutaneous adipose tissue of grazing heifers supplemented with plant oil-enriched concentrates. Journal of Animal Science, 85, 509-518.

Noci, F., Kiely, P.O., Monahan, F.J., Stanton, C., Moloney, A.P. (2005): Conjugated linoleic acid concentration in M. Longissimus dorsi from heifers offered sunflower oil based concentrates and conserved forages. Meat Science, 69, 509-518.

Nuernberg,K., Fischer, A., Nuernberg, G., Ender, K., Dannenberger, D. (2008): Meat quality and fatty acid composition of lipids in muscle and fatty tissue of Skudde lambs fed grass versus concentrate. Small Ruminant Research, 74, 279-283.

Osorio, M.T., Zumalacárregui, J. M., Figueira, A., Mateo, J. (2007): Fatty acid composition in subcutaneous, intermuscular and intramuscular fat deposits of suckling lamb meat: Effect of milk source. Small Ruminant Research, 73, 127-134.

Swanson, D., Block, R., Mousa, S.A. (2012): Omega-3 Fatty Acids EPA and DHA: Health Benefits Throughout Life. Advances in Nutrition, 3, 1-7.

Urrutia, O., Soret, B., Insausti, K., Mendizabal, J.A., Purroy, A., Arana, A. (2015): The effects of linseed or chia seed dietary supplementation on adipose tissue development, fatty acid composition, and lipogenic gene expression in lambs. Small Ruminant Research, 123, 204-211.

Vasta, V., Nudda, A., Cannas, A., Lanza, M., Priolo, A. (2008): Alternative feed resources and their effects on the quality of meat and milk from small ruminants. Animal Feed Science and Technology, 147, 223-246.

Wood, J.D., Richardson, R.I., Nute, G.R., Fischer, A.V., Campo, M.M., Kasapidou, E., Sheard, P.R., Enser, M. (2003): Effects of fatty acids on meat quality: a review. Meat Science, 66, 21-32.

FATTY ACIDS PROFILE OF MAIZE SILAGES OF DIFFERENT HYBRIDS

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ABSTRACT

The aim of this research was to analyse fatty acid profile of maize silages of different hybrids – FAO 470 and FAO 490. Fatty acids were analyzed by gas chromatography with a flame ionization detector (FID) using the Agilent 6890A GC system. For statistical analysis of fatty acid content was used statistical program SPSS 20.0. The polyunsaturated fatty acid (PUFA) content was 52.56 $g.100g^{-1}$ in hybrid FAO 470 and 55.30 $g.100g^{-1}$ in hybrid FAO 490. The content of monounsaturated fatty acid (MUFA) was 24.36 $g.100g^{-1}$ in FAO 470 and 23.30 $g.100g^{-1}$ in FAO 490. Saturated fatty acid content (SFA) was 17.95 $g.100g^{-1}$ in hybrid FAO 470 and 18.21 $g.100g^{-1}$ in hybrid FAO

490. The highest content in both hybrids was linoleic acid (FAO 470 46.46 g.100g^{-1}, FAO 490 47.59 g.100g^{-1}), oleic acid (FAO 470 23.91 g.100g^{-1}, FAO 490 22.71 g.100g^{-1}), palmitic acid (FAO 470 13.42 g.100g^{-1}, FAO 490 13.55 g.100g^{-1}), α-linolenic acid (FAO 470 5.12 g.100g^{-1}, FAO 490 6.71 g.100g^{-1}) and stearic acid (FAO 470 2.62 g.100g^{-1}, FAO 490 2.53 g.100g^{-1}). Other fatty acids (lauric acid, myristic acid, heptadecanoic acid, γ-linolenic acid, arachidic acid, cis-11-eicosenoic acid and behenic acid) were present in corn silage below 1 g.100g^{-1}. Statistically significant differences ($p<0.05$) were found between hybrids in the content of polyunsaturated and monosaturated fatty acid, in the content of palmitoleic acid, oleic acid, α-linolenic acid and 11-cis-eicosenoic acid.

Keywords: fatty acids; PUFA; MUFA; maize silage; hybrids

INTRODUCTION

The fatty acids are the most important structure unit of all the lipids (Keresteš et al., 2011). Fatty acids form a carbon chain terminated on the one side with a methyl group and at the other end with a carboxyl group (Rustan - Devon, 2005). Chow (2008) states that there are so called the essential fatty acids that the body can´t produce and function properly must be taken in the diet. They are needed, but the organism has not require synthesis of enzymes. Lawrence (2010) adds, that these acids are precursors for the synthesis of a number of necessary substances that have a significant biological function. There are two such acids: linoleic acid and α-linolenic acid. Conditionally it also includes y-linolenic, lauric, palmitoleic fatty acids. Depending on the level of saturation, they are divided into saturated and unsaturated (Chow, 2008). Saturated fatty acids (SFA) are fatty acids without double bonds and have unbound (straight) chain. The disadvantage is that increased intake of these acids is a risk factor for cardiovascular diseases. Unsaturated fatty acids (UFA) are divided according to whether they have one (monounsaturated (MUFA) or more double bonds (polyunsaturated - PUFA) in their chain (Chow, 2008). Author also adds that, the most important polyunsaturated fatty acids are essential fatty acid - linoleic, linolenic, arachidonic, docosahexaenoic and eicosapentaenoic. The basic polyunsaturated fatty acids are separated from the position of the first double bond from the terminal methyl group of

the fatty acids omega 3 (n-3) and omega 6 (n-6).In the case of PUFA n-3, the first double bond is located on the third carbon in the molecule from the methyl end (Holub, 2002). This includes α-linolenic acid, eicosapentaenoic acid, decosapentaenoic acid and docosahexaenoic acid (Hayat et al., 2009). The PUFA n - 6, the first double bond is located at the sixth carbon from the methyl end. This includes, for example, linoleic acid and arachidonic acid (Sommer, 1999). Corn silage is a carbohydrate feed, among the easily digestible feeds with a low content of degradable nitrogenous substances. The low content of nitrogenous substances must be compensated in feed rations by protein or concentrate feed (Zeman et al., 2006). The most suitable date for harvesting maize maize is in the phase of dairy-waxy to waxy maturity of the grain. A significant factor determining the quality of maize silage is the impact of the hybrid (Bíro et al., 2008; Gálik et al., 2016).

MATERIAL AND METHODS

In our experiment, fatty acids in silage of different corn hybrids (two maize hybrids – FAO 470 (n=3) and FAO 490 (n=3) were determined. Both two maize hybrids with stay – green maturation type were planted in a 200 m above sea level on anthrosolic chernozems (Malženice, Mojmírovce) in 2013. The maize silage was ensilaged without the addition of additives and samples were taken 4 weeks after ensiling. Laboratory analysis on the fatty acid profile in silages was carried out in the Laboratory of Quality and Nutrition of Foods at the Department of Animal Nutrition at the Slovak Agricultural University. The fatty acids in maize silage by standard laboratory methods and procedures was determined. For the characteristic of lipid fraction triglycerides to glycerol and free fatty acids were hydrolyzed. Fatty acids were then derivated to methylesters. After their preparation were separated on the basis of carbon number and level of unsaturation by using gas chromatography fitted with a flame-ionization detector (FID). For the identification column 37 components mixture (Supelco 47885-U) was used. Standard solution was diluted with 10 ml of hexane with 1 ml supplementation of 2 N potassium hydroxide in methanol. Analytic tube was heated 30 seconds at 60°C in a water bath. After 1 minute 2 ml of 1 N hydrochloric acid was added. The top layer was transferred in an amount 2 ml to autosampler vial with

ninhydrin (Na_2SO_4). Injection of samples was performed by injection autosampler Agilent. The content of fatty acids on machine Agilent 6890A GC (Agilent Technologies, USA) as a percentage in crude fat was determined. The statistical program IBM SPSS 20.0 to statistically evaluate the fatty acid content of corn silage was used. Differences between groups were analyzed with one-way analysis of variance (ANOVA). Values with upper index [a] and [b] are statistically significant (P <0.05) - based on the Post Hoc Tukey test.

RESULTS AND DISCUSSION

In our analysis was found that between the maize silages of different hybrids there were statistically significant differences ($p<0.05$) in both mono and polyunsaturated fatty acids (Table 1). For monounsaturated fatty acids, in silage of hybrid FAO 470 had a content of 4.35% higher than in silage of hybrid FAO 490. On the other hand, for polyunsaturated fatty acids, the content of FAO 470 was 4.95% lower. The most represented fatty acid in the silage was linolenic acid. In silage of hybrid FAO 490, a 2.43% higher content of linoleic acid, than in silage of hybrid FAO 470 was found. However, this difference was not statistically significant. The fatty acid second most substituted fatty acid was oleic acid. In silage of FAO 490 hybrid it was 5.02% lower than in silage of FAO 470 hybrid. The difference was statistically significant ($p<0.05$). The third highest content in silage was found in palmitic acid. In silage of hybrid FAO 490, a higher content of this acid by 0.97% was found. On the other hand the lower content of 5-7% in silage α-linolenic acid was found. In silage of FAO 470 hybrid, this acid had a 23.69% lower content. This difference was statistically significant ($p<0.05$). Stearic acid in silage of FAO 470 hybrids by 3.56% more than in silages of FAO 490 hybrid was present. Other investigated fatty acid in small amounts in silage were found. The statistically significant difference ($p<0.05$) was still demonstrated in palmitoleic acid (FAO 490 by 43.47% more) and cis-11-eicosenoic acid (in silage of hybrid FAO 470 by 11.53% less). The ratio of n-3/n-6 and the n-6/n-3 fatty acid ratio was statistically not significant. However the ratio of n-3 / n-6 in silage of hybrid FAO 490 was 23.01% more and the n-6/n-3 ratio by 15.93% less than in silage of FAO 470 hybrid.

Table 1. The content of individual fatty acids in the maize silages of different hybrids

Fatty acid (g.100g^{-1})	**Maize silages**			
	Hybrid FAO 470		**Hybrid FAO 490**	
	Mean	**SD**	**Mean**	**SD**
lauric acid	0.16	0.03	0.20	0.04
myristic acid	0.20	0.03	0.23	0.04
palmitic acid	13.42	0.47	13.55	0.61
palmitoleic acid	0.23^{a}	0.22	0.33^{b}	0.05
heptadecanoic acid	0.00	0.00	0.04	0.07
stearic acid	2.62	0.10	2.53	0.12
oleic acid	23.91^{a}	0.35	22.71^{b}	0.42
linoleic acid	46.46	1.45	47.59	1.63
γ-linolenic acid	0.00	0.00	0.22	0.20
α-linolenic acid	5.12^{a}	0.33	6.71^{b}	0.58
arachidic acid	0.66	0.03	0.69	0.04
cis-11- eicosenoic acid	0.22^{a}	0.00	0.26^{b}	0.00
behenic acid	0.33	0.02	0.40	0.05
polyunsaturated fatty	52.56^{a}	1.04	55.30^{b}	1.01
monounsaturated fatty	24.36^{a}	0.34	23.30^{b}	0.38
saturated fatty acids	17.95	0.72	18.21	0.87
ratio Σn3/Σn6	0.13	0.01	0.16	0.02
ratio Σn6/Σn3	7.66	0.78	6.44	0.92

Ferlay et al. (2006) in the experiments carried out in France found the following fatty acid content in maize silage: palmitic acid 15.6 g.100g^{-1}, stearic acid 2.4 g.100g^{-1}, oleic acid 23.7 g.100g^{-1}, linoleic acid 48.6 g.100g^{-1}and α-linolenic acid content 3.4 g.100g^{-1}. AbuGhazaleh et al. (2007) in the USA in experiments of maize silage found that the palmitic acid content was of16.6 g.100g^{-1}, stearic acid 2.9 g.100g^{-1}, 18.8 g.100g^{-1}of oleic acid, linoleic acid 48.5 g.100g^{-1}and α- inolenic acid content 11.1 g.100g^{-1}. The greatest deviations from our results were recorded in Whitlock et al. (2006), which found in the USA experiments the following fatty acid contents in corn silage: palmitic acid content 29.5 g.100g^{-1}, stearic acid 3.5 g.100g^{-1}, oleic acid 4.0 g.100g^{-1}, linoleic acid 18,7 g.100g^{-1}, and the α - linolenic acid content was 4.9 g.100g^{-1}.

Alezones et al. (2010) found in 12 maize hybrids grown in Venezuela the content of monounsaturated fatty acids in the range 31,1 - 36,4 g.100g^{-1} (average 34,6 g.100g^{-1}) and the content of polyunsaturated fatty acids from 46,1 - 51,2 g.100g^{-1} (average 47.7 g.100g^{-1}). They further indicate the content of some particular fatty acids in these hybrids:

- palmitic acid from 13.4 to 14.5 g.100g^{-1} (average 14 g.100g^{-1}),
- palmitoleic acid from 0.20 to 0.27 g.100g^{-1} (average 0.2 g.100g^{-1}),
- oleic acid ranging from 30.5 to 35.8 g.100g^{-1} (average 33.9 g.100g^{-1}),
- linoleic acid from 45.1 to 50.2 g.100g^{-1} (average 46.8 g.100g^{-1}),
- arachidic acid from 0.60 to 0.87 g.100g^{-1} (average 0.9 g.100g^{-1}).

The authors also reported a significant differences between hybrids for stearic acid, oleic and linoleic acid ($p>0.01$), as well as the palmitic acid and arachidic ($p>0.05$). No statistically significant differences for the other investigated fatty acids were found.

CONCLUSION

The aim of this research was to determine the fatty acid content of maize silage of different hybrids. Among FAO 470 and FAO 490 examined hybrids were slightly different in the content of the individual fatty acids. From the content of the saturated fatty acids in silages of hybrid FAO 470 and also FAO 490 had the highest proportion palmitic acid, then stearic acid, arachidic acid, behenic acid, myristic acid, lauric and heptadecanoic acid. From monounsaturated fatty acids (MUFA) in silages of both hybrids had the highest content oleic acid, palmitoleic and cis-11-eicosenoic acid. Of polyunsaturated fatty acids (PUFA), the highest content in silages of hybrid FAO 470 and also in silages of 490 hybrid was observed in linoleic acid, α-linolenic acid and γ-linolenic acid. Compared with other studies of various authors, changes in the content of fatty acid in maize silages are related from different kinds hybrid maize and also from the environment in which the maize hybrids are grown (for example Slovak Republic, the USA, Venezuela).

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REFERENCES

AbuGhazaleh A.A., Felton D.O., Ibrahim S.A. (2007): Milk conjugated linoleic acid response to fish oil and sunflower oil supplementation to dairy cows managed under two feeding systems. Journal of Dairy Science, 90, 4763–4769.

Alezones J., Ávila M., Chassaigne A., Barrientos V. (2010): Caracterización del perfil de ácidos grasos en granos de híbridosde maíz blanco cultivados en Venezuela. Archivos Latinoamericanos de Nutricion, 60, 397–404.

Bíro D., Gálik B., Juráček M., Šimko M., Michálková J., Gyöngyová E., Taška M. (2008): Fermentation process characteristic of different maize silage hybrids. Journal of Central European Agriculture, 9, 463–468.

Ferlay A., Martin B., Pradel P., Coulon J.B., Chilliard Y. (2006): Influence of grass-based diets on milk fatty acid composition and milk lipolytic system in Tarentaise and Montbéliarde cow breeds. Journal of Dairy Science, 89, 4026–4041.

Gálik B., Bíro D., Šimko M., Juráček M., Horniaková E., Rolinec M. (2016): Nutritional characteristics of feed. 1st edition. Nitra: Slovak Agriculture University. 101 p. (in Slovak).

Holub, B.J. (2002): Clinical nutrition: omega-3 fatty acids in cardio- vascular care. Canadian Medical Association Journal, 166, 608-615.

Chow, C.K. (2008): Fatty Acids In Foods And Their Health Implications. 3rd edition. CRC Press: Boca Raton, 1298 p.

Keresteš, J., Bíreš J., Ebringer L., Ďuračková, Z. et al. (2011). Health and human nutrition. Bratislava: CAD PRESS, 1037 p.

Lawrence, G.D. (2010): The fats of life: essential fatty acids in health and disease. London: Rutgers University Press, 297 p.

Rustan, A.C, Drevon, CH.A. (2005): Fatty acid: Structure and properties. Available from: https://www.uio.no/studier/emner/matnat/ farmasi/FRM2041/v06/undervisningsmateriale/fatty_acids.pdf

Sommer, A. (1999): Fats and fatty acids in animal nutrition. 1st edition. Nitra: Výskumný ústav živočíšnej výroby. 57 p. (in Slovak).

Whitlock L.A., Schingoethe D.J., AbuGhazaleh A.A., Hippen A.R., Kalscheur K.F. (2006): Milk production and composition from cows fed small amounts of fis oil with extruded soybeans. Journal of Dairy Science, 89, 3972–3980.

Zeman, L. et al. (2006): Nutrition and feeding of farm animals. 1st edition. Praha : Profi Press. 360 p. (in Czech)

THE EFFECT OF HUMIC SUBSTANCES ON GROWTH ABILITY AND FEED UTILIZATION IN FARM PHEASANTS FATTENING

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ABSTRACT

The aim of the present research was to analyze evaluate the effect of humic substances on growth ability and feed utilization of pheasants fatted up to the 90 day of age. Totally 200 one-day-old pheasants were divided to four dietary groups (n=50) namely control and three experimental groups with supplementation of humic substances in the total feed mixtures at the dosage 0.50%, 0.75% and 1.00%. Pheasants were feeding ad libitum with feed mixtures and drinking water was provided ad libitum. In the 1, 10, 20, 30, 40, 50, 60, 70, 80, 90 day of fattening were weighed individually and average body weight gains were calculated. It was found that the body weight of pheasants was increasing with the bird age, however there were insignificant noted no significant differences among groups from 1 to 60 days of fattening. Significantly the highest body weight ($P \leq 0.05$) we recorded from 70 to 90 day of age in benefit of experimental groups with addition of 0.50 and 0.75% humic substances. In contrast, experimental group with supplementation of 1.00% humic substances had insignificant lower body weight ($P > 0.05$) compared with control from 40 to 90 day of fattening. The highest average body weight gains were observed in pheasants in period from 40 to 50 ages of fattening in control and experimental groups. We recorded highest feed conversion ratio control

group (6.07 kg), lowest feed conversion ratio was achieved in experimental group with addition of 0.50% humic substances (5.73 kg).

Keywords: pheasant; fattening; humic substances; body weight; feed conversion

INTRODUCTION

After the application of antibiotics as feed additives in order to enhance growth in production animals has lately been restricted (Enberg et al. 2000), researchers have looked for new feed additives that are not harmful to human health. Humates, a part of fertilizers, are derived from plant matter decomposed by bacteria and contain humus, humic acid, fulvic acid, ulmic acid and some microelements (Yörük et al., 2004). Humic acid based mixtures have the potential to be an alternative to antibiotic growth promoters in broiler diets (Ceylan et al., 2003). Humic acids are organic compounds naturally present in water and soil. They form three-dimensional structure molecules, containing aromatic nuclei with oxygen and nitrogen heterocycles. In the side chains, bound to an aromatic nucleus, hydroxyl, carbonyl, carboxyl, amine and sulfhydryl functional groups are present (McCarthy 2001; Zralý et al., 2008). Due to different humic acids structures, the content of functional groups and various qualities (colloidal, spectral, electrochemical and ion exchange) their considerable adsorption capacity is assumed (Alvarez Puebla et al., 2005). In many studies were observed the positive effect of humic substances on the growth of animals, feed conversion (Ozturk et al., 2012; Mirnawati and Marlida, 2013), hatchability of hens (Yörük et al., 2004; Kucukersan et al., 2005) and the viability of animals (Karaoglu et al., 2004).

The aim of this study was to analyze valuate the effect of different doses of humic substances in feed mixture on growth ability and feed utilization of fatted pheasants.

MATERIAL AND METHODS

A total 200 farm pheasants were used in the trial. The experiment started with 1 day-old chicks and lasted 90 days. Pheasants were housed to 42 day of fattening in private pheasant farm with a deep litter with controlled temperature in area of 7.36 m^2, from 43 days to end of fattening period pheasants were placed in outdoor cages (each group separately), partly covered aviaries, with area of 18.00m^2. Microclimatic parameters in

the experimental enclosure were as follows: air temperature ranged from 35°C to 23°C on average depending on age; average relative humidity was 70-75%; light regimen during the whole period of feeding was set to 24 light hours. The humic substances used in our experiment consist of 62.00 % humic acid, 9.00% fulvic acid and 9.00% minerals. Animals were divided into 4 groups, control (without humic substances) and experimental groups with supplementation of humic substances in the total feed mixtures (E1: basal+0.50%; E2: basal+0.75% and E3: basal+1.00%). Pheasants were fed by feed mixtures: starter (to 5 week of fattening) in form of crushed granules and grower (from 6 week to end of fattening) in form of granules. Feeding and watering were ad libitum. The nutritive values of the feed mixtures are presented in Table 1.

Table 1. Diet composition of feed mixtures

Nutrients	Units	Feed Mixture	
		Starter	Grower
Crude protein	%	23.50	20.00
Crude fat	%	3.00	3.30
Crude fibre	%	2.80	3.00
Ash	%	8.10	5.80
Lysine	%	1.43	1.01
Methionine	%	0.57	0.47
Calcium	%	1.50	0.90
Phosphorus	%	0.87	0.66
Sodium	%	0.15	0.14
Copper	mg/kg	30.00	18.00
Zinc	mg/kg	141.00	84.00
Manganese	mg/kg	121.00	72.00
Iron	mg/kg	80.00	48.00
Selenium	mg/kg	0.60	0.40
Iodine	mg/kg	2.00	1.00
Vitamin A	IU/kg	20101.00	12060.00
Vitamin D_3	IU/kg	5025.00	3015.00
Vitamin E (alfa-tokoferol)	mg/kg	50.00	30.00
Phytase	FTU/kg	402.00	402.00

The content of nutrients were analysed in the Laboratory of the Quality and Nutritive Value of Feeds (Department of Animal Nutrition, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture) by standard laboratory principles and methods. The body weight of birds was determined by weighing on the 1^{st}, 10^{th}, 20^{th}, 29^{th}, 40^{th}, 50^{th}, 70^{th} and 90^{th} day of fattening. Weight gains were evaluated on the basis of body weight over the whole period of fattening.

All data were statistically analyzed by ANOVA using the SAS system (SAS, 2001). Significant differences between the treatment means were compared by using Duncan Multiple Range test (Duncan, 1955).

RESULTS AND DISCUSSION

The effect of different levels of humic substances on body weight of pheasants during the fattening period is shown in Table 2.

Table 2. Average body weight of pheasants (g)

Age (days)	Control	E1	E2	E3
1	21.76±1.87	22.08±1.69	21.54±1.85	21.88±1.83
10	64.98±7.53	65.73±7.71	65.21±7.48	65.07±7.62
20	147.53±12.68	149.97±13.08	149.56±12.97	148.62±12.75
30	257.38±21.87	260.07±22.11	259.84±21.96	257.72±21.74
40	393.94±46.69	398.76±47.23	397.29±47.31	391.88±46.88
50	537.36±65.28	546.93±66.76	543.76±65.78	532.19±64.83
60	663.82±84.52	691.91±85.93a	688.32±85.88[a]	658.06±84.21[ab]
70	783.11±99.75	833.28±101.76[a]	829.97±100,65[b]	773.39±97.43 [ab]
80	898.42±126.07	961.95±129.61[a]	959.72±128.87[b]	881.48±127.69 [ab]
90	1007.86±179.76	1078.29±188.89[a]	1076.38±189.63[b]	988.74±181.76 [ab]

Values shown are mean±SD (standard deviation)
[a,b] means in a row with different superscript differ significantly (P≤ 0.05)

The average body weight of pheasants increased with the age. There were insignificant differences detected among groups from 1 to 60 days of fattening. A significant increasing in body weight (P≤0.05) was recorded in pheasants which were fed with addition of 0.50 and 0.75% humic substances from 70 to 90 ages of fattening. We found, that experimental group with supplementation of 1.00% humic substances had insignificantly lower body weight (P>0.05) in comparison with control from 40 to 90 ages of fattening. The highest average body weight at the end of fattening was achieved in experimental group with level of 0.50% humic substances with value 1078.29±188.89 grams, while the lowest weight was observed in the experimental group with level of 1.00% humic substances (988.74±181.76 grams).

Ceylan et al. (2003) and Bailey et al. (1996) reported that addition level of 0.25% humate enhanced body weight gain of broilers and these results supported results of our study. But Kocabaglı et al. (2002); Karaoglu et al. (2004) and Yalcin et al. (2005) have

reported that 0.1-0.25% humate additions did not affect body weight gain of broilers. On the contrary, supplementation of 0.5-2.5% humic acids in ration decreased body weight gain of broilers (Rath et al., 2006).

Differences in body weights between our study and other studies might be attributed to the fact that humic acids sources and levels were different.

As shown Table 3, the highest body weight gains were observed period from 40 to 50 ages of fattening for all groups.

Table 3. Average body weight gains (g/10 days)

Age (days)	Control	E1	E2	E3
1-10	4.32	4.37	4.37	4.32
10-20	8.26	8.42	8.44	8.36
20-30	10.99	11.01	11.03	10.91
30-40	13.66	13.87	13.75	13.42
40-50	14.34	14.82	14.65	14.03
50-60	12.65	14.50	14.46	12.59
60-70	11.93	14.14	14.17	11.43
70-80	11.53	12.87	12.98	10.91
80-90	10.94	11.63	11.67	10.73

As Table 4 indicates that the better average feed conversion ratio was found in experimental group with addition of 0.50% humic substances, namely 4.04 kg. The worst feed conversion ratio was achieved in control group (3.89 kg).

In comparison with our results, Suchý et al. (2003) found feed conversion 3.2 kg in 50 days of age and Mikundová et al. (2005) recorded feed conversion 5.0 kg in 90 days of age. Similarly Sarıca and Karacay (1994) found that feed conversion ratio was recorded 4.66, 4.78 and 5.04 for the 12, 13 and 14 weeks of age.

Table 4. Feed conversion ratio (kg)

Age (days)	Control	E1	E2	E3
1-10	1.72	1.69	1.64	1.71
10-20	2.12	2.11	2.11	2.09
20-30	2.66	2.62	2.69	2.68
30-40	3.37	3.52	3.52	3.39
40-50	3.97	4.17	4.13	4.04
50-60	4.88	5.08	5.06	4.93
60-70	5.06	5.29	5.31	5.11
70-80	5.49	5.76	5.74	5.54
80-90	5.73	6.06	6.07	5.78
Average	3.89	4.04	4.03	3.92

CONCLUSION

On the base of observed results, humic substances are able to affect the performance of farm pheasants. Our results suggest that pheasants fed diets with addition of 0.50% humic substances had higher average body weight and average body weight and better feed conversion ratio. In contrast, on the basis of the achieved parameters, highest level of humic substances (1.00%) indicated as ineffective.

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REFERENCES

Alvarez-Puebla, R.A., Goulet, P.J.G., Garrido, J.J. (2005): Characterization of the porous structure of different humic fractions. Colloids Surf A Physicochem Eng Asp, 256, 129-135.

Bailey, C. A., White, K.E., Domke, S.L. (1996): Evalution of menefe humate tm on the performance of broilers. Poultry Science, 75, 84.

Ceylan, N., Ciftci, I., Ilhan, Z. (2003): The effects of some alternative feed additives for antibiotic growth promoters on the performance and gut microflora of broiler chicks. Turkish Journal of Veterinary and Animal Science, 27, 727-733.

Duncan, D.B. (1955): Multiple Range and Multiple F-test. Biometrics, 11, 1-42.

Enberg, R.M., Hedemann. M.S., LESER, T.D., JENSEN, B.B. (2000): Effect of Zinc Bacitracin and Salinomycin on Intestinal Microflora and Performance of Broilers. Poultry Science 79, 1311-1319.

Karaoglu, M., Macit, M., Esenbuga, N., Durdag, H., Turgut, L., Bilgin, O.C. (2004): Effect of suplemental humate at different levels on the growth performance slaughter and carcass traits broilers. International Journal of Poultry Science, 3, 406-410.

Kocabagli, N., Alp, M., Acar, N., Kahraman, R. (2002): The effects of dietary humate supplementataion on broiler growth and carcas yield. Poultry Science, 81, 227-230.

Kucukersan, S., Kucukersan, K., Colpan, I., Goncuoglu, E. (2005): The effects of humic acid on egg production and egg traits of laying hen. Veterinary Medicine, 50, 406-410

McCarthy, P. (2001): The principles of humic substances. Soil Science, 166, 738-751

Mikundová, M., Straková, E., Suchý, P. (2005): Růstová intenzita bažantích kuřat při intenzivním výkrmu a chemické složení svaloviny bažantů. VII. Konference mladých vědeckých pracovníků s mezinárodní účastí. Brno: VFU Brno, 128-131.

Mirnawati, Y.R., Marlida, Y. (2013): Effects of humic acid addition via drinking water on the performance of broilers fed diets containing fermented and non-fermented palm kernel cake. Archiva Zootechnica, 16, 41-53.

Ozturk, E., Ocak, N., Turan, A., Cankaya, S. (2012): Performance, carcass, gastrointestinal tract and meat quality traits and selected blood parameters of broilers fed diets supplemented with humic substances. Journal of the Science of Food and Agriculture, 92, 59-65.

Rath, N.C., Huff, E.W., Huff, G.R. (2006): Effects of humic acid on broiler chickens. Poultry Science, 85, 410-414.

Sarica, M., Karacay, N. (1994): A research on the growth performance and carcass traits of pheasants. Turkish Journal of Veterinary and Animal Sciences, 18, 371-376.

SAS User's Guide: Statistics version 6.12 Edn., SAS Institute, Inc., Cary, NC., 2001, USA.

Suchý, P., Pažout, V., Straková, E., Slezáčková, I. (2003): Srovnání kvality jatečného těla u vykrmovaných kuřat a bažantů. Současnost a perspektivy chovu drůbeže. Praha: ČZU v Praze, 71-82.

Yörük, M.A., Gül, M., Hayirli, A., Macit, M. (2004): The effects of supplementation of humate and probiotic on egg production and quality parameters during the late laying period in hens. Poultry Science, 83, 84-88.

Yalcin, S., Ergun, A., Erol, H., Yalcin, S., Ozsoy, B. (2005): Use of L-carnitine and humate in laying quail diets. Acta Veterinaria Hungarica, 53, 361-370.

Zralý, Z., Písaříková, B., Trčková, M., Navrátilová, M. (2008): Effect of humic acids on lead accumulation in chicken organs and muscles. Acta Veterinaria Brno, 77, 439-445.

EFFECT OF PROBIOTIC FEED ADDITIVES ON THE FUNKCIONAL STATUS OF THE RUMEN

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ABSTRACT

In our study we have examined the influence of the probiotics *Bifidobacterium* sp. on the functional status of the cattle´s rumen. Two adult cows of Aberdeen-angus were used in this experiment. They were treated with a permanent cannula, which served for daily administration of probiotics *Bifidobacterium* sp.. Samples of rumen fluid were analysed for the amount of volatile fatty acids, protozoans, pH and quantity of ammonia. When we tested the effect of the probiotics on each variable, the fixed effect of the influence of an individual has not been proved. When we tested the data without the effect of the individual in a linear model, the butyric and acetic acids were the variables best describing our data. The amount of protozoans increased as their levels grew. There is a strong effect of the individual as only two individuals were used. However, our results indicate that the influence of the probiotics *Bifidobacterium* sp., on the functional status of the rumen is low. These results could have been affected by the low number of experiment-replication as well as by a small quantity of tested animals.

Keywords: *Bifidobacterium* sp.; protozoa; feed supplements; fistula; cattle

INTRODUCTION

The main objective of animal husbandry is to ensure provision of quality and healthy products for people. Other important factors being good living condition for the animals and respect the natural environment. Zootechnical research puts a great effort to improve the quality and harmlessness of meat for the human health. Antibiotics used to be a part of the feed ration as growth additives in the past (Dibner and Richards, 2005). Then a fear of development of a resistance to antibiotics arose, as it can be transferred to human microflora (Mathur and Singh, 2005). This has led to a ban on using antibiotics as a growth stimulators within the EU from the 1st of January 2006 (EC, 2003, 2001). This prohibition in the EU countries has caused the research to concentrate on finding other ways to modulate the rumen microflora and enhance the growth of livestock. Applying of probiotics, prebiotics and synbiotics can have a positive effect on animal health and subsequently also profitability of the husbandry (Rada and Šplíchal, 2010). Even though the knowledge of the impacts of such feed supplements has increased, the

basic information and the influence on individuals are still incomplete (Gaggìa et al., 2010). The results of applying probiotics on various species and class of animals has often been contradictory so far. Positive effect on good health is explained as an increased immune reaction or decreasing in diarrhoea occurrence in calfs and piglets. There are more than 240 preparation currently approved in the EU. Using of probiotics in livestock animals presents the contrary to nutritional using of antibiotics (Zelenka, 2015).

The aim of this experiment was to find out how does the administration of probiotical feed supplements influences the rumen microflora and microfauna and also how they will affect the basic chemical and biological processes in the rumen of the cannulated cattle.

MATERIAL AND METHODS

The testing took place in an accredited stable of a purpose-built facility of the university agricultural farm in Čtyři Dvory from the 25th August 2015 till 29th January 2016.

Two adult cows Aberdeen-angus breed were used. They were treated with a permanent cannula (ø 13 cm) to enable evaluation of the administration of probiotics *Bifidobacterium* sp. (10^7.g $^{-1}$). Tested animals were accommodated in box stalls with unlimited access to drinking basin and salt lick. The average body weight during the experiment was 799 ± 7 .1 kg and 594 ± 9 kg, respectively. The *Bifidobacterium* sp. probiotics had been administered in a freeze-dried form. Two grams were diluted in 100 ml of drinking water and then applied through a fistula into the rumen every day at 9 am during both habituating and testing periods. This experiment had been repeated twice with the same activity scheme (tab. 2). Both cows were gradually engaged to the testing period within the first repetition. In the third control period the individuals were fed the basic feed ration (BFR). A datalogger was used to record the microclimate during the whole experiment.

Organizational scheme of particular periods of the experiment:

a) Preliminary (14 days)

- during this time the animals got only the BFR enabling both animals´ microflora to get on the same physiological condition

- samples of rumen fluid and feces were taken and analysed

b) Habituating period (14 days)

- cows had been fed with BFR and were getting the probiotic supplement

- the goal was to accustom the rumen microflora to the supplement

- samples of rumen fluid and feces were taken and analysed

c) Testing period (21 days)

- both individuals were getting BFR together with probiotics, samples of rumen fluid and feces were taken and analysed

Table 1. The design of our experiment with periods when was the experiment running and when the animals were acclimated.

