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CONTENT

THE INFLUENCE OF GRAZING AND MOWING FREQUENCY ON THE BASIC NUTRIENT CONTENT IN PASTURE VEGETATION

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Abstract

The aim of this study was to assess the effect of the frequency of pastoral herding in experimental plots at the content of the nutrients in pasture growth. To further evaluate when forages in terms of nutrient content, and in particular the most suitable fiber for cattle, and to compare the results obtained regarding the content studied nutrients in pasture vegetation, with optimal values.

For a basic evaluation of the nutrient content in pasture samples was used the method of NIR with device InfraXact. InfraXact works on the principle of reflectance and transflectance close to infrared range (NIR) at wavelengths from 570 to 1850 nm. This method can determine the content of basic nutrients in the feed in a few minutes.

The results lead to the conclusion that increased grazing in the same area slightly increases the nutrient content in the biomass of pasture vegetation on the surface. Therefore, it is recommended practice to graze cattle in smaller plots and during the grazing season make many turns (do many changes between pastures plots).

Keywords: grazing, pasture vegetation, grass mowing, nutrients

Grazing is a natural way to obtain food for ruminants. It positively influences the overall economy breeding, but also the health of the herd. The result is as good as production, but also the reproductive performance of the herd.

Grazing management should notably regulate the pasture composition. Good pasture and grazing management can improve nutrients and botanical structure (Čermák et al., 2004). A high botanical biodiversity expressed by the presence of many herbs is especially characteristic for the pastures located at higher altitudes. Herbs contribute to the improved pasture quality having a beneficial influence on the digestion process, animal health and quality of animal products (Søegaard *et al.,* 2009). There are appreciable differences in preference or deprecation various plant species out at pasture. Some of plant species have positive influence on animal health and digestion (Hejduk, 2007). Grasslands fertilizing is used for increasing of forage yields and quality and simultaneously for soil fertility maintenance (Hejduk, 2011). The yield and quality of forage is determined primarily by fertilization and intensity of use (Nawrath et al, 2012).

The yield of grassland is different. The annual production of dry feed is about 1.0 to 15.0 $t.ha^{-1}$ dry weight (Hejduk et al, 2012). During the grazing season in a sward reduced crude protein content while increasing fiber content (Volfová et al, 2012).

Material and methods

Samples were collected in areas for research. The experiment took place at two areas in piedmont areas of Mountain Šumava (Vysoký Chuchelec and Rojov) for two years (2011, 2012). Biomass samples were weighed, dried in a drying at 60 ° C to constant weight. After drying, the samples were scrapped particle size of 2 mm, and then used for further analytical processing.

For evaluation of the obtained samples was applied NIR method. This method quickly provides information on the content of essential nutrients in a given sample. NIR

spectroscopic analysis is a method which utilizes naturally occurring electromagnetic spectrum. NIR region of the spectrum is defined wavelengths between 700 nm and 2500 nm. This area proved to be ideal spectral region for the production of natural products and measurement of chemical properties of various samples (solid, liquid or gaseous). InfraXact device was used that works on the principle of reflectance and transflektance in the near infrared (NIR) at wavelengths from 570 to 1850 nm. The samples were evaluated using the device InfraXact for ash, fat, neutral detergent fibre (NDF), crude protein (NL). The nutrient content in the samples was compared with the results reported in the literature (Zeman, 1995).