Animal	preliminary	habit	experiment	habit	experiment	habit	experiment	habit	experiment
1	BFR	BFR + P	BFR + P	BFR	BFR	BFR + P	BFR+P	BFR	BFR
2	BFR	BFR	BFR	BFR + P	BFR + P	BFR	BFR	BFR + P	BFR + P

BFR – basic feeding ration
BFR – control
BFR + P – experiment – basic feeding ration + probiotic

Animals have been fed twice a day (06:00 a 15:00). The basic feeding ration comprised of unlimited amount of drinking water and also hay. The feeding ration has been computed based on the weight. For the first one it was 7–7.5 kg of hay (7.25 ± 0.16 kg). For the second animal it was 5–5.5 kg (5.3 ± 0.15 kg). The volume of consumed water had been measured within the morning feeding. The first cow drank 31 ± 7.8 $l.day^{-1}$ on average whilst the second one 33 ± 6.7 $l.day^{-1}$.

Samples of the rumen liquid were taken three time a week three hours after the morning feeding (on Monday, Wednesday and Friday) during all three periods of the experiment. Collection of the fluid had been carried out through the rumen cannula with a probe

connected to a hand operated suction pump (Hofírek and Dvořák, 2002). Obtained fluid had been immediately transferred to the laboratory. Samples were filtered through a gauze prior to the analyses. The rumen fluid has been used to determining of the pH, nitrogenous particles content, VFA (volatile fatty acids) and the amount of protozoans.

RESULTS AND DISCUSSION

60 samples of rumen fluid were collected from each individual altogether. The pH, the amount of VFA (acetic acid, butyric acid, propionic acid), ammonia (NH_3) and number of protozoans were measured from this fluid.

Statistical analysis

Linear model was used to test the efects of pH, the amount of VFA, ammonia and protozoans of the experimental group (control, probiotics). We chose to use the effect of individual animal as a fixed variable. The results were insignificant for all groups (see Tab.2). Data were analysed with the R software, version 3.1.2 (R Development Core Team, 2014).

Table 2. The results of final linear model for the effects of pH, acetic, butyric and propionic acid, ammonia and the number of protozoans in two different groups of animals tested.

	Group				
	Df	Sum Sq	Mean Sq	F value	p
pH	1	0.00027	0.00027	0.0599	0.80714
Acetic acid	1	10.33	10.33	0.4277	0.5144
Butyric acid	1	0	0	0.0001	0.991
Propionic acid	1	0.95	0.948	0.1163	0.7337
Ammonia	1	0.585	0.58451	1.1943	0.2767
Protozoans	1	42.6	42.64	0.4995	0.4811

Temperature in the stable was measured during the experiment. The values of temperature were partially correlated with protozoans level in both animals and groups. According to the works of Gianesella et al., (2012) and Tajima et al. (2007) is lower temperature of environment related to higher rumen fermentation and so with the increased level of volatile fatty acids and ammonium ions. The other factor participating

in the rumen fermentation, apart from the outside temperature is the humidity. When the higher temperature combines with higher humidity, the fermentation characters could be also higher compared to a period with lower temperature. Both temperature and humidity also affects the amount and composition of the rumen microflora (Tajima et al., 2007).

Tables 3. and 4. are showing the average values of each variable obtained from the samples of rumen fluid. The figures are divided to the ones from the period prior the experiment (7 days before), after 14 days (at the end of habituating period) and after 21 days (the end of testing period).

Table 3. All data with their mean values ± SD obtained from the rumen fluid of animal 1.

	Animal 1				
		Control		Experiment	
	before	14 days	21days	14days	21days
acetic acid [mmol.l^{-1}]	58.14 ± 3.2	56.44 ± 5.2	60.81 ± 5.6	58.52 ± 5.8	58.26 ± 4.6
propionic acid [mmol.l^{-1}]	16.1 ± 6.5	16.46 ± 3.7	15.89 ± 2.6	16.92 ± 3.1	17.85 ± 3.4
butyric acid [mmol.l^{-1}]	9.53 ± 1.0	9.25 ± 1.3	10.09 ±1.3	9.8 ± 1.6	9.44 ± 2.1
ammonia [mmol.l^{-1}]	2.98 ± 0.7	3.89 ± 0.6	3.5 ± 0.9	3.34 ± 0.5	3.75 ± 0.45
pH	6.99 ± 0.03	6.96 ± 0.06	6.94 ± 0.07	6.97 ± 0.06	6.92 ± 0.04

Table 4. All data with their mean values ± SD obtained from the rumen fluid of animal 2.

	Animal 2				
		Control		Experiment	
	before	14 days	21days	14days	21days
acetic acid [mmol.l^{-1}]	48.18 ± 6.3	54.16 ± 5.6	54.02 ± 3.1	51.82 ± 4.6	52.66 ± 5.2
propionic acid [mmol.l^{-1}]	12.13 ± 3.1	14.26 ± 1.9	15.61 ±2.9	14.61 ±3.0	13.05 ±2.5
butyric acid [mmol.l^{-1}]	7.14 ± 0.9	8.26 ± 0.9	7.81 ± 1.2	7.99 ±0.8	8.19 ± 1.2
ammonia [mmol.l^{-1}]	3.02 ± 0.3	3.15 ± 0.7	3.49 ± 0.4	3.89 ± 0.64	3.47 ± 0.7
pH	7.05 ± 0.05	6.99 ± 0.04	6.96 ± 0.05	6.99 ± 0.08	6.99 ± 0.09

Our work did not reveal the effect of probiotics on the pH, it only revealed the effect of individual. That is in agreement with the work of Ritz et al. (2014). It is quite probable that the significance is influenced by the kind of probiotics. Qadis et al. (2014) had used a probiotic combination of *Lactobacillus plantarum*, *Enterococcus facium* and

Clostridium butyricum in their experiment and Wang et al. (2016) used *Bacillus subtillis natto*. Unlike our work in which we used the *Bifidobacterium* sp.. (Russell et al., 2011) already proved their effect, but there is no study using this probiotics in animalas fed only by hay.The probiotics did not have any effect on the amount of VFA, same as in the study of (Qadis et al., 2014). Wang et al. (2016) state, that the probiotics decrease the concentration of acetic and propionic acids. This is in contrast with the study made by Beauchemin et al. (2003). The only significant variable for the VFA in our study was the effect of individual. That was very likely caused by the physical differences between the cows (size, weight). The amount of ammonium ions had not been affected by the probiotics.

Li et al. (2009) found out that the figures for ammonium ions are conditional on the precise spot of harvesting the rumen fluid. The values for samples obtained from the center of rumen can be up to ten times lower than of the ones gotten from the cranial parts. As we can see in tables 3 and 4, the average figures for ammonium ranged from 2.89 ± 0.7 to 3.89 ± 0.6 mmol.l^{-1}. Optimal value for the microbial synthesis is 2.9 - 3.5 mmol.l^{-1} (Firkins et al., 2007; Jallow and Hsia, 2011). We can also see that the average figures had been arising compared to the pre-experimental period. But the rise in values also occurred in control periods with both individuals. That could have been caused by the alternation of the testing and control periods within individuals. The rumen fluid usually contains 10^6 protozoans (Saleem et al., 2013). These microorganisms are highly sensitive to changes in diet and react very quickly to changes in conditions, especially pH (Pfeffer and Hristov, 2005). In the second animal there was a mild increase of the protozoans after the probiotics were applied. But the statistical analysis was still insignificant for both animals. The insignificance could have been a consequence of the low number of tested animals caused by the lack of space and finances as well. It is also quite difficult to get the permit to cannulate and manipulate the animals. In experiments like this is not at all rare to have such a low number of tested animals. For example Qadis et al. (2014) and Lee et al., (2004) used only 12 cannulated individuals for their research and Guedes et al. (2008) only three. Both numbers beeing still too low for fine statistical analyses.

CONCLUSION

The linear model analyses of the obtained data did not provide any significant result and so the effect of probiotics *Bifidobacterium* sp. was not proved in this study.

These results might have been affected by the low number of animals used for testing and insufficient repeating. That was caused by very high expenses on their maintenance, the difficulty brought by application of the cannula and finally also by the complicated acquiring of the permission to manipulate with farm animals. The period for which we applied the probiotics might have been too short and the alternation of testing and control periods might have been to rapid. And last but not least the insignificance could have been affected by the kind of used probiotics *(Bifidobacterium* sp.*)* and the magnitude of the dose.

It would be very convenient to monitor the effect on the amino acids in the rumen within follow-up research as their increase would have a positive effect on digestibility of the feed. Another kinds of probiotics and their different dosage should be tested as well.

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REFERENCES

Beauchemin, K. a., Yang, W.Z., Morgavi, D.P., Ghorbani, G.R., Kautz, W., Leedle, J.A., (2003): Effects of bacterial direct-fed microbials and yeast on site and extent of digestion, blood chemistry, and subclinical ruminal acidosis in feedlot cattle. Journal of Animal Science 81, 1628–1640.

Dibner, J.J., Richards, J.D., (2005): Antibiotic growth promoters in agriculture: history and mode of action. Poultry Science 84, 634–643.

EC, (2003): Commission of the European Communities, Commission Regulation (EC) No. 1831/2003. Official Journal of European Union L268, 29–43.

EC, (2001): Commission of the European Communities, Commission Recommendation, 2001/459/EC. Official Journal of European Union L 161, 42–44.

Firkins, J.L., Yu, Z., Morrison, M., (2007): Ruminal nitrogen metabolism: Perspectives for integration of microbiology an nutrition for diary. Journal of Dairy Science 90, 1–16.

Gaggìa, F., Mattarelli, P., Biavati, B., (2010): Probiotics and prebiotics in animal feeding for safe food production. International Journal of Food Microbiology 141, 15–28.

Gianesella, M., Piccione, G., Cannizzo, C., Casella, S., Morgante, M., (2012): Influence of temperature and humidity on rumen pH and fatty acids in dairy cows. Journal of Environmental Biology 33, 1093–1096.

Guedes, C.M., Gonçalves, D., Rodrigues, M.A.M., Dias-da-Silva, A., (2008): Effects of a *Saccharomyces cerevisiae* yeast on ruminal fermentation and fibre degradation of maize silages in cows. Animal Feed Science and Technology 145, 27–40.

Hofírek, B., Dvořák, R., Němeček, L., Doležal, R., Pospíšil, Z., (2009): Nemoci skotu. Noviko a.s., Brno,1149. (in Czech).

Jallow, D.B., Hsia, L.C., (2011): Effect of six feed supplements on ruminal degradation characteristics and amino acid profile of sheep. International Journal of Animal and Veterinary Advances 3, 367–373.

Lee, S.S., Kim, H.S., Moon, Y.H., Choi, N.J., Ha, J.K., (2004): The effects of a non-ionic surfactant on the fermentation characteristics, microbial growth, enzyme activity and digestibility in the rumen of cows. Animal Feed Science and Technology 115, 37–50.

Li, M., Penner, G.B., Hernandez-Sanabria, E., Oba, M., Guan, L.L., (2009): Effects of sampling location and time, and host animal on assessment of bacterial diversity and fermentation parameters in the bovine rumen. Journal of Applied Microbiology 107, 1924–1934.

Mathur, S., Singh, R., (2005): Antibiotic resistance in food lactic acid bacteria - a review. International Journal of Food Microbiology 105, 281–295.

Pfeffer, E., Hristov, A.N., (2005): Nitrogen and phosphorus nutrition of cattle. CABI Publishing, Wallingford, 288.

Pinloche, E., McEwan, N., Marden, J.-P., Bayourthe, C., Auclair, E., Newbold, C.J., 2013. The effects of a probiotic yeast on the bacterial diversity and population structure in the rumen of cattle. PLoS One 8, e67824.

Qadis, A.Q., Goya, S., Ikuta, K., Yatsu, M., Kimura, A., Nakanishi, S., Sato, S., 2014. Effects of a bacteria-based probiotic on ruminal pH, volatile fatty acids and bacterial flora of holstein calves. J. Vet. Med. Sci. 76, 877–885.

Rada, V., Šplíchal, I., 2010. Probiotika, prebiotika, synbiotika: Současný stav a perspektivy využití v živočišné produkci, in: Přírodní látky a jejich biologická aktivita. Karolinum, Praha: Univerzita Karlova, pp. 331–368. (in Czech).

R Core Team. (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Ritz, J., Codron, D., Wenger, S., Rensch, E., Hatt, J.-M., Braun, U., Clauss, M., (2014): Ruminal pH in cattle (*Bos primigenius* f. *taurus*) and moose (*Alces alces*) under different feeding conditions: a pilot investigation. Journal of zoo and aquarium research 2, 44–51.

Russell, D. a., Ross, R.P., Fitzgerald, G.F., Stanton, C., (2011): Metabolic activities and probiotic potential of bifidobacteria. International Journal of Food Microbiology 149, 88–105.

Saleem, F., Bouatra, S., Guo, A.C., Psychogios, N., Mandal, R., Dunn, S.M., Ametaj, B.N., Wishart, D.S., (2013): The bovine ruminal fluid metabolome. Metabolomics 9, 360–378.

Tajima, K., Nonaka, I., Higuchi, K., Takusari, N., Kurihara, M., Takenaka, A., Mitsumori, M., Kajikawa, H., Aminov, R.I., (2007): Influence of high temperature and humidity on rumen bacterial diversity in Holstein heifers. Anaerobe 13, 57–64.

Wang, Z., He, Z., Beauchemin, K. A., Tang, S., Zhou, C., Han, X., Wang, M., Kang, J., Odongo, N.E., Tan, Z., 2016. Comparison of two live *Bacillus* species as feed additives for improving in vitro fermentation of cereal straws. Animal Science Journal 87, 27–36.

Zelenka, J. (2015): Krmná aditiva. Inovace bez legrace 13/018/1310b/164/000695. Program rozvoje venkova Ministerstva zemědělství.

EFFECT OF BACILLUS AMYLOLIQUEFACIENS ON THE APPARENT JEJUNAL DIGESTIBILITY OF CHICKENS

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ABSTRACT

The aim of study was to determine the effects of the probiotic *Bacillus amyloliquefaciens* CECT 5940 (10^9 CFU / g) applied as a feed additive Ecobiol (Evonic Nutrition and Care GmbH, Nemecko) on the apparent jejunal digestibility of dry matter (DM), crude protein (CP) and ash of broiler chickens. 7800 one-day-old male broiler chickens (Ross 308) were divided randomly into 4 treatments (TRT) with 15 repetitions (n = 130). Birds were used in the feeding experiment for 35 days. Three feed mixtures, the starter (BR1), grower (BR2) and finisher (BR3) were used for feeding in 3 periods. BR3 was based on wheat and soybean meal (SBM) for TRT 1 and 3, as well as on maize and SBM for TRT 2 and 4 in the 3rd period. The feed additive was applied into feeds at the dosage 1 kg / 1000 kg for TRT 3 and 4. 105 birds from each TRT were randomly selected on day 35 of age. After stunning of chickens with an alternating current, the samples of chymus were taken from the jejunum. The minimal weight of one sample from the particular pen was 5.0 g. There were quantified DM, CP, fat, fibre, ash in the feed BR3 as well as DM, CP and ash in the chymus of jejunum. The apparent jejunal digestibility of nutrients (%) was calculated according to equation: 100 – (% indicator in feed / % indicator in chymus) * (% nutrient in chymus / % nutrient in feed) * 100. The ash insoluble in HCl was used as an internal indicator which was analysed in the feed mixtures and in the jejunal chymus. The values of the DM, CP and ash digestibilities (%) decreased in TRT 3 or 4 by 49.10 ($p < 0.01$), 40.19 ($p < 0.01$) 65.54 ($p < 0.01$) and 25.05, 40.33 ($p < 0.01$), 26.10, respectively. The supplementation of diets with *B. amyloliquefaciens* had not any stimulating effect on the jejunal digestibility of analysed parameters in the finishing period of poultry.

Keywords: poultry; digestion; intestine, *Bacillus*

INTRODUCTION

The significant modification of digestibilities of proteins and amino acids is characteristic for the caecal fermentation. It ismore effective to determine the digestibility at the intestinal level than in the excreta (Ravindran et al., 1999). In spite of

the mentioned fact, the most of currently published papers about the poultry digestibility are based on excreta analysis (Sibbald, 1986, NRC, 1994). The attraction of the determination at the excreta level is simplicity and the experiments can be carried out on a large number of birds without sacrificing them. However, the published results on intestinal digestibility of feed ingredients are limited. The intestinal digestibility is expressed as apparent when the values are not corrected for endogenous losses (Sibbald, 1982, 1986). The digestibility is affected by the commensal intestinal microflora too, which can be influenced by the probiotic bacteria. The probiotics are providing several health benefits to poultry after peroral intake such as the maintaining of favourable gut microflora with the positive effect on the gut health. The strain *Bacillus amyloliquefaciens* CECT 5940 is characteristic with the production of proteolytic and amylolytic enzymes and the lactic acid which is causing a decrease of the intestinal pH value. The scientific hypothesis, used in the process of preparation of the described experiment, was based on the fact that the bacteria *B. amyloliquefaciens* CECT 5940 is producing a quantity of enzymes which allow the animals to improve the degradability of the feed nutrients. Therefore, we expected that this microorganism can enhance digestibility of observed nutrients in the small intestine.

The aim of the presented study was to determine the effect of the feed additive based on *B. amyloliquefaciens* CECT 5940 strain on the jejunal digestibility of dry matter (DM), crude protein (CP) and ash of broiler chickens.

MATERIAL AND METHODS

Chickens and diets

7800 one-day-old male broiler chickens of hybrid Ross 308, were delivered from a commercial hatchery XAVERgen a.s. (Habry, RCH Dvérce). They were divided at random into 4 treatments. The treatments had 15 repetitions and the each of them with 130 animals. The chickens from repetitions were housed into identical floor pens with the deep bedding. Birds were located in the air-conditioned halls of an experimental farm (International testing of poultry, government enterprise) in Ustrašice (Czech Republic). The feed and water were provided *ad libitum*. The chickens were fed with

the pelleted feed mixtures for 35 days in 3 periods. The starter (BR1), grower (BR2) and finisher (BR3) diets were prepared and formulated with aminovitans AMV BR1-plus-F N+N, AMV BR2-plus-F NAR, AMV BR3-plus F (Trouw nutrition Biofaktory s.r.o., Czech Republic) without antibiotics and produced in the mentioned farm. The anticoccidial agents with phytase were added into BR1 (Narasin and Nicarbasin) and BR2 (Narasin) only. The methionine was used as the first limiting amino acid. The feed mixtures based on the wheat with the soybean meal (SBM) (B1) or maize with SBM (B2) were used for feeding of the treatment 1 and 2, respectively. The treatments 3 (B1 + probio) and 4 (B2 + probio) were fed with the same feed mixtures with the addition of probiotic additive Ecobiol (Evonic Nutrition and Care GmbH, Nemecko) at the dosage 1 kg / 1000 kg feed. The additive contained the rapid growing bacteria *B. amyloliquefaciens* CECT 5940 (10^9 CFU / g).

Feed analysis

The samples of finisher feed mixtures (BR3) for four treatments were analysed (Table 1) according to the official methods of the Association of Official Analytical Chemists (Cunniff, 1995). DM, CP, crude fat and ash were determined in the feeds. Crude fibre was analysed by the common method (Van Soest et al., 1991). The insoluble portion of ash in HCl was determined in the feed mixture as the residue of ash, after dissolving ash in diluted hydrochloric acid by weighing (Daněk et al., 2005, Commission Regulation EC (2009).

Analysis of intestinal contents from the jejunum

105 birds from each treatment with 15 pens were randomly selected on

Table 1. Chemical composition of the finisher compound feeds

Component (g/kg)	Compound feeds (BR3)			
	B1	**B2**	**B1 + probio**	**B2 + probio**
Dry matter	919.40	915.20	918.10	918.00
Crude protein	221.10	201.80	215.80	195.90
Crude fat	75.20	73.30	79.10	77.40
Crude fibre	36.50	30.40	35.50	29.00
Ash	54.00	48.70	56.20	51.00
Ash insoluble in HCl	0.50	0.50	1.60	1.40

B1 – diet based on wheat with the soybean meal (SBM), B2 – diet based on maize with SBM, probio – feed probiotic additive with *Bacillus amyloliquefaciens* CECT 5940 (1000 g / 1000 kg feed)

day 35 of age. The stunning was performed with an alternating current with the frequency 50 Hz for 4 seconds. The samples of the jejunal chymus (15 samples from one treatment) with the minimal weight 5.0 g were placed into sterile boxes immediately after necropsy and placed on the surface of solid CO_2 at the temperature -56.4 ^oC for transportation. The storage of samples was performed in deep freezer at the temperature – 55.0 ^oC till the analyse. The analyses of DM, CP, ash and the insoluble portion of ash in HCl were determined in the feed mixtures by weighing. The analytical methods used for the jejunal chymus were the same as for feed analyses.

Check of the apparent digestibility

The apparent digestibility of nutrients in the jejunum was determined by calculating the analysed content of nutrients in the feed mixtures and in the jejunal chymus concerning the content of the portion of ash insoluble in HCl (Johnson et al., 2014). The ash insoluble in HCl was used as the internal indicator.

The apparent digestibilities of nutrients (DM, CP, ash) in the jejunum were calculated with the following equation:

Apparent digestibility of nutrients (%) = 100 – (% indicator in feed / % indicator in chymus)*(% nutrient in chymus / % nutrient in feed)*100.

Statistical analysis

The data are expressed as means ± standard deviation (SD) of single values. Means of the results from the treatments were compared by one-way analysis of variance. Significance was declared at $P < 0.05$, $P < 0.01$, and $P < 0.001$.

RESULTS AND DISCUSSION

The digestibility data of DM, CP and ash in the jejunum on day 35 are summarized in Table 2. The values of the DM digestibility decreased in the treatment 3 by 49.10 % ($p < 0.01$) or treatment 4 by 25.05 % in comparison to treatment 1 and 2, respectively. The decrease of CP digestibilities was observed in the treatment 3 by 40.19 % ($p < 0.01$) and 4 by 40.33 % ($p < 0.01$) compared to treatment 1 and 2. The statistically significant differences of ash digestibilities were observed as for treatment 3 when the value decreased by 65.54 % ($p < 0.01$) in comparison to treatment 1. On the other hand, in the case of comparison of the treatments 2 and 4, the decrease was lower by 26.10 % in the 4th one.

The achieved results of the jejunal digestibility of DM and CP demonstrated the decrease of these parameters in the treatments 3 or 3 and 4 after intake of feed mixture based on maize with soybean meal combined with the probiotic additive containing the bacterial strain *B. amyloliquefaciens* CECT 5940.

One of the characteristics of the increased digestibility is the decrease of viscosity of the analysed intestinal chymus (Günal et al., 2004). Similarly, Geeraerts at al. (2016) observed significant reduction of viscosity of supernatant from chymus for all non-maize diets. There were used diets containing maize, wheat, rye, barley and oat in the *in vitro* digestive model experiment.

Table 2. Digestibility of nutrients in the jejunum of broiler chickens in the finishing period of fattening

Nutrient (%; mean ± SD)	Compound feeds (BR3)			
	B1 (n = 15)	B2 (n = 15)	B1 + probio (n = 15)	B2 + probio (n = 15)
Dry matter	80.85^{a} ± 5.416	80.32^{a} ± 8.481	41.15^{b} ± 12.939	60.20^{ab} ± 18.293
Crude protein	89.85^{a} ± 2.426	86.93^{a} ± 6.532	53.74^{b} ± 13.626	51.87^{b} ± 12.000
Ash	70.50^{a} ± 7.797	71.70^{a} ± 11.254	24.29^{b} ± 9.274	52.99^{ab} ± 12.621

B1 – diet based on wheat with the soybean meal (SBM), B2 – diet based on maize with SBM, probio – feed probiotic additive with *Bacillus amyloliquefaciens* CECT 5940 (1 kg / 1000 kg feed), SD – standard deviation; the means with different superscript letters in the same row differed significantly ($p < 0.01$).

The values of the digestibility of mineral elements, measured as the ash, decreased in the treatment 3. However, the enzymatic activity of the used probiotic bacteria probably had not any effect on this parameter. Very important effect of probiotics is the protection of chicken intestinal apparatus against pathogenic bacteria with the subsequent favourable influence on the gut health. However, there were not observed the beneficial effects of B. amyloliquefaciens on the protection against necrotic enteritis caused by the pathogenic Clostridium perfringens (Jerzsele et al., 2012, Geeraerts et al., 2016).

CONCLUSION

The supplementation of diets, based on wheat or maize combined with soybean meal, with the probiotic bacteria *B. amyloliquefaciens* CECT 5940 for broiler chickens had not any stimulating effect on the jejunal digestibility of DM, CP and ash in the finishing period.

ACKNOWLEDGEMENT

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REFERENCES

Commission regulation EC (2009): Commission regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed. Official Journal of the European Union, L54.

Cunniff P. (1995): Official Methods of Analysis of Association of Analytical Chemists, 16thedn.AOAC International, Arlington, Va., USA

Daněk P., Paseka A., Smola J., Ondráček J., Bečková R., Rozkot M. (2005): Influence of lecithin emulsifier on the utilisation of nutrients and growth of piglets after weaning. Czech Journal of Animal Science, 50, 459-465.

Günal M., Yasar S., Forbes J.M. (2004): Performance and some digesta parameters of broiler chickens given low or high viscosity wheat-based diets with or without enzyme supplementation. Turkish Journal of Veterinary and Animal Science. 28, 323-327.

Geeraerts S., Delezie E., Ducatelle R., Haesebrouck F., Devreese B., Van Immerseel F. (2016): Vegetative Bacillus amyloliquefaciens cell do not confer protection against necrotic enteritis in broilers despite high antibacterial activity of its supernatant against Clostridium perfringens in vitro. British Poultry Science, 57, 324-329.

Jerzsele, A., Szeker K., Csizinszky R., Gere E., Jakab C., Mallo J.J., Galfi P. (2012): Efficacy of protected sodium butyrate, a protected blend of essential oils, their combination, and Bacillus amyloliquefaciens spore suspension against artificially induced necrotic enteritis in broilers. Poultry Science, 91, 837-843.

Johnson L.A., Deep A., Classen H. (2014): Digestibility and performance responses of broiler chickens fed a pea-based diet with different levels of dietary microbial phytase. University of Saskatchewan Undergraduate Research Journal, 1, 39-44.

NRC (1994): Nutrient requirements of domestic animals. Nutrient requirements of poultry, 9th rev. ed. National Research Council, National Academy Press, Washington D.C., USA.

Ravindran V., Bryden W.L. (1999): Amino acid availability in poultry-in vitro and in vivo measurements. Australian Journal of Agricultural Research, 50, 889-908.

Sibbald I. R. (1982): Measurement of bioavailable energy in poultry feeding stuffs: a review. Canadian J of Animal Science, 62, 983-1048.

Sibbald I.R. (ed) (1986): The T.M.E. System of feed evaluation: Methodology, Feed

composition data and bibliography. Technical Bulletin 1986-4E, Animal Research Centre Contribution 85-19, Canada, Agriculture Canada, Research Branch, Otawa, Canada.

Van Soest P. J., Robertson J.B., Lewis B.A. (1991): Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583-3597.

CORNELL SYSTEM IN RUMINANT NUTRITION

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ABSTRACT

The nutritional value of typical feeds (n = 6) for ruminants was evaluated with chemical analysis and procedures for determination of individual nitrogen fractions by Cornell system. Three samples of roughages and three samples of concentrates were dried, milled, analysed for individual nutrients and then analysed for individual nitrogen fraction. Nitrogen fractions A, B1, B3, B3 and C were from 12.5 to 25.9 % of crude protein (CP), 5.0 to 32.8 % of CP, 3.9 to 60.8 % of CP, 4.3 to 39.5 % of CP and 4.1 to 11.2 % of CP, respectively. The knowledge of the protein degradability of feed rations is important for understanding the ruminants' digestibility processes.

Keywords: ruminants; nutrients; crude protein; nitrogen fractions

INTRODUCTION

The evaluation of individual fractions of crude protein (CP) using detergent analysis takes into account the requirements of the animal on the feed nutrient compositions. This gives a broader view on the ruminants´ digestibility process. For good health,

welfare and animal production is important to offer optimal feed ration with balanced components influencing feed utilization (Fox et al., 2004; Ghoorchi et Arbabi, 2010; Cornell University, Department of Animal Science, 2014). Fractionation of CP have been characterized by A (non-protein nitrogen), B1 (rapidly degradable protein), B2 (intermediately degradable protein), B3 (slowly degradable protein) and C (indigestible protein) fractions using Cornell system (CNCPS; Cornell Net Carbohydrate and Protein System) (Ghoorchi at Arbabi, 2010). The first mention about CNCPS has been in years 1992 and 1993 (Fox et al., 1992; Russell et al., 1992; Sniffen et al., 1992; O´Connor et al., 1993) and was developed at Cornell University in Ithaca (New York) (Fox et al., 2004). Since this time is CNCPS constantly evaluated (Valderrama et Anrique, 2011; Chrenková et al., 2014 and other publications).

For this purpose, the study was aimed to determine the nutritional value of various feeds using chemical analysis and procedures for determination of the nitrogen fractions, i.e. A, B1, B2, B3, C, NPN (non-protein nitrogen), SOLP (soluble protein), ADIN (acid detergent insoluble nitrogen) and NDIN (neutral detergent insoluble nitrogen).

MATERIAL AND METHODS

Dataset of roughages and concentrate samples (n = 6) was used for this study for evaluation of nitrogen fractions in ruminant nutrition according to model of CNCPS. The fresh material was dried at 50°C. The dried material was subsequently milled and passed through a 1 mm sieve for laboratory analysis and then was analysed for individual nutrients: CP was analysed according to the Kjeldahl method (nitrogen × 6.25); ether extract was determined using Soxtec extraction with petroleum ether; ash was determined after 4.5 h of combustion at 550°C (AOAC, 2005). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to the methods described by Van Soest et al. (1991).

The individual CP fractions were determined by the following methods of Licitra et al. (1996):

1. non-protein nitrogen was measured using trichloroacetic acid (TCA),
2. determination of soluble nitrogen and protein,

3. determination of nitrogen insoluble in acid detergent using the Fibertec apparatus,
4. determination of nitrogen insoluble in neutral detergent using the Fibertec apparatus.

Individual nitrogen fractions were calculated according to Kelzer et al. (2010) (Table 1).

Table 1. Calculations of individual nitrogen fractions according to Kelzer et al. (2010):

Fraction A: same as NPN
Fraction B1: difference of NPN from SOLP
Fraction B2: CP without SOLP and NDIN
Fraction B3: ADIN minus NDIN
Fraction C: same as ADIN

ADIN = acid detergent insoluble nitrogen, CP = crude protein, NDIN = neutral detergent insoluble nitrogen, NPN = non-protein nitrogen, SOLP = soluble protein

RESULTS AND DISCUSSION

The chemical composition of estimated samples is in Table 2. CP ranged from 115.1 to 314.6 g/kg of dry matter (DM). The highest value of CP (314.6 g/kg of DM) was found for rapeseed cake. It corresponds with fact that this feed is typically a protein feed. Values of ether extract and ash were from 26.0 to 128.3 and from 29.9 to 296.8 g/kg of DM, respectively. The values of ADF and NDF show values common for roughage and concentrate feeds. ADF values for roughages were from 278.7 to 355.5 g/kg of DM and NDF values from 565.8 to 579.5 g/kg of DM. Concentrates were from 74.8 to 166.3 g/kg of DM for ADF and 147.1 to 195.4 g/kg of DM for NDF.

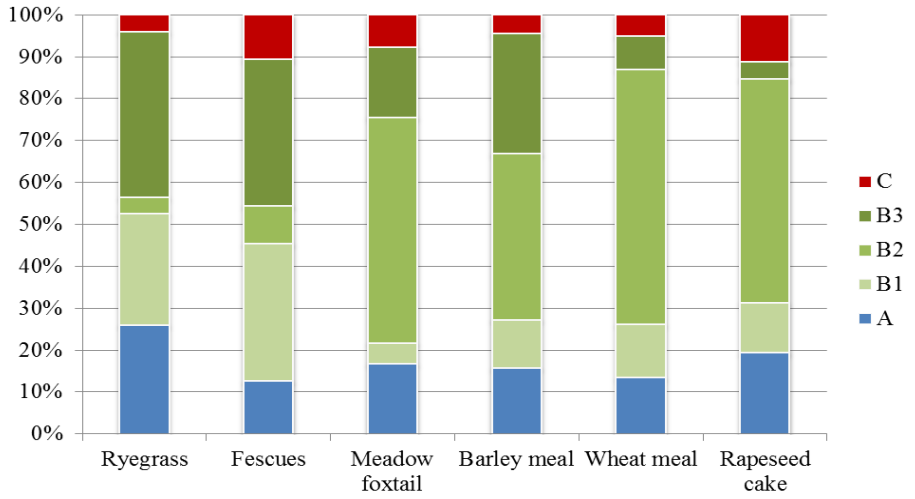
Table 2. Chemical composition (g/kg of DM) of the estimated samples

	DM	CP	EE	Ash	ADF	NDF
Ryegrass	933.2	120.8	30.0	194.2	352.0	565.8
Fescues	929.9	139.1	26.1	193.1	355.5	577.1
Meadow foxtail	918.2	205.2	34.1	76.8	278.7	579.5
Barley meal	885.2	115.1	26.0	29.9	77.4	195.4
Wheat meal	962.9	144.1	128.3	296.8	74.8	147.1
Rapeseed cake	910.0	314.6	109.9	85.6	166.3	188.5

ADF= acid detergent fibre, CP = crude protein, DM = dry matter, EE = ether extract, NDF = neutral detergent fibre
The values are arithmetic means (n = 2) based on dry matter

The values of nitrogen fractions are expressed in % of CP, while the final value should be 100 %. The resulting values of individual nitrogen fractions express potential degradability in the rumen. In this study we found the fastest degradability (fraction A) for ryegrass (25.9 % of CP) and the lower A fraction was found for fescues (12.5 % of CP). These feeds have more or less similar values of this non-protein nitrogen (fraction A) for roughages and concentrates. Similar values have a study of Moreira et al. (2012). Next fast degradable nitrogen fraction is B1, which is rapidly degradable protein. This fraction was from 5.0 to 32.8 % of CP for roughages feeds and from 11.5 to 12.8 % of CP for concentrates feeds. Fraction B2 represents intermediately degradable protein. This fraction was from 3.9 to 60.8 % of CP. The small value was found for ryegrass (3.9 % of CP) and fescues (9.0 % of CP). Very high value was found for barley meal, rapeseed cake and wheat meal (39.6, 53.4 and 60.8 % of CP, respectively). The high value of rapeseed cake corresponds with authors Shannak et al. (2000). This authors (Shannak et al., 2000) confirm the high value of this fraction for samples of roughages, it corresponds with our results for meadow foxtail. Fraction B3, which represents slowly degradable protein, was from 4.3 to 39.5 % of CP. It shows different values, same as publication of Shannak et al. (2000). Indigestible protein (fraction C) was from 4.1 to 11.2 % of CP. This fraction represents usually the smallest part of CP. Especially small values of concentrates corresponds with authors Polat el al. (2014).

Figure 1. Proportion of individual nitrogen fraction (% of CP)



A = non-protein nitrogen, B1 = rapidly degradable protein, B2 = intermediately degradable protein, B3 = slowly degradable protein, C = indigestible protein, CP = crude protein

CONCLUSION

Evaluation of individual fractions of CP using detergent analysis takes into account the requirements of the animal on the feed nutrient compositions. This gives a broader view on the ruminants' digestibility process of feeds/feed rations. The study describes the nutritional value of roughages and concentrate feeds. There were determined the basic chemical composition and the fractions of CP for estimating samples. Fractions of crude protein have been characterized by NPN, SOLP, NDIP, ADIP, A, B1, B2, B3 and C. The results of these determinations describe different representation of these fractions in various samples.

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REFERENCES

AOAC. (2005): Official Methods of Analysis, 18th edition. AOAC, Gaithersburg, MD, USA.

Chrenková M., Čerešňáková Z., Weisbjerg M. R., Formelová Z., Poláčiková M. and Vondráková M. (2014): Characterization of proteins in feeds according to the CNCPS and comparison to in situ parameters. Czech J. Anim. Sci., 59, 288–295.

Cornell University, Department of Animal Science: Cornell Net Carbohydrate and Protein System [online]. [Cited 2014-06-15]. Available from: http://www.cncps.cornell.edu/

Fox D.G., Sniffen C.J., O'Connor J.D., Russell J.B. and Van Soest P.J. (1992): A net carbohydrate and protein system for evaluating cattle diets. III. Cattle requirements and diet adequacy. J. Anim. Sci., 70, 3578–3596.

Fox D.G., Tedeschi L.O., Tylutki T.P., Russell J.B., Van Amburgh M.E., Chase L.E., Pell A.N. and Overton T.R. (2004): The Cornell Net Carbohydrate and Protein System model for evaluating herd nutrition and nutrient excretion. Anim. Feed Sci. Technol., 112, 29–78.

Ghoorchi T. and Arbabi A. (2010): Study of protein characteristic of five feeds by CNCPS model. Asian J. Anim. Vet. Adv., 5, 584–591.

Kelzer J.M., Kononoff P.J., Tedeschi L.O., Jenkins T.C., Karges K. and Gibson M.L. (2010): Evaluation of protein fractionation and ruminal and intestinal digestibility of corn milling co-products. J. Dairy Sci., 93, 2803–2815.

Licitra G., Hernandez T.M. and Van Soest P.J. (1996): Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Tech., 57, 347–358.

Moreira A.L., Berchielli T.T., Reis R.A., Ruggieri A.C., Simili F.F., Fagundes J.L. and de Miranda Gomide C.A. (2012): Nitrogen and carbohydrate fractions on Tifton-85 pastures overseeded with annual winter and summer forage species in different seasons. Revista Brasileira de Zootecnia, 41, 1806–9290.

O'Connor J.D., Sniffen C.J., Fox D.G. and Chalupa, W. (1993): A net carbohydrate and protein system for evaluating cattle diets. IV. Predicting amino acid adequacy. J. Anim. Sci., 71, 1298–1311.

Polat M., Şayan Y. and Özelçam H. (2014): Estimating in situ effective crude protein degradability with Cornell Net Carbohydrate and Protein System parameters in energy-rich feedstuffs for ruminants. Kafkas Univ. Vet. Fak., 20, 259–265.

Russell J.B., O'Connor J.D., Fox D.G., Van Soest P.J. and Sniffen C.J. (1992): A net carbohydrate and protein system for evaluating cattle diets. I. Ruminal fermentation. J. Anim. Sci., 70, 3551–3561.

Shannak S, Südekum K.-H. and Susenbeth A. (2000): Estimating ruminal crude protein degradation with in situ and chemical fractionation procedures. Anim. Feed Sci. Tehnol., 85, 195–214.

Sniffen C.J., O'Connor J.D., Van Soest P.J., Fox D.G. and Russell, J.B. (1992): A net carbohydrate and protein system for evaluating cattle diets. II. Carbohydrate and protein availability. J. Anim. Sci., 70, 3562–3577.

Valderrama X. and Anrique R. (2011): In situ rumen degradation kinetics of high-protein forage crops in temperate climates.Chil. J. Agr. Res., 71, 572–577.

Van Soest P.J., Robertson J.B. and Lewis B.A. (1991): Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74, 3583–3597.

MONITORING OF THE ORGANIC MATTER DIGESTIBILITY OF CEREAL CROPS DURING GROWING SEASON

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ABSTRACT

The aim of this experiment was the monitoring of the organic matter digestibility of 4 species of cereal forage during the growing season. The experiment was conducted in collaboration with cultivation station Slechtitelska Stanice Vetrov (Oseva UNI, a.s. Chocen). Analyses were performed in the laboratory of Faculty of Agriculture, University of South Bohemia in Ceske Budejovice. Organic matter digestibility was

determined by pepsin-cellulase in vitro method according to Míka et al. (2009) with using inexpensive Czech cellulases in Ankom Daisy Incubator II.

Statistical significant differences were found among cereal crops and sampling dates. Wheat forage had the highest organic matter digestibility and oat had the lowest. Cereals reported the highest organic matter digestibility in the first sampling date (13.06.14) and the lowest in the fourth sampling date (03.07.14). Organic matter digestibility of cereals was affected by vegetation stages. The highest organic matter digestibility was achieved before inflorescence emergence, the lowest during inflorescence emergence, heading, flowering, anthesis and development of fruit. Slight increase of organic matter digestibility was detected during the ripening. It was also found that the organic matter digestibility changed differently for each cereal plant during the season.

Keywords: digestibility; cereals; forage; oat; triticale; wheat; barley

INTRODUCTION

Cereals are the crops often used to the feeding of livestock. Cereals are grown not only to the gaining of grain but also to fodder purpose, especially for cattle nutrition. The forage can be fed fresh or can be preserved. It was discovered that cows fed only with forage from cereals showed a worse body condition and lower milk yield but their milk had better quality with wholesome ratio of fatty acids (Schroeder et al., 2010).

Oat (*Avena sativa L.)* is historically the youngest cultivated cereal crop that is not exigent for soil conditions. Oat is distinguished by very fast growth, growing season till the fully ripe takes only 120 days. Oat forage is suitable for feeding in green state, for the production of hay and silage. The yield of fresh green forage is 30-50 t/ha (Skládanka, 2005). Atego is shorter, yellow-grained variety with good resistance to lodging (Oseva UNI). This Czech variety is very popular even abroad. The grain yield of Atego was a 10% higher than another varieties of oat in Germany (Brouma, 2004). Triticale (*Triticosecale*) is fertile hybrid of wheat and rye. It was cultivated in Germany in 1888 (Petr, 2001a). Triticale is interesting forage crop because of large height and copious leafs (Petr, 2001b). The harvest area of triticale is on the increase in Europe because of forage is convenient for biogas production, and vice versa it is on the

decrease in the Czech Republic (Nesvadba et al., 2017). Kargo is higher variety with good resistance to lodging (Oseva UNI). Wheat (*Triticum aestivum L.*) is one of the oldest cultivated crops. It is demanding of soil conditions. It provides tasty forage that grows old slowly. Green forage yield is 25 t/ha (Skládanka, 2005). Tercie is short variety with good resistance to lodging (Oseva UNI). Barley (*Hordeum sativum L.*) is not exigent for climatic conditions but it is very demanding of soil qualities. Winter barley can be used for forage purpose, vegetation grows old slowly. The green forage yield of spring barley is very low (15 t/ha) and it is not suitable for forage purpose (Skládanka, 2005). Heris is spring variety for animal feeding. The growing of this variety is economical effective because of resistance to diseases (Oseva UNI).

The determination of the nutrients content and digestibility of feed is necessary for correct feed doses composition (Beever and Mould, 2000). The digestibility can be determined using in vivo or in vitro methods or can be reckoned by prediction equations (Horrock and Vallentine, 1999; Undersander and Moore, 2002), but these estimations are not too exact. In vivo methods are generally considering as the most accurate, but high cost, laboriousness and necessity of live animals complicate these methods (Huhtanen et al., 2006). Robinson et al. (2004) questioned exactness of in vivo methods for using as a reference methods. Very common in vivo method is the incubation of samples in rumen fluid and in pepsin - hydrochloric acid solution (Tilley and Terry, 1963). Popular method without the necessity of live animals is pepsin-cellulase method. While older sources did not recommend this method for the determination of organic matter digestibility of individual crops, but rather for the comparison among the plants (Nocek, 1988), new researches shows high correlation between in vitro and in vivo methods and recommend pepsin-cellulase method as reliable method for the determination of digestibility (Nousiainen et al., 2003; Forejtová et al., 2005; Jančík, 2007) According to Barchiesi-Ferrari et al. (2011) the discovery of equations for making this method more accurate is important, because not only crop species but also vegetation stage should be take into consideration. NIRS (near infrared spectroscopy) is the secondary method for digestibility determination, but it need high number of calibration samples and reliable reference methods for results verify (Rinne et al., 2006).

MATERIAL AND METHODS

The experiment was conducted on the samples of four cereal species: oat variety Atego, triticale variety Kargo, wheat variety Tercie and barley variety Heris. This experiment was conducted in collaboration with cultivation station Slechtitelska Stanice Vetrov (Oseva UNI, a.s. Chocen) where the cereals were grown on a parcel Pahorky: LPIS (Land Parcel Identification System) 8607/2, altitude 610 m, potato production type, oat production subtype, brown soil type, light soil, pH 6.1, seeding 17.04.14, fertilization dates: 16.04.14 LOVOFERT LAV 27 150 kg nitrogen per ha and 15.05.14 LOVOFERT LAV 27 200 kg nitrogen per ha. The sampling was conducted at eight dates during the growing season in 2014: 13.06., 20.06., 27.06., 03.07., 11.07., 17.07., 25.07. and 01.08.. Overall 96 samples were collected - 3 samples of each cereal in 8 sampling dates.

Analyses were done in the laboratory Faculty of Agriculture, University of South Bohemia in Ceske Budejovice. The determination of Enzymatically soluble organic matter (ELOS) was carried by pepsin-cellulase method according to Mika et al. (2009). Organic matter digestibility by pepsin-cellulase method (OMDcel) was calculated from ELOS. The samples were dried, milled on the mill with a sieve of 1 mm and exposed to the pepsin-HCl solution for 24 hours at 40 °C. Thereafter the samples were exposed to cellulase solution in acetate buffer for 24 hours at 40 °C. The incubation of samples was done in filter bags F57 in Daisy Incubator II (Ankom Technology Corp., Macedon, NY). The samples were dried to constant weight at 105 °C, weighed and incinerated at 550 °C after incubation.

The results was evaluated using software MS Excel and Statistica function Anova (repeated measures design). The results evaluation was done with regard to the date of sampling and also to vegetation stages - BBCH (Enz and Dachler, 1997). Samples were divided to 4 groups by vegetation stages: I. stem elongation / booting, II. inflorescence emergence, heading / flowering, anthesis, III. development of fruit and IV. ripening.

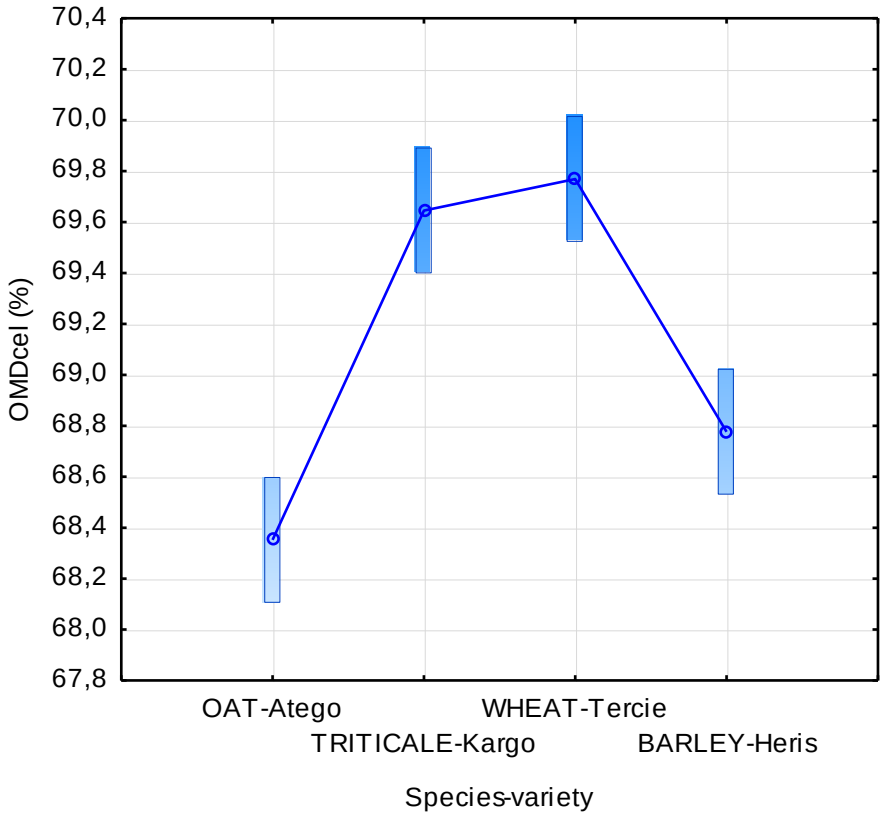
RESULTS AND DISCUSSION

The vegetation stages BBCH was detected during the sampling, they are entered in Tab.1.

Table 1. – The BBCH phases of tested cereals

Date – 2014	13.06.	20.06.	27.06.	03.07.	11.07.	17.07.	25.07.	01.08.
OAT Atego	33	50	57	59	75	78	86	87
TRITICALE Kargo	49	59	61	65	71	74	75	80
WHEAT Tercie	47	55	65	67	73	75	77	86
BARLEY Heris	49	54	57	59	75	78	86	89

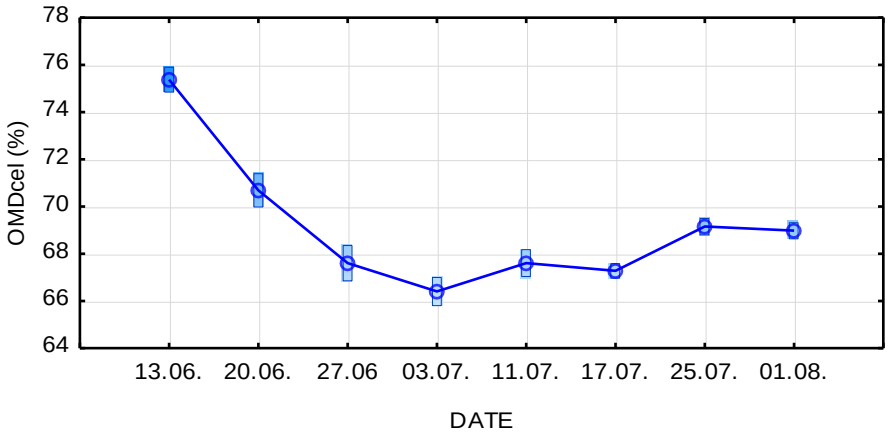
Figure 1. Organic matter digestibility (OMDcel) of tested cereals



Statistical significant differences were detected in the values of OMDcel among cereal species (p=0.001). Figure 1. shows OMDcel means of cereals from all sampling dates. The highest OMDcel had wheat 69.77% (SD - standard deviation 3.15). Minimum was 66.11% (27.06.14) and maximum 75.84% (13.06.14). Phillips and Horn (2008) detected

the digestibility of wheat in vivo. The value of digestibility was 61.8% when experiment was done with sheep and 64.6% with cattle. This value is close to OMDcel of Tercie in flowering stage. The second highest OMDcel had triticale 69.64%. Minimum was 67.06% (27.06.14) and maximum 74.09% (13.06.14). Triticale evinced the smallest SD (2.53), it shows the least pronounced variation of OMDcel during the growing season. The digestibility of triticale was researched by Lithourgidis et al. (2006), he discovered digestibility value 60.48% (mean over whole growing season). Lower value is probably caused by used method. Digestibility was detected using prediction equation (Horrock and Vallentine, 1999). Average OMDcel of barley forage was 68.78% (SD 2.87). Minimum was 65.17% (03.07.14) and maximum was 74.66% (03.06.14). The lowest OMDcel had oat, only 68.35% (SD 4.47). Minimum was 63.84% (11.07.14) and maximum 76.91% (13.06.14). Lithourgidis et al. (2006) stated average digestibility 60.27%, this value is lower because of used method prediction equation. Aydin et al. (2010) observed that oat seeded in Spring had higher digestibility and better nutrients content. It could be assumed even worse OMDcel when the seeding would be done in Autumn.

Figure 2. Effect of sampling dates on OMDcel



Highly significant difference was also found among harvest dates (p = 0.001). The development of OMDcel during the growing season is evident from Figure 2. Cereals showed the highest OMDcel value on 13.06.14 (75.37%), then the value dropped. Minimum was reached on 03.07.14 (66.40%) and then the value slightly increased. Differences between dates are noticeable and the right harvest date is very important for

good quality of forage which is consistent with the statement Collar and Askland (2001), Tatarčíková (2007).

Figure 3. Organic matter digestibility (OMDcel) of cereals at consecutive harvest dates

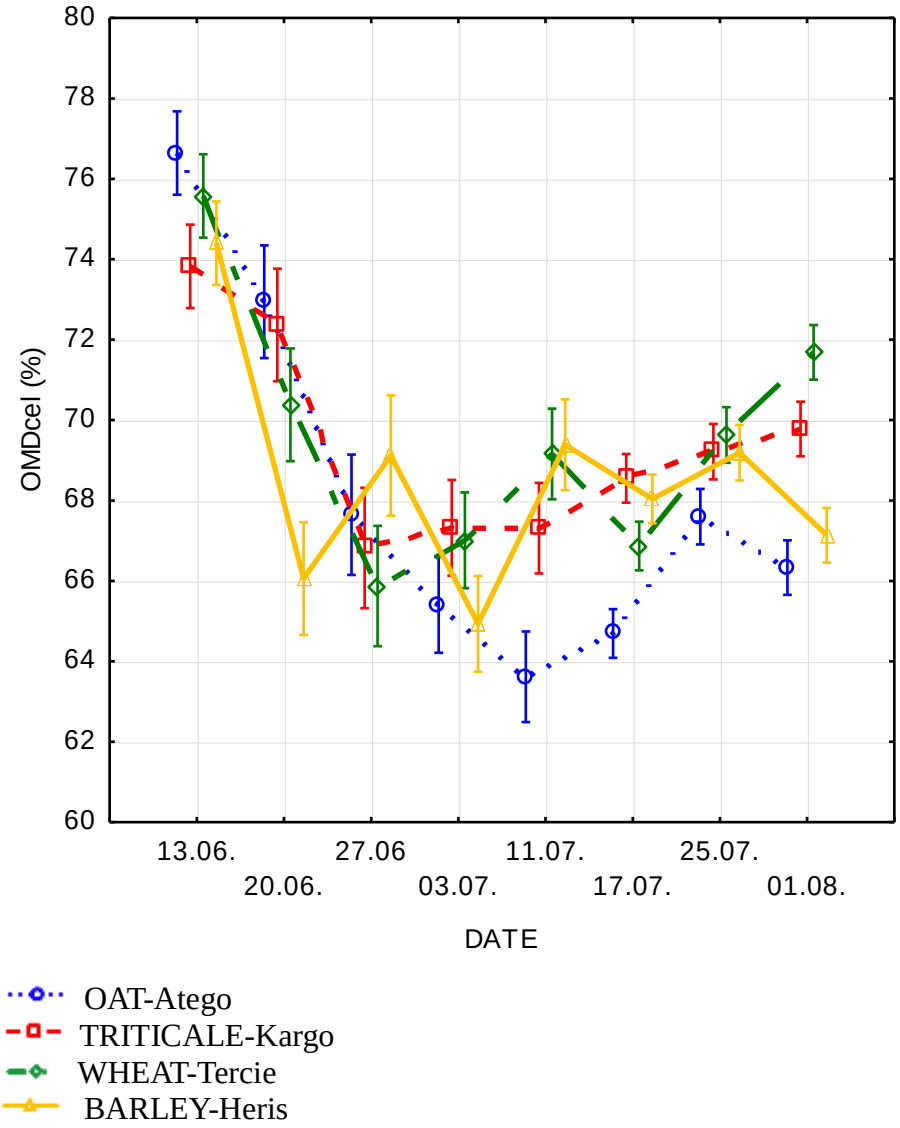
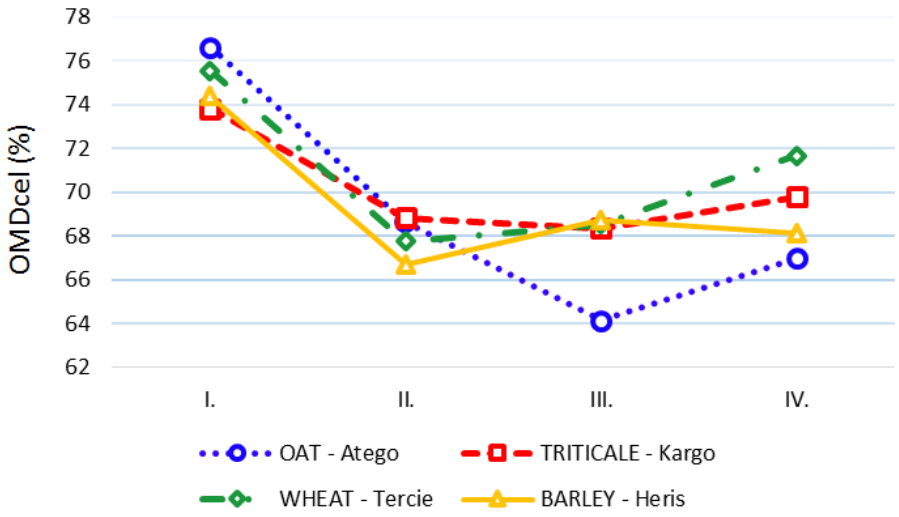


Figure 3. shows OMDcel of each cereal during the growing season, where the different course was found (p 0.001).

OMDcel was different for each BBCH stage (p=0.001). The different OMDcel among vegetation stages is obvious from Figure 4. OMDcel in the I. period corresponded with vegetation stages – the lower BBCH the higher digestibility. The influence of species became obvious in the II. period. Oat distinguished strongly from other cereals in the

III. period. While the OMDcel of wheat and barley increased during the development of fruit and the OMDcel of triticale stagnated, the OMDcel of oat fell considerably. OMDcel of all cereals expect barley rised in the period IV. because of grain ripeness and increase in starch content (Kačicová and Přikryl, 2016). Gill et al. (2013) founded out dry matter digestibility of barley 64.80% in stage dough ripeness and Gill and Omokanye (2016) discovered dry matter digestibility of triticale 62.90% in the same stage. The values are lower probably because of using only prediction equation for digestibility detection (Undersander and Moore, 2002).

Figure 4. Effect of vegetation phases on OMDcel cereals



CONCLUSION

Statistical significant differences in OMDcel among cereals, sampling dates and vegetation stages followed from this experiment. Cereals reached the highest OMDcel on the first date of sampling when crops were in vegetative phase before the heading. OMDcel sharply fell down during inflorescence emergence, heading, flowering, anthesis and development of fruit. Slight increase was detected during the ripening. Wheat and triticale had the best results in digestibility, oat was the worst at it. Well-chosen harvest term is necessary for the gaining of good quality forage, because differences between two sampling dates with seven days interval was up to 8.39%.

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REFERENCES

Aydin, N., Mut Z., Mut H. and Ayan I. (2010): Effect of Autumn and Spring Sowing Dates on Hay Yield and Quality of Oat (Avena sativa L.) Genotypes. Journal of Animal and Veterinary Advances. 9(10).

Barchiesi-Ferrari, C., Alomar D, and Miranda H. (2011). Digestibilidad mediante Pepsina-Celulasa de Ensilajes de Pradera: Efectos del Tipo de Pradera, Estado de - 51 - Madurez y Variaciones en el Metodo Enzimatico. Chilean journal of agricultural research, 71(2), 249-257.

Beever, D.E., and Mould F.L. (2000): Forage evaluation for efficient ruminant livestock production. In Givens, D.I., Owen E., Axford R.F.E, and Omed H.M. (eds.) Forage evaluation in ruminant nutrition. CABI Publishing, Wallingford, Oxon, UK, 15-42

Brouma, D. (2004): Atego uspel i v zahranici. Uroda [online]. Available from: www.uroda.cz

Collar, C. and Askland, G. (2001): Harvest stage effects on yield and quality of winter forage. In: Proceedings of the 31st California alfalfa and forage symposium, Modesto, CA, UC Cooperative Extension University of California, Davis: 12-13.

Enz, M., Dachler, Ch. (1997): Compendium of Growth Stage Identification Keys for Mono-and Dicotyledonous Plants Extended BBCH scale 2nd Edition .

Forejtová, J., Lád F., Třináctý J., Richter M., Gruber L., Doležal P., Homolka P and Pavelek L. (2005): Comparison of organic matter digestibility determined by in vivo and in vitro methods. Czech Journal of Animal Science. 50(2).

Gill, K.S., Omokanye A.T. (2016) Spring Triticale Varieties Forage Yield, Nutrients Composition and Suitability for Beef Cattle Production. Journal of Agricultural Science, 8(10).

Gill, K.S., Omokanye A.T., Pettyjohn J.P. and Elsen M. (2013). Evaluation of Forage Type Barley Varieties for Forage Yield and Nutritive Value in the Peace Region of Alberta. Journal of Agricultural Science, 5(2).

Horrock, R.D., Vallentine J.F. (1999): Harvested Forages. 1. Academic Press,

Huhtanen, P., Nousiainen J., and Rinne M. (2006): Recent developments in forage evaluation with special reference to practical applications. Agricultural and Food Science, 15, 293-323.

Jančík, F. (2007): Ruminal degradation of cell walls selected grasses harvested at different stages of ripeness and preserved by drying and silage. PhD Diss. Jihočeská univerzita v Českých Budějovicích.

Kačicová, L., Přikryl J. (2016). Škrob a stravitelnost objemnýych krmiv. Úroda. Available from: www.uroda.cz

Lithourgidis, A.S., Vasilakoglou I.B., Dhima K.V., Dordas C.A. and Yiakoulaki M.D. (2006): Forage yield and quality of common vetch mixtures with oat and triticale in two seeding ratios. Field Crops Research, 99, 106-113.

Míka, V. (2009): Determination of ELOS (enzymatic soluble organic matter) in forage using Czech cellulases from Trichoderma reesei: certified methodic. 1. Praha: Vyzkumny ustav rostlinne vyroby.

Nesvadba, Z., Ustak S. and Hermuth J. (2017): Moznosti vyuziti ozimeho tritikale na produkci biomasy a bioplynu. Agromanual [online]. Available from: www.agromanual.cz

Nocek, J.E. (1988): In situ and Other Methods to Estimate Ruminal Protein and Energy Digestibility: A Review. American Dairy Science Association, 71(8), 2051–2069.

Nousiainen, J., Rinne M., Hellamaki M., and Huhtanen P. (2003): Prediction of the digestibility of the primary growth of grass silages harvested at different stages of maturity from chemical composition and pepsincellulase solubility. Animal Feed Science and Technology 103, 97-111.

Petr, J. (2001a) Tritikale v ceskem obilnarstvi. Uroda. Available from: www.uroda.cz

Petr, J. (2001b) Moznosti vyuziti tritikale. Uroda. Available from: www.uroda.cz

Phillips, W.A., Horn G.W. (2008). Intake and digestion of wheat forage by stocker calves and lambs. Journal of Animal Science, 86(9), 2424-2429.

Rinne, M., Olt A., Nousiainen J, Seppa, Tuori M., Paul C., Fraser M., and Huhtanen P. (2006): Prediction of legume silage digestibility from various laboratory methods. Grass and Forage Science 61, 354-362.

Robinson, P.H., Givens D.I., and Getachew G. (2004): Evaluation of NRC, UC Davis and ADAS approaches to estimate the metabolizable energy values of feeds at maintenance energy intake from equations utilizing chemical assays and in vitro determinations. Animal Feed Science and Technology 114, 75-90.

Schroeder, G.F., Couderc J.J., Bargo F. and Rearte D.H. (2010): Milk production and fatty acid profile of milkfat by dairy cows fed a winter oats (Avena sativa L.) pasture only or a total mixed ration. New Zealand Journal of Agricultural Research, 48(2), 187-195.

Skládanka J. (2005): Multimedialni ucebni texty picninarstvi: Ústav výživy zvířat a pícninářství MZLU v Brně [online]. Available from: http://web2.mendelu.cz

Tatarčíková, L. (2007): O úspěchu často rozhodují detaily. *Náš chov*. Available from: www.naschov.cz

Tilley, J., Terry R. (1963): A two stage technique for the in vitro digestion of forage crops. Journal of the British Grassland Society 18, 104-111.

Undersander, D., Moore, J.E. (2002): Relative forage quality (RFQ) indexing legumes and grasses for forage quality. University of Wisconsin Extension. Available from: www.uwex.edu

THE OCCURRENCE OF ISOFLAVONES IN DAIRY FEEDSTUFFS AND ASSESSMENT OF THEIR TRANSFER INTO MILK

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ABSTRACT

The aim of the study was to determine the intake of isoflavones from commonly used dairy diets and to quantify the transfer of the isoflavones from feed into the bovine milk under on-farm conditions. The study was performed on individual milk samples collected from four dairy farms breeding Holstein (Farm 1 and 2) and Czech Fleckvieh x Holstein cows (Farm 3 and 4). Milk samples were taken from five average yielding cows per herd and were analysed on the content of basic constituents and isoflavones. Samples of feedstuffs were taken at the same time as milk samples and were analysed on the content of dry matter (DM) and isoflavones. In all farms feeding mixtures were identified as the only source of isoflavones in the diets with concentration of total isoflavones ranging between 143.1 and 986.7 mg/kg of DM resulting in total isoflavones intake from 1071 to 8393 mg/d. Daidzein, genistein, glycitein and metabolite equol were detected in milk of all farms. The total carry-over rate of isoflavones from feed into the milk ranged from 0.3 μg/mg (Farm 4) to 3.6 μg/mg (Farm 1).

Keywords: dairy cows; isoflavones; equol; milk

INTRODUCTION

Isoflavones are substances that naturally occur in plants. It is a constantly studied group of phytoestrogens, which is characterized by strong estrogenic effects (COT, 2003; Duncan et al., 2003; Adler et al., 2014). Like endogenous estrogens, phytoestrogens have a phenolic ring in their chemical structure that allows them to bind to estrogen receptors (Setchell and Cassidy, 1999).

The most frequent plant isoflavones are genistein, daidzein, glycitein, formononetin and biochanin A. All mentioned are common components of dairy feedstuffs, in the form of glycosides they are found in fodder crops (especially in red clover, white clover and lucerne) (Kalač, 2013) and soybean (Flachowsky et al., 2011). During the digestion process, glycosides are hydrolyzed, then released isoflavones undergo a number of transformations (Kalač, 2013). The final metabolites are excreted in the urine or faeces, some of which are also tranferred to the milk (Höjer et al., 2012). The results of many studies proved an increase in isoflavone concentrations in milk at elevated isoflavone levels in the feedstuffs (Hoikkala et al., 2007; Steinshamn et al., 2008; Adler et al., 2014).

At present, the positive effect of isoflavones on human health is being discussed. Significant effects are observed mainly with daidzein metabolite equol which allegedly helps in cardiovascular diseases, osteoporosis, menopausal symptoms, or even in breast and prostate tumors (Wroblewski Lissin and Cooke, 2000; Setchell, 2004; Kalač, 2013). With the exception of soy foods, cow's milk can be a good source of isoflavones in human nutrition (Adler et al., 2014, Hoikkala et al., 2007).

The aim of the study was to determine the intake of isoflavones from commonly used dairy diets and to quantify the transfer of the isoflavones from feed into the bovine milk under on-farm conditions.

MATERIAL AND METHODS

The study was performed on individual milk samples collected from four private dairy farms breeding Holstein (Farm 1 and 2) and Czech Fleckvieh x Holstein cows (Farm 3

and 4). Within each herd, five average yielding cows in midlactation were selected. Samples of evening and morning milk were mixed into one representative sample per cow and were analysed on the content of basic constituents and isoflavones daidzein, genistein, glycitein and metabolite equol.

The composition of diets that were used on farms are given in Table 1. Samples of feedstuffs were taken at the same time as milk samples and were analysed on the content of dry matter (DM) and isoflavones (daidzein, genistein and glycitein).

Table 1. Composition of the diet of individual groups of cows

Items	**Units**	**Farm 1**	**Farm 2**	**Farm 3**	**Farm 4**
Maize silage	kg/d	38	15	30	25
Lucerne haylage	kg/d	-	6	-	5
Peas haylage	kg/d	-	4	-	-
Sugarbeet chippings silage	kg/d	-	15	-	-
Wheat bran	kg/d	-	3	-	-
Lucerne hay	kg/d	2	-	3	2
Supplemental mixture	kg/d	8	4	8	8
Composition of supplemental mixtures					
Barley	g/kg	266	-	200	100
Oat	g/kg	266	-	-	-
Wheat	g/kg	-	225	250	276
Peas	g/kg	-	85	-	-
Maize grain	g/kg	-	95	100	150
Rapeseed	g/kg	-	85	-	110
Sugarbeet chippings	g/kg	96	-	-	-
Soybean meal	g/kg	-	500	-	-
Extruded full-fat soya	g/kg	336	-	-	-
Soybean meal, peeled, toasted	g/kg	-	-	-	190
Linseed	g/kg	-	-	30	-
Malt sprouts	g/kg	-	-	40	76
Maize germs	g/kg	-	-	40	-
Extracted sunflower	g/kg	-	-	50	-
Pumpkin seed cake	g/kg	-	-	250	-
Blend of vitamines	g/kg	0.5	-	0.5	5
Blend of minerals	g/kg	35.5	10	39.5	37
Blend of energy supplements	g/kg	-	-	-	56

In feed samples, levels of isoflavones were determined after their releasing from bonded forms (Třináctý et al., 2009) using HPLC–DAD. Milk samples were prepared and analysed by LC-MS-(TOF) as described in Kašparovská et al. (2016).

The isoflavone transfer from feed into milk expressed as total carry-over rate (tCOR) was calculated according to Flachowsky et al. (2011) as follows:

tCOR = total excretion of isoflavones in milk (μg/day) / isoflavone intake (mg/day)

RESULTS AND DISCUSSION

The average daily intake of DM and isoflavones is presented in Table 2. In all farms feeding mixtures that contained soybean components (see Table 1) were identified as the only source of isoflavones in the diets. The concentration of total isoflavones in mixtures ranged from 143.1 to 986.7 mg/kg of DM (data not shown) resulting in total isoflavones intake from 1071 to 8393 mg/d. Our findings are in agreement with Třináctý et al. (2009), Flachowsky et al. (2011) or Kašparovská et al. (2016) that also detected isoflavones in feeding mixtures, however concentration of isoflavones in the diets and daily isoflavones intake differed considerably between farms and above mentioned studies. This discrepancy can be explained by a wide range of total isoflavones concentration in soybeans that can vary from 1.2 up to 4.2 mg/kg (Kurzer and Xu, 1997; Nakamura et al., 2000) in dependence on the origin of soybeans, environmental factors, growth, harvesting and processing (Flachowsky et al., 2011).