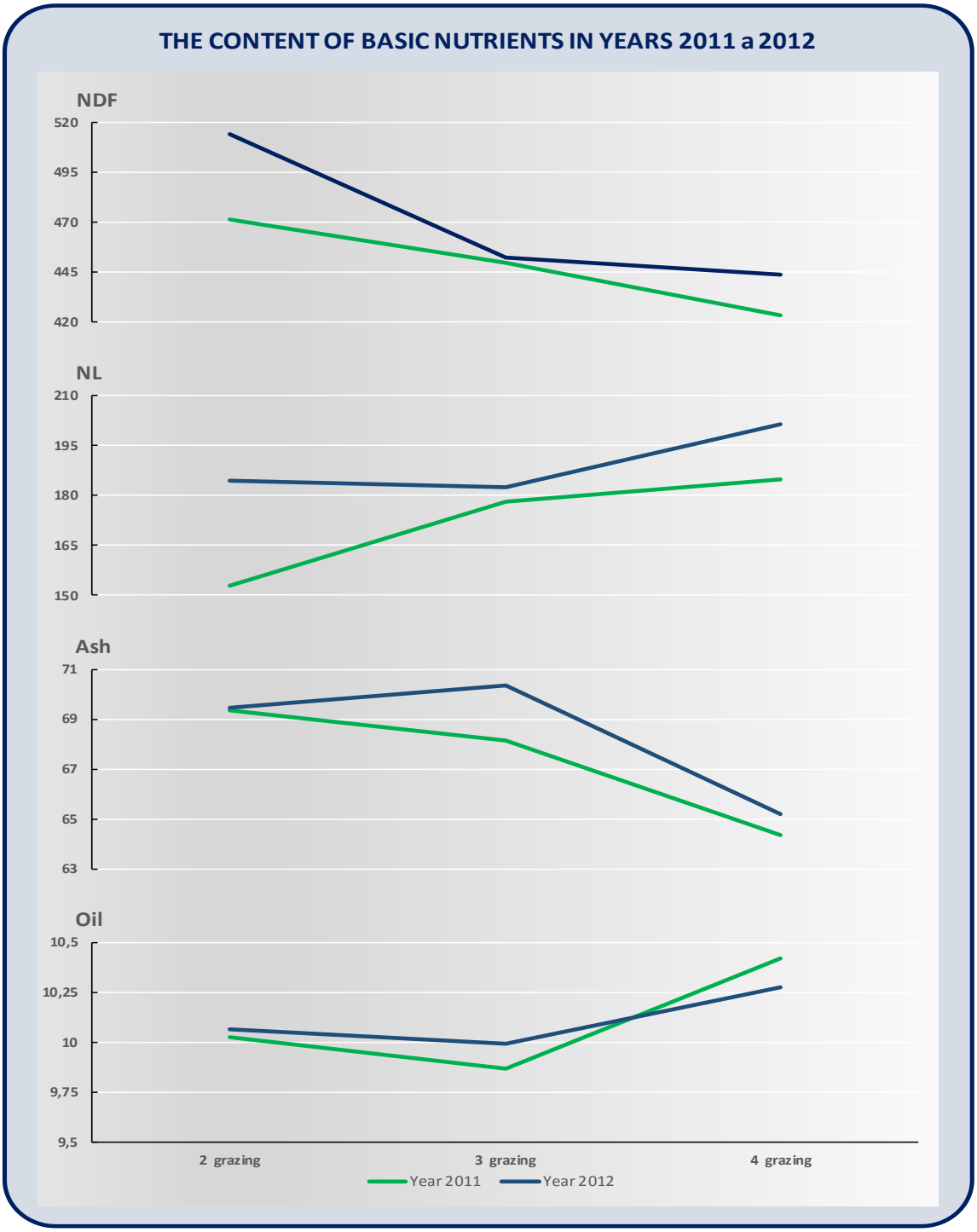
Results and discussion

The table 1 shows the nutrient content in samples which were grazed or mown. The content of ash, oil, NDF and NL was determined by device InfraXact with NIR method. There are samples from two areas (Rojov, V. Chuchelec) and the results from two years. Numbers 1, 2, and 3 means how many time was each of plots mown (cut) or grazed. he results show that multiple grazing livestock (cattle) in the same area slightly increases the nutrient content in the biomass of pasture vegetation. The values found correspond to the values of nutrients reported in the literature (Zeman, 1995).

Table 1: The nutrient content in samples of vegetation son pasture grazed and mown plots in locations Rojov and Vysoký Chuchelec in years 2011 and 2012

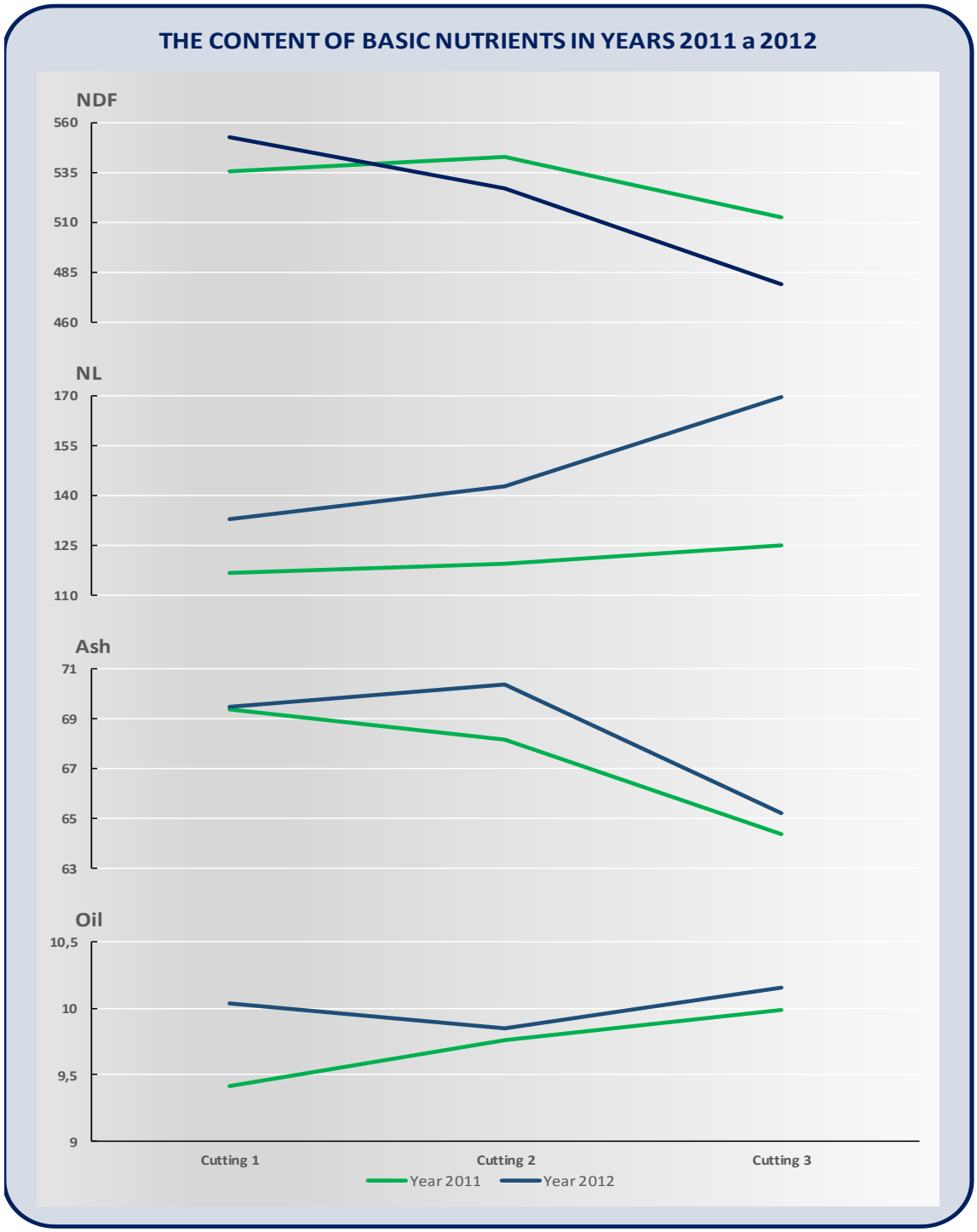
Area	Nutrients	Year	Cutting frequency				Grazing frequency			
			1	2	3		2	3	4	
Rojov	**Ash**	2011	52,70	54,27	55,66		64,63	66,15	62,10	
		2012	48,51	56,94	63,77		67,35	68,46	62,92	
		Avarage	**50,61**	**55,60**	**59,71**		**65,99**	**67,30**	**62,51**	
	Oil	2011	9,50	9,85	10,04		9,55	9,42	9,93	
		2012	10,05	9,74	9,81		9,84	9,74	9,85	
		Avarage	**9,78**	**9,79**	**9,92**		**9,70**	**9,58**	**9,89**	
	NDF	2011	530,25	515,95	497,77		472,53	456,62	446,26	
		2012	573,86	475,10	472,34		504,77	443,48	448,40	
		Avarage	**552,06**	**495,52**	**485,05**		**488,65**	**450,05**	**447,33**	
	NL	2011	123,43	125,33	129,96		137,36	169,37	175,95	
		2012	126,92	134,96	159,87		172,42	162,84	181,45	
		Avarage	**125,18**	**130,14**	**144,91**		**154,89**	**166,10**	**178,70**	
V. Chuchelec	**Ash**	2011	56,30	64,36	79,24		74,13	70,18	66,59	
		2012	55,41	58,80	70,18		71,64	70,31	67,55	
		Avarage	**55,86**	**61,58**	**74,71**		**72,88**	**70,25**	**67,07**	
	Oil	2011	9,32	9,67	9,93		10,51	10,33	10,91	
		2012	10,02	9,97	10,51		10,30	10,26	10,70	
		Avarage	**9,67**	**9,82**	**10,22**		**10,40**	**10,29**	**10,81**	
	NDF	2011	540,36	570,04	527,36		470,62	442,22	400,41	
		2012	532,23	579,69	485,74		522,90	461,01	438,55	
		Avarage	**536,30**	**574,86**	**506,55**		**496,76**	**451,61**	**419,48**	
	NL	2011	110,31	113,30	120,18		168,17	186,66	193,24	
		2012	138,65	150,67	179,30		196,33	201,90	221,36	
		Avarage	**124,48**	**131,98**	**149,74**		**182,25**	**194,28**	**207,30**	