Table 2. Intake of dry matter and isoflavones

Intake of	**Units**	**Farm 1**	**Farm 2**	**Farm 3**	**Farm 4**
Dry matter	kg/d	18.1	21.6	18.5	22.2
Isoflavones					
Daidzein	mg/d	454	639	451	3404
Genistein	mg/d	705	1129	305	4380
Glycitein	mg/d	175	333	315	610
Isoflavones total	mg/d	1335	2101	1071	8393

Milk yield and composition and concentration of isoflavones in milk is given in Table 3. The milk yield ranged from 19.7 to 24.6 kg/d. Daidzein, genistein, glycitein and metabolite equol were detected in milk of all farms. The concentration of individual isoflavones was very diverse across the farms. For example concentration of genistein ranged from 5.2 µg/l (Farm 4) to 158.6 µg/l (Farm 1). Concentration of equol varied between 16.6 µg/l and 73.6 µg/l and the highest value was found on Farm 4. In general, our results are in accordance with findings of Třináctý et al., (2009), Křížová et al., (2011), Flachowsky et al. (2011) or Kašparovská et al. (2016) that studied transfer of soybean-derived isoflavones from feed into milk as well as with e.g. Kuhnle et al. (2008) or Antignac et al. (2004) that reported low content of isoflavones and equol in samples of various commercially available milk.

Total carry-over rate ranged from 0.3 µg/mg (Farm 4) to 3.6 µg/mg (Farm 1). The lowest value is in agreement with findings of Flachowsky et al. (2011) and the highest one is in accordance with Křížová et al. (2011). The relatively wide range in total carry-over rate can be caused by differences in rumen degradability of soybean components in the diet (Křížová, Němcová, unpublished data). Furthermore, according Flachowsky et al. (2011) and Steinshamn et al. (2008) to the carry-over rate is rate-limiting, diminishing the amount of isoflavones transferred to the milk with increasing content of isoflavones in the feed.

Table 3. Yield and composition of milk

Items	**Units**	**Farm 1**	**Farm 2**	**Farm 3**	**Farm 4**
Milk yield	kg/d	19.7	23.1	22.4	24.6
Composition of milk and isoflavones carry-over rate					
Fat	g/100g	4.5	4.5	3.9	6.1
Protein	g/100g	3.4	3.0	3.1	3.0
Daidzein	µg/l	40.0	22.9	15.4	11.7
Genistein	µg/l	158.6	50.1	33.4	5.2
Glycitein	µg/l	28.7	6.4	9.7	3.9
Equol	µg/l	16.6	24.8	26.1	73.6
t-COR*	µg/mg	3.6	1.2	1.8	0.3

* total carry-over rate

CONCLUSION

Feeding mixtures commonly used in dairy diets containing various soybean feeding componets are a source of isoflavones that can be excreted into the milk. Although transfer of isoflavones from feed into milk is low, bovine milk can be considered as a source of isoflavones in human nutrition.

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REFERENCES

Adler, S. A., Purup, S., Hansen-Møller, J., Thuen, E., Gustavson, A. M., Steinshamn, H. (2014): Phyto-oestrogens and their metabolites in milk produced on two pastures with different botanical compositions. Livestock Science, 163, 62–68.

Antignac, J. P., Cariou, R., Le Bizec, B., Andre, F. (2004): New data regarding phytoestrogens content in bovine milk. Food Chemistry, 87: 275–281.

COT (Committe on toxicity of chemicals in food, consumer products and the environment) (2003): COT-report. Phytoestrogens and Health. Food Standards Agency.

Duncan, A. M., Phipps, W. R., Kurzer, M. S. (2003): Phyto-oestrogens. Best Practice & Research Clinical Endocrinology and Metabolism, 17, 253–271.

Flachowsky, G., Hünerberg, M., Meyer, U., Kammerer, D. R., Carle, R., Goerke, M., Eklund, M. (2011): Isoflavone concentration of soybean meal from various origins and transfer of isoflavones into milk of dairy cows. Journal für Verbraucherschutz und Lebensmittelsicherheit, 6, 449–456.

Hoikkala, A., Mustonen, E. Saastamoinen, I., Jokela, T., Taponen, J., Saloniemi, H., Wähälä, K. (2007): High levels of equol in organic skimmed Finnish cow milk. Molecular Nutrition & Food Research, 51, 782–786.

Höjer, A., Adler, S., Purup, S., Hansen-Møller, J., Martinsson, K., Steinshamn, H., Gustavsson, A. M. (2012): Effects of feeding dairy cows different legume-grass silages on milk phytoestrogen concentration. Journal od Dairy Science, 95, 4526–4540.

Kalač, P. (2013): Fresh and ensiled forages as a source of estrogenic equol in bovine milk: a review. Czech Journal of Animal Science, 58, 296–303.

Kašparovská J., Křížová L., Lochman J., Dadaková K., Kašparovský T. (2016): Soybean-derived isoflavone determination in rumen fluid and milk by LC–MS-(TOF). Journal of Chromatographic Science, 54, 997–1003.

Křížová, L., Veselý, A., Třináctý, J., Schulzová, V., Hurajová, A., Hajšlová, J., Kvasničková, E., Havlíková, Š. (2011): Changes in isoflavones concentrations in cheese during processing and ripening. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 59, 153–162.

Kuhnle, G. G. C., Delaqulla, C., Aspinall, S. M., Runswick, S. A., Mulligan, A. A., Bingham, S. A. (2008): Phytoestrogen content of foods of animal origin: dairy products, eggs, meat, fish, and seafood. Journal of Agricultural and Food Chemistry, 56, 10099–10104.

Kurzer, M. S., Xu, X. (1997): Dietary phytoestrogens. Annual Review of Nutrition, 17, 353–381.

Nakamura, Y., Tsuji, S., Tonogai, Y. (2000): Determination of the levels of isoflavonoids in soybeans and soy-derived foods and estimation of isoflavonoids in the Japanese daily intake. The Journal of AOAC International, 83, 635–650.

Setchell, K. D. R. (2004): Equol – Origins, actions, and clinical relevance of this specific soy isoflavone metabolite. Journal of Nutrition, 134, 1235S-1236S.

Setchell, K. D. R., Cassidy, A. (1999): Dietary isoflavones: Biological effects and relevance to human health. Journal of Nutrition. 129, 758S–767S.

Steinshamn, H., Purup, S., Thuen, E., Hansen-Møller, J. (2008): Effects of clover-grass silages and concentrate supplementation on the content of phytoestrogens in dairy cow milk. Journal of Dairy Science, 91, 2715–2725.

Třináctý J., Hanuš O., Křížová L., Schulzová V., Hajšlová J. (2009): The effect of feeding soybean-derived phytoestogens on their concentration in plasma and milk of lactating dairy cows. Archives of Animal Nutrition, 63, 219–229.

Wroblewski Lissin, L., Cooke, J. P. (2000): Phytoestrogens and cardiovascular health. Journal of the American College of Cardiology. 35, 1403–1410.

EFFECT OF LINSEED AND SUNFLOWER SEEDS IN PIG DIET TO FATTY ACID CONTENT IN THE PORK FROM MANGALITSA

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ABSTRACT

The aim of study was to evaluate the effect of linseed and sunflower seed in diet of fattening Mangalitsa pigs on fatty acid content in the meat. Eighteen Mangalitsa pigs were divided into two groups: diet with 10 % of sunflower seed addition (group S) and diet with 10 % of linseed addition (group L). Pigs received feed mixture and water by *ad libitum system.* The fattening period lasted from 30 kg to 100 kg of body weight. The diet with linseed addition significantly increased proportion of oleic acid, vaccenic acid and DHA compared to diet with sunflower seed addition ($P < 0.05$). However, the total percentage of saturated fatty acid as well as polyunsaturated fatty acid in meat was increased by sunflower seed diet, but the total percentage of monounsaturated fatty acid was decreased compared with linseed diet. It can be concluded, that there are not significance differences in total proportion of fatty acids in meat between diets with sunflower seed addition and linseed addition for fatteners of Mangalitsa with exception of differences in oleic acid, vaccenic acid and DHA.

Keywords: linseed; Mangalitsa; MUFA; PUFA; SFA; sunflower

INTRODUCTION

The Mangalitsa is a rustic pig breed and it is a typical representative of the fatty pig breeds. The average total mass consists of 30 % - 35 % meat and 65 % -

70 % fat tissue (Egerszegi et al., 2003). The fat of Mangalitsa is softer and easier to digest by human with higher content of polyunsaturated fatty acids compared to fat from pig meat breeds (Parunović et al., 2013).

Generally, pork meat is an excellent source of nutritive compounds which are essential in human nutrition (Cordis et al. 2015). Numerous factors such as genetic factors, breed, sex, energy intake as well as fatty acids composition of the diet influence the fatty acids content of the fatty and muscle tissues of pigs (Petrović et al. 2014). The fatty acid content of aliments is of highly great importance with respect to healthy human nutrition. While saturated fatty acids are regarded a risk factor for cardiovascular diseases, the polyunsaturated fatty acids are considered as assisting in the prevention of cardiovascular diseases (Csápo and Salamon, 2013).

Fatty acid content of pork can be easily manipulated trough the feeding regime. In pig diet, an emphasis is laid on the omega -3 fatty acid vegetable oils such as soy, olive, linseed, sunflower or rapeseed (Václavková et al. 2015). The human nutritionists recommend a higher intake of polyunsaturated fatty acids (PUFA), especially n-3 PUFA at the expense of n-6 PUFA (Raes et al. 2004). The imbalance fatty acid intake such as ratio of PUFA: SFA or the ratio of n-6:n-3 PUFA, is a risk factor in cancer and coronary heart diseases. The recommended ratio of PUFA to SFA (P:S) should be increased to above 0,4 and ratio of n-6:n-3 PUFA less than 4 (Wood et al. 2003). According to Raes et al. (2004), several animal feeding goals have been carried out using different breeds aiming at bringing the PUFA/SFA ratio of meat closer to the recommended values more than 0.7 and for the n-6/n-3 ratio less than 5. Nowadays nutritionists have focussed on the type of PUFA and the balance in the diet between n-3 PUFA and n-6 PUFA(Wood et al. 2003).

The aim of study was evaluate the effect of diet with linseed addition and the diet with sunflower seed addition for pig fatteners in relation to fatty acid content in the pork from Mangalitsa breed.

MATERIAL AND METHODS

The experiment was carried out in the Experimental center of Animal at Slovak University of Agriculture in Nitra (SUA). The experimental material comprised of 18 pigs of Mangalitsa breed. The pigs were divided into two groups: group S (n=8), which received diet with 10 % of sunflower seed addition and group L (n=10), which received diet with 10 % of linseed addition. The composition of diets is presented in Table 1. The fatty acids composition of diets is shown in Table 3. The pigs were reared in the same outdoor intensive conditions and they received feed mixture and drinking water by *ad libitum* system. The pen was consisted of concrete floor and the straw was used as bedding.

Table 1. Composition of diets for fatteners

Ingredients (%)	Diet S	Diet L
Corn	50	50
Barley	10	10
Wheat	10	10
Soybean meal	10	10
Sunflower seed	10	-
Linseed	-	10
Granuled alfalfa	7	7
Mineral and vitamin supplement[1]	3	3

[1]retihol 200 000 m.j., cholecalciferol 30 000 m.j., α-tocopherol 400 mg, riboflavin 80 mg, pyridoxine 30 mg, cyanocobalamin 1000 mcg, niacinamide 300 mg, folic acid 2 mg, pantothenic acid 300 mg, cholinchlorid 4000 mg, Cu 600 mg, Fe 3400 mg, Zn 1000 mg, Mn 1000 mg, I 30 mg, Se 8 mg.

Table 2. Fatty acids profile of diets for fatteners

Fatty acids profile (%)	Diet S	Diet L
PUFA	53,46	68,38
MUFA	32,95	18,54
SAFA	11,97	11,66
C:16 (palmitic)	8,3	8,7
C18:0 (stearic)	2,6	2,6
C18:1cis n9 (oleic)	32,6	18,3
C18:2n-6 (linoleic)	52,7	63,9
C18:3 n3 (alfa-linolenic)	0,8	4,5

Diet S: diet with 10 % of sunflower seed, Diet L: diet with 10 % of linseed

The fattening period lasted from 30 kg to 100 kg of body weight. Then pigs were slaughtered in the slaughterhouse of Experimental center of Animals. The day after the slaughter, the samples of *Musculus longissimus dorsi* (MLD) were taken from right half carcass for analyses of fatty acids profile. The fatty acids profile was analysed by FT IR (Fourier Transform InfraRed) method. FT IR is method of infrared spectroscopy. An infrared spectrum represents a fingerprint of a homogenized sample with absorption peaks which correspond to the frequencies of vibrations between the bonds of the atoms making up the material.

The parameters of fatty acids profile were statistically analysed by the analyses of variance (ANOVA) using the Statistical Analysis System (SAS 9.2. using of application Enterprise guide 5.1, 2012). The means and standard error of mean (SEM) were calculated. Tukey's test was applied to compare the mean values of the groups with different feed mixture.

RESULTS AND DISCUSSION

The results of fatty acids profile in *Musculus longissimus dorsi* (MLD) are shown in Table 3. The percentage of lauric acid, palmitic acid and stearic acid were slightly increased in Group S compared to Group L. On the contrary, myristic acid was found negligible higher in Group L than in Group S. The total content of saturated fatty acids (SFA) was higher in Group S compared to Group L. Differences were not statistically significant, due to this fact the content of SFA was not influenced by the diet.

The diet with linseed significantly increased proportion of oleic acid and vaccenic acid in MLD compared to diet with addition of sunflower seed ($P < 0.05$), which is shown by total content of monounsaturated fatty acid (MUFA) in MLD. However differences of MUFA in MLD between groups were not statistically significant.

The linoleic acid, CLA, EPA, DPA as well as DHA ($P < 0.05$) were found higher in group L than in group S. However, the total percentage of polyunsaturated fatty acid (PUFA) was increased by the sunflower diet compared to linseed diet, but the differences between groups were not statistically significant. The content of n-6 PUFA was found higher in the group S, but n-3 PUFA was lower than in group L.

Table 3. The effect of two diets of fatty acids content of *m. longissimus dorsi*

Fatty acids profile (%)	Group S (n=8) Mean	Group L (n=10) Mean	SEM	P-values
C12:0 (Lauric)	0,068	0,066	0,002	n.s.
C14:0 (Myristic)	1,27	1,28	0,006	n.s.
C16:0 (Palmitic)	24,47	24,41	0,039	n.s.
C18:0 (Stearic)	11,24	11,12	0,056	n.s.
C18:1cis-9 (Oleic)	42,29	43,91	0,355	*
C18:1trans-11 (Vaccenic)	4,47	4,55	0,019	*
C18:2n-6 (Linoleic)	0,047	0,048	0,001	n.s.
CLA (Conjugated linoleic acid)	0,125	0,126	0,003	n.s.
C18:3n-3 (α linolenic)	0,266	0,266	0,006	n.s.
C20:5n-3 (EPA)	0,090	0,095	0,003	n.s.
C22:5n-3 (DPA)	0,137	0,141	0,002	n.s.
C22:6n-3 (DHA)	0,039	0,043	0,001	*
Total SFA	36,91	36,69	0,213	n.s.
Total MUFA	50,71	50,98	0,351	n.s.
Total PUFA	12,35	11,85	0,223	n.s.
Total n-3 PUFA	0,604	0,630	0,011	n.s.
Total n-6 PUFA	10,89	10,39	0,228	n.s.
Ratio n6 : n3	18,10	16,54	0,424	n.s.
Ratio PUFA : SFA	0,336	0,322	0,007	n.s.

Group A: diet with 10 % sunflower seeds Group B: diet with 10 % of linseed
SEM: Standard error of mean, SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids
n.s.: non-significant, *: $P < 0,05$

It can be concluded that total content of SFA, PUFA, MUFA in MLD were not significantly influenced by different diets for fatteners. While the diet with linseed addition significantly increased oleic and vaccenic acids as well as DHA in MLD of Mangalitsa. In the studyof Cordis et al. (2015) was found lower percentages of total content of SFA, MUFA as well as PUFA in MLD from Mangalitsa compared to our results. The pigs were reared extensively and were fed only by grass and cereals without concentrates. According to Habeanu et al. (2014), the Mangalitsa breed fed by diet with linseed addition had higher content of SFA, PUFA in the intramuscular fat of MLD, but lower composition of MUFA compared to our study. However the profile of each fatty acid such as myristic acid, palmitic acid, stearic acid, oleic acid, EPA, DPA and

DHA were similar with our results. Petrović et al. (2014) compared rustic pig breeds Moravka
and Mangalitsa. The pigs received the diet with 5% sunflower oil meal addition. They determined decreased percentage of SFA as well as PUFA, but increased proportion of MUFA in MLD compared to Moravka. However, they achieved in their study higher proportion of SFA, MUFA, but lower content of PUFA than in our study. Research by Tomović et al. (2016) has shown that MLD of Mangalitsa had lower percentage of SFA and PUFA, but higher proportion of MUFA compared with Large White pigs. The pigs received diet with sunflower meal addition. The Mangalitsa breed from our study had higher content of SFA, PUFA, but lower percentage of MUFA.

In our study, the diet with linseed addition decreased the PUFA/SFA ratio and n-6/n-3 ratio compared to the diet with sunflower addition. However the PUFA/SFA ratio as well as n-6/n-3 ratio was not significantly influenced by diets. The PUFA/SFA ratio was less than 4, how it is recommended according human nutritionist. However, n-6/n-3 ratio was higher than it is recommended. Similarly in the research of Parunović et al. (2012) and Parunović et al. (2013) was observed that Mangalitsa had in MLD PUFA/SFA ratio less than 4, but n-6/n-3 was higher than 30. The study of Kouba et al. (2003) showed that diet for pigs containing 6 % of linseed reduced the n-6/n-3 PUFA ratio in MLD to 3,9 compared to ratio 7,9 in control group of pigs. In contrary, in our study was observed more higher n-6:n-3 PUFA ratio.

CONCLUSION

From the results obtained it can be concluded, that the diet with linseed addition significantly increased oleic and vaccenic acids as well as DHA in MLD of Mangalitsa. However, the total content of SFA, PUFA as well as MUFA was not significantly influenced by different diet for fatteners.

ACKNOWLEDGEMENT

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REFERENCES

Cordis, I., Mihaiu, M., Tabaran, A., Mihaiu, R., Dan, S.D., Reget, O., Cordea, D., Muresan, C. (2015). Compositional Studies on Mangalitsa Meat Products for Public Consumption. Bulletin UASVM Veterinary Medicine, 72, 57-61.

Csápo, J., Salamon, R. V. (2013). Fatty acid composition and cholesterol content of the fat of pigs of various genotypes. Acta Univ. Sapientiae, Alimentaria, 6, 23-33.

Egerszegi, I., Ratky, J., Solti, L., Brussow, K. P. (2003). Mangalica – an indigenous swine breed from Hungary (Review). Arch Tierz Dummerstorf, 46, 245-256.

Habeanu, M., Lefter, N., Gheorghe, A., Nagy, A., Marin, D., Ropota, M. (2014). Effects of dietary flaxseed oil on the muscle fatty acid composition in Mangalitsa pigs in an extensive rearing systém. South African Journal of Animal Science, 44, 240-244.

Kouba, M., Enser, M., Whittington, F. M., Nute, G. R., Wood, J. D. (2003). Effect of a high-linolenic acid diet on lipogenic enzyme activities, fatty acid composition, and meat quality in the growing pig. Journal of Animal Science, 81, 1967-1979.

Parunović, N., Petrović, M., Matekalo-Sverak, V., Trbović, D., Mijatović, M., Radović, Č. (2012). Fatty acid profile and cholesterol content of m. longissimus of free-range and conventionally reared Mangalitsa pigs. South African Journal of Animal Science, 42, 101-113.

Parunović, N., Petrović, M., Matekalo-Sverak, V., Radović, Č., Stanišić, N. (2013). Carcass properties, chemical content and fatty acid composition of the Musculus longissimus of different pig genotypes. South African Journal of Animal Science, 43, 123-136.

Petrović, M., Wähner, M., Radović, Č., Radojković, D., Parunović, N., Savić, R., Brkić, N. (2014). Fatty acid profile of m. longissimus dorsi of Mangalitsa and Moravka pig breeds. Archiv Tierzucht, 57, 1-12.

Raes, K., De Smet, S., Demeyer, D. (2004). Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review. Animal Feed Science and Technology, 113, 199-221.

SAS Institute Inc. (2008) SAS® 9.2. Cary, NC: SAS Institute Inc.

Tomović, V. M., Šević, R., Jokanović, M., Šojić, B., Škaljac, S., Tasić, T., Ikonić, P., Polak, M. L., Polak, T., Demšar, L. (2016). Quality traits of longissimus lumborum muscle from White Mangalica, Duroc x White Mangalica and Large White pigs reared under intensive conditions and slaugtered at 150 kg live weight: a comparative study. Arch. Anim. Breed, 59, 401-415.

Václavková, E., Bělková, J., Rozkot, M. (2015). Effect of linseed in pig diet on fatty acid content in backfat of Prestice Black-Pied pigs. Research in pig breeding, 9, 16-19.

Wood, J.D., Richardson, R.I., Nute, G.R., Fisher, A.V., Campo, M.M., Kasapidou, E., Sheard, P.R., Enser, M. (2003). Effects of fatty acdis on meat quality: a review. Meat Science, 66, 21-32.

CHANGES IN THE BLOOD BIOCHEMICAL PROFILE OF CALVES WITH FEEDS SUPPLEMENTS

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ABSTRACT

The pre-weaning period is critical for calves health and growth. Adding probiotics, prebiotics and homeopathic feed additives may help assist postnatal development by improving physiological processes and thereby health condition calves. The aim of this study was to assess influence of selected food supplements in particular farm on biochemical parameters in the blood of calves. In experiment the calves were divided in to five groups – four experimental and one control. Experimental groups were filed feed supplements to support active immunity and improving health according to the methodology for period of time five weeks. Results were statistically evaluated and compared with each other. This experiment was performed from March to August 2016.

Keywords: probiotics; prebiotics; homeopathic; *Bifidobacterium*; succinic acid

INTRODUCTION

Calf morbidity and mortality is associated with high costs for the farmer, such as compensation for calf losses, the costs for medical treatment (Mohd et al., 2012). Moreover, calf morbidity and mortality are important animal welfare issues (Mee, 2013).

The physiological processes of a livestock animal, including the immune system, can be largely influenced by the availability of nutrient and trace minerals that are essential for multiple biochemical processes, including immune response, cell replication, and

skeletal development, and are particularly relevant for the newborn (Carroll and Forsberg, 2007).

In addition to the impact of post-natal feeding intensity on short-term constitution, nutritional stimuli during a sensitive period of development affect the long-term metabolic performance of the adult organism. This phenomenon, called 'nutritional programming', 'developmental programming' or 'metabolic imprinting' (Guilloteau et al., 2009; Kaske et al., 2010) also permanently affects the release of hypothalamic neuropeptides controlling feed intake and long-term weight gain due to the plasticity of the regulatory system (Taylor and Poston, 2007).

Immunity is the ability of the body to resist the pathogenic invasion. Insufficient immunity threatens the health and overall survival and so its maintenance and improvement are of primary importance (Patel et al., 2015). Appropriate development of the innate immune system is essential so that newborn calves would survive, especially when they face the pressure of infectious diseases that are responsible for high morbidity and mortality. In the first several months of life newborn calves have a weakened immune system because the function of granulocytes and their complete activity are low (Cervenak et al., 2009, Cortese, 2008) and the specific immunity of calves has not developed sufficiently yet (Boysen et al., 2006).

Neonatal ruminants are unique in that, at birth, they are physically and functionally two different types of animal with respect to their gastrointestinal system (Heinrichs and Lesmeister, 2005). The gastrointestinal trackt of a newly born calf is sterile, and colonization of the gastrointestinal tract begins immediately after birth. Thereafter, a complex and dynamic microbial ecosystem with high densities of living bacteria is established in the large intestine as animals grow to maturity (Stewart et al. 1988).

Probiotics/prebiotics have the ability to modulate the balance and activities of the gastrointestinal microbiota, and are, thus, considered beneficial to the host animal and have been used as functional foods. Numerous factors, such as dietary and management

constraints, have been shown to markedly affect the structure and activities of gut microbial communities in livestock animals (Uyeno et al. 2015).

The term “probiotics” has been amended by the FAO/ WHO to “Live microorganisms, which, when administered in adequate amounts, confer a health benefit on the host” (Fuller, 1989). Several lactic acid bacteria (LAB) strains, species belonging to the genera Lactobacillus, Bifidobacterium, and Enterococcus, are considered beneficial to the host and have, thus, been used as probiotics and included in several functional foods. Probiotics have the ability to enhance intestinal health by stimulating the development of a healthy microbiota (predominated by beneficial bacteria), preventing enteric pathogens from colonizing the intestine, increasing digestive capacity, lowering the pH, and improving mucosal immunity (Uyeno et al. 2015).

Succinic acid is a four-carbon dicarboxylic acid, which has attracted much interest due to its abroad usage as a precursor of many industrially important chemicals in the food, chemicals, and pharmaceutical industries (Jiang et al., 2017). It has been previously shown that succinic acid ester can be taken up and metabolized by pancreatic cells, leading to increased pro-insulin biosynthesis (Alarcon and Wicksteed, 2002) insulin secretion and lowered blood glucose (Zawalich et al., 1992; Juan et al., 1998)

The proper function of certain physiological processes can be determined by using haematologicals and biochemicals blood analysis. The knowledge about normal values of biochemical variables in blood plasma and other physiological variables is important for assessment of damage of organs and tissues in different diseases and for assessment of development from the welfare aspect (Steinhardt and Thilescher, 2000).

The most common parameters determined by biochemical blood analysis include, for example: urea, alkaline phosphatase, gamaglutamyltransferase, total protein, cholesterol, triglycerides, glucose, calcium and phosphorus.

MATERIAL AND METHODS

In experiment were included 72 experimental and eighteen controls calves. The calves were divided into five groups: 1st group – Probiotics (*Bifidobacterium species*); 2nd group – Prebiotics (Succinic acid); 3rd group – Probiotics and Prebiotics (*Bifidobacterium species* and Succinic acid); 4th group - Homeopathic (PVB for the prevention and treatment of parasitic diseases) and 5th group - Controls.

The first blood samples were taken from day 2 to day 5 of age after birth, the next blood samples were collected every week for time five week. The calves were received colostrum 5 day after birth, then a milk replacer was administered and its composition is shown in Table No. 1. From day 7 the calves received a starter that is produced by the agricultural enterprise concerned.

Table 1. The formulation of milk replacer

Analytical ingredients		**Trace elements**		**Vitamins (per 1 kg)**	
Crude protein	20%	Potassium iodide	0.25 mg	Vitamin A	25 000 IU
Crude fibre	0%	Cobalt	0.2 mg	Vitamin D_3	6 000 IU
Crude oils and fats	20%	Manganese	30 mg	**Antioxidants**	
Crude ash	8%	Copper	10 mg	BHT	150 mg
Calcium	0.80%	Selenium	0.4 mg	**Preservative**	
Sodium	0.50%	Iron	80 mg	Citric acid	1 000 mg
Phosphorus	0.70%	Zinc	50 mg		

All calves had the same stabling. In the Probiotics group was served every day 2 g clean culture *Bifidobacterium species* in concentration 10^7. The Prebiotics group was administered Succinic acid in an amount of 2 g/head/day. The Probiotics and Prebiotics group received 2 g *Bifidobacterium species* and the same amount Succinic acid. The fourth experimental group Homeopathic was received 20 ml mixture homeopathic. These additives were dissolved in colostrum, later in milk replacer. All these groups were administered feed supplements from day 2 after birth. The feed supplements were administered over a period 5 weeks once daily. The control group received unchanged feeding dose. The sample analyses were performed in the laboratory of the Faculty of Agriculture in Czech Budejovice, always just the next morning after the withdrawal.

Statistical analysis used Statistica 12 (ANOVA). Due to continuous variables being analyzed, results are presented with standardized mean differences (SMD) between the additives supplements and controls with 95% confidence intervals.

RESULTS AND DISCUSSION

The mean values of measurements from the experiment were compared with the reference values of Clinic for ruminants in Kosice. In Table 2 are typify the obtained average values of measurements with standard deviations and reference values.

Table 2. Average values of measurements with standard deviations and reference values.

	Homeopathic	Probiotics	Prebiotics	Pro + Pre	Control
Urea (mmol/l)					*RV =2 - 5.5 mmol/l
1. week	4.26 ± 0.8	4.6 ± 0.89	4.41 ± 0.71	3.69 ± 1.15	4.37 ± 1.13
2. week	3.36 ± 0.65	3.41 ± 1.09	3.45 ± 0.89	3.15 ± 1.00	3.31 ± 0.66
3. week	3.29 ± 0.56	3.21 ± 0.61	3.44 ± 0.63	3.52 ± 0.8	3.14 ± 0.73
4. week	3.61 ± 0.92	3.47 ± 0.69	3.27 ± 0.74	3.3 ± 0.72	3.43 ± 0.82
5. week	3.31 ± 0.68	3.18 ± 0.67	3.33 ± 0.62	3.21 ± 0.73	3.06 ± 0.58
Alkaline phosphatase (µkat/l)					RV= to 8 µkat/l
1. week	5.92 ± 2.06	6.41 ± 1.7	7.4 ± 2.61	6.46 ± 1.63	6.01 ± 2.24
2. week	3.92 ± 0.84	4.98 ± 1.63	4.59 ± 1.35	4.79 ± 1.31	4.35 ± 1.63
3. week	4.53 ± 1.51	4.86 ± 2.5	4.67 ± 1.93	4.46 ± 1.56	3.92 ± 1.68
4. week	4.46 ± 1.24	4.69 ± 1.75	4.52 ± 2.02	4.15 ± 1.44	4.71 ± 1.95
5. week	4.13 ± 1.46	4.52 ± 1.56	4.85 ± 2.09	3.95 ± 1.67	4.28 ± 1.54
Gamma-glutamytransferase (µkat/l)					RV = individual
1. week	7.23 ± 4.28	10.26 ± 5.95	7.2 ± 5.18	9.88 ± 7.43	7.99 ± 6.57
2. week	2.25 ± 1.15	2.5 ± 1.11	2.13 ± 1.23	2.48 ± 1.4	2.12 ± 1.10
3. week	1.18 ± 0.14	1.29 ± 0.55	1.07 ± 0.45	1.23 ± 0.74	1.03 ± 0.48
4. week	0.73 ± 0.27	0.73 ± 0.3	0.65 ± 0.22	0.7 ± 0.31	0.67 ± 0.27

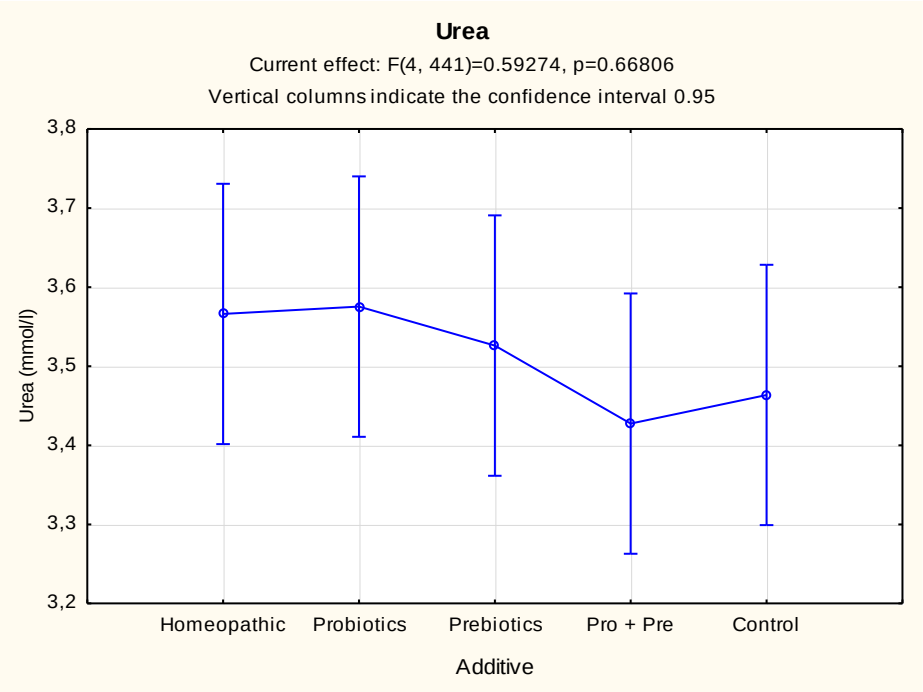
5. week	0.51 ± 0.16	0.54 ± 0.17	0.46 ± 0.17	0.45 ± 0.18	0.45 ± 0.18
Total protein (g/l)					RV = 50 - 70 g/l
1. week	66.15 ± 4.71	66.23 ± 6.85	64.09 ± 6.55	64.78 ± 4.77	62.53 ± 6.15
2. week	61.82 ± 5.01	62.41 ± 6.78	59.87 ± 6.45	61.74 ± 7.13	60.19 ± 4.45
3. week	61.51 ± 5.47	59.6 ± 5.7	60.67 ± 4.00	61.59 ± 5.2	59.15 ± 6.19
4. week	62.34 ± 6.27	60.73 ± 5.05	62.01 ± 6.15	60.71 ± 4.83	62.24 ± 6.94
5. week	62.56 ± 7.84	62.1 ± 6.38	63.23 ± 5.57	60.7 ± 8.08	63.88 ± 6.18
Cholesterol (mmol/l)					RV = 1.3 - 3.9 mmol/l
1. week	1.97 ± 0.52	1.69 ± 0.68	1.92 ± 0.54	2.02 ± 0.69	1.93 ± 0.62
2. week	1.91 ± 0.58	1.66 ± 0.61	1.7 ± 0.44	1.52 ± 0.48	2.04 ± 0.57
3. week	2.46 ± 0.51	2.07 ± 0.56	2.16 ± 0.58	2.43 ± 0.59	2.31 ± 0.55
4. week	2.39 ± 0.56	2.09 ± 0.59	2.18 ± 0.55	2.12 ± 0.46	2.09 ± 0.39
5. week	2.27 ± 0.53	2.1 ± 0.53	2.25 ± 0.48	2.13 ± 0.42	2.3 ± 0.43
Triglycerides (mmol/l)					RV = 0.17 - 0.51 mmol/l
1. week	0.54 ± 0.27	0.57 ± 0.28	0.49 ± 0.24	0.51 ± 0.21	0.47 ± 0.25
2. week	0.33 ± 0.14	0.29 ± 0.19	0.23 ± 0.09	0.25 ± 0.12	0.27 ± 0.13
3. week	0.39 ± 0.24	0.35 ± 0.19	0.35 ± 0.19	0.4 ± 0.22	0.36 ± 0.13
4. week	0.27 ± 0.17	0.21 ± 0.14	0.24 ± 0.13	0.29 ± 0.09	0.2 ± 0.1
5. week	0.22 ± 0.1	0.21 ± 0.11	0.17 ± 0.07	0.18 ± 0.08	0.18 ± 0.07

Urea

Urea is a waste product of the organism, but it also plays an important role in re-exchange in the nephrons, where it helps to re-absorb the water and some ions into the bloodstream. Urea is used as a renal function indicator. Concentration of urea in the blood depends on nutrition or is a manifestation of kidney disease and urinary tract damage, as reported by Ulrich Von Bock und Polach (1994). Urea values ranged within the reference range. Statistical differences were not found when compared to the control

group (Homeopathic p = 0.48; Probiotics p = 0.35; Prebiotics p = 0.59; Probiotics and Prebiotics p = 0.78).

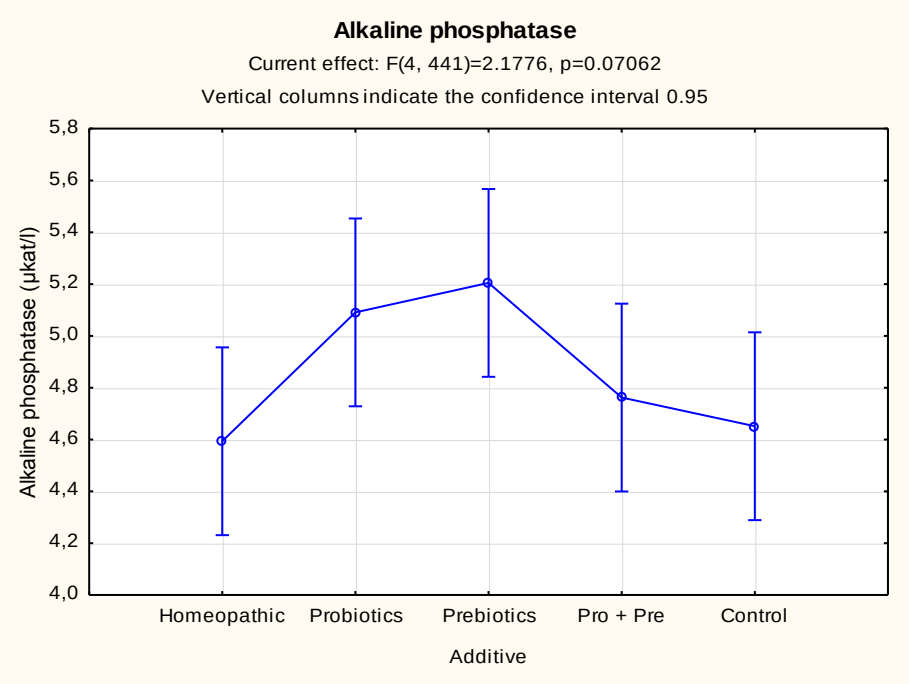
Figure 1. The statistical differences between observed feed supplements and urate values in calves blood from 1 to 5 weeks after birth.



Alkaline phosphatase

Alkaline phosphatase is an enzyme that cleaves phosphate esters into free phosphates in the alkaline medium (Jelínek et al., 2003). It is found in almost all organs and tissues (Ulrich Von Bock und Polach 1994). Despite this, alkaline phosphatase activity is a very useful serum biochemical indicator of liver disease, particularly cholestatic disease. However, increases in the activity of alkaline phosphatase in serum and other body fluids may reflect physiologic or pathologic changes beyond those of hepatic origin (Fernandez, 2007). It is most often seen as part of liver, bone, and intestinal examination. All observed values were within the range of reference values also by Jain (1986) and Radostits et al. (1994). Higher values were found from 1 to 3 in probiotics, prebiotics, probiotics + prebiotics compared to the control group. However, these are not statistically significant differences (Homeopathic p = 0.07; Probiotics p = 0.11; Prebiotics p = 0.57; Probiotics and Prebiotics p = 0.66).

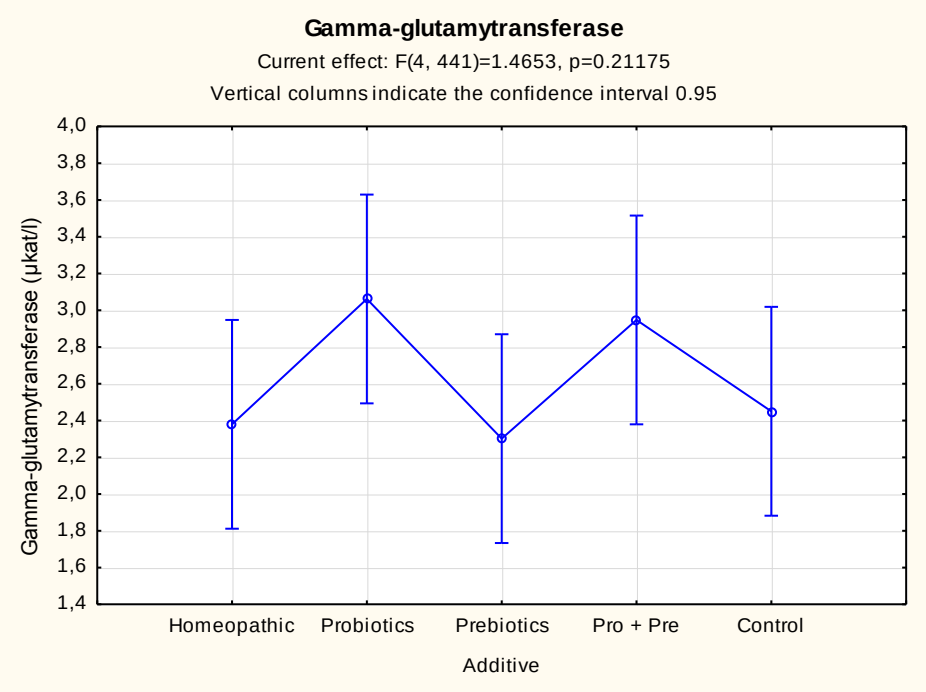
Figure 2. The statistical differences between observed feed supplements and calf alkaline phosphatase values from 1 to 5 weeks after birth.



Gamma-glutamyltransferase

The enzyme GGT is accumulated in increased amounts in the colostrum (Zanker et al., 2001) and is absorbed through the intestinal wall after colostrum intake. So the activity of GGT in the serum of newborn calves is increased in this period and can be used for indirect estimation of colostrum intake (Bostedt, 1983; Schlerka and Bucher, 2003). All results met the reference values also according to Jain, (1986) and Radostits et al. (1994). Almost all groups showed slightly higher values than the control group. (Homeopathic p = 0.85; Probiotics p = 0.15; Prebiotics p = 0.71; Probiotics and Prebiotics p = 0.3).

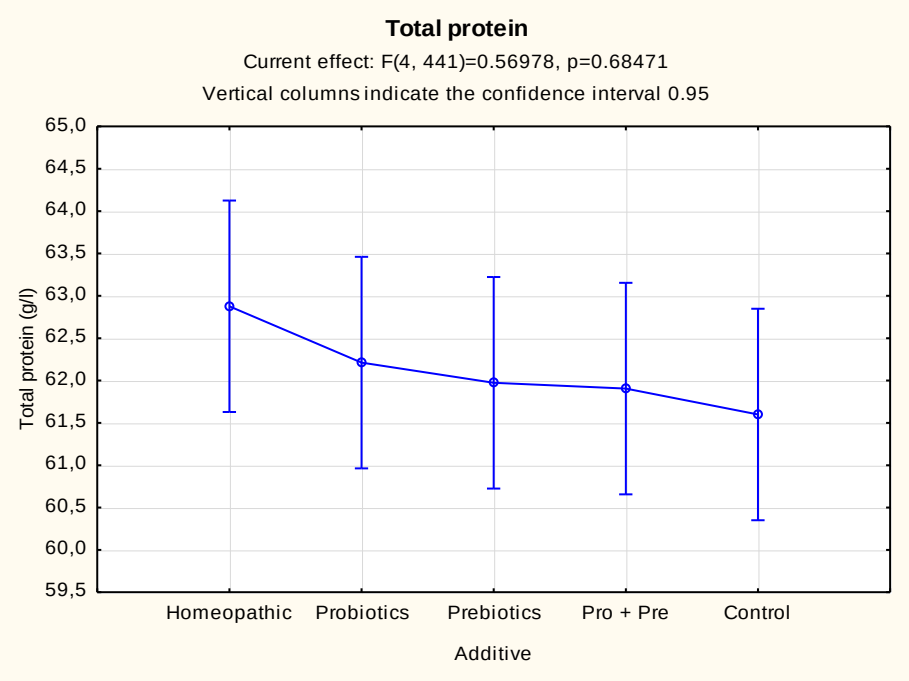
Figure 3. The statistical differences between observed feed supplements and gamma-glutamyltransferase values in calves blood from 1 to 5 weeks after birth.



Total protein

Total blood plasma proteins include albumin, globulin and proteins involved in blood clotting (fibrinogen, prothrombin and other clotting factors). The total serum or plasma protein level depends not only on the amount of protein but also on the water content of the blood (Bod'a et al., 1990). Measuring of total Protein concentration in the 1st week of age can be used as indirect indicator of colostrum supply (Tyler et al., 1998, 1999 According to Jain (1986) and Radostits et al. (1994), some total protein values were slightly lower. Also, total blood protein levels up increased in all groups into the 3rd weeks. There was no statistically significant effect (Homeopathic p = 0.16; Probiotics p = 0.50; Prebiotics p = 0.67; Probiotics and Prebiotics p = 0.74).

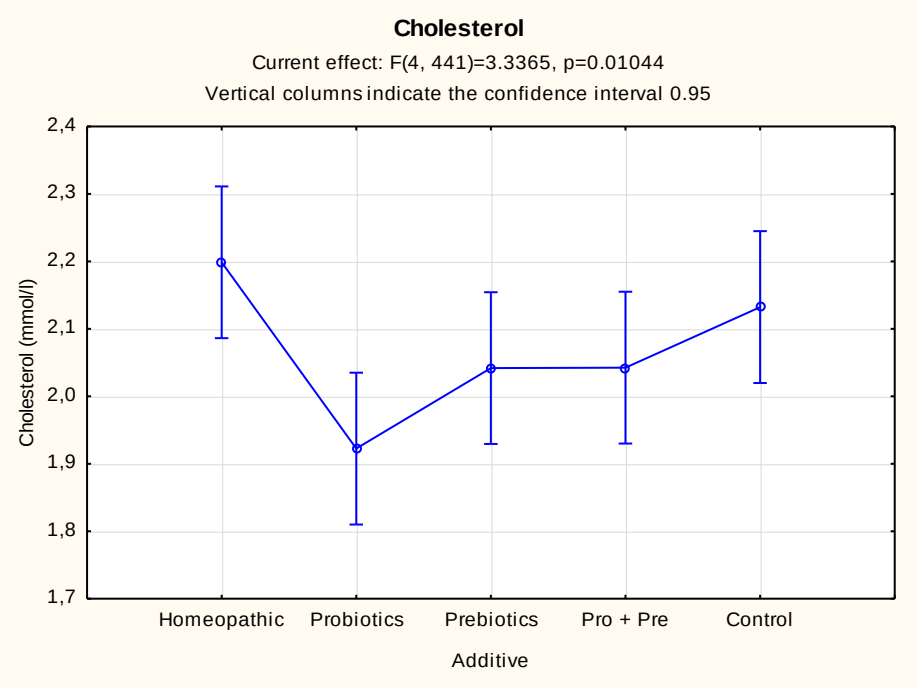
Figure 4. The statistical differences between observed feed supplements and total calf protein values from 1 to 5 weeks after birth



Cholesterol

A very important role of cholesterol is in metabolic processes, the most important of which is that they are involved in vitamin D3 formation, is the basis of some hormones (adrenal cortex, sex hormones), is used to inactivate poisonous substances, participate in fat resorption and stimulate fat storage In the liver. (Reece et al., 1998, Racek et al., 2006). The increase in values occurred only in the third week of the Homeopathic and Probiotics + Prebiotics groups, in the fourth week also in prebiotics compared to the control group. The statistically significant effect on cholesterol levels in the blood had only probiotics compared to the control group ($p = 0.01$). (Homeopathic $p = 0.40$; Prebiotics $p = 0.25$; Probiotics and Prebiotics $p = 0.27$).

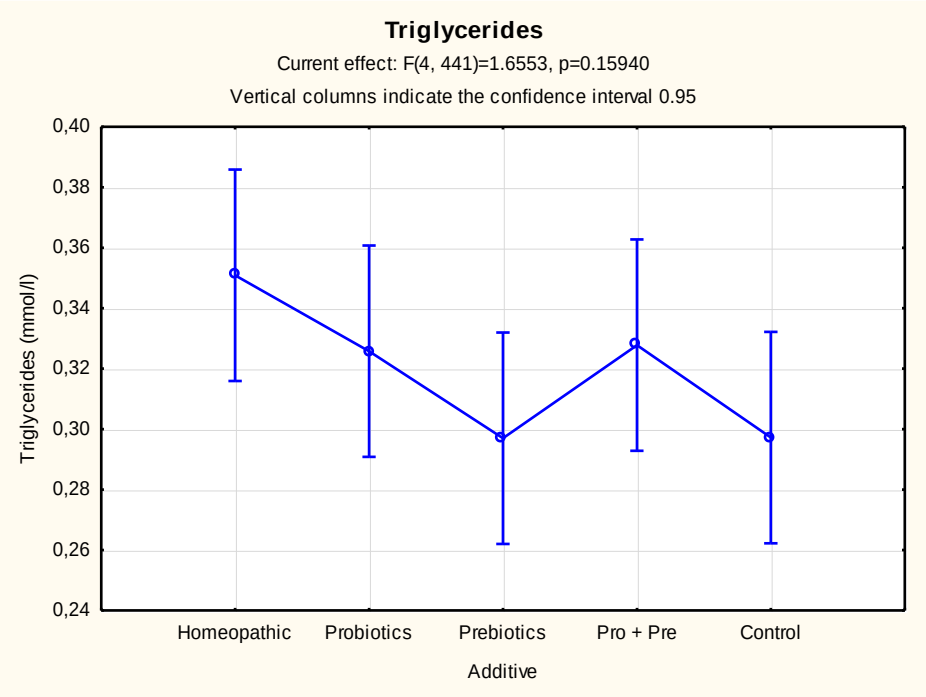
Figure 5. The statistical differences between observed feed supplements and cholesterol values in calves blood from 1 to 5 weeks after birth



Triglycerides

Triglycerides are fats which found in circulating blood. Lipids play a crucial role in mammals' metabolism, in fact these biological molecules function as the storage form of energy (triglycerides) (Arfuso et al., 2017). Someone calves were found triglyceride values to be higher than the reference values. According to Ulrich Von Bock und Polach (1994) this could be due to blood sampling being carried out a few hours after the drinking of milk. In the homeopathic group, a statistically significant effect on the level of triglycerides in the blood of the calves was observed over the control group ($p = 0.04$). (Probiotics $p = 0.27$; Prebiotics $p = 0.99$; Probiotics and Prebiotics $p = 0.18$).

Figure 6. The statistical differences between observed feed supplements and triglyceride values in calves blood from 1 to 5 weeks after birth



CONCLUSION

The investigation of the effects of probiotics (*Bifidobacterium sp.*), prebiotics (Succinic acid) and homeopathic (PVB) on biochemical parameters in the blood showed certain trends of the studied values of blood parameters in the blood of calves compared to the control group. The statistically significant effect were detected of probiotics on cholesterol levels in blood, which is very important for certain metabolic processes ($p = 0.01$) and too were statistically significant effect of homeopathic on triglycerides in blood calves ($p = 0.04$), which are important as an energy source. However, other no statistically significant difference between the control and experimental groups was observed in any of the studied parameters. Therefore it is to conclude that in these experiments these substances did not have a significant important effect on the dynamics of selected biochemical parameters in the blood of calves from 3 to 40 days of age.

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REFERENCES

Alarcon, C., Wicksteed, B. (2002): Succinate is a preferential metabolic stimulus-coupling signal for glucose-induced proinsulin biosynthesis translation, Diabetes 51, 2496.

Arfuso, F., Fazio, F., Panzera, M., Giannetto, C., Di Pietro, S., Giudice, E., & Piccione, G. (2017): Lipid and lipoprotein profile changes in newborn calves in response to the perinatal period. Acta Veterinaria, 67(1), 25-32.

Boďa, K., Bouda, J., Doubek, J., Dvořák, R., Hofírek, B., Konrád, J., Kóňa, E., Kučera, A., Michnová, E., Surynek, J., Tomáš, J. (1990): Patologická fyziológia hospodářských zvierat. Bratislava, Príroda, 386 s.

Bostedt H. (1983): Vergleichende Untersuchung über die Entwicklung des Enzymprofils im Blut von Kälbern und Lämern in der neonatalen Adaptationsperiode, Berlin Münch Tierärztl Wochenschr, 96, 431-8

Boysen, P., Olsen, I., Berg, I., Kulberg, S.,Johansen, G.M., Storset, A.K. (2006): Bovine CD2-/NKp46+ cells are fully functional natural killer cells with a high activation status. BMC Immunology 27, pp. 7–10.

Carroll, J. A., and N. E. Forsberg. (2007): Influence of stress and nutrition on cattle immunity. Veterinary Clinics of North America: Food Animal Practice. 23:105–149.

Cervenak, J., Kacskovics, I. (2009): The neonatal Fc receptor plays a crucial role in the metabolism of IgG in livestock animals. Veterinary Immunology and Immunopathology, 128, pp 171–177.

Cortese, V.S. (2008): Neonatal immunology. The Veterinary Clinics of North America. Food Animal Practice 25, pp. 221–227.

Fernandez, N. J., Kidney, B. A. (2007): Alkaline phosphatase: beyond the liver. Veterinary Clinical Pathology, 36.3: 223-233.

Fuller, R. (1989): Probiotics in man and animals. Journal of Applied Bacteriology, 66, s. 365 – 378.

Guilloteau, P., Zabielski, R., Hammon, H. M., Metges, C. C. (2009): Adverse effects of nutritional programming during prenatal and early postnatal life, some aspects of regulation and potential prevention and treatments. Journal of Physiology and Pharmacology 60 (Suppl. 3), 17–35.

Heinrichs, A.J. and K.E. Lesmeister (2005): Rumen development in the dairy calf, p.53-65. In PC Garnsworthy (ed.), Calf and Heifer Rearing. Nottingham University Press

Jain, N. C. (1986): Schalm's Veterinary Hematology. 4th edn. Philadelphia, Lea & Febiger

Jelínek, P., Koudela, K., Doskočil, J., Illek, J., Kotrbáček, V., Koudela, K., Kovářů, F., Kroupová, V., Kučera, M., Kudláč, E., Trávníček, J., Valent, M., Boďa, K., Konrád, J. (2003): Fyziologie hospodářských zvířat. Brno, MZLU Brno, 409 s.

Jiang, M., Ma, J., Wu, M., Liu, R., Liang, L., Xin, F., Zahang, W., Jia, H., Dong, W. (2017): Progress of succinic acid production from renewable resources: Metabolic and fermentative strategies. Bioresource Technology. Https://doi.org/10.1016/j.biortech.2017.05.209

Juan, A., Martinez, G., Maria, L., Penacarrillo, V., Valverd,e I., Bjorkling, F., Malaisse, W. J. (1998) Potentiation of the insulinotropic and hypoglycemic action of gliquidone by succinic acid esters, Eur. J. Pharmacol. 325, 65–68.

Kaske, M., Wiedemann, S., Kunz, H. J. (2010): Metabolic programming: background and potential impact for dairy cattle. Flemish Veterinary Journal 79, 445–451.

Mee, F. John (2013): Why Do So Many Calves Die on Modern Dairy Farms and What Can We Do about Calf Welfare in the Future? Animals, 3(4), 1036-1057

Mohd Nor, N., Steeneveld, W., Mourits, M.C.M., and Hogeveen, H. (2012): Estimating the costs of rearing young dairy cattle in the Netherlands using a simulation model that accounts for uncertainty related to diseases. Preventive Veterinary Medicine. 2012; 106: 214–224

Patel, S., Shukla, R., Goyal, A. (2015): Probiotics in valorization of innate immunity across various animal models. Journal of functional foods 14, pp.549-561

Racek, J., Eiselt, J., Fridecký, B., Holeček, V., Nekulová, M., Pittrová, H. and Verner, M. (2006): Klinická biochemie, druhé, přepracované vydání. Galén, Praha, 107, 142-143.

Radostits, 0. M., Blood, D. C. and Gay, C. C. (1994): Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. London, Bailliere Tindall

Reece, W. O. (1998): Fyziologie domácích zvířat. Praha, Grada Publishing, spol. s r.o., 456 s

Schlerka, G., Bucher, A., (2003): Über den Verlauf der -Glutamyltransferase-Aktivität und des Gesamteiweißgehaltes im Blutserum von neugeborenen Kälbern, Tierärztl Umsch, 58, 146-52

Steinhardt, M. and H. H. Thielscher (2000): Tiergerechte Haltung und physiologische Funktionen von Tieren. Tierkrztliche Umschau .55: 189- 198.

Stewart, C.S., G. Fonty and P. Gouet (1988): The establishment of rumen microbial communities. Animal Feed Science and Technology 21:69-97.

Taylor, P. D. and Poston, L. (2007): Developmental programming of obesity in mammals. Experimental Physiology 92, 287–298

Tyler, J. W., S. M. Parish., T.E.Besser., D.C .Van Metre., G.M .Barrington and J.R. Middleton (1999): Detection of low serum mmunoglobulin concentrations in clinicaly ill calves. Journal of Veterinary Internal Medicine. 13: 40-43.

Ulrich Von Bock und Polach (1994): Směrné hodnoty důležitých laboratorních vyšetření pro domácí zvířata. Vetpres – vydavatelství a. s. Biopharm – VÚBVL, Jílové u Prahy, 127 s.

Uyeno, Y., Shigemori, S., Shimosato, T. (2015): Effect of probiotics/prebiotics on cattle health and productivity. Microbes and environments, 30.2: 126-132.

Zanker, I. A., Hammon, H. M., Blum, J. W. (2001): Activities of γ-Glutamyltransferase, Alkaline Phosphatase and Aspartate-Aminotransferase in Colostrum, Milk and Blood Plasma of Calves Fed First Colostrum at 0–2, 6–7, 12–13 and 24–25 h after Birth. Transboundary and Emerging Diseases, 48.3: 179-185.

Zawalich, W.S., Zawalich, K.C. (1992): Biochemical mechanisms involved in monomethyl succinate-induced insulin secretion, Endocrinology 131, 649–654.

EFFECTS OF HUMIC ACIDS ON PRODUCTION PARAMETERS OF PHEASANTS

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ABSTRACT

The effects of the addition of humic substances on the production parameters and the eggs quality were observed in the experiment with pheasant hens, during 61 days of laying period. The aditivum was applied into feed at the level of 0.5 %. There were not observed any significant differences between the control and experimental groups as far as mortality, feed conversion, laying intensity, egg weight, egg size, proportion of shells, egg yolks and egg whites, content of cholesterol and colour of yolk are

concerned. The significant difference was ascertained in the case of percentage of hatchability. 1759 chickens were hatched from 2412 hatching eggs of the control group which represented 72.9 % hatchability. From 2261 eggs incubated in the hatchery, 1886 pheasant chickens were hatched in the experimental group with the hatching percentage 83.4 %.

Keywords: humic acids; pheasant; production; eggs

INTRODUCTION

Humic substances are very suitable for the use in various areas of economic activity because of their properties. They are utilized for the detoxication of substances contaminating the environment (Skybová, 2006) as well as in industry, pharmacology, agriculture, veterinary and human medicine. They are used as supporting substances for the treatment of diarreha, malnutrition, dyspepsia and acute intoxication. There were observed the effects of humic substances on mycotoxins (Arafat et al., 2017) and the modification of rumen fermentation (Galip et al., 2010; McMurphy et al., 2011) were observed. There were observed in various experiments the effects of humic acids on production parameters of livestock, on feed conversion of poultry and the quality of products (Herzig et al., 2009; Gomes-Rosales et al., 2015; Lala et al., 2016; Arpášová et al., 2016), on broiler chickens when the mentioned substances were combined with probiotics or plant extracts (Demeterová et al., 2009; Pistová et al., 2016), on pigs (Wang et al., 2008; Chang et al., 2014), on rabbits (Ondruška et al., 2012) and on the egg-laying hens (Yörük et al., 2004; Kucukersan et al., 2005).

The aim of our work was the observation of effects of humic substances on the production parameters and the egg quality of pheasant hens.

MATERIAL AND METHODS

One hundred and forty pheasant hens were used in the experiment. The production parameters were observed during period of 79 days. The laying period took time for 61 days. Ten laying flocks (1 cock and 7 hens in 1 flock) were kept under standard rearing conditions throughout the whole laying cycle in the control group. Pheasants were fed ad libitum with a complete feed mixture for laying hens ad libitum with the free access

to drinking water. The same number of laying flocks of pheasants with the same sex ratio was in the experimental group. The experimental group was fed with the complete feed mixture for laying hens with the addition of 0.5 % natural humic substances. The humic substances was added in the form of oxihumolite with the content 68% total humic acids (free humic acids 48%) and minerals 18% (locality Dudar, Hungary).

The analyses of feed mixtures were performed for the quantification of nutrients at the beginning and in the course of experiment (Table 1).

Table 1. Content of nutrients in feed mixture (in 1 kg of dry mater)

Dry mater	$g.kg^{-1}$	896.8	Ca	$g.kg^{-1}$	19.68
CP	$g.kg^{-1}$	196.1	Mg	$g.kg^{-1}$	2.64
Fat	$g.kg^{-1}$	51.9	Na	$g.kg^{-1}$	1.0
Ash	$g.kg^{-1}$	96.3	K	$g.kg^{-1}$	7.97
NFE	$g.kg^{-1}$	601.4	P	$g.kg^{-1}$	8.25
ME	MJ	12.29	Cu	$mg.kg^{-1}$	18.29
CF	$g.kg^{-1}$	54.3	Zn	$mg.kg^{-1}$	174.73

CP – crude protein, NFE – nitrogen free extract, ME metabolizable energy, CF - crude fibre

As for the production parameters, the consumption of compound feed, the feed conversion, the weight of pheasant hens at the start and at the end of laying period, daily produced eggs and the weight and size of eggs. The egg size, the shape index, the percentage of shell weight, egg white and egg yolk, the content of cholesterol in yolk as well as the shell strength were monitored in a representative samples of eggs from the experimental and control groups (n = 40). The shape index was calculated according to equation SI = w/l x 100 (w – width; l – lenght). The concentration of cholesterol was determined spectrophotometri-cally by the Bio-La tests (Pliva-LaChema Brno Ltd., Czech Republic).

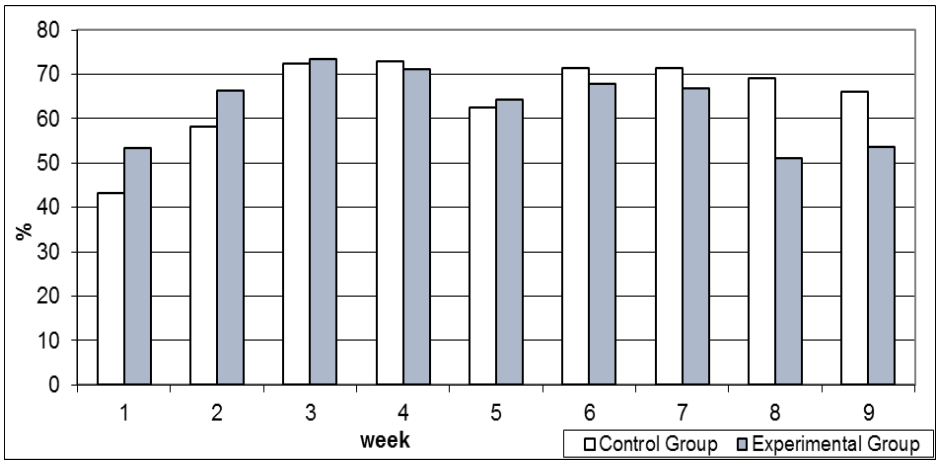
There were used for the test of hatchability 2412 eggs from the control group and 2261 eggs from the experimental group. The statistical evaluation of the monitored parameters was performed by the unpaired t-test.

RESULTS AND DISCUSSION

The average weight of the pheasant hens in the control group was 1.073 kg at the beginning of the laying period. This parameter decreased by 9.5% to 0.971 kg at the end of the laying period. The average weight of hens from the experimental group was 1.041 kg and the decrease by 1.6 % to 1.024 kg was observed at the end of the laying period. Two pheasant hens died in the control group during 61 days long laying period, which was 2.8 % mortality. In the experimental group, four hens died (5.6% mortality) during the same period. The average feed consumption per one hen a day was 63.57 g in the control group. The feed intake of pheasant hens of experimental group was higher by 5.0 g per one hen a day. The egg production was at the level 2730 in the control group and 2381 in the experimental group. The average value of egg-laying of one hen was 39.8 in the control group and 38.9 eggs in the experimental group.

The egg production was increased in the experimental group for the first 3 weeks of laying period. The values of this parameter were balanced in both groups within 4 to 6 weeks. Later in the last 3 weeks, it was kept at the same level in the control group whereas the decrease was observed in the experimental group, which negatively influenced total egg production in comparison to control group (Graph 1).

Graph 1. Percentage of egg-laying in the particular weeks



The feed consumption per one egg produced in the control group was 125.1 g but it was by 7.2 g higher in the experimental group. The feed consumption per 1 kg of eggs produced in the control group was 3.47 kg and this parameter was at the level 3.74 kg in the group of pheasant hens where humic acids are added into feed. The differences were

statistically no significantly. The content of cholesterol in the egg yolk was 3.127 $mg.g^{-1}$ in the control group whereas the values were no significantly lower by 0.264 $mg.g^{-1}$ (2.863 $mg.g^{-1}$) in the group when the humic substances were added into feed. In several studies dealing with the observation of the influence of humic substances on laying hens at laying period and on the production parameters and the egg quality, humic substances were applied at concentrations of 30 - 90 mg per kg of feed or in grams per ton of feed (Kucukersan et al., 2005). The unambiguously favourable results after the application of 10 and 30 ml per kg of feed are demonstrated by Ozturk et al. (2009) too. Yörük et al. (2004) observed the administration of humates and probiotics at a later stage of laying of hens. They found that administration of humates at a concentration of 0.1 or 0.2 % into feed mixture had not any effect on the mortality of hens. They recorded the improvement of feed conversion and the increase of production compared to control group with not any differences of the egg quality. Hayirly et al. (2005) observed the beneficial effect of humic substances added into feed mixture at concentrations 0.3 % in the case of hens kept in cages with higher stocking density. There was registered lower feed consumption while maintaining production, egg quality and some metabolic parameters.Eggs from the experimental group were by 1.11 g lighter in our experiment in comparison to control group. At the same width of eggs in both groups (control 34.5 mm, experimental 34.8 mm), the eggs from the control group were by 1.4 mm longer. The mean egg-shape index was 76.54 % in the control group and in the experimental group 79.56 %. The average weight and percentage weight ratios of shell, white and yolk in eggs are demonstrated in Table 2. The weight of the shell in the control group was 3.01 g and after addition of humic substances 3.05 g. The control group had by 0.017 mm thinner eggshell (0.331 versus 0.348 mm). The strength of shell was almost identical in the both groups (3.90 versus 3.88).

Table 2. Weight of eggs and portion of weight of shell, yolk and white

Weight	Control group	Experimental group	
eggs g	30,75 ± 0,70	29,86 ± 0,66	NS
shell (%)	9,29 ± 0,14	9,65 ± 0,22	NS
yolk (%)	29,99 ± 0,55	30,04 ± 0,66	NS
white (%)	60,72 ± 0,51	60,31 ± 0,62	NS

± - SD; NS – no significant difference

The percentage weight ratio of shell, yolk and white corresponds to the average ratio for chicken eggs. The shell is on average 10%, yolks and whites make up 30% and 60% of the egg weight, respectively (Nagy et al., 2009a; 2009b). One thousand seven hundred and fifty-nine chickens were hatched in the control group, which represented 72.9% of hatchability. On the other hand, 1886 pheasants were hatched in the experimental group with the hatching rate of 83.4%.

CONCLUSION

The effects of humic substances on the production and quality of eggs of laying hens are positively appreciated by many authors. We did not confirm the beneficial effect on feed consumption, feed conversion per one kg of produced eggs and on the amount of produced eggs in our experiment with pheasant chickens after the addition of 0.5% humic substances into feed mixture. The significant improvement of hatchability was observed after intake of humic substances.

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REFERENCES

Arafat R.Y., Khan S.H., Saima I. (2017): Evaluation of humic acid as an aflatoxin binder in brojler chickens, Annual Animal Science, 17, (1), 241-255.

Arpášová H., Pospišilová D., Pistová V. (2016): Využitie huminových kyselín vo výkrme brojlerových kurčiat (Use of humic acids in broiler chickens), Slovenský Chov, (8), 32-33.

Demeterová M., Mariščaková R., Pistl J., Naď P., Šamudovská A. (2009): The effect of the probiotic strain Enterococcus faecium DSM 7134 in combination with natural humic substances on performance and health of broiler chickens, Berliner und Munchener Tierarztliche Wochenschrift 122, (9-10), 364-369.

Galip N., Polat U., Biricik H. (2010): Effects of supplemental humic acid on ruminal fermentation and blood variables in rams, Italian Journal of Animal Science, 9, 390 - 393.

Gomes-Rosales S., Angels M.L. (2015): Addition of a worm leachate as source of humic substances in the drinking water of broiler chickens, Asian-Australas Journal of Animal Science, 28, (2), 215-222.

Hayirli A., Esenbuga N., Macit M., Lacin E., Karaoglu M., Karaca H., Yildiz L. (2005): Nutrition practice to alleviate the adverse effects of stress on laying performance, metabolic profile, and egg quality in peak producing hens I. The Humate Supplementation, Asian-Australian Journal of Animal Science 18, (9), 1310-1319.

Herzig I., Navrátilová M., Totušek J., Suchý P., Večerek V., Bláhová J., Zralý Z. (2009): The effect of humic acid on zinc accumulation in chicken broiler tissues, Czech J. of Animal Science, 54, (3), 121-127.

Chang Q., Lu Z., He M., Gao R., Bai H., Shi B., Shan A. (2014): Effects of dietary supplementation of fulvic acid on lipid metabolism of finishing pigs, American Society of Animal Science, 20, 4921 – 4926.