Table 2: The content of nutrients from the grazing samples



The table 2 and No. 3 shows a graphical representation of the individual nutrients with changes their contents depending on the frequency of mowing or grazing. The amount of NL is directly proportional to the number of mowing and grazing.

Table 3: The content of nutrients from the mowing samples



Conclusion

For the determination of nutrients in the samples pasture was used NIR method by a machine InfraXact. The samples were evaluated using the device InfraXact for ash, fat, neutral detergent fibre (NDF), crude protein (CP).

The amount of CP is directly proportional to the number of mowing and grazing, in contrast to NDF and ASH, wherein the amount of the increasing number of mowing and grazing decreases. Based on the results we can not make a definite conclusion about the dependence of the amount of fat (oil) and the number of mowing and grazing.

From the obtained results we can formulate the conclusion that in these locations (Rojov and Velký Chuchelec) is optimal in terms of nutrient content in the biomass of pasture vegetation appears to be the most appropriate method of grazing livestock, because multiple grazing livestock (cattle) in the same area slightly increases the nutrient content in the biomass of pasture vegetation.

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References

1. Čermák, B., Donald, M., Ball, C., Hoveland, S., Garry, D., Lacefield, Frelich, J.: Vliv kvality krmiv na produkci a zdravotní nezávadnost mléka a masa. Vědecko-odborná publikace, České Budějovice 2004, pp. 167, ISBN 80-7040-744-1
2. Hejduk, S., Sochorec, M., Raus, J.: Ekosystémové funkce travních porostů, Mendelova univerzita v Brně, 2012, pp. 25
3. Hejduk, S.: Changes of soil agrichemical characteristics in pastures influenced by mineral fertilizing. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis.* 2011. sv. 59, n. 1, pp. 113–119, ISSN 1211-8516.
4. Hejduk, S.: Kvalita píce při extenzivním využívání pastvin. Náš chov, 2007, n. 3, pp. 102-106.
5. Nawrath, A., Skládanka, J., Hrabě, F.: Vliv hnojení a intenzity využívání na produkci, druhovou diverzitu a kvalitu travního porostu, Mendelova univerzita v Brně, MendelNet 2012, pp. 126-132
6. Søegaard K., Eriksen J., Askegaard M.: Herbs in the grassland. ICROFS news, n. 3, pp. 5-6, 2009.
7. Volfová, K., Frelich, J., Čermák, B., Petrášková, E. Kobes, M.: Kvalitativní parametry pastevních porostů v různých nadmořských výškách, Jihočeská univerzita v Českých Budějovicích, 2012, pp. 40-45, ISBN 978-80-7394-345-5.
8. Zeman, L. et al.: Katalog krmiv (Tabulky výživné hodnoty krmiv), VÚVZ Pohořelice, 1995, pp. 465, ISBN 80-901598-3-4

EFFECTS OF FLAX SEED SUPPLEMENTATION ON THE CONTENT OF POLYUNSATURATED FATTY ACIDS IN GOAT´S MILK

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Abstract

Goat's milk is very valuable and desirable food for their high digestibility and unique composition. It contains only a small amount of milk protein casein and is therefore suitable for people with allergy to casein. The aim of this study was to assess the influence of added flax seed on fatty acids content in goat´s milk. Two groups of goats were fed *ad libitum* intake of meadow hay and concentrate, which contained for the experimental group 0.1 kg of linseed. The experiment lasted two months with a 14-day preparatory phase. The results of analyzes of the experimental group showed increasing content of PUFA, the most important was a significant increase in CLA content. This fact has considerable importance in human´s nutrition and health.

Keywords: goat, milk, flax seed, fatty acids, PUFA, CLA

Goat is one of the oldest domesticated animals, and probably the first livestock bred by human (Fantová et al., 2000). Goats are bred all around the world for their good productive qualities, hardiness and excellent ability to adapt to challenging conditions. Their milk has an extra special dietary and sensory properties, for which is prized in many countries. Goat´s milk was the first milk used in human nutrition (Vejčík and Král, 1998) because is very similar to breast milk and is easily digestible (Gilbert, 2003). It is suitable for people with digestive problems, because is slightly alkaline - compared to cow's milk, therefore suitable for people suffering from acidity and stomach ulcers. It is also suitable for people suffering from eczema, asthma, migraines, colitis, constipation and stress syndromes (Jandal, 1996).

In comparison to cow´s milk are proteins of goat´s milk easily digestible and also basic amino acids are better absorbed (Jandal, 1996 and Ceballos et al., 2009). In addition, goat´s milk contains six out of ten essential amino acids in higher amounts (Haenlein, 2004).

One of milk proteins, casein, may cause allergy to milk. However, goat milk contains this protein only in small amount and for this reason is suitable for people suffering from casein allergy (Raynal-Ljutovac et al., 2008).

Fatty acids profile of goat´s milk is significantly different from other ruminant animals (Park et al. 2007). Goat´s milk contains more essential fatty acids (linoleic, arachidonic acid) than other milk (Goat's Milk [web] 1997). Also there are more unsaturated fatty acids than in cow´s milk. Park et al. (2007) reported that most of the fat (75 %) consists of five fatty acids: oleic (C18:1-n9), palmitic acid (C16:0), stearic (C18:0), myristic (C14:0) and capric (C10:0).