Kucukersan S., Kucukersan K., Colpan I., Goncuoglu E., Reisli Z., Yesilbag D. (2005): The effects of humic acid on egg production and egg traits of laying hen. Veterinary Medicine, 50, (9), 406-410.

Nagy J., Danko J., Jevinová P., Kožárová I., Marcinčák S., Nagyová A., Pipová M., Popelka P., Turek P. (2009a): Hygiena a technológia hydiny a vajec. 1. vyd. Košice: Edičné stredisko Univerzity veterinárskeho lekárstva, s. 268 – 333. ISBN 978-80-8077-132-4

Nagy J. et al. (2009b): Hygiena mäsa hydiny, vajec a zveriny – I. diel. 1. vyd. Košice: Edičné stredisko Univerzity veterinárskeho lekárstva, 291–338. ISBN 978-80-8077-179-9

McMurphy C.P., Duff G.C., Sanders S.R., Cuneo S.P., Chirase N.K. (2011): Effects of supplementing humates on rumen fermentation in Holstein steers, South African J of Animal Science, 41, (2), 134 – 140.

Ondruška Ľ., Chrastinová Ľ., Rafay J., Pospišilová D., Parkanyi V. (2012): Effect of humic substances and probiotics on growth performance and meat quality of rabbits, Potravinárstvo, 6, (2), 39 – Ozturk E., Coskun I., Ocak N., Erener G. (2009): Effects of dietery humic substances on egg production and egg shell quality of hens after peak laying period, African J of Biotechnology 8, (6), 1155-1159.

Pistová V., Arpášová H., Hrnčar C., Kačaniová M., Haščík P. (2016): The effect of the humic acid and herbal additive supplement on production parameters of broiler chicken, Scientific Papers: Animal Science and Biotechnologies, 49, (2), 166-169.

Skybová M. (2006): Huminové kyseliny – prínos pre environmentálny výskum (Humic acids – a benefits for environmental research), Acta Montanistica Slovaka, 11, (2), 362 – 366.

Yörük, M.A., Gül, M., Hayirli, A., (2004): The effects of supplementa-tion of humate and probiotic on egg production and quality parameters during the late laying period in hens. Poultry Science, 83, (1), 84-88.

Wang Q., Chen Y.J., Yoo J.S., Kim H.J., Cho J.H., Kim I.H. (2008): Effects of supplementation humic substances on growth performance, blood characteristics and meat quality in finishing pigs, Livestock Science, 117, 270 – 274.

EFFECTS OF HUMIC SUBSTANCES ON THE CONTENT OF SELECTED MINERALS IN BLOOD AND FAECES IN FATTENING PIGS

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ABSTRACT

The aim in this work was to investigate the effect of the addition of humic substances on the level of mineral elements (calcium, copper, zinc) in the blood serum and in faeces of fattening pigs. The addition of humic substances was realized in test group in dose 5g per 100g of the prepared complete feed mixture during 35 days. Blood collection was performed in day 7, 14, 21, 35 of experiment in both groups. Samples of faeces were collected in day 14 and 35. The levels of selected mineral elements detected by atomic absorption spectrometry pointed to small changes in the mineral concentration in blood and excrements. The level of copper in the serum of test group increased ($P<0.01$) in comparison with serum of pigs from control group. The serum zinc levels were significantly lower ($P<0.01$) in day 7 and 14 in the test group. Excrements from pigs in test group contained significantly lower copper and zinc concentration ($P<0.01$) compared to control group. The results of the present study indicated that preparation of humic acids caused a decrease in concentration of metals (copper and zinc) in the faeces and slightly increased the copper serum level in fattening pigs without increasing the values above the physiological range.

Keywords: pig; humic acid; blood serum; excrement; calcium; copper; zinc

INTRODUCTION

Humic substances (HS) are a class of compounds that are generated from the decomposition of organic matter in the soil. Their oral use is permitted in horses, ruminants, swine and poultry for the treatment of diarrhoea, dyspepsia and acute intoxications (EMEA, 1999). HS in pig diet was also shown to improve growth performance, meat quality, increase the nutrient digestibility and reduce ammonia emission from manure (Ji et al., 2006; Wang et al., 2008; Pisarikova et al., 2010). Scientific articles about the effect of HS on intestinal health and composition of intestinal microflora are rather scarce (Shermer et al. 1998; Aksu and Bozkurt, 2009).

In pig species, most dietary Zn and Cu is excreted in manure and eventually spread on soils with growing risks for heavy metals "phytotoxicity", especially in areas of intensive pig production (Heo et al., 2013; Liu et al., 2014). The formation of chelate complexes is an important aspect of the biological role of humic acids in regulating bioavailability of metal ions. Copper is essential and potentially toxic element. Zinc is an essential element for animals. The role of zinc on the metabolism of the body resulting from its participation in the structure of the different enzymes. The other enzymes acts as co-factor (Matte et al., 2017). Antagonistic relationship was observed between Cu and Zn. High doses of zinc in a ration causes decrease of copper in the liver and the decrease in calcium and sodium in the body.

We investigated the effect of preparation of humic acid to changes of minerals level in serum and faeces of fattening pigs.

MATERIAL AND METHODS

Twelve fattening pigs (meat hybrid) were divided in two groups (six in control group and six animals in test group). The experiment was conducted during 35 days, with initial mean body weight (BW) 17.90 ± 2.25 kg in control group (CG) and 18.15 ± 2.26 kg in test group (TG). The commercial preparation of humic substances (Humac Natur AFM, Humac ltd., Slovak Republic) was mix into prepared complete feed mixture in

TG during experimental period in dose 0.5g HS / 100g feed. The characteristics of the applied HS were the following: the size of particles up to 100 μm, max. moisture 15%, the content of humic acids in DM min. 65%, fulvic acid min. 5%, minerals Ca 42.28 g/kg, Mg 5.10 g/kg DM and microelements Cu 15, Zn 37, Mn 142, Co 1.24, Se 1.67 as well as Mo 2.7 mg/kg DM. Composition of diet in test and control group and analyzed nutrients from the diets are shown in Table 1. The pigs were fed twice a day. The chemical analyses of diets were performed according to Commission Regulation (EC) No. 152/2009. Blood collection for determination of selected minerals was performed 4 times at weekly intervals (in day 7, 14, 21, 35 of experiment) in the CG and TG, 4–5 hours after morning feeding from sinus ophthalmic. The concentrations of calcium, zinc and copper in serum were determined by means of flame atomic absorption spectrometry using a Unicam Solar 939 (Great Britain). Before measuring, serum samples were deproteinized by supplementing trichloroacetic acid at a 1:1 ratio. After centrifugation, were analysed in the supernatant. The content of Ca, Cu and Zn in blood were determined according to the methodology used by the Official lists methods and laboratory diagnosis of food and feed Bulletin of the Ministry of Agriculture SR (2004) Listing the Official Methods of Laboratory Diagnosis of Food and Feed.

Samples of faeces (excrements) were collected individually in CG and TG in day 14 and 35. Minerals were assayed with atomic absorption spectrophotometry. The differences between means were determined, according to the unpaired t-test using GraphPad Prism 6 software.

Table 1. Composition and contribution of the diets

Feeds	**control (-) and test (+ 0.5g HS/100g feed) diet in %**	
corn	35	
wheat	20	
barley	18.2	
soybean meal	23	
premix VM	3	
synthetic AA	0.8	
	Analysis calculated in % DM	
Dry mater %	88.38	88.20
CP	19.88	19.81
Fat	2.66	2.70

Ash	6.03	6.10
Starch	47.5	47.9
Ca	0.59	0.68

VM – vitamins and minerals, AA – amino acids, CP – crude protein

RESULTS AND DISCUSSION

One aspect to consider is the fact that, once taken up, humic substances (HS) are able to migrate to organs (Steinberg et al., 2003). They have both non-specific and specific effects. The non-specific effects are physical and chemical. All organisms have the means to rid themselves of chemical burdens. The second aspect to be considered is the presence of other HS features, such as chelation. Sanmanee and Areekijseree (2010) have shown that fulvic acid treatment reduces the toxicity of Cu in the mammalian cells. However, Fe, Zn, Mn and Cu are included in the group of essential trace elements required for maintaining cellular function and are integral components of numerous metal-containing enzymes (Rajkowska and Protasowicki, 2013).

The levels of selected mineral elements detected by atomic absorption spectrometry pointed to small changes in the amounts of mineral present in serum (Table 2) and excrements (Table 3).

After humic acid addition in TG was find decreasing the zinc levels in the serum of pigs in comparison with pigs that were not fed with humic acid in day 7 and 14. The serum zinc levels were significantly lower ($P<0.01$) in day 7 and 14 in pigs fed with HS. Contrast, the concentration of zinc in blood serum in day 21 and 35 was slightly higher in TG. On the other hand, the level of copper in the serum increased in comparison with serum that not fed with HS in all collect intervals. Levels of copper in serum were significantly higher in day 7, 14 ($P<0.01$) and day 21 ($P<0.05$) after application HS in feed. The application of HS in fed for fattening pigs had no effect on changes in calcium in the serum levels in pigs. The detected amounts of calcium and copper serum in pigs in both groups were within the physiological range (Ca 1.80 – 3 mmol/l; Cu 18 – 34 μmol/l, respectively). The levels of zinc serum were slightly below the physiological range or on the bottom level of physiological range (Zn 17 – 36 μmol/l). It was found out that the humic acid additive caused Ca level increase in rabbit's meat (Miśta et al., 2012). On the other hand, Abdel-Mageed (2012) recorded that birds fed diets

supplemented with HS at the low levels had significant decrease in serum Ca and P concentrations. The results obtained by Zralý and Písaříková (2010) confirmed that feeding sodium humate to animals had no significant adverse effect on the Cu or Zn content in the investigated organs and tissues and cited many other authors with the same findings.

Table 2. Levels of selected mineral elements in blood serum (Mean ± SD)

element	7 day	14 day	21 day	35 day
	Control group			
Ca mmol/l	2.48 ± 0.05	3.03 ± 0.13	2.50 ± 0.29	2.48 ± 0.45
Cu µmol/l	25.09 ± 3.89	24.95 ± 3.38	20.60 ± 1.25	20.99 ± 3.12
Zn µmol/l	17.50 ± 2.85 **	17.23 ± 3.21 **	15.77 ± 3.55	16.08 ± 4.09
	Test group			
Ca mmol/l	2.60 ± 0.07	2.69 ± 0.12	2.54 ± 0.08	2.67 ± 0.09
Cu µmol/l	29.38 ± 3.11 **	29.02 ± 2.29 **	23.74 ± 3.38 *	21.22 ± 2.43
Zn µmol/l	15.28 ± 1.63	14.56 ± 1.81	16.40 ± 4.08	17.76 ± 1.24

Mean – the mean levels of serum; SD – standard deviation; significant differences ** ($P \leq 0.01$); * ($P \leq 0.05$)

Faeces of pigs fed diet with HS contained significantly less copper ($P<0.01$) in both collect interval compared to pigs in the control group. Significantly increased zinc ($P<0.01$) was found in faeces from pigs that fed without HS in day 35. Similarly, levels of minerals in excrements for pigs reported Heugten et al. (2004).

The genetic potential of animals must be considered in order to explain the differences in mineral concentration in pig excrements and the composition of the diets and the level of trace mineral inclusion influences the mineral composition of faeces too, as showed by Shaw et al. (2002). The positive effect of humic substances can be explained by an enhancement of the metabolic activity of cell membranes by acceleration of oxidative processes due to increased nutrient uptake, which stimulates vital functions (Islam et al. 2005).

Table 3. Levels of selected mineral elements in faeces (Mean ± SD)

Element	Control group		Test group	
	14 day	35 day	14 day	35 day
Ca g/kg DM	9.48 ± 3.21	10.46 ± 2.99	10.54 ± 1.89	12.54 ± 2.35
Cu mg/kg DM	291.92 ± 23.78	265.78 ± 15.48	238.08 ± 36.54 **	223.28 ± 15.03 **
Zn mg/kg DM	304.83 ± 39.68	401.93 ± 33.45 **	297.75 ± 62.45	353.90 ± 42.73

significant differences ** ($P \leq 0.01$).

CONCLUSION

In conclusion, the preparation of humic acids caused a decrease in concentration of metals (copper and zinc) in the faeces and slightly increased the level of copper in blood serum level of fattening pigs.

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REFERENCES

Abdel-Mageed M. A. A. (2012): Effect of dietary humic substances supplementation on performance and immunity of Japanese quail. Egyptian Poultry Science, 32(3), 645-660.

Aksu, T. Bozkurt A. S. (2009): Effect of dietary essential oils and/or humic acids on broiler performance, microbial population of intestinal content and antibody titres in the summer season. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 15(2), 185-190.

Bulletin of the Ministry of Agriculture SR (2004) Listing the Official Methods of Laboratory Diagnosis of Food and Feed, XXXVI, p. 339.

COMMISSION REGULATION (EC) No 152/2009 of January 17, 2009 laying down the methods of sampling and analysis for the official control of feed. Official J of the European Union. February 26, 2009.

EMEA (1999): Committee for veterinary medical products. Humic acids and their sodium salts. Http:/www.emea.europa.eu/pdfs/vet/ mrls/055499en.pdf (accessed February 1999).

Heo J.M., Opapeju F.O., Pluske J.R., Kim J.C., Hampson D.J., Nyachoti C.M. (2013): Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. Journal of animal physiology and animal nutrition, 97, (2), 207-237.

Heugten, E., O´Quinn P.R., Funderburke D.W., Flowers W.L., Spears J.W. (2004): Growth performance, carcass characteristics, plasma minerals, and fecal mineral excretion in grower-finisher swine fed diets with levels of trace minerals lower than common industry levels. Journal of Swine Health and Production, 12, (5), 237-241.

Islam K.M.S., Schuhmacher A., Gropp J.M. (2005): Humic acid substances in animal agriculture. Pakistan Journal of Nutrition, 4, (3), 126-134.

Ji F., McGlone J.J., Kim S.W. (2006): Effects of dietary humic substances on pig growth performance, carcass characteristics, and ammonia emission. Journal of animal science, 84, (9), 2482-2490.

Liu, Y., Ma Y.L., Zhao J.M., Vazquez-Añón M., Stein H.H. (2014): Digestibility and retention of zinc, copper, manganese, iron, calcium, and phosphorus in pigs fed diets containing inorganic or organic minerals. Journal of animal science, 92, (8), 3407-3415.

Matte J. J., Girard C. L., Guay F. (2017): Intestinal fate of dietary zinc and copper: Postprandial net fluxes of these trace elements in portal vein of pigs. Journal of Trace Elements in Medicine and Biology, 44, 65-70.

Miśta D., Rząsa A, Wincewicz E., Zawadzki W., Dobrzański Z., Szmańko T., Gelles A. (2012): The effect of humic-fatty acid preparation on selected haematological and biochemical serum parameters of growing rabbits. Polish journal of veterinary sciences, 15, (2), 395-397.

Písaříková B., Zralý Z., Herzig I. (2010): The effect of dietary sodium humate supplementation on nutrient digestibility in growing pigs. Acta Veterinaria Brno, 79, (3), 349-353.

Rajkowska M., Protasowicki M. (2013): Distribution of metals (Fe, Mn, Zn, Cu) in fish tissues in two lakes of different trophy in Northwestern Poland. Environmental monitoring and assessment, 185, (4), 3493-3502.

Sanmanee N., Areekijseree M. (2010): The effects of fulvic acid on copper bioavailability to porcine oviductal epithelial cells. Biological trace element research, 135, (1-3), 162-173.

Shermer C. L., Maciorowski K.G., Bailey C.A., Byers F.M., Ricke S.C. (1998): Caecal metabolites and microbial populations in chickens consuming diets containing a mined humate compound. Journal of the Science of Food and Agriculture, 77, (4), 479-486.

Steinberg, C. E., Paul, A., Pflugmacher, S., Meinelt, T., Klocking, R., Wiegand, C. (2003): Pure humic substances have the potential to act as xenobiotic chemicals-A review. Fresenius Environmental Bulletin, 12, (5), 391-401.

Shaw D.T., Rozeboom D.W., Hill G.M, Booren A.L., Link J.E. (2002): Impact of vitamin and mineral supplement withdrawal and wheat middling inclusion on finishing pig growth performance, fecal mineral concentration, carcass characteristics, and the nutrient content and oxidative stability of pork. Journal of Animal Science, 80, (11), 2920-2930.

Zralý, Z., Písaříková, B. (2010): Effect of sodium humate on the content of trace elements in organs of weaned piglets. Acta Veterinaria Brno, 79, (1), 73-79.

Wang Q., Chen Y.J., Yoo J.S., Kim H.J., Cho J.H., Kim I.H. (2008): Effects of supplemental humic substances on growth performance, blood characteristics and meat quality in finishing pigs. Livestock Science, 117, (2), 270-274.

NUTRITIONAL VALUE AND PRODUCTION OF GREEN MASS BY THREE VARIETIES OF WHITE LUPINE

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ABSTRACT

The objective of the study was to compare the production ability and nutritional value of three varieties of white lupin (Amiga, Dieta and Zulika), grown at Nový Jičín university farm of the Veterinary and Pharmaceutical University Brno. Each variety was grown on an area about 10 ha, under identical soil and climate conditions. Sampling of green mass was carried out random selection of 10 samples from the area of 1 m^2 of each variety. Sampling, were made at the age of 15 weeks of crops when the crops were at the stage of fully developed green pods. Compared production per hectare, of

individual varieties tested lupine white grown in the identical soil and climatic conditions, we concluded that the highest production potential in the age of 15 weeks showed a variety Zulika, in comparison with the variety Dieta and Amiga. From a nutritional point of view, the variety Zulika can be assess very positive, because it contained the green mass most of crude protein.

Keywords: white lupin; Amiga; Dieta; Zulika; production of green mass; chemical analysis

INTRODUCTION

The Lupinus genus includes approximately 300 species of annual and perennial herbs. At the beginning of the last century, new, so-called "sweet" lupin varieties low in alkaloids (bitter substances) and high in proteins were developed. That was an impulse for renewed interest in its utilization and lupin became the source of proteins in the nutrition of both humans and animals (Dijkstra et al., 2003). So in the 20th century, lupin became part of modern agriculture and food systems. In Europe, mainly two varieties are grown: *Lupinus luteus* and *L. albus*, while in Australia it is *L. angustifolius* (Cowling et al., 1998). The development of varieties with solid pods and varieties low in alkaloids allowed that these varieties stopped being used as green manure and soiling crops and became legumes grown for seeds. The nutrient composition of lupin is exceptional, it has a high content of proteins and soluble fibre and, unlike cereals, a low content of starch (Petterson et al., 1997). *Lupinus albus, L. angustifolius*, and *L. luteus* have a relatively low content of oil, lupins do not contain anti-nutritional factors such as trypsin inhibitors and saponins. As the source of energy they compete with cereals and as the source of proteins with oil seed meals. The lupin value is enhanced by the capacity to supplement other food components to achieve an overall balance of nutrients (Straková et al., 2006).

MATERIAL AND METHODS

The objective of the study was to compare the production capabilities of three varieties of white lupine in the same soil and climatic conditions at Nový Jičín university farm in 2015. Three promising varieties was selected for the experiment, variety Dieta, Amiga and Zulika. Each of these varieties were grown on an area of 10 ha. Each variety was sown simultaneously 11. 4. 2015 in the amount of 2 q/ha. Harvest of all three varieties was realized in the period 28. 8. 2015. Sampling of green matter was conducted by random selection of 10 samples of 1 m^2 area of each variety. Samplings were carried out in the age 15 week of crops, the crops were at the stage of fully developed green pods. In the following period, also did not increase the volume of vegetation, followed by aging and shrinking green pods of green mass. The collected samples of vegetation were subsequently dried and homogenized for analytical analysis.

Within chemical analysis were collected in green mass samples these nutrients: dry matter by drying the sample at 105 ◦C to constant weight, crude protein by the Kjeldahl method Buchi analyzer (produced Centec automatic spol. Ltd.), fat by device ANKOM^{XT10} Fat Analyzer (company OK SERVICE BioPro®), crude fiber, and the individual fiber fractions (ADF, ADL, NDF) device Ankom^{220} Fiber Analyzer (produced by OK SERVICE BioPro®), starch was determined by polarimetry, ash gravimetric method after ashing the sample at 550 °C under specified conditions, gross energy (GE) calorimetrically AC 500 instrument (LECO), nitrogen-free substances and organic matter were determined by calculation. The results were processed by statistical methods using statistical software Unistat 5.6 for Excel. Were evaluated mean values and their difference multiple comparison using Tukey-HSD test, at a significance level of $P \leq 0.01$ and $P \leq 0.05$. Each indicator is presented of diameter (x) and standard deviation (± SD).

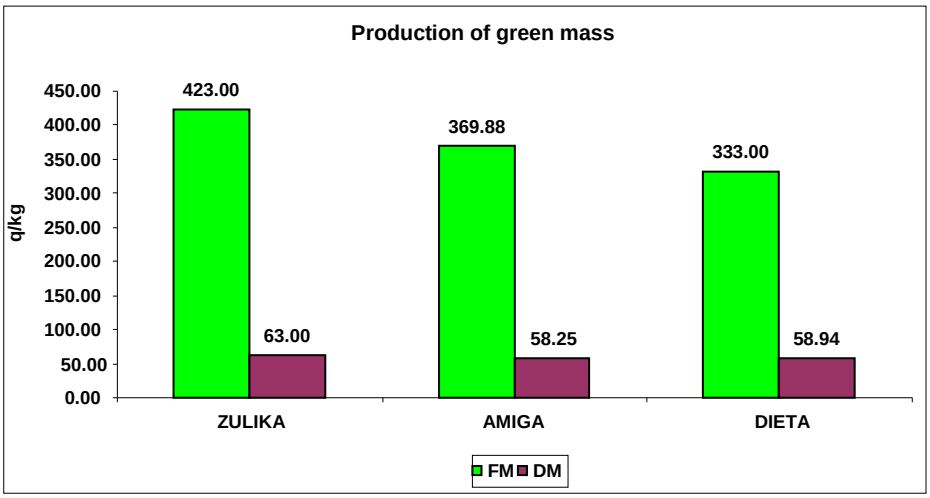
RESULTS AND DISCUSSION

Average values of green mass yields (DM) at the age of 15 weeks crops indicate that productive variety Zulika is 4.23 kg/m^2 (0.63 kg/m^2), Amiga 3.70 kg/m^2 (0.58 kg/m^2) and the lowest production was variety Dieta 3.33 kg/m^2 (0.59 kg/m^2). The production of

green mass per 1 m^2 has been proven highly significantly higher average value of the variety Zulika compared with Dieta. In this crops solids relevance is not proven, it is due to differences in water content of the fresh crop.

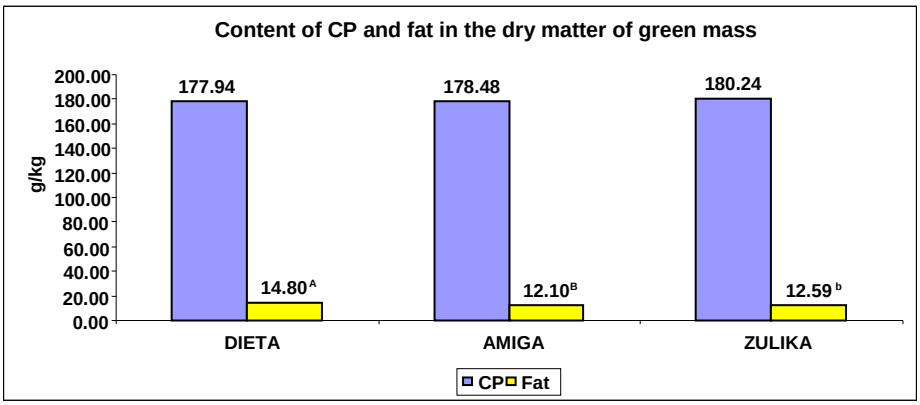
From the results of the average production of green matter (dry matter), we calculated the yield of green matter and dry matter as shown in figure 1. In assessing the hectare yield of fresh green matter, the highest production for the Zulika variety was lower, the lowest for the Amiga and the lowest for the Dieta. In the hectare yield of dry matter, Zulika was the most productive of the varieties tested.

Figure 1. Hectare yields of green mass in fresh matter (FM) and dry matter (DM) in three tested varieties of white lupine



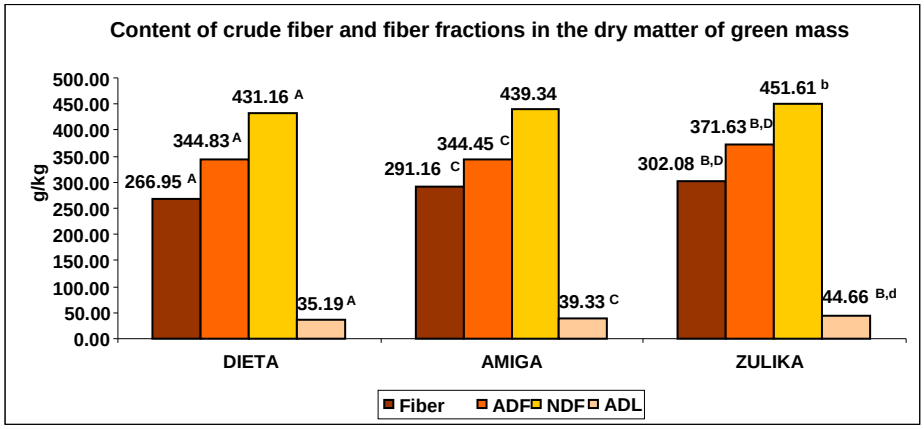
In the present study we were interested not only the quantity of production, but also its quality, evaluated based on the nutrient composition of dry matter green mass in the tested varieties. Figure 2 shows the content of crude protein (CP) and fat in dry matter of green mass at the three tested varieties of lupine. Between the average values CP were not statistically significant differences between the varieties, despite the variety Zulika showed the highest content of CP. Conversely, the variety diet was statistically significant $P \leq 0.01$ high fat content compared with the Amiga and Zulika.

Figure 2. The average content of crude protein and fat in the three varieties tested white lupine (AB, CD highly significant difference between the average values $P \leq 0.01$)



Interestingly as documented in figure 3 the differences in crude fiber and fiber between factions of the complex. Statistically, the highest average fiber $P \leq 0.01$ was demonstrated in a variety Zulika compared Dieta and Amiga varieties. Similarly, even in the ADF was highly significantly $P \leq 0.01$ the highest average value in the variety Zulika compared Dieta and Amiga varieties. For variety Zulika was determined and the highest average value for NDF, which is significantly $P \leq 0.05$ differed from the average value of the NDF in the Dieta variety. Also Zulika variety proved the highest average value of the ADL, which is highly significantly $P \leq 0.01$ different from the average value of the ADL of the variety Dieta and conclusively $P \leq 0.05$ of Amiga variety.

Figure 3. Average fiber and fiber fractions content of 3 tested varieties (AB, CD highly detectable difference $P \leq 0.01$, Ab and Cd apparent difference between mean $P \leq 0.05$)



CONCLUSION

In terms of production per hectare compared to the individual tested varieties of white lupine grown in the same soil and climatic conditions, we concluded that the highest production potential at the age of 15 weeks of crops showed the variety Zulika, compared with the variety Dieta and Amiga. The variety Zulika produced per 1 ha the most: the green mass of fresh and dry matter, crude protein, fiber and fiber fractions, organic matter, ash and gross energy. Also from the point of view of nutrition variety Zulika is very positive, because contained in the green mass the most of crude protein.

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REFERENCES

Cowling, W.A.; Huyghe, C.; Swiecicki W. Lupin breeding. In: Gladstones, J.S.; Atkins, C.A.; Hamblin, J. (Eds.): Lupins as crop plants: Biology, production and utilization. CAB International, Oxon, UK, 1998, p. 93-120.

Dijkstra, D.S.; Linnemann, A.R.; Van Bockel, T.A. Towards sustainable production of protein-rich foods: Appraisal of eight for Western Europe. PART II: Analysis of the technological aspects of the production chain. Crit Rev Food Nutr 43, 2003: p. 481 – 506.

Petterson, D.S.; Sipsas, S.; Macintosh, J.B. The chemical composition and nutritive value of Australian pulses, Greins Research and Development Corporation, Canberra, Australia, 1997, 68 p.

Straková, E.; Suchý, P.; Večerek, V.; Šerman, V.; Mas, N.; Juzl, M. 2006: Nutritional composition of seeds of the genus *Lupinus*. Acta Vet Brno 75: 489-493.

THE INFLUENCE OF MILK THISTLE SEED CAKES ON LAYING HENS PERFORMANCE

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ABSTRACT

The experiment was performed with Bovans Brown hens (n = 30) after 69 weeks of live. The experiment ran for 11 weeks – from the age of 69 weeks to the 80th week of the animals. Hens were divided into 2 groups. The experimental group of poultry (n = 15) received feed containing 7% of milk thistle seed cakes (group MT7) and control group (n = 15) received feed mixture without milk thistle seed cakes. In experiment was found significantly ($P < 0.05$) higher quantity of eggs per group in experimental MT7 group. This is also related to a higher number of eggs per hen and day that was also in MT7 group ($P < 0.05$). The total weight of eggs per group was found statistically higher ($P < 0.05$) in experimental group, as well. Hens in the experimental group contains milk thislte seed cakes laying more eggs whose production has eaten more feed.

Keywords: poultry nutrition; layers; egg; *Silybum marianum*

INTRODUCTION

Milk thistle (*Silybum marianum* L.) have been used for almost two thousand years as a natural treatment for the liver diseases (Ding et al., 2001). The main active substances occurring in milk thistle are flavonolignans, which are hepatoprotective substances. The seeds of milk thistle contain flavonoids quercetin, taxifolin, and particularly flavonolignans in an amount of 1.5–3%. The mixture of silydianin (10%), silychristin (20%) and silybin (50–60%) is known as silymarin (Opletal and Skrivanova 2010, Ding et al., 2001, Zahid and Durrani 2007). The anthocyanins, mainly cyanidin-3-glucoside

(CG) respectively, have been reported to be bioavailable (Miyazawa et al., 1999). CG decreased obesity and circulating triglycerides in an in vivo study (Wei et al., 2011).

Some trials showed that silymarin addition in diet or silymarin administration increased productive and reproductive performances and improved livestock health status of animals (Tedesco 2001).

This study was conducted to evaluate influence of the milk thistle seed cakes at dose 7% in feed mixture on performance parameters of hens after 69th weeks of live.

MATERIAL AND METHODS

An experiment was performed with Bovans Brown hens (n = 30) after 69 weeks of live. The experiment ran for 11 weeks – from the age of 69 weeks to the 80th week of age of the animals. Hens were divided into 2 groups. The experimental group of poultry (n = 15) received feed containing 7% of milk thistle seed cakes (group MT7) and control group (n = 15) received feed mixture without milk thistle seed cakes. The Table 1 and the Table 2 shows composion and chemical compositon of the diets. Laying hens were housed in cubicles on a litter of wood shavings. They had roosts, laying nests, automatic nipple drinkers and self-feeding feeders with a capacity corresponding to a given number of animals. The room was artificially controlled by temperature, relative humidity, and light mode to match the requirements of the relevant animal category. The light mode was set to 18 hours of light and 6 hours of darkness with gradual dimming and decay. The hens were marked with numbered circles on the legs.

The feed mixtures were assembled isotonitrogen and isocalorically, so it´s nutrient composition corresponds to the recommended nutrient needs of the appropriate category according to the Recommended Nutrient Content in Poultry Feeding Compounds (Zelenka et al., 2007). The feed mixtures were fed in unformed mixture. Layers had *ad-libitum* access to feed and water. Daily weight of feed residues was weighted to calculate a total feed consumption. The new feed was weighed daily. The hens in the experiment were repeatedly weighted to determine live weight. The health status experimental animals of was regularly monitored. During the experiment, the weaned eggs was evaluated quantitatively. The eggs were weighing and the total weight of the

weaned eggs were recorded every day (n = 62 days). Subsequently, the average weight of one egg in each group and the feed consumption per hen and egg were calculated. The eggs were collected and weighed in period from 69 weeks to 80 weeks of layer´s age.

Table 1. Composition of the diet in individual groups (g/kg)

Components	**C**	**MT7**
Wheat	600	600
Soybean meal	200	168
Milled Limestone	74	74
Milk thistle seed cakes	0	70
Maize	54.1	0.8
Rapeseed oil	31.7	39
Premix*	30	30
Monocalcium phosphate	5	5.4
Wheat gluten	4.7	10.8
Methionin	0.5	1
Lysin	0	1

* Premix content (in 1 kg): Methionin 0.35 %, Ca 3.50 %, P 0.55 %, Na 0.19 %. Cu 8.00 mg, Zn 40.00 mg, Mn 60.00 mg, I 1.20 mg, Se 0.10 mg. Retinol 10000.00 IU (international units), vitamin D_3 2500.00 IU, tocopherol 11.00 mg., vitamin K_3 2.00 mg, vitamin B_1 2.50 mg, vitamin B_2 6.50 mg, vitamin B_6 3.00 mg, vitamin B_{12} 10.00 mg, niacinamid 20.00 mg, calcium panthotenate 6.00 mg, choline chloride 200.00 mg. Butylhydroxyanisol (BHA) (E320) 100.00 mg, butylhydroytoluen (BHT) (E321) 500.00 mg, ethoxyquin (E324) 1000.00 mg.

The milk thistle seed cakes contain in dry matter 217 g/kg crude protein, 100 g/kg ether extract and 292 g/kg crude fibre. The used seed cakes also contained 129.83 mg/kg cyanidin-3-glucoside measured by Varga et al. (2013).

Table 2. Chemical composition of diets in kg (as fed basis)

Nutrient	**C**	**MT7**
Dry matter (g)	880	800
AME (MJ) *	11.46	11.41
Crude protein (g)	159.49	162.29
Ether extract (g)	50.58	54.39
Crude fibre (g)	43.92	53.23
Ash (g)	130.50	118.80

* Apparent metabolizable energy – calculated value

Data has been processed by Microsoft Excel (USA) and Statistica version 12.0 (CZ). We used one-way analysis of variance (ANOVA). To ensure evidential differences Scheffe's test was applied and differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The mean live weight of layers is not change during the experiment. On the start of experiment average live weight of hens was 1.81 kg in C group and 1.79 kg in MT7 group. Compare to that, the mean live weight on the end of experimental period was 1.74 kg for all two groups. During the experimental period 2 deaths were recorded in each group.

The mean quantitative assessment of eggs and the mean feed consumption shows Table 3. It was found significantly ($P < 0.05$) higher quantity of eggs per group in experimental MT7 group. This is also related to a higher number of eggs per hen and day that was also in MT7 group ($P < 0.05$). The total weight of eggs per group was found statistically higher ($P < 0.05$) in experimental group, as well.

Compare to that, the experimental group had statistically ($P < 0.05$) lower total feed consumption for a day per group and per hen too.

Table 3. The average quantitative assessment of eggs and the mean feed consumption

	C			MT7		
	mean	SD		mean	SD	
Mean number of eggs per group and day (pcs)	10.58	1.584	a	11.48	1.667	b
Mean number of eggs per hen and day (pcs)	0.75	0.113	a	0.80	0.118	b
Mean total eggs weight per day (g)	680	104	a	740	114	b
Mean weight of one egg (g)	64.81	1.044	a	64.79	1.094	a
Mean total feed consumption per group and day (kg)	1.56	0.207	a	1.69	0.256	b
Mean total feed consumption per hen and day (g)	110.56	14.705	a	116.91	17.822	b
Mean total feed consumption per one egg (g)	150.83	31.586	a	150.15	37.140	a

[a, b] different letters in one line means statistically defferent differences $P < 0.05$.
SD – standard deviation; pcs – pieces

Hashemi Jabali et al. (2017) found in their study with hens decrease in feed intake during whole experiment with inclusion of 30 g/kg milk thistle meal. Feed conversion ratio was significantly reduced in this group, too. The broiler chickens feed mixture contained 15 g/kg milk thistle seeds significantly reduced feed conversion ratio (Zahid and Durrani, 2007). In the same experiment provide by Hashemi Jabali et al. (2017) inclusion of 3% milk thistle seed meal significantly increased egg production and due to this egg weight was significantly increased, as well. This finding confirmed results of Schiavone et al. (2007).

On the other hand, Cullere et al. (2016) observed that inclusion of milk thistle herb in the diets of growing rabbits did not affect their performance, feed intake or feed conversion ratio.

However, Erisir et al. (2016) observed significantly higher a total egg production of Quails in experimental group contain 10 g/kg of milk thistle seed.

CONCLUSION

Hens in the experimental group contains milk thislte seed cakes laying more eggs whose production has eaten more feed.

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REFERENCES

Cullere, M., Dalle Zotte, A., Celia, C., Renteria-Monterrubio, A.L., Gerencsér, Z., Szendrő, Z., kovács, M., Kachlek M.L., Matics, Z. (2016): Effect of Silybum marianum herb on the productive performance, carcass traits and meat quality of growing rabbits. Livestock Science 194, 31-36.

Ding, T.M., Tian, S.J., Zhang, Z.X., Gu, D.Z., Chen, Y.F., Shi, Y.H., Sun, Z.P. (2001): Determination of active component in silymarin by RP-LC and LC/MS. Journal of Pharmaceutical and Biomedical Analysis 26, 155-161.

Erisir, Z., Iflazoglu Mutlu, S., Simsek, U.G., Ciftci, M., Azman, M.A., Benzer, F., Erisir, M. (2016): Effects of Milk Thistle (Silybum marianum) Seed Supplementation to High-Calorie Basal Diets of Quails on Egg Production, Egg Quality Traits, Hatchability and Oxidative Stress Parameters. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 22, 577-584.

Hashemi Jabali, N.S., Mahdavi, A.H., Ansari Mahyari, S., Sedghi, M., Akbari Moghaddam Kakhki, R. (2017): Effects of milk thistle meal on performance, ileal bacterial enumeration, jejunal morphology and blood lipid peroxidation in laying hens fed diets with different levels of metabolizable energy. Journal of Animal Physiology and Animal Nutrition 101, 1-11.

Miyazawa, T., Nakagawa, K., Kudo, M., Muraishi, K., Someya, K. (1999): Direct intestinal absorption of red fruit anthocyanins, cyanidin-3-glucoside and cyanidin-3,5-diglucoside, into rats and humans. Journal of Agricultural and Food Chemistry 47, 1083-1091.

Opletal, L., Skrivanova, V. (eds.) (2010): Natural substances and their biological activity (in Czech). Prague: Karolinum.

Schiavone, A., Righi, F., Quarantelli, A., Bruni, R., Serventi, P., Fusari, A. (2007): Use of Silybum marianum fruit extract in broiler chicken nutrition: Influence on performance and meat quality. Journal of Animal Physiology and Animal Nutrition 91, 256-262.

Tedesco, D. (2001): The potentiality of herbs and plants extracts as feed additives in livestock production. Zootecnia e Nutrizione Animale 27, 111-133.

Varga, M., Bánhidy, J., Cseuz, L., Matuz, J. (2013): The anthocyanin content of blue and purple coloured wheat cultivars and their hybrid generations. Cereal Research Communications 41, 284-292.

Wei, X., Wang, D., Yang, Y., Xia, M., Li, D., Li, G., Zhu, Y., Xiao, Y. Ling, W. (2011): Cyanidin-3-O-β-glucoside improves obesity and triglyceride metabolism in KK-Ay mice by regulating lipoprotein lipase activity. Journal of the Science of Food and Agriculture 91, 1006-1013.

Zahid, R., Durrani, F. (2007): Biochemical, hematological, immunological and growth promotant role of feed added milk thistle (Silybum marianum) in broiler chicks. M. Sc. (Hons) thesis, NWFP Agricultural University, Peshawar, Pakistan.

Zelenka J., Heger J., Zeman L. (2007): Recommended Nutrient Content in Poultry Feeding Compounds (in Czech). MZLU in Brno, 78 p.

Feed supplements and their effect on the incidence of coccidia oocysts in the digestive tract of pheasants

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ABSTRACT

The occurrence of coccidiosis is an enormous problem of pheasant farming. The aim of this study is to show, that this disease can be cured by unconventional dietary supplements. Dietary supplements which were used in this study were as follows: prebiotics (*Ascophyllum nodosum*), probiotics (*Lactobacillus thermophilus*) and homeopathics. Four groups of pheasants were made - three experimental groups and one control group. All groups were consisted of 12 individuals. The pheasant *Phasianus colchicus* was used in this experiment. Dietary supplements were served for 30days. The favourable effect of homeopatics ($P < 0.05$) and *Lactobacillus thermophilus* ($P < 0.05$) on the intestinal tract of pheasant was statistical significant.

Keywords: Eimeria; homeopatics; prebiotics; probiotics

INTRODUCTION

Coccidiosis remains a major economically important disease for the poultry industry, including intensively reared pheasants. The risk of clinical outbreaks is directly proportional to the concentration of bird populations. Young poultry show transitional predisposition to infectious diseases during the first week of life due to qualitative damage of bird in wild nature and obtaining the host defence (Lowenthal et al., 1997). Coccidiosis is one of the most frequently occurring mass disease in young pheasants, partridges and turkeys and may significantly limit the success of the whole breeding. Coccidia are unicellular parasites that in birds, like in mammals, primarily attack the

intestinal mucosa and evoke inflammatory changes, known as coccidiosis (Gassal, 2003). Young pheasants are most sensitive to Eimeria infection during the first four days, after which they become more resistant. This period of a "transitional immunoincompetence" is caused by a general failure of T-cells to proliferate and secrete cytokines and functional immaturity of heterophils during the first week of defence (Cacho et al., 2012). Coccidiosis is a disease that has a large economic impact on the poultry industry causing high mortality, poor growth and high medical costs (Williams, 1998). In poultry, coccidiosis is caused by parasites of the genus Eimeria (Coccidia subclass). Currently, the use of anti-coccidial drugs is one common means to prevent and treat coccidiosis. However, massive and long-time use of anti-coccidial drugs has led to the presence of drug-resistant parasites and residual drugs in poultry products, raising concerns about public health and food safety (Orengo et al., 2012). In European countries, the use of anti-coccidial and anti-histomonas drugs as feed additives has been strictly limited since 2006 (Regulation 1831/2003 of the European Parliament) and a full ban has been proposed to be effective in 2021 by the Council Directive of 2011/50/EU published in the Official Journal of the European Union, L 104 of 19 April 2011. The utilization of anti-coccidial vaccines is an alternative means to prevent coccidiosis. Despite the significant progress made over recent years, efficacy, safety and cost effectiveness are still challenges for anti-coccidial vaccines in poultry (Sharman et al., 2010).

In this study the aim of the experiment was to reduce the incidence frequency of coccidia oocysts in the intestinal tract of pheasants while administering different additives. Chosen additives were homeopatics, probiotics and prebiotics. Homeopathy usually uses medicaments extremely diluted. Their effectiveness depends on bio-energy mechanisms. It uses extracts of plants and minerals for their medicine and adds derivatives of various modern drugs and chemicals in extreme dilution. It is also to be used in the fight against infectious diseases (Day, 2007). Several studies have shown that the use of homeopathy is appropriate under organic farming, while other alternative therapies seem to be relatively rare. The effectiveness of alternative treatments is generally poorly documented, especially in the case of homeopathy. The use of homeopathy therefore led to concerns that its use may have a negative impact on animal

health (Hektoen, 2007). Homeopathy does not cure the disease directly, but the individual itself is able to fight against it. It is important that the environment allowed to keep the balance of the patient and thus improving of the environmental factors has a great importance for successful homeopathic treatment (Verdone, 2000).

Prebiotics are non-digestible food ingredients that promote the growth or activity of intestinal microflora and improve the health of the consumer. This usually involves hardly digestible or non-digestible oligosaccharides. These ones become in the colon a substrate for certain desirable bifidobacteria, which ferment them - main waste products are butyric, propionic and acetic acid (Kalac, 2003). Biopolym is a hydrolyzate of brown seaweed Ascophyllum nodosum, which is obtained in the cold coastal waters, especially near Iceland, but also in coastal areas of Norway and Canada (Vostoupal et al., 2005). Probiotics are live microorganisms that beneficially affect the health status of intestine by modifying of intestinal microflora, especially in young animals (Streitz, 2006). Handling of the intestinal microflora using dietary supplement of real microbe is a new approach not only from a nutritional point of view, but also as an alternative treatment to overcome the adverse effects of antibiotics and drugs. These beneficial microorganisms are usually referred as "probiotics", which are capable to colonize and proliferate in the gut of the host and execute numerous beneficial effects by modulating various biological systems of the host (Cross, 2002). Over the years, a succession of strategies were conducted how to modulate the composition of intestinal microflora for better growth, digestion and immunity. Medical host immunity was investigated in various animals as well as humans (Burr, 2007). Duration of dosing of probiotics is another important factor which may influence the occupancy of the intestinal tract, persistence and subsequent induction of immune response in the host (Choi & Yoon, 2008). Probiotics microbes and their supplementation in the poultry diet is suggested to improve the productive performance (Ayed & Chaoui, 2011; Dibaji et al., 2012). The enhanced performance of the birds is associated with beneficial effects of the probiotics balancing microflora (Dibaji et al., 2012; Panda et al., 2003; Houndonougbo et al., 2011).

MATERIAL AND METHODS

The experiment took place from April 2014 to July 2014. The experiment included a total of 180 pieces of pheasants. These pheasants were involved in the experiment continuously. In April 2014, the first group of pheasants created, that was divided into five subgroups of 15 pieces - the first group *Ascophyllum nodosum*, the second group of homeopathy, the third group *Lactobacillus thermophilus* and the control group. The same groups and subgroups were created in May and June 2014. All groups were given feed mixture BŽ from the producer Velas a.s. that did not content anticoccidics (composition: 36 % corn, 32 % wheat, toasted soybean extraction meal 10 %, wheat bran 10 %, fish-flour 4 % yeast 2 % lucerne flour, ground limestone, 1.2 % dicalcium phosphate 0.5 % sodium chloride 0.3 %, vitamins A, D3, E) for a period of 26 days.

The first group "*Ascophyllum nodosum*" was administered orally with 40 ml of the hydrolyzate from brown seaweed in water for four weeks daily.

The second experimental group "homeopathics" recieved orally 20 ml homeopathics mixed in water for four weeks daily.

The third group "*Lactobacillus thermophilus*" was given one tablet of probiotics in water for four weeks daily.

The fourth group was a control one and received a feed ration unchanged.

During the experiment, the data logger was located to the barn scanning temperature differences every hour and subsequently an average daily temperature, depending on the frequency of occurrence of coccidia oocysts was formed. After 56 days, all pheasants from each of the group were weighed, then slaughtered and then in their intestines the frequency of coccidia oocysts´ occurrence was analyzed. In order to confirm the incidence of coccidia oocysts in the intestinal tract and to identify better the type and number of coccidia, a detailed survey of all sections of intestines from slaughtered animals was made by veterinarian specialized in poultry health matters.

The data were analyzed using a General Linear Model ANOVA (four ways with the interactions) of the statistical package STATISTICS 10 (Analytical Software, Tallahasee, FL, USA).

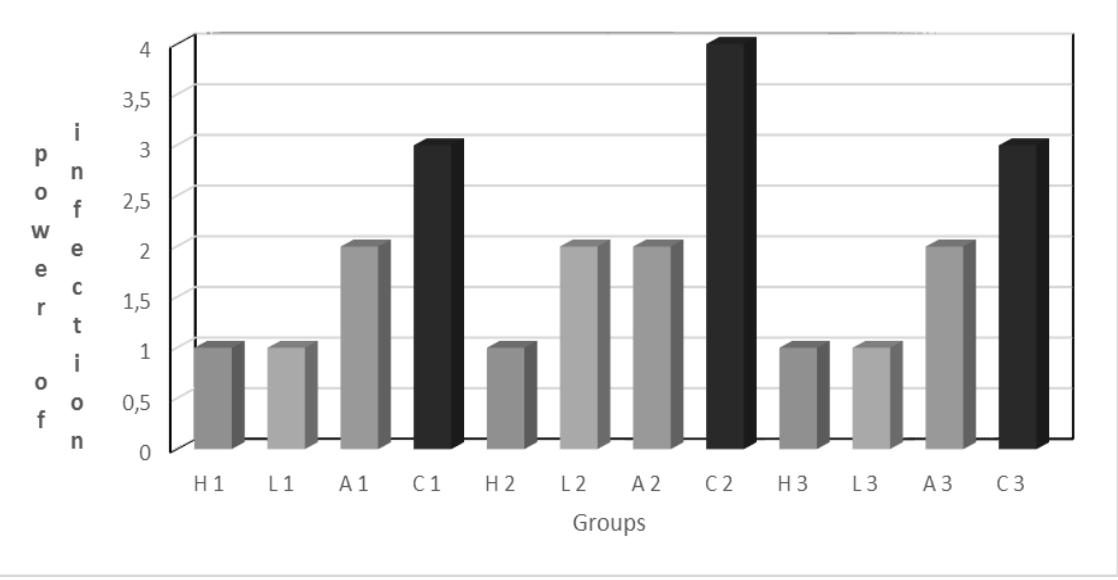
RESULTS AND DISCUSSION

Based on the processed data, we can confirm that after three repetitions performed from April to July 2014 statistically positive effects of homeopathic were proved compared with the control group. The incidence rate of oocysts of coccidia in the intestinal mucosa of pheasants reduced $P = 0.036$. The positive effect of homeopathic remedies on health status and reduction of pathogens incidence confirm also studies in poultry (Velkers et al., 2005; Berchieri et al., 2006) and sheep (Rocha et al., 2006).

Statistically significant was also the influence of probiotic *Lactobacillus thermophilus* $P = 0.047$. Improving health by means of probiotics administration confirm in their studies (Ayed & Chaoui, 2011; Dibaji et al., 2012; Panda et al., 2003; Houndonougbo et al., 2011; Khan et al., 2012; Fuller, 1989; Sojoudi et al., 2012).

In the group with prebiotic product *Ascophyllum nodosum* a positive trend of its efficacy on incidence of coccidia oocysts in the digestive tract of pheasants was observed ($P = 0.065$), but the effect did not reach any statistical significance. *Ascophyllum nodosum* demonstrated a positive influence on the creation of natural immunity during the whole period of feaces testing. Coccidia infection was at very low level and therapeutic breaks did not have any effect on the extent of infection.

Figure 1. The mean power of infection oocysts coccidia in the intestines of pheasants



H 1 = group homeopatic in April (N = 15), H 2 = group homeopatic in May (N = 15), H 3 = group homeopatic in June (N = 15), L 1 = group *Lactobacillus thermophilus* in April (N = 15), L 2 = group *Lactobacillus thermophilus* in May (N = 15), L 3 = group

Lactobacillus thermophilus in June (N = 15), A 1 = group *Ascophyllum nodosum* in April (N = 15), A 2 = group *Ascophyllum nodosum* in May (N = 15), A 3 = group *Ascophyllum nodosum* in June (N = 15), C 1 = control group in May (N = 15), C 2 = control group in April (N = 15), C 3 = control group in June (N = 15)

Table 1. Economic evaluation of the use of feed supplement in US dollars

Groups	Cost of feed per day	Cost of feed for 56 days	Cost of feed attachment	Losses - death in crowns	Total costs	Total profit
Lactobacillus	0.11	89.83	12.14	0	101.97	**32.73**
Homeopatics	0.11	89.83	3.93	0	93.76	**40,93**
Ascophyllum	0.11	89.83	12.80	-8.98	111.61	**23.09**
Controls	0.11	89.83	0	-17.96	107.79	**26.91**

Experiments have shown profitability in three groups and the control sample (always 15 pieces) for fattening 56 days (selling price 1 piece is 8.98 USD).

Also the natural effects were proved, such as temperature and humidity of environment in which the pheasant chicken were reared. It is known that in humid and warm weather the oocysts do well and sporulate easily. On the other hand at low temperatures and in a dry environment sporulation is slow.

CONCLUSION

When testing designated biopreparations a beneficial effect on the incidence of oocysts was demonstrated in two out of three samples. It shows the possibilities for further use in pheasants breeding and it is a promise even for rearing of more resistant individuals and improvement of the overall status of pheasants in natural conditions because the administration of homeopathic and probiotics appear to promote the creation of natural immunity of reared pheasants. In large-scale breeding of pheasants anticoccidics are often used which although contributing to the breeding of pheasants later released into the wild, at the same time being less capable in terms of developing the natural immunity against infection by different parasites, in our case, coccidia.

These preparations should also gain popularity for other reasons, one of which is a lower purchase price, whether prebiotics and homeopathic remedies. The other reason

could be ecological breeding of pheasants and subsequent production of pheasant meat in “bioquality”.

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References

Ayed, M.H. & Ghaoui, F. (2011): Efficiency of supplementing Saccharomyces cerevisiae var. ellipsoideus for improved growth performance and carcass yield in broilers, Research Opinions in Animal and Veterinary Sciences, 1, 371–374.

Berchieri, A., Turco, W.C.P., Paiva, J.B., Oliveira, G.H. & Sterzo, E.V. (2006): Evaluation of isopathic treatment of Salmonella enteritidis in poultry, Homeopathy, 95, 4–97.

Burr, G. (2007): Microbial ecology of the gastrointestinal tract of fish and the potential application of prebiotics and probiotics in fish aquaculture, Journal of the World Aquaculture Society, 36(1), 425-436.

Cross, M.L. (2002): Microbes versus microbes: immune signals generated by probiotic lactobacilli and their role in protection against microbial pathogens. Immunology Medical and Microbiology, 34(4), 245-253.

Day, C,H, (2007): Aternative veterinary Medicine, Natural.

Del Cacho, E., Gallego, M., Lee, S.H., Lillehoj, H.S., Quilez, J., Lillehoj, E.P. & Sánchez-Acedo, C. (2012): Induction of protective immunity against Eimeria tenella, Eimeria maxima, and Eimeria acervulina infections using dendritic cell-derived exosomes, Infection and immunity, 80(5), 1909-1916.

Dibaji, S.M., Seidavi, A. & Asadpour, L. (2012): Effect of dietary inclusion of the synbiotic Biomin IMBO on broilers’ some blood metabolites, Research Opinions in Animal and Veterinary Sciences, 2, 10–13.

Fuller, R. (1989): Probiotics in man and animals, Journal of Applied Bacteriology, 66, 365–378.

Gassal, S. (2003): Untersuchungen zum Ekto- und Endoparasitenbefall von Fasanhähnen (Phasianus colchicus), Dissertation, Veterinärmedizinische Fakultät der Universität Leipzig.

Hektoen, L. (2007): Use of alternative veterinary medicine in organic dairy farming. Proceedings of the fifth workshop NAHWOA a positive impact on health: preventive measures and alternative strategie, 42-49.

Houndonougbo, M.F., Chrysostome, C.A.A.M. & Amoussa, Z.L.A.O. (2011): Tchoukoutou residue and yogurt as feed additives in broilers feed, Research Opinions in Animal and Veterinary Sciences, 1, 597–600.

Choi, S.H. & Yoon, T.J. (2008): Non-specific immune response of rainbow trout (Oncorhynchus Mykiss) by dietary heat-inactivated potential probiotics. Immune Network, 8(3), 67-74.

Kalac, P. (2003): Functional foods - Steps to Health, Czech Budejovice.

Khan, R.U., Rahman, Z., Javed, I. & Muhammad, F. (2012): Effect of vitamins, probiotics and protein on semen traits in post-molt male broiler breeders, Animal Reproduction Science, 135(4), 85-90.

Lowenthal, J.W., York, J.J., O'Neil, T.E., Rhodes, S., Prowse, S.J., Strom, A.D.G. & Digby, M.R. (1997): In vivo effects of chicken interferon-γ during infection with Eimeria, Journal of Interferon and Cytokine Research, 17(9), 551-558.

Orengo, J., Buendia, A.J., Ruiz-Ibanez, M.R., Madrid, J., Del Rio, L. & Catala-Gregori, P. (2012): Evaluating the efficacy of cinnamaldehyde and Echinacea purpurea plant extract in broilers against Eimeria acervulina, Veterinary Parasitology, 185, 158–163.

Panda, A.K., Reddy, M.R., Rama, S.V. & Praharaj, N.K. (2003): Production performance, serum/yolk cholesterol and immune competence of white leghorn layers as influenced by dietary supplementation with probiotic, Tropical Animal Health and Production, 35, 85–94.

Rocha, D.R.A. & Pacheco, R.D.L. (2006): Amarante AFT, Efficacy of homeopathic treatment against natural infection of sheep by gastrointestinal nematodes, Brasilia Journal Parasitology Veteterinaria, 16(1), 24-27.

Sharman, P.A., Smith, N.C., Wallach, M.C. & Katrib, M. (2010): Chasing the golden egg: vaccination against poultry coccidiosis, Parasite Immunology, 32, 590–598.

Sojoudi, M.R., Dadashbeiki, M. & Bouyeh, M. (2012): Effects of different levels of symbiotic. TechnoMos on broilers performance, Research Opinions in Animal and Veterinary Sciences, 2, 243–248.

Streitz, E. (2006): Alternative additives - an area with great potential. DGS Magazin, 26(1), 30–33.

Velkers, F.C., Loo, A.J.H., Madin, F. & Eck, J.H.H. (2005): Isopathic and pluralist homeopathic treatment of commercial broilers with experimentally induced colibacillosis, Research Veterinary Science, 78, 77–83.

Verdone, M. (2000): Homeopathy and organic livestock production. Workshop Livestock Husbandry and Homeopathic Approach, Milano.

Vostoupal, B., Soch, M., Novak, P., Gjurov, V., Jelinek, A., Dedina, M. & Pliva, P. (2005): Options sub-purpose redevelopment bioclimate rural areas using plant bio-algens series. Prague, 105 – 108.

Williams, R.B. (1998): Epidemiological aspects of the use of live anticoccidial vaccines for chickens, International Journal for Parasitology, 28, 1089–1098.

SELECTED BLOOD BIOCHEMICAL INDICATORS OF YOUNG DWARF LOP RABBIT FEMALES IN RELATION TO THE DIFFERENT DIETS

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ABSTRACT

The aim of the study was to evaluate the dietary effect of the white lupin seeds (*Lupinus albus*) on biochemical indicators in the dwarf rabbits. The study was conducted on a total of 16 young rabbit females belong to the Dwarf Lop breed. These rabbits were divided into 2 dietary groups. The control group received a foreign commercial pelleted diet. The rabbits of the experimental group were fed a complete pelleted diet containing the white lupin seeds. The blood samples were taken at the age of 8 weeks. Obtained values of the biochemical examination were within physiological reference ranges for rabbits. With respect to the dietary effect, we found the significant decrease in the content of albumin ($P < 0.01$), urea ($P < 0.05$) and activity of alkaline phosphatase ($P < 0.05$). The study brings preliminary data concerning the dietary effect of the white lupin seeds on the rabbit´s biochemical profile. It can be concluded that white lupin seeds can be suitable ingredient in the diets for the dwarf rabbits. However, further studies will be necessary to perform the optimization of the diets and the explanation of dietary effects of the white lupin seeds on dwarf rabbit´s physiology.

Keywords: lupin seeds; rabbit nutrition; normal biochemical indicators

INTRODUCTION

The progress and research of rabbit nutrition is predominantly focused on the meat-type rabbits sphere (Maertens, 2010), while the dwarf rabbit's nutrition requirements show

distinct differences (Proença and Mayer, 2014). Feeding incorrect diets has been linked with amounts of health problems (Prebble and Meredith, 2014).

The blood examination can provide valuable insights into the rabbit health and it influences the veterinarion´s decision. However, there is a lack of accurate information concerning normal physiology and clinical pathology in the pet rabbits (Meredith, 2014). Our previous research on the dwarf rabbits revealed that the blood indicators can be affected also by the breed (Šimek *et al.*, 2017). Recently, dietary inclusion of the lupin seeds showed health benefits in selected biochemical indicators in laboratory hamsters (Fontanari *et al.*, 2012), rats (Sirtori *et al.*, 2004) and pigs (Martins *et al.*, 2005). Regarding laboratory rabbits, Marchesi *et al.* (2008) found positive dietary effects of the white lupin seeds on their lipid metabolism. However, no finginds have existed concerning the dietary effect of the white lupin seeds on the basic biochemical profile of the dwarf rabbits.

The aim of the present study was to evaluate dietary inclusion of the white lupin seeds on the biochemical indicators in young females of the Dwarf Lop breed.

MATERIAL AND METHODS

The experimental procedures were approved by the Animal Welfare Committee of the University of Veterinary and Pharmaceutical Sciences Brno (no. 66/2016/2230/FVHE).

Animals

The study was carried out on a total of 16 young rabbit females of the Dwarf Lop breed. The purebreds originated from a common pet stock. The rabbit kits were housed together with the lactacing does up to their 8th week of age. The rabbits were housed in outdoor cages (65×60×45 cm, wide×high×deep) with additional elevated platform. The hutch was sheltered against unfavourable weather condition. The rabbit´s health status was monitored once a day.

Nutrition and experimental design

The rabbits with the lactating does were divided into 2 dietary groups. In the experiment, two types of the pelleted complete diets were used. Rabbit kits in the

control group received a foreign commercial feed for dwarf rabbits (Berkel-Futter Light 6008, Coesfeld, Germany). Rabbits of the experimental group received an experimental diet containing the white lupin (*Lupinus albus*) seeds var. Dieta. Ingredient and chemical composition of the diets is presented in Table 1. The young rabbits received daily amounts of approx. 30 g of the pelleted feed per kg of live weight. Moreover, meadow hay was offered three-times a week, and the rabbits had free access to drinking water.

Table 1. Ingredient and chemical composition of the used rabbit diets.

Item	Units	Diets	
		Control	Experimental
Ingredient in 1 kg of the diet			
Alfalfa meal	g/kg	417.0	340.0
Barley	g/kg	85.0	100.0
Wheat bran	g/kg	226.0	100.0
Oat	g/kg	0	100.0
Oat bran	g/kg	60.0	0
Lupin seeds	g/kg	0	250.0
Malt sprouts	g/kg	151.0	50.0
Sugar beet pulp	g/kg	29.00	0
Mollasses	g/kg	19.00	30.0
Premix	g/kg	0	10.0
Monocalcium phosphate	g/kg	1.0	7.0
Calcium carbonate	g/kg	8.5	10.0
Sodium chloride	g/kg	3.5	3.0
Chemical composition in 1 kg of dry matter			
Dry matter	g/kg	1000.0	1000.0
Crude protein	g/kg	160.5	201.5
Crude fibre	g/kg	173.2	161.4
ADF	g/kg	233.6	252.5
NDF	g/kg	420.0	347.6
ADL	g/kg	53.0	57.2
Ether extract	g/kg	26.8	47.4
Crude starch	g/kg	151.9	180.0
Ash	g/kg	86.2	87.6
Ca	g/kg	11.20	14.41
P	g/kg	5.7	5.9
DE	MJ/kg	9.84	9.90

ADF, acid detergent fibre; NDF, neutral detergent fibre; ADL, acid detergent lignin; DE, digestible energy

Blood sampling and biochemical examination

The blood samples were taken at the age of 8 weeks. With respect to the ear length, a *vena saphaena lateralis* was used. Obtained blood samples were relocated to sample tubes with heparin and transported to the laboratory. The blood samples were centrifuged to obtain a blood plasma. These samples were analysed using a DPC Konelab 20i Analyzer® (Thermo Fisher Scientific, Finland). In the blood plasma, we determinted following biochemical indicators: total protein, albumin, glucose, total cholesterol, triacylglycerols (TAG), creatinine, urea, calcium, inorganic phosphorus, sodium, potassium, chloride and activities of the enzymes alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Statistical analysis

Statistical analyses were performed using the STATISTICA CZ version 10 software. One-way ANOVA was used to determine differences in the biochemical indicators. When ANOVA showed significant differences between the dietary groups, Tuckey´s HSD test was used. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The results of the biochemical examination are given in Table 2. In our study, we found no significant difference in the total protein content between the evaluated dietary groups. However, we found a significant decrease in the albumin concentration ($P < 0.01$) in the experimental group. Al-harbi *et al.* (2014) found in their toxicological study that young laboratory rabbits fed a lupin seeds monodiet showed a decrease in the albumin concentration. Our values of the total protein and albumin are consistent with physiological reference ranges (Quesenberry, 2000; Wesche, 2014), however we found rather lower values of the total protein in our study than Martinec *et al.* (2012) in 3-month old medium-sized rabbit breeds (range of 38.0-54.4 g/L). According to Campbell (2012), the albumin constitutes approximately 40-60% of the total protein content, which is consistent with findings in present study.

Table 2. Biochemical indicators of the Dwarf Lop females at the age of 8 weeks.

Indicator	Units	Control group (n=8)		Experimental group (n=8)		*P*
		x	95% CI	x	95% CI	
Total protein	g/L	51.65	46.97-56.33	52.68	47.81-56.96	ns
Albumin	g/L	27.71^{B}	25.65-29.77	23.50^{A}	21.12-25.88	**
Glucose	mmol/L	7.88	7.52-8.54	7.45	6.92-7.98	ns
Total cholesterol	mmol/L	1.61	0.82-2.41	0.94	0.70-1.18	ns
Triacylglycerols	mmol/L	1.63	1.00-2.25	1.48	1.00-1.96	ns
Creatinin	µmol/L	65.60	60.56-70.64	63.37	58.86-67.88	ns
Urea	mmol/L	5.78^{b}	4.90-6.67	4.25^{a}	2.88-5.62	*
ALP	µkat/L	3.00^{b}	2.31-3.69	2.07^{a}	1.47-2.67	*
ALT	µkat/L	0.45	0.25-0.65	0.46	0.38-0.53	ns
AST	µkat/L	0.49	0.25-0.73	0.65	0.39-0.92	ns
Ca	mmol/L	3.04	2.84-3.23	2.93	2.80-3.06	ns
P_i	mmol/L	1.67	1.42-1.92	1.79	1.29-2.30	ns
Na	mmol/L	142.99	140.80-145.17	140.81	134.36-147.26	ns
K	mmol/L	4.42	4.16-4.67	4.66	4.39-4.93	ns
Cl	mmol/L	111.28	108.63-113.92	110.76	108.41-113.12	ns

^{a,b}: Means within a row with different superscripts letters differ ($P < 0.05$); ^{A,B}: Means within a row with different superscripts letters differ ($P < 0.01$); x, arithmetic mean; CI, confidence interval; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Ca, calcium; P_i, inorganic phosphorus; Na, sodium; K, potassium; Cl, chloride

In our study, we found also no significant dietary effect on the plasmatic concentration of glucose, total cholesterol, TAG and creatinine. The observed values were in the physiological ranges for healthy rabbits (Quesenberry, 2000; Wesche, 2014). The urea is an end-product of nitrogenous metabolism (Harcourt-Brown, 2002). In our experiment, the rabbits of the experimental group showed the lower urea content ($P < 0.05$). On the other hand, Al-harbi *et al.* (2014) found the higher values of the urea in laboratory rabbits fed only lupin seeds monodiet. ALP is a membrane-bound enzyme, while its normal plasma activity in rabbits varies with age, breed and strain (Campbell, 2012). We found a significant decrease in the ALP activity in rabbits of the experimental group ($P < 0.05$). Generally, the ALP levels in our study were higher than reference range (Quesenberry, 2000), however Harcourt-Brown (2002) states that there

is a wide variation between the physiological reference ranges. Besides that, Sanaa *et al.* (2012) found that dietary inclusion of the selected legume species had a lowering effect on ALP activity in rats. The values of the ALT, AST, calcium, inorganic phosphorus, sodium, potassium and chloride found in our study were in physiological reference ranges (Quesenberry, 2000; Wesche, 2014), and did not vary between the evaluated dietary groups.

CONCLUSION

The different diet prescription had significant effects on the albumin concentration, urea concentration and activity of the ALP. Based on our findings, it seems that dietary inclusion of the white lupin seeds affects mainly the protein metabolism of the young dwarf rabbits. Obtained results of the biochemical examination were within normal reference ranges for rabbits. With respect to the good general health state of the monitored rabbits, it can be concluded that white lupin seeds at the rate of 250 g/kg of the diet can be suitable ingredient in diets for dwarf rabbits. The study brings preliminary data concerning the dietary effect of the white lupin seeds on biochemical profile of the dwarf rabbits. However, further studies will be necessary to perform the optimization of the diets and explanation of dietary effects of the white lupin seeds on dwarf rabbit´s physiology.

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REFERENCES

Al-harbi, M.S., Al-Bashan, M.M., Abu Amrah, K.A.F. (2014): Impacts of feeding with *Lupinus albus* (White lupin), and *Lupinus termis* (Egyptian lupin) on physiological activities and histological structures of some rabbit´s organs, at Taif Governorate. Wourld Journal of Zoology, 9, 166-177.

Campbell, T.W. (2012): Clinical chemistry of mammals: Laboratory animals and miscellaneous species. In: Thrall, M.A., Weiser, G., Allison, R.W., Campbell, T.W.: Veterinary hematology and clinical chemistry. 2nd edition. Wiley-Blackwell, Ames, USA, 571-581.

Fontanari, G.G., Batistuti, J.P., da Cruz, R.J., Salvida, P.H.N., Arêas, J.A.G. (2012): Cholesterol-lowering effect of whole lupin (*Lupinus albus*) seed and its protein isolate. Food Chemistry, 132, 1521-1526.

Harcourt-Brown, F.M. (2002): Textbook of rabbit medicine. 1st edition. Butterworth Heinemann, Oxford, UK.

Maertens, L. (2010): Feeding systems for intensive production. In: de Blas, C., Wiseman, J. (ed.): Nutrition of the rabbit. 2nd edition. CAB International, Wallingford, UK, 253-266.