Materials and methods

The aim of this study was to assess the effect of addition of flaxseed to the diet on fatty acids in goat´s milk. This experiment took place at organic farm in LFA (Less Favoured Areas). The animals were divided into two groups. Both, the control and experimental group consisted of eight goats. Goats were fed by *ad libitum* intake of meadow hay and concentrate. Concentrate for the experimental group contained 0.5 kg barley/oat meal (1:1) and 0.1 kg linseed, for the control group it was 0.7 kg barley/oat meal. The experiment lasted for 2 months with 14 days of preparatory phase.

Analysis of fatty acids was made by Department of Applied Chemistry at University of South Bohemia in České Budějovice by gas chromatography. The results were statistically evaluated by program Statistics 9th.

Results and discussion

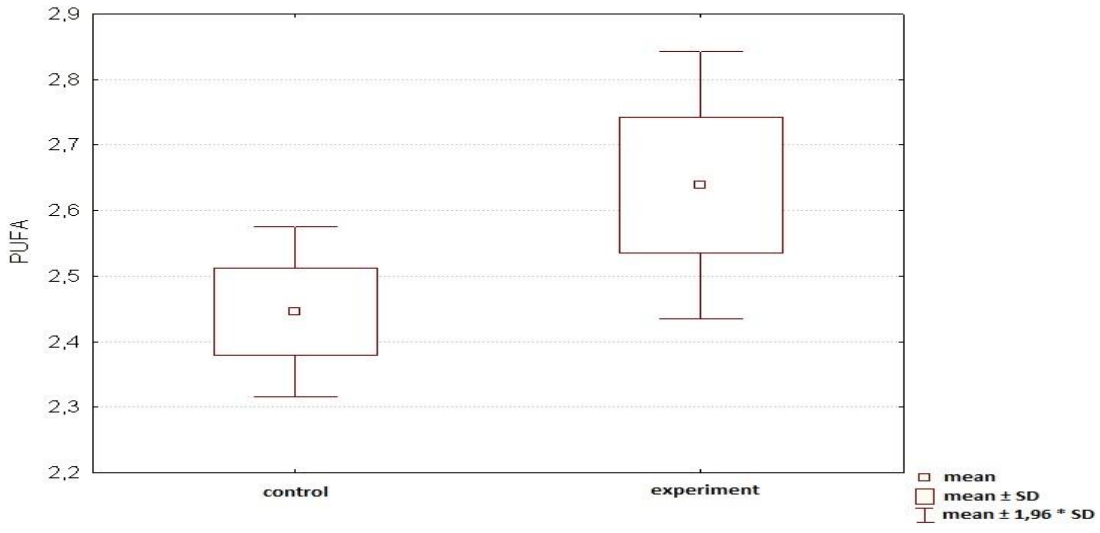
Average values of the fatty acids in goat´s milk for experimental and control groups, divided according to saturation, are listed in Table 1. Fatty acids are divided into saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). The results showed increasing content of PUFA and MUFA, and also reduced SFA.

Table 1: Content of fatty acids in goat´s milk according to saturation

	Control group		**Experimental group**			
	mean	**SD**	**mean**	**SD**	**t**	**p**
SFA	69,75635	1,915516	67,04438	4,588959	1,56556	0,135875
MUFA	26,45634	1,770894	28,77922	4,59397	-1,35033	0,194615
PUFA	2,44554	0,186708	2,63884	0,344815	-1,43278	0,170054
C18:2 CLA	0,36767	0,050160	0,523844	0,104128	-3,90346	0,001143

The most significant was the concentration of C18:2 – conjugated linoleic acid (CLA) was significantly higher ($P < 0.05$) in the milk of goats fed by flax seed diet compared to control diet. CLA has provably anticancer effects (Haenlein, 2004; Samková et al., 2008; Park, 2009), reduces the ratio of body fat to proteins (Kalač, 2003; Park, 2009), is also anti-inflammatory and prevention of cardiovascular diseases (Park, 2009). Park et al. (2010) and Kalač (2003) suggest that it may also reduce the risk of stroke. Differences in content of PUFA and CLA between the experimental and control group are also shown in Graph 1 and Graph 2.

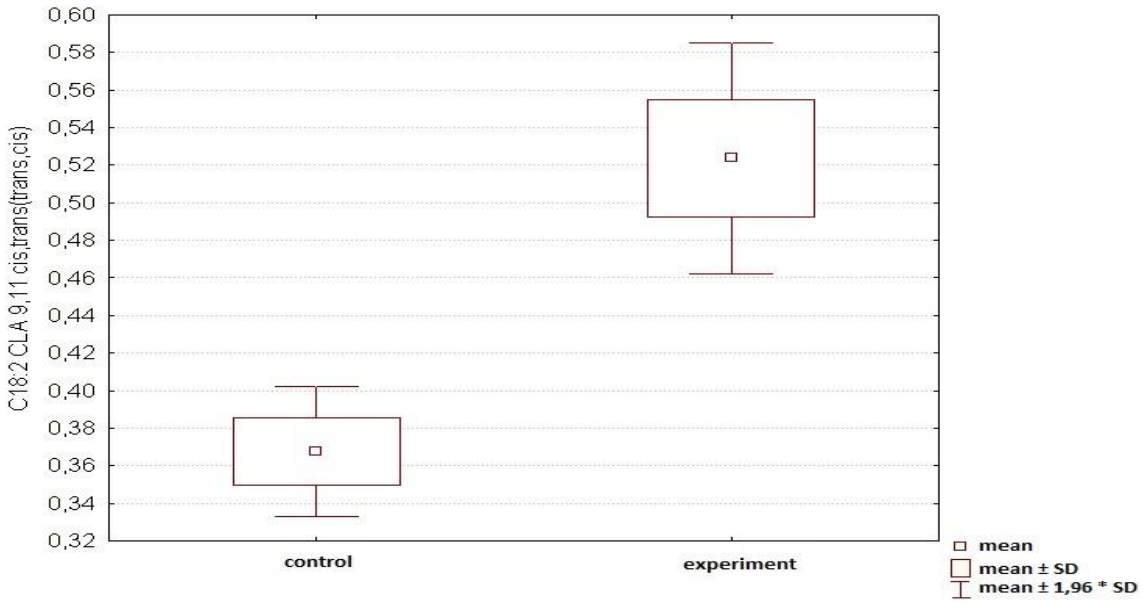
Graph 1. Mean of PUFA content in milk of control and experimental group



PUFA belong to fatty acids with positive effect on human´s health, however correct ratio of n-

3/n-6 acid groups should be maintained, with the maximum value being 1/5 (Samková et al., 2008).

Graph 2. Mean of CLA content in milk of control and experimental group



Flax seed contains a high oil level (40 % of total seed weight), and α-linolenic acid account for 55 % of total fatty acids of the oil (Gao et al., 2009).

Park et al. (2007) presents the mean value of CLA in goat´s milk fat is 0,65 %, while sheep´s milk contains 1,08 % and cow´s milk 1,08 % CLA in milk fat. Addition of flax seed to the diet of goats resulted in increasing of unsaturated fatty acids content in milk. Similar values were founded by Král (2010). Our results are the same as Rusníkova et al. (2012) and Ryšavý et al. (2012) reported. The positive effect of flax seed supplementation to lactating ruminants confirmed also Zhang et al. (2006). In their experiment were used lactating ewes. In experiment by Čermák (2013) which was used on grazing goats were found similar results - during grazing the content of SFA in goat´s milk fat continuously decreased. Opposite trend was recorded for MUFA. A significant increase of herb proportion contributed to a highest content of PUFA (including CLA) in milk fat. The pasture had similar effect on the milk composition as supplementation of flax seed.

Conclusion

Supplementation of flax seed to lactating goats has significant effect on milk fatty acids. The results of the above analysis clearly shows that flax seed can be used as a supplement of a feed ratio of goats, for its positive effect on the reduction of saturated fatty acids while also increase polyunsaturated fatty acids in milk. Of these polyunsaturated fatty acids has been the most significant increase in the CLA content.

Flax seed to lactating goats can be used as nutritional supplement increasing polyunsaturated fatty acids in milk. This fact has significant effect for human´s nutrition and health.

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References

1. Ceballos, L.S., et al. (2009). Utilization of nitrogen and energy from diets containing protein and fat derived from either goat milk or cow milk. *The Journal of Dairy Research.* 76 (4), 497-505.
2. Čermák, B., Král, V., Frelich, J., Boháčová, L., Vondrášková, B., Špička, J., Samková, E., Podsedníček, M., Węglarz, A., Makulska, J., Zapletal, P. (2013). Quality of goat pasture in less-favoured areas (LFA) of the Czech Republic and its effect on fatty acid content of goat milk and cheese. *Animal Science Papers and Reports.* 31 (4), 331-346
3. Fantová, M. et al. (2000) *Chov koz.* 1. vyd. Praha : Brázda s. r. o., 2000. 192 s. ISBN 80-209-0290-2.
4. Gao, Y., Sun, t., Li, J. (2009). Effect of oilseeds rich in linoleic and linolenic acids on milk production and milk fatty acid composition in dairy cows. *Front. Agric. China*. 3 (3), 311-318.
5. Gilbere, G. (2003). Everything was not "within normal range". *Total Health.* 25 (1), 27-30.
6. Goat's Milk. A Natural Alternative for Milk Sensitive Patients. Dynamic Chiropractic [online]. 1997, vol. 15, is. 25, [cit. 2010-02-23]. Dostupný z WWW: <http://www.chiroweb.com/mpacms/dc/article.php?id=38646>.
7. Haenlein, G.F.W. (2004). Goat´s milk in human nutrition. *Small Ruminant Research*. 51(2), 155-163.
8. Jandal, J. M. (1996). Comparative aspects of goat and sheep milk. *Small Ruminant Research.* 22 (2), 177-185.
9. Kalač, P. (2003). Funkční potraviny:kroky ke zdraví. České budějovice : DONA s.r.o., 2003. 130 s. ISBN 80-7322-029-6.
10. Král, V. (2010). *Ověření vlivu krmiv na příjem krmných dávek koz a složení jejich mléka.* Diplomová práce, Zemědělská fakulta JU, Česká republika.
11. Park, Y. W., Juarez, M., Ramos, M., Haenleinen, G.F.W. (2007). Physico-chemical characteristics of goat and sheep milk. *Small Ruminant Research.* 68 (1-2), 88-113.
12. Park, Y. (2009). Conjugated linoleic acid (CLA): Good or bad trans fat?. Journal of Food Composition and Analysis. 2009, vol. 22, Supplement 1, s. S4-S12.
13. Park, Y., et al. (2010). Effects of dietary conjugated linoleic acid (CLA) on spontaneously hypertensive rats. Journal of Functional Foods. 2010, vol. 2, 1, p.54-59.
14. Raynal-Ljutovac, K., Lagriffoul, G., Paccard, P., Guillet, I., Chilliard, Y. (2008). Composition of goat and sheep milk products : An update. *Small Ruminant Research.* 79 (1), 57-72.
15. Rusníková, L., Straková, E., Suchý, P. (2012). Monitoring the quality of vegetace oils. In Proceeding of international Animal Nutrition PhD Conference Brno November 21.11.2012 (pp 199-204). ISBN 978-80-7375-667-3
16. Ryšavý, J., Křížová, L., Janštová, B. (2012). The effect of feeding rumen-protected CLA to lactating dairy cos on fatty acid profile of milk fat. In Proceeding of international Animal Nutrition PhD Conference Brno November 21.11.2012 (pp 205-214). ISBN 978-80-7375-667-3
17. Samková, E., Pešek, M., Špička, J. (2008). *Mastné kyseliny mléčného tuku skotu a faktory ovlivňující jejich zastoupení.* 1. České Budějovice : Jihočeská univerzita v Českých Budějovicích Zemědělská fakulta, 2008. 90 s. ISBN 978-80-7394-104-8
18. Vejčík, A., Král, M. (1998). *Chov ovcí a koz.* 1. vyd. České Budějovice: Jihočeská univerzita v Českých Budějovicích Zemědělská fakulta, 1998,145 s. ISBN80-7040-297-0

ASSESSMENT OF DIGESTIBILITY OF MEADOW HAY AND GRASS HAYLAGE FOR HORSES

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Abstract

The aim of this study was to compare the nutrient content in meadow hay and grass haylage, fed to horses, and then to compare dry matter (DM) and crude fibre (CF) digestibility, obtained from *in vivo* experiments. The both test feeds were produced in the National Stud Kladruby nad Labem, there were purchased and transported to the location of experiment. Eight adult mares were used in experiments to determine *in vivo* digestibility of meadow hay and grass haylage. Horses were individually housed and fed. All the diets, refusals and faecal samples were collected for chemical analyses and calculation of *in vivo* digestibility. Samples were analyzed for contents of DM, crude protein (CP), ether extract (EE) and CF. Data were analyzed using t-test.