Marchesi, M., Parolini, C., Diani, E., Rigamonti, E., Cornelli, L., Arnoldi, A., Sirtori, C.R., Chiesa, G. (2008): Hypolipidaemic and anti-antherosclerotic effects of lupin proteins in a rabbit model. British Journal of Nutrition, 100, 707-710.

Martinec, M., Härtlová, H., Chodová, D., Tůmová, E., Fučíková, A. (2012): Selected haematological and biochemical indicators in different breeds of rabbits. Acta Veterinaria Brno, 81, 371-375.

Martins, J.M., Riottot, M., de Abree, M.C., Viegas-Crespo, A.M., Lança, M.J., Almeida, J.A., Freire, J.B., Bento, O.P. (2005): Cholesterol-lowering effects of dietary blue lupin (*Lupinus augustifolius* L.) in intact and ileorectal anastomosed pigs. Journal of Lipid Research, 46, 1539-1547.

Meredith, A. (2014): The value of clinical pathology in pet rabbit medicine. Veterinary Record, 174, 552-553.

Prebble, J.L., Meredith, A.L. (2014): Food and water intake and selective feeding in rabbits on four feeding regimes. Journal of Animal Physiology and Animal Nutrition, 98, 991-1000.

Proença, L.M., Mayer, J. (2014): Prescription diets for rabbits. Veterinary Clinic of North America: Exotic Aminal Practice, 17, 485-502.

Quesenberry, K.E. (2000): Rabbits. In: Birchard, S.J., Sherding, R.G. (eds.): Saunders manual of small animal practice. 2nd edition. WB Saunders, Philadelphia, USA, 1493-1511.

Sanna, A.M., Shahenda, M.E., Hassan, Z.H. (2012): Effects of some legume on hypercholesterolemia in rats. Journal of American Science, 8, 1453-1460.

Sirtori, C.R., Lovati, M.R., Manzoni, C., Castiglioni, S., Duranti, M. Magni, C., Moranti, S., D´Agostina, A., Arnoldi, A. (2004): Proteins of the white lupin seed, a naturally isoflavone-poor legume, reduce cholestrolemia in rats and increase LDL receptor activity in HepG2 cells. Journal of Nutrition, 134, 18-23.

Šimek, V., Zapletal, D., Straková, E., Pavlík, A., Suchý, P. (2017): Physiological values of some blood indicators in selected dwarf rabbit breeds. World Rabbit Science, 25, 27-36.

Wesche, P. (2014): Clinical pathology. In: Meredith, A., Lord, B. (eds.): Rabbit medicine. 1st edition. BSAVA, Gloucester, UK, 124-137.

ILEAL DIGESTIBILITY OF DOUBLE HAPLOID WHEAT PROTEINS

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ABSTRACT

The aim of our study was to evaluate the potential positive impact of the digestibility of crude protein. In the trials were used males of ROSS 308 aged 35 days, which was fed by mixture containing wheat meal. The used wheat differed by baking value. Sixteen double haploid (DH) winter wheat lines were monitored. Affecting of digestibility were studied using methods ileal digestibility. For the determination of digestibility was chosen indicator method using chromium oxide. Crude protein digestibility was observed only for the diet of pure wheat.

Keywords: wheat; ileal digestibility of protein; broiler chicken; chromium oxide

INTRODUCTION

The importance of wheat in the Czech Republic results from its dominant position among cereals (PRUGAR et al, 2008). Of the total produced wheat in the Czech Republic (5,426.9 thousand tons / year) (SZIF, 2016), the majority share of the produced wheat is used for animal feeding purposes. Cereals are not only a source of energy in animal nutrition but also an important source of protein (ZEMAN et al, 2003).

The feed value of any feed is determined by the content individual nutrients and specifically active substances, their accessibility, balance, energy value, dietetic, specific and other properties, but also the possible presence of undesirable linkages and substances, antinutrients and other depressants. The low nutritional value of cereal proteins is due in particular to the high proportion of prolamine protein fractions characterized by a low content of essential amino acids, especially lysine, tryptophan,

methionine and arginine (STEINBACHOVÁ, 2011). Selective breeding has increased the content of CP from 11,1 % to 13,2 % (CHLOUPEK et al, 2005).

The aim of our experimental research was to determine the ileal digestibility of the nitrogenous substances present in selected DH lines of wheat.

MATERIAL AND METHODS

Into the experimental monitoring were included sixteen DH lines of wheat set in different baking quality. These varieties of wheat have been deliberately chosen because wheat for the purpose of feeding is taken wheat that does not correspond to the quality of wheat processed for human nutrition. Wheat samples were scraped. The experimental diet was composed of wheat and chromium oxide supplement, as an exoindicator for determining digestibility by the indicator method.

The nutritional composition of diets from the varieties used was very balanced. Our goal was to monitor the digestibility of CP, so we were more interested in their content. We assume that differences in plain CP content will not be a factor that will significantly affect the overall digestibility of CP. It will rather be the individual protein fractions determining the baking quality of wheat that can be the interaction between nutrients in the feed.

For the experimental monitoring were used cock of ROSS 308 at 35 days. Chickens were placed in balance cages at 2 pieces. Feeding and infeeding of chickens was a form of *ad libitum*. The diets used for broilers were composed only of wheat scrap with chromium oxide supplement. An experimental monitoring was started after the habitual periods of chicken. The organization and management of the experiments was consistent with recognized chicken experimentation methods as well as requirements for determining ileal digestibility. After 3 days of feeding in the experimental period, chickens were slaughtered and ileum removed. Ileum is a small intestine section of *Diventriculum meckeli* - a residual yolk sac, after branching blind pouches. From the last third of the ileum, 3 cm in front of the branching of the blind pouches, the contents of the ileus were slightly pushed out. For one determination has always been the content of 5 chickens ileum pushed out. This experiment was 2 times repeating. The

digestibility of CP was determined as ileal digestibility when chromium oxide was used as an indicator. The digestibility calculation was according to the formula:

$$\text{Digestible CP} = \frac{\text{I feed} \times \text{N fec}}{\text{I fec} \times \text{N feed}} \times 100$$

I feed - *Indicator in feed*
N fec - *Nutrient in feces*
I fec - *Indicator in feces*
N feed - *Nutrient in feed*
(feces - ileum content)

The removed ileum content was lyophilized nad the CP content was determined in dry matter. All analyzes were carried out in accordance with accepted methods, in feed laboratories by commonly used methodologies.

RESULTS AND DISCUSSION

Table 1. Digestibility of CP (%)

Line of wheat	Digestibility of CP [%]
DH 08 102	82,78
DH 08 104	70,32
DH 08 108	58,05
DH 08 109	75,37
DH 08 113	50,87
DH 08 118	77,54
DH 08 120	78,34
DH 08 121	83,04
DH 08 123	82,23

DH 08 128	88,70
DH 08 137	76,25
DH 08 139	87,78
DH 08 145	75,49
DH 08 116	84,42
Waxy pen	66,80
Waximum	76,76

All results are in dry matter.

This table shows that the best results were achieved at lines DH 08 128 and DH 08 139. On the other hand, the worst results can be seen at lines DH 08 113 and DH 08 108.

CONCLUSION

From the theoretical knowledge and the results of our own work, some conclusions can be proposed which will be of general validity in the use of wheat varieties and hybrids with different baking quality for feed purpose. Also for possible technological modifications of these wheat prior to their use as components of compound feeds.

The results of our experimental observations are more or less only part of the research, as we only evaluated the feed itself - wheat in the biological experiments.
We will continue with other biological experiments, in particular to demonstrate the potential to improve the production value of used feeds and compound feeds for monogastric animals.

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REFERENCES

Chloupek, O., Procházková, B., Hrudová, E. (2005): Plant cultivation and quality. Mendel University in Brno. Brno. 181 p. ISBN: 80-7157-897-5. (in Czech)

Prugar, J. a kol. (2008): Quality of plant products on the 3rd millennium threshold. Research Institute of Brewing and Malting in cooperation with the Plant Products Quality Commission CAAS. Prague. 327 p. ISBN: 978-80-86576-28-2. (in Czech)

Steinbachová, E. (2011): Verification of the nutritional value of grain protein from prospective feed lines and wheat varieties. PhD Diss. Prague: Czech University of Life Sciences Prague. 145 p. Available from: http://www.agrobiologie.cz/pds/dp/steinbachova.pdf. (in Czech)

SZIF (2016): Process of Harvesting of Cereals and Canola in the Czech Republic as of 12 September 2016 (final report) [online]. Available from: https://www.szif.cz/cs/CmDocument?rid=%2Fapa_anon%2Fcs%2Fzpravy%2Fnarodni_dotace%2Fznove_zpravodajstvi%2F1473669339101.pdf. (accessed Aug 12, 2017).

Zeman, L., Procházková, J., Pipálová, S., Klecker, D. (2003): Crop varietal evaluation form in tern of food science and technology. In. Questions of feeding value. Breeding seminary. VÚRV Praha. 20-23.

DAILY RUMEN TEMPERATURE COURSES OF HOLSTEIN DAIRY COWS

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ABSTRACT

The aim of this research was to find daily courses of rumen temperature (RT) and differences in RT between animals using rumen fermentation continuous monitoring with boluses in cooperation with University Experimental farm in Oponice during 24 weeks of lactation. Totally, 7 Holstein cows had implemented bolus for monitoring RpH and RT every 15 minutes with accuracy ± 0.1. Animals were fed once daily with Total Mix Ratio *ad libitum* between 4:00 and 5:00 and milked three times per day at 6:00, 12:00 and 18:00. Statistically significant differences between cows in RT was observed. The difference between maximal and minimal measured RT was 10.2% (from

32.0 °C to 42.2 °C). However, the minimal measured value was limited by bolus capabilities. The difference between maximal and minimal RT monitored for whole period was 2.20% between animal 1049 and 1205. Daily courses of RT by drinking regime, feeding regime and milking were affected. During the feeding day is RT fluctuating. Generally, before milking and feeding RT increased and after the milking and feeding decreased.

Keywords: bolus; cattle; continous monitoring; daily regime; rumen environment

INTRODUCTION

Heat stress in rumminats is manifested by reduction of feed intake, increase in maintenance requirements, milk yeld decrease and quality of milk is declining. It is clear that heat stress has an effect on milk protein and casein production and composition that is greater than the indirect effect of reduced intake (Cowley et al., 2015). Different physiological, lactational, and nutritional responses to heat stress have been reported in ruminants: by Baumgard and Rhoads (2013) in dairy cows, and by Hamzaoui et al. (2013) and Salama et al. (2014) in dairy goats. Attempts to measure the body temperature of cattle have been made at various locations, including the rectum, ear (tympanic), vagina, reticulorumen, intraperitoneal cavity, and udder (milk) (Hicks et al., 2011; Aalseth, 2005).The reticuloruminal temperature may have more variation because of equipment errors, the influence of water intake, more within- or among-animal variation, changes in VFA production within the reticulorumen, greater sensitivity to ambient conditions, or increased ability to detect physiological changes. Moreover, there is a strong relationship between ruminal and rectal temperature (Rose-Dye et al., 2011) and between the reticular temperature and intake water temperature or rectal temperature in healthy cows. When cows consume large quantities of cold water, the effect of water intake is sizable and sustained (Bewley, 2008ab). The aim of this research was to find daily courses of RT and differences in RT between animals using rumen fermentation continuous monitoring with boluses.

MATERIAL AND METHODS

Experiment in cooperation with the University Experimental Farm in Oponice during 24 weeks of lactation was realised. Selected 7 cows of Holstein breed (average age 3.57) had average milk production 10 175 kg per lactation with 3.94% of fats, 3.10% of crude proteins and 4.7% of lactose. From 7 cows were 3 in the 2nd lactation and 4 in the 3rd lactation. Experimental cows were loose housed with laying boxes system and automatic manure scraper in the manure corridor in the groups with another dairy cows together. Daily diet on the feeding table was folded. For 20 dairy cows two drinkers in one section were available. Animals were milked 3 times per day at 6:00, 12:00 and 18:00 in the fishbone milking parlour for 20 cows.

Feeding

Animals were fed once daily with Total Mix Ratio (TMR 1: 16,81 kg DM; 99,87 MJ NEL; 16,44 % CP; 25,85 % NDF; 20,42 % starch; TMR 2: 25,45 kg DM; 153,86 MJ NEL; 15,74 % CP; 24,35 % NDF; 25.39 % starch) ad libitum between 4:00 and 5:00. TMR consisted of corn silage, alfalfa silage, feed mixture, high moisture corn, cotton seed and straw. Feed mixture in the daily diet consisted of soybean meal, rapeseed meal, oat grain, corn grain, DDGS and by pass fat. Corn silage acidity (pH 3.85) and alfalfa silage acidity (pH 4.85) with Sodium Bicarbonate (daily 550 g.head-1) and Magnesium Oxide (daily 51 g.head-1) were neutralised. Daily diet was automatically reeled 3 times per day.

Data measuring, data collecting and statistical evaluation

Every dairy cow had implemented farm bolus for continual data measuring which was implemented through esophagus orally with the use of special balling gun. Ruminal pH and temperature values were measured every 15 minutes (96 data points per day) with accuracy ± 0.1 for pH. Used boluses (eCowDevon, Ltd., Great Britain) are characteristic with its small dimensions (135 x 27 mm) and weight 207 g. Data with the handset with antenna and dongle connected with USB dongle connector with the radio frequency 434 MHz in the milking parlour were downloaded. Collected data were summarized with HathorHBClient v. 1.8.1 and statistically evaluated with IBM SPSS v. 20.0 (One-way ANOVA, Tukey Test, Pearson correlation test).

RESULTS AND DISCUSSION

Daily courses of RT by drinking regime, feeding regime and milking were affected (Table 1). The peaks of water intake mainly after the return from milking parlour were found. The first decrease of RT at the time of feeding (1.01%, $p<0.01$ at 4:00; 1.89%, $p<0.01$ at 5:00 and 0.22% at 6:00) was found. After that an increase of RT (0.64% at 7:00, $p<0.01$ and 0.25% at 8:00) was found. Furthermore, this increase continued to the time of the first milking except 9:00. After the returning from the milking parlour were dairy cows drinking. It is demonstrated on the fall of RT at 12:00 (0.16%) and 13:00 (0.55%; $p<0.01$). However, another increase of RT after this declining of RT was found. At the time before third milking RT values rose by 0.37% (14:00), 1.14% (15:00; $p<0.01$), 0.36% (16:00; $p<0.01$). After that, a decrease of RT for 5 consecutive hours was determined. Moreover, at the time of third milking (18:00), a fall by 0.77% was found. Resting period of animals by lower water intake was accompanied. It is noticeable in the growth of average RT from 22:00 to 3:00. At 22:00 (0.70%) and 23:00 (0.91%) statistically significant increase of RT ($p<0.01$) was found. Then, this rise of RT continued to the time of the morning feeding. The biggest difference between 3:00 and 6:00 (3.08%; $p<0.01$) was found. Ammer et al. (2016) in their research found the average mean of RT 38.5°C in range from 36.9°C to 41.5°C. The highest RT in the morning (38.8–38.9°C), followed by evening (38.4–38.8°C) and midday (38.2–38.5°C) were found (Ammer et al., 2016).

Table 1- Daily courses of the ruminal temperature in °C

H	$\bar{x}$	SD	Min	Max
0	39.18^{ab}	1.04	32.3	41.5
1	39.31^{b}	0.90	33.3	41.5
2	39.47^{c}	0.76	34.5	41.8
3	39.49^{c}	0.75	32.0	41.6
4	39.09^{a}	1.13	32.5	41.3
5	38.35^{dehl}	1.39	32.1	41.9
6	38.27^{e}	1.33	32.0	42.2

7	38.51^{fghjmq}	1.15	32.2	41.5
8	38.61^{ghikqrs}	1.18	32.3	41.5
9	38.49^{hijkmt}	1.24	32.5	41.3
10	38.55^{fijq}	1.20	32.2	41.2
11	38.60^{gjkqrsu}	1.21	32.0	41.1
12	38.54^{fkmq}	1.20	32.3	40.9
13	38.33^{el}	1.31	32.2	41.1
14	38.47^{djlm}	1.28	32.2	41.3
15	38.91^{n}	1.07	32.3	41.4
16	39.05^{ao}	1.14	32.9	41.5
17	38.93^{nop}	1.35	32.1	41.4
18	38.63^{qr}	1.38	32.2	41.6
19	38.53^{fhmr}	1.28	32.1	41.4
20	38.48^{dfhs}	1.25	32.4	41.3
21	38.44^{dflt}	1.28	32.0	41.3
22	38.71^{qu}	1.15	32.2	41.3
23	39.06^{ap}	1.01	34.0	41.4
DA	38.75	1.23	32.0	42.2

Different letters in the columns indicate significant differences. The mean difference is significant at the 0.05 level (Tukey Test).

abbreviations: H – hour, $\bar{x}$ – sample mean, SD – standard deviation, Min – minimal value, Max – maximal value, DA – daily average

Statistically significant difference between cows in RT was found (Table 2). There was found a great impact of water intake on RT. This resulted in a wide range (10.2°C) between minimal and maximal RT measured during monitored period. Furthermore, the difference between maximal and minimal detected RT was 24.17%. Overall, minimal detected values of ruminal RT by the temperature sensor of bolus were limited. Finally, the maximal difference between animal 1049 and 1205 2.20% ($p<0.01$) was found. RT between 38 and 40°C are optimal for rumen microbial fermentation. Peak microbial fermentation occurs after feeding, and RT may rise as high as 41°C (Dehority, 2004).

RT are related to animals activities like water intake, feeding and milking (Ammer et al., 2016). It is important to note that normal core body temperature has been reported to range from 38.0 to 39.17°C. Thus, cows with core body temperature exceeding 39.1°C were defined as febrile or abnormal (Divers and Peek, 2008). Gasteiner et al. (2009) found in their experiment average RT from 38.12 ± 0.80°C to 38.55 ± 0.83°C. Bodas (2014) found RT from 34.57°C to 39.78°C with average 38.77°C.

Table 2. Differences in ruminal temperature between dairy cows in °C

Cow ID	$\bar{x}$	SD	Min	Max
1023	38.84^{ac}	1.36	32.1	42.2
1038	38.49^{b}	1.18	32.1	40.6
1049	38.45^{b}	1.10	32.0	41.8
1053	38.81^{ac}	1.28	32.0	41.5
1203	38.89^{c}	1.11	32.1	41.3
1204	38.77^{a}	1.13	32.1	41.7
1205	39.32^{d}	1.23	32.0	41.6
Total	38.75	1.23	32.0	42.2

Different letters in the columns indicate significant differences. The mean difference is significant at the 0.05 level (Tukey Test). Abbreviations: $\bar{x}$ – mean, SD – standard deviation, Min – minimal value, Max – maximal value, TA – total average

CONCLUSION

Statistically significant differences between cows in RT was observed. The difference between maximal and minimal measured RT was 10.2% (from 32.0 °C to 42.2 °C). However, the minimal measured value was limited by bolus capabilities. The difference between maximal and minimal RT monitored for whole period was 2.20% between animal 1049 and 1205. Daily courses of RT by drinking regime, feeding regime and milking were affected. During the feeding day is RT fluctuating. Generally, before milking and feeding RT increased and after the milking and feeding decreased.

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REFERENCES

Aalseth E. (2005): Fresh cow management: What is important, what does it cost, and what does it return. In Proc. Western Dairy Management Conf., Reno, NV, 1-12.

Ammer S., Lambertz C., Gauly M. (2016): Comparison of different measuring methods for body temperature in lactating cows under different climatic conditions. Journal of Dairy Research, 83, 2, 165–172.

Baumgard L. H., Rhoads R. P. (2013): Effects of Heat Stress on Postabsorptive Metabolism and Energetics. Annual Review of Animal Biosciences, 1, 1, 311–337.

Bewley J. M., Einstein M. E., Grott M. W., Schutz M. M. (2008a): Comparison of Reticular and Rectal Core Body Temperatures in Lactating Dairy Cows. Journal of Dairy Science, 91, 12, 4661–4672.

Bewley J. M., Grott M. W., Einstein M. E., Schutz M. M. (2008b): Impact of Intake Water Temperatures on Reticular Temperatures of Lactating Dairy Cows. Journal of Dairy Science, 91, 10, 3880–3887.

Cowley F. C., Barber D. G., Houlihan A. V., Poppi D. P. (2015): Immediate and residual effects of heat stress and restricted intake on milk protein and casein composition and energy metabolism. Journal of Dairy Science, 98, 4, 2356–2368.

Dehority B. A. (2004). Cellulose digesting rumen bacteria. In Dehority BA (ed) Rumen microbiology. Nottingham University Press, Nottingham, pp 177–208.

Divers T.J., Peek S.F. (2008): Rebhun's Diseases of Dairy Cattle (2nd Ed.), Saunders Elsevier, St. Louis, Missouri. ISBN 978-1-4160-3137-6

Gasteiner J., Fallast M., Rosenkranz S., Häusler J., Schneider K., Guggenberger T. (2009): Measuring rumen pH and temperature by an indwelling and wireless data transmitting unit and application under different feeding conditions. In 4th European Conference on Precision Livestock Farming, 127-133.

Hamzaoui S., Salama A. A. K., Albanell E., Such X., Caja G. (2013): Physiological responses and lactational performances of late-lactation dairy goats under heat stress conditions. Journal of Dairy Science, 96, 10, 6355–6365.

Hicks L. C., Hicks W. S., Bucklin R. A., Shearer J. K., Bray D. R., Soto P., Carvalho V. (2001): Comparison of methods of measuring deep body temperatures of dairy cows. In Livestock Environment VI, Proceedings of the 6th International Symposium 2001, 432, American Society of Agricultural and Biological Engineers.

Rose-Dye T. K., Burciaga-Robles L. O., Krehbiel C. R., Step D. L., Fulton R. W., Confer A. W., Richards C. J. (2010): Rumen temperature change monitored with remote

rumen temperature boluses after challenges with bovine viral diarrhea virus and Mannheimia haemolytica. Journal of Animal Science, 89, 4, 1193–1200.

Salama A. A. K., Caja G., Hamzaoui S., Badaoui B., Castro-Costa A., Façanha D. A. E., Bozzi R. (2014): Different levels of response to heat stress in dairy goats. Small Ruminant Research, 121, 1, 73–79.

THE EFFECT OF APPLYING OF *LACTOBACILLUS PLANTARUM* WITH *LACTOBACILLUS BREVIS* TO THE FERMENTATION OF GRASS SILAGES

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ABSTRACT

The aim of the experiment was to analyze the effect of the addition of *Lactobacillus plantarum* with *Lactobacillus brevis* on the fermentation quality of grass silages from permanent grassland after 180 days of ensilage in silage bags. The phytomass with the prevalence of *Arrhenantherumelatius* (L.) (36%) was ensiled. Grass matter was harvested at early boom stage and wilted 24 hours. Subsequently was wilted matter chopped with a self-propelled forage harvester at 50 mm length. Wilted grass matter was ensiled into the white plastic silage bags with diameter 2.7 m using silage press in two variants: variant C without additive and variant A with addition of biological additive (*Lactobacillus plantarum* and *Lactobacillus brevis* $2x10^5$ $cfu.g^{-1}$). After 180 days of ensilage, nutrients and parameters of the fermentation process were determined. Addition of a biological additive containing *Lactobacillus plantarum* and *Lactobacillus brevis* influenced the fermentation process of silages from permanent grassland

with a significantly higher concentration of acetic acid and significantly lower alcohols content, acidity of water extract and value of pH after 2 months of fermentation. Application of biological additive containing homo and heterofermentative lactic acid bacteria nonsignificantly decreased butyric acid content and degree of proteolysis.

Keywords: grass silage; permanent grassland; fermentation; *Lactobacillus brevis*; *Lactobacillus plantarum*

INTRODUCTION

Nutritive value of phytomass of permanent grassland is very variable and depends on many factors: spread species, cultivar differences, maturity stage, the number of cuttings, fertilization, soil and climate conditions, management system (Vozár et al., 2012). Grasses have wide use in animal nutrition both for direct feeding (fresh pasture) but also as conserved forage (hay or silage) (Jendrišáková et al., 2011). The versatile use of grasses for feeding results from their chemical composition, the ratio and structure of nutrients, but also from their biological value and dietetic properties. In comparison with legumes, grasses have lower crude protein and Ca content, but higher fat, nonstructural carbohydrates content and hence higher energy value. From the point of ensilability grasses belong to the group with the hard ensilability due to the higher buffering capacity and lower water-soluble carbohydrates content. Grasses are silaged with multi-phase harvesting technology with wilting (Bíro et al., 2014). One of the possibilities for improve the silage quality is to use silage additives (Doležal and Hejduk, 2002; Lád et al., 2006). Bacterial inoculants usually contain homofermenative lactic acid bacteria which are used to influence optimal fermentation and some inoculants contain heterofermentative lactic acid bacteria for improve aerobic stability of silage (Majlát et al., 2016). *Lactobacillus brevis* is obligate heterofermentative lactic acid bacteria, which hexoses ferment to lactic acid, carbon dioxide and ethanol (or acetic acid in the presence of an alternative electron acceptor) and pentoses convert to lactic and acetic acid (Holzer et al., 2003). The aim of the experiment was to analyze the effect of the addition of *Lactobacillus plantarum* with *Lactobacillus brevis* on the fermentation quality of grass silages from permanent grassland after 180 days of ensilage in silage bags.

MATERIAL AND METHODS

The experiment was carried out in farm Klenovec, located in a sub-mountain area in Rimavská Sobota district (middle part of Slovakia). The phytomass from permanent grassland with the prevalence of *Arrhenantherumelatius* (L.) (36%) was ensiled. Grass matter was harvested at early boom stage and wilted 24 hours. Subsequently was wilted matter chopped with a self-propelled forage harvester at 50 mm length and inoculated with biological additive which contained homo and heterofermentative lactic acid bacteria (*Lactobacillus plantarum* and *Lactobacillus brevis* $2x10^5$ $cfu.g^{-1}$) in dose 1 liter per 1 ton (with the recommended dose of 4 g of additive per 1 liter). Wilted grass matter was ensiled into the white plastic silage bags with diameter 2.7 m using silage press in two variants. Variant C was without silage additive and variant A with biological additive. We have taken samples (n=3) for laboratory analysis to determine the nutrients and parameters of fermentation process of grass silages after 180 days of ensilage. Dry matter was determined by drying at 103±2°C and Crude protein by using the Kjeldahl's method. Silage extracts were prepared from 200 g of sample and overflowed by 2000 ml of distilled water, after 20 hours stained. Contents of fermentation acids (lactic, acetic, butyric) was detected on analyzer EA 100 (Villa Labeco, SR) using the ionic electrophoresis method. Content of alcohols and NH_3 were determined by microdiffusion method, acidity of water extract by alkalimetric titration to pH 8.5 and active acidity (pH) by electrometric method. Fermentation products were calculated by count of fermentable acids with alcohols. The degree of proteolysis was calculated according to following formula: degree of proteolysis (%) = $N\text{-}NH_3$ / total N x 100. Collected data were statistically evaluated with IBM SPSS v. 20.0 (One-way ANNOVA, Tukey Test).

RESULTS AND DISCUSSION

Silage without additive had average dry matter content 352.2 g/kg and silage with biological additive 365.5 g/kg, after 180 days of ensilage. Dry matter content was optimal for grass silage. According to Skládanka et al. (2014) is target value of dry matter for grass silages from 350 to 400 g/kg, and according to Čunderlíková et al. (2003) 350-400 g/kg. After 6 months of fermentation, lower content of lactic acid in the variant with additive was found, but differences

were not statistically significant (Table 1). This is consistent with the previous finding that inoculation with heterofermentative lactic acid bacteria (^{he}LAB) decreased the concentrations of lactic acid (Kleinschmit and Kung, 2006; Rabelo et al., 2016). Silages with biological additive (*L. plantarum* + *L. brevis*) had significantly ($P<0.05$) higher content of acetic acid. Our results confirmed that addition of *L. brevis* increase content and ratio of acetic acid, when ratio of lactic to acetic acid in C silages was 8.95:1 and in A silages 7.3:1. These results are in agreement with Nishino and Touno (2005), Tabacco et al. (2011), Knicky et al. (2014). In the control silages, the percentage share of acetic acid from the total content of fermentation acids, was 9.96% and in experimental silages 12.03%. Butyric acid as main product of *Clostridium* is undesirable fermentation product in silages. Non-significantly ($P>0.05$) lower content of butyric acid was identified in silages treated with biological additive. Butyric acid concentration was decreased in silages with ^{he}LAB in study of Nishino and Touno (2005) and Jatkauskas et al. (2013), but differences were significant. Our results confirmed that alcohol production was significantly reduced ($P<0.05$) by addition of the biological additive containing the *Lactobacillus brevis*. Silages A had lower content of alcohols by 38.5% in comparison with control. Nishino and Touno (2005) also reported lower content of alcohols in grass silages with *L. casei*. Driehuis et al. (2001) observed that *L. buchneri* (^{he}LAB) with homofermentative LAB decreased content of ethanol in grass silage after 90 days ensilage in jars. By contrast, Kleinschmit and Kung (2006) reported, that concentrations of ethanol in silages treated with *L. buchneri* (^{he}LAB) were greater than in untreated silage. Inoculation decreased content of fermentation products, what is in agreement with results of Kang et al. (2009). Silages with additive were characterized by significantly lower ($P<0.05$) acidity of water extract than untreated silages (C). Non-significantly ($P>0.05$) lower value of degree of proteolysis was reported in silages with biological additive. Silages inoculated with *L. buchneri* plus *P. pentosaceus* and *L. plantarum* had lower concentrations of ammonia-N than uninoculated silage in experiments of Driehuis et al. (2001) too. The decreased production of butyric acid resulted in significantly ($P<0.05$) lower pH value in silages with biological additive.

Table 1. Fermentation quality of grass silages

Variant /SP		LA	AA	BA	OH^-	FP	AWE	DP	pH
		g/kg of dry matter					mg*	%	
C	$\bar{x}$	74.74	8.35[a]	0.72	6.31[a]	87.58	1790.82[a]	7.66	4.21[a]
	S.D.	3.50	0.08	0.13	1.19	7.50	25.85	0.07	0.09
A	$\bar{x}$	74.65	10.23[a]	0.18	3.88[a]	93.89	1473.38[a]	7.11	3.91[a]
	S.D.	7.73	0.08	0.02	0.41	2.68	53.91	0.56	0.18

C - control, A - with additive, SP - statistical parameters, LA - lactic acid, AA - acetic acid, BA - butyric acid, OH^- - alcohols, FP -fermentation products, AWE - acidity of water extract, DP - degree of proteolysis, values with the same index in column are significant at $P<0.05$, *mg KOH/100 g

CONCLUSION

Addition of a biological additive on the base *Lactobacillus plantarum* and *Lactobacillus brevis* influenced the fermentation process of silages from permanent grassland with a significantly higher concentration of acetic acid and significantly lower alcohols content, acidity of water extract and value of pH after 180 days of ensilage. Application of biological additive containing homo and heterofermentative lactic acid bacteria non-significantly decreased butyric acid content and degree of proteolysis.

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REFERENCES

Bíro D., Juráček M., Šimko M., Gálik B., Rolinec M. (eds) (2014): Conservation and adjustment of feeds. Slovak University of Agriculture, Nitra, Slovakia. (in Slovak)

Čunderlíková M., Polák M., Kunský M. (2003): Nutritive value and digestibility of grass and grass/lucerne silage preserved in big bales. In: Forage conservation, Nitra, RIAP, Slovakia, 170–171.

Doležal P., Hejduk S. (2002): The effect of inoculation on fermentation quality of wilted grass silage. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 50, 67–74. (in Czech)

Driehuis F., OudeElferink S.J.W.H., Van Wikselaar P.G. (2001): Fermentation characteristics and aerobic stability of grass silage inoculated with *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria. Grass and Forage Science, 56, 330–343.

Holzer M., Mayrhuber E., Danner H., Braun R. (2003): The role of *Lactobacillus buchneri* in forage preservation. TRENDS in Biotechnology, 21, 282–287.

Jatkauskas J., Vrotniakiene V., Ohlsson Ch., Lund B. (2013): The effect of three silage inoculants on aerobic stability in grass, clover-grass, lucerne and maize silages. Agricultural and Feed Science, 22, 137–144.

Jendrišáková S., Kováčiková Z., Vargová V., Michalec M. (2011): The impact of cattle and sheep grazing on grassland in Veľká Fatra National Park. Journal of Water and Land Development, 15, 83–90.

Kang T.W., Adesogan A.T., Kim S.C., Lee S.S. (2009): Effects of an esterase-producing inoculant on fermentation, aerobic stability, and neutraldetergent fiber digestibility of corn silage. Journal of Dairy Science, 92, 732–738.

Kleinschmit D.H., Kung L. (2006): A meta-analysis of the effects of *Lactobacillus buchneri* on the fermentation and aerobic stability of corn and grass and small-grain silages. Journal of Dairy Science, 89, 4005–4013.

Knicky M., Wiberg H-G., Eide F., Gertzell B. (2014): Dynamics of gas formation during ensilage. In: Proc. of the 5th Nordic Feed Science Conference, Uppsala, Sweden, 41–46.

Lád F., Čermák B., Jančík F., Kadlec J. (2006): The influence of silage additives for qualitative parameters of clover-grass silages. Journal of Central European Agriculture, 7, 313–318.

Majlát M., Juráček M., Bíro D., Šimko M., Gálik B., Rolinec M., Herkeľ, R. (2016): The effect of aerobic exposure on nutritive value and fermentation parameters of maize silage. Journal of Central European Agriculture, 17, 335–345.

Nishino N., Touno E. (2005): Ensiling characteristics and aerobic stability of direct-cut and wilted grass silages inoculated with *Lactobacillus casei* or *Lactobacillus buchneri*. Journal of the Science of Food and Agriculture, 85, 1882–1888.

Rabelo C.H.S., Basso F.C., McAllister T.A., Lage J.F., Gonçalves G.S., Lara E.C., Oliveira A.A., Berchielli T.T., Reis R.A. (2016): Influence of *Lactobacillus buchneri* as silage additive and forage: concentrate ratio on the growth performance, fatty acid profile in longissimus muscle, and meat quality of beef cattle. Canadian Journal of Animal Science, 96, 550–562.

Skládanka J., Cagaš B., Doležal P. et al. (eds) (2014): Fodder crops production. Mendel University in Brno, Czech Republic. (in Czech)

Tabacco E., Piano S., Revello-Chion A., Borreani G. (2011): Effect of *Lactobacillus buchneri* LN4637 and *Lactobacillus buchneri* LN40177 on the aerobic stability, fermentation products, and microbial populations of corn silage under farm conditions. Journal of Dairy Science, 94, 5589–5598.

Vozár Ľ., Jančovič J, Kovár P., Bačová S. (2012): Adaptability of permanent grassland to drought. Journal of Life Sciences, 6, 1057–1060.

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