In both tests of feed very low digestibility of DM of meadow hay (39.98 %) and of grass haylage (40.83 %) was found. The digestibility of CF was very low, of meadow hay (33.91 %) and of grass haylage (39.88 %) was very low as well. These results indicate that the forages were harvested in the same locality (Kladruby) in the equal vegetation period. Forages were harvested since late till very late stage at the end of flowering. Feeds of this quality are not suitable for the horse nutrition.

Keywords: horses, digestibility, meadow hay, grass haylage

Material and methods

Tested feed. In two experiments the nutrient content and the digestibility of DM and CF in two different rations were monitored. Two treatments were evaluated: grass haylage in wrapped bales and field-dried meadow hay. In the first experiment the feeding of meadow hay was examined. The second test ration was containing the grass haylage. The both test feeds were produced in the National Stud Kladruby nad Labem, there were purchased and transported to the location of experiment.

In vivo experiments. The experiments were performed between the months of November and December in accredited barn in Netluky owned by Institute of Animal Science, Praha - Uhřiněves. Eight adult Czech Warmblooded mares with mean age of 9 yr (range 4 to 16 yr) and a mean initial body weight (BW) of 554 kg (range 509 to 609 kg) were used. The mares were kept in individual stables with the area 3.5 m x 4.0 m with a litter of wood shavings and had ad libitum access to fresh water and salt blocks.

The experiments were divided into two periods, to the preparation and the trial. In preparation the feed intake by individual mares was tested. The experimental period was performed with a number of mares that were selected on the basis of feed intake. Before the beginning of each experiment and at the end, were recorded the live weights of mares were recorded.

In the first experiment the feeding of meadow hay was examined. The ration contained 12 kg of meadow hay and was divided into two dosages per day. The 7 day preparation period precede the 7 day trial period. The preparation was limited to a short period of time because of normal feeding of this diet, the meadow hay has been traditionally used as a basal diet for

horses. The mares were fed from nonmesh nylon hay bags hung at a heigh of 1 m above the ground. All bedding was removed to minimize the contamination of samples during the experimental period with mares. In the second experiment the feeding of grass haylage was examined.

The second ration contained 20 kg of grass haylage, which was again divided into two dosages per day. For this ration 14 days long preparatory period was carried out, again preceded by the experimental period lasting for 7 days. The mares were again fed from nonmesh nylon hay bags hung at a heigh of 1 m from the ground. During both experiments, the mares carried out light work (walk, trot) for one hour. Feed, residues and faeces were weighed with the accuracy of 0,01 kg. Faeces were collected immediately after excretion and were stored daily for total weight determination and then a 10% of representative sample for individual animals. All the diets, refusals and faecal samples were preserved in polyetylene bags stored until chemical analyses.

Chemical analysis. Samples of feed, residues and faeces were dried for 48 hours at 50 °C**.** Dried samples were milled, the size of particles was 1 mm (CYCLOTEC 1093 Sample Mill) and were analysed according to AOAC (2005) methods. The residual moisture of the samples was determined by oven drying for 6h at 105°C. Ash was determined after 6h at 550°C. Ether extract (EE) after 6 h extraction with petroleum-ether. Nitrogen was determined using a Kjeldahl method according to method 976.05 by Association of Official Analytical Chemists (AOAC, 2005) and crude protein (CP) was calculated as N x 6.25. CF was determined with a Fibertec. The DM and the CF digestibility were calculated then. The *in vivo* digestibility coefficients of horses were calculated as follows:

$$\% D = (\text{Intake} - \text{Faecal Excretion}) / \text{Intake} \times 100$$

Statistical analysis. Because of the small data pool, the t-test (Statistica, 2013; STATSOFT, Inc.) was used to compare differencies of digestibilities between two feeds for horses (meadow hay and grass haylage).

Results and discussion

The aim of the experiment was to study nutrient content in meadow hay and grass haylage, fed to horses, and then compare DM and CF digestibility, obtained from in vivo experiments. The chemical composition of the meadow hay and grass haylage is shown in Table 1. The DM content in grass haylage was lower as in hay. DM of hay (90 %) determined by the autors Bergero and Peiretti (2011) was similar than found in our study. DM of haylage (50.91 %) was in this study same than was determined by the previous authors (58.5 %). In general, forages preserved by ensiling are usually harvested at an earlier stage of maturity than forages preserved for hay (Ragnarsson and Lindberg 2010). However, in this study, the haylage was harvested at the late stage (end of flowering). Crude protein (CP) content of the grass haylage was higher than that of the meadow hay. This difference could be ascribed to the presence of legumes in the permanent meadow and to the greater loss of leaves as a consequence of haymaking. The content of CF was higher in the meadow hay (39.85 %) than in the haylage (30.73%). The content of CF higher than 30 %, generally show a low digestibility of feeds.

Table.1: Content (% in dry matter) of feed nutrients

	DM	CP	EE	CF
Meadow hay	93.80	7.74	1.05	39.85
Grass haylage	50.81	9.85	2.23	30.73

DM – dry matter; CP – crude protein; EE – ether extract; CF – crude fibre

Good quality of forage is the basis for feeding of horses. Hay is the most common type of forage when pasture is unavailable. Hay production depends to a great extent on weather conditions. Dry storage after harvest is crucial to achieve and maintain quality and to avoid mold growth. The quality of hay determines species composition in addition to a growth phase, during which the crop is harvested. The highest nutrient content is at the stage of early flowering in the case of clovers, as for grasses at the beginning of earing. In the late harvest plants are already depleted by nitrogen compounds, on the other hand a higher proportion of fibre and hence the low digestibility and utilization of energy and nutrients (Müller, 2005).

The quality problems concerning a dry hay are usually connected to dust. In general, a mouldy hay, and also a good quality hay, contains a high level of dust (plant fragments, fungal spores, mites, and bacteria) that can lead to recurrent health problems. That is partly why the use haylage bales as a replacement for hay has increased in horses in recent years (Müller, 2005; Ragnarsson and Lindberg, 2010). Moore-Coyler and Longland (2000) suggested that increasing numbers of owners are feeding grass haylage to horses with no detrimental effects. In fact, haylage has proved to be a dustless and palatable feed with an enhanced nutritive value. Ragnarsson and Lindberg (2010) in their study reported that due to weather conditions during the harvesting period, haylage have become increasingly popular, and are gradually replacing hay as the major forage source for horses.

The use of haylages is becoming more widespread, even in the horse sector, but only on one condition that the knowledge relative to the quality of conservation and the nutritive value of these products which vary considerably according to the type of farming and the vegetative stage, degree of drying, ensiling technique, and so forth be studied in detail by the breeders and experts. The nutritive value of fermented forages is often better than that of dry hay because of the lower growth stage and smaller losses of leaves (Peiretti and Bergero, 2004; Bergero and Peiretti, 2011).

Haylages can offer a valid alternative to hay on condition that these products are well conserved, have maintained a nutritive value that is similar to that of the original grass, are without risks for the health of the horses, and are available in different sizes according to the requirements of the stables where the conserved silages must be consumed in the shortest possible time to prevent aerobic deterioration (Peiretti and Bergero, 2004).

There are many factors, which could affect feed digestibility in diet for horses, such as diet composition, level of intake, content of digestible and non-digestible nutrients and others (Gálik *et al*, 2011). In this study the determination of the digestibility of feeds *in vivo* method was used. Quantitative determination of feed intake and faeces collection are considered accurate to determine the total digestibility in horses (Schurg, 1981; Bergero et al., 2009; Sales, 2012).

Digestibility coefficients between the meadow hay and grass haylage is shown in Table 2. The hay DM digestibility (39.98 %) was lower compared to the study (Bergero and Peiretti, 2011), in this study was DM digestibility (57,8 %). The haylage DM digestibility (53.9 %) determined by the authors Bergero and Peiretti (2011) was higher than in our study. The haylage DM digestibility determined by the previous authors was similar to that found in study of Moore-Coyler and Longland (2000). We conclude that the DM digestibility of both feed is very low. The feed were harvested in the same locality (Kladruby) in the equal growth phase. According to the low digestibility of the DM of hay and haylage it is suggested that this type of feed was harvested at the late to very late stage at the end of flowering. The digestibility of DM was not influenced by the way of harvesting.

The hay CF digestibility was lower than haylage CF digestibility. Särkijärvi *et al*, (2010) in their study reported CF digestibility of haylage in the case of early cut was higher (63.4%) than at the late cut (35.5 %). From these results we can conclude that the haylage was harvested late growth phase.

Table.2: Digestibility of DM (dDM, %) and CF (dCF, %).

	Meadow hay	Grass haylage	P
dDM (%)	39.98±4.30	40.83±2.13	0.647
dCF (%)	33.91±5.51	39.88±4.64	0.046

Conclusion

The feed (meadow hay, grass haylage) were harvested in late to very late stage at the end of flowering. The feed of this quality was not suitable for horse nutrition. Increased nutritional requirements for horses in relation to reproduction, growth, recreational or athletic performance requires a balanced ration that allows to perform their full potential. It is achieved only by feeding of quality feed produced from forages harvested at the optimal stage of maturity. Good quality forage is the basis of horse feeding. Hay is the most common type of forage when pasture is unavailable. If haylage is good quality and presents the same nutritive value as hay obtained from the same field and it can be used as a good alternative to replace hay in diets for equids.

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References

1. AOAC (2005). Official Methods of Analysis, AOAC International. 18th Edition. Gaithersburg, USA, ISBN 0-935584-75-7
2. Bergero, D., Peiretti, P. G. (2011). Intake and Apparent Digestibility of Permanent Meadow Hay and Haylage in Ponies. Journal of Equine Veterinary Science. 31, 67-71.
3. Bergero, D., Préfontaine, C., Miraglia, N., Peiretti, P. G. (2009). A comparison between the 2 N and 4 N HCl acid – insoluble ash methods for digestibility trials in horses. Animal 3, 1728 – 1732.
4. Gálik, B, Bíro, D, Halo, M., Juráček, M., Šimko, M., Vaščáková, V., Rolinec, M. (2011). The effect of phytoaditives on macroelemets digestibility of sport horses. Journal of Central European Agrikulture. 12, 390-397
5. Moore-Colyer, M. J. S., Longland, A. C. (2000). Intakes and in vivo apparent digestibilities of four types of conserved grass forage by ponies. Anim Sci. 71, 527–534
6. Müller, C.E. (2005) Fermentation patterns of small-bale silage and haylage produced as a feed for horses. Grass Forage Sci, 60, 109–118
7. Peiretti, P.G.,Bergero D. (2004). Grass silages as feedstuff for horses. J Food Agr Environ, 2, 182–185
8. Ragnarsson, S., Lindberg, J. R. (2010). Nutritional value of mixed grass haylage inIcelandic horses. Livestock Science**.** 131**,** 83–87
9. Sales, J. (2012). A review on the use of indigestible dietary markers to determine total tract apparent digestibility of nutrients in horses. Animal Feed Science and Technology. 174, 119–130
10. Särkijärvi, S., Sormunen-Cristian, R., Heikkilä, T., Rinne, M., Saastamoinen, M. (2012). Effect of grass species and cutting time on *in vivo* digestibility of silage by horses and Wheel. Livestock Science. 144, 3. 230–239
11. Schurg W.A. (1981): Compilation of data evaluating various techniques for determining digestion of equine rations. In: Proceedings of the 7th Equine Nutrition and Physiology Society Symposium, Warrenton, VA, USA, pp. 1–2.

EFFECT OF PARENTERAL AND PERORAL APPLICATION OF SELENIUM ON SELECTED ANTIOXIDANT PARAMETERS IN DAIRY COWS DURING PERIPARTAL PERIOD

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Abstract

In this study we observed the effect of peroral and parenteral supplementation of Selenium (Se) on selected antioxidant parameters in the blood of dairy cows during peripartal period. Forty Holstein cows between 2nd and 4th lactation-gestation cycle were randomly allocated to four groups (n=10 per group): control group (C) and three experimental groups (D1, D2, D3). Experimental groups were parenteral or peroral supplemented by selenium (Se) or with the addition of α-Tocopherol at different time intervals. Dairy cows in D1 group were peroral supplemented with 0.3 mg Se/kg/DM in the form of Na_2SeO_3 during 42 days. Cows in D2 group received subcutaneous injections of Selevit inj. a.u.v at the dose of 20 ml/cow on 21st and 12th day before calving. In D3 group, cows were injected at the same dose of Selevit inj. a.u.v but only on 21st day before calving. Then we determined concentration of Se and activity of GPx in the blood. The blood samples from *vena jugularis* were collected on 21st day before calving (control sampling), 3rd day after calving and 21st day after calving. The significant changes in the concentration of Se and activity GPx (glutathione peroxidase) in blood were determined in D1 group on the 3rd day and 21st day after calving. The changes concentration of Se in plasma of cows from D2 group have been recorded only on the 3rd day after calving. In D3 group were not observed changes in monitored parameters in comparison with the control group.

Keywords: selenium, application, glutathione peroxidase, dairy cows, plasma levels

Determination of the selenium status of livestock has improved considerably during the past few years with the discovery that Se in blood closely correlates with GPx activity. Selenium is an essential component of GPx and is incorporated into erythrocyte GPx during erythropoeisis. Each molecule of GPx contains four atoms of Se (Pavlata et al., 2002).

On the territory of the Member States of the EU, there is very variable the presence of Se in the soil, from an average of 0.05-0.1 ppm. Territory of Nordic countries, France, the Balkans and England are given as Se-deficient soils. In recent years, it was confirmed that the Central Europe is area with very low concentrations of Se in the soil (Grešáková et al., 2013).

The dry period is the most important phase of a dairy cow's lactation cycle. Good nutrition and management in the dry period have been shown to minimise calving problems and early lactation metabolic disorders, maximise subsequent lactation performance and udder health and optimise fertility and overall cow productivity. Dietary supplementation with vitamin E and selenium during the last 3 weeks of the dry period had a substantial positive effect on mammary gland health. (Slavik et al., 2006).

Diets containing Se under 0.3 mg/kg of DM and 500 IU of vitamin E/cow of day during the dry period can cause decrease of immunostimulation and antioxidant activity (Petrovič et al., 2005).

Material and methods

Study was carried out in herd of 300 Holstein dairy cows in east of Slovakia. Dairy cows were kept in freely housed in three reconstructed cowshed milked a twice a day. In farms they used seasonal feeding with total mix ration (TMR) according to actually request during dry period and lactation. Milking took place in the tandem milking shed Boumatic 2 x 10 Xpressway (Wisconsin, USA). Before drying was applied intramammary antibiotic preparation Orbenin Dry cow a.u.v. (Pfizer, IT) to every quarter of udder.

Experimental design and sampling

During dry period 40 dairy cows were selected between 2nd and 4th lactation in last stages of pregnancy. Selected dairy cows were divided into 3 experimental and one control group of 10 pieces. At the time of expected delivery (21 days before) in dairy cows were taken blood from *vena jugularis* into two 12 ml and 2 ml heparinised test tubes. After the withdrawal and mixing the samples were transported at a temperature of +4°C, in a cool box to the laboratory. All samples of blood plasma, together with 2 ml of heparinized blood samples were stored at -54°C until the their analysis.

After the withdrawal of blood, at the time of 21 days before expected calving, selected groups of dairy cows were treated as follows:

- The first group (D1) was peroral supplemented with addition of 0.3 mg Se/kg of DM in the form of Na_2SeO_3 during 42 days.
- The second group (D2) was on 21st and 12th day before calving, subcutaneously administered by Selevit. inj. a.u.v. (Biotika, SR) at a dose of 20 ml/PCs, (44.4 mg/Se and 500 IU/α-Toc/cow).
- The third group (D3) was only on 21st day before calving, subcutaneously once filed applied Selevit inj. a.u.v. (Biotika, SR) on the same dose as the group D2.
- The fourth group (C) was without parenteral and peroral supplementation was fed with the diet containing 197 - 211 μg of Se per kg/DM.

Monitoring of plasma concentration of Se and GPx activities in the blood of dairy cows was carried out on 3rd and 21st day after calving.

Laboratory analyses

Glutathione peroxidase activity per gram of haemoglobin in the erythrocytes (U/g of Hb) was determined by the Ransel - Randox RS 505. Haemoglobin was analyzed by Haemoglobin kits **(**Randox-Ransel, UK). The concentration of the Se in sample of feed was determined after wet mineralization by atomic absorption spectrometer Zeman 4100 (Perkin Elmer, USA) equipped with generating device system procedure according to Kováč et al. (2012). The concentration Se in the blood plasma was evaluated in duplicate using the fluorimetric methods according to Rodriguez et al. (1994).

Statistical Analysis

One-way analysis of variance (Anova) with the *post hoc* Dunett´s Multiple Comparison Test was subsequently used to compare the control group to the peroral and parenteral supplemented groups. Differences between the mean values of the different treatment groups were considered significant when P values were smaller than 0.05. Values in tables are means (M) and standard deviation (SD).

Results and discussion

The effect of peroral and parenteral supplementation of Selevit inj. a.u.v. on value of Se (μg/L) in blood plasma of dairy cows during peripartal period is described in table 1. In D1 group were increased concentration of Se on 3rd and 21st ($P \leq 0.05$) day after calving and in

D2 group was increased concentration of Se on 3rd day after calving in compare to control group. In D3 group not showed increased plasmatic concentration during following period.

Table 1: Effect of peroral and parenteral supplementation of Selevit. inj. a.u.v. on the averaged concentrations of Se (μg/L) in blood plasma of dairy cows before and after calving

Period	Groups			
	C M±SD	D1 M±SD	D2 M±SD	D3 M±SD
21st day a. p.	**64.11** ± 1.82	**64.72** ± 2.34	**65.14** ± 2.75	**64.35** ± 1.72
3rd day p. p.	**63.24** ± 2.55[a]	**78.13** ± 4.43[b]	**77.32** ± 3.67[c]	**64.21** ± 2.71
21st day p. p.	**77.14** ± 2.37 [a]	**89.8** ± 3.56 [b]	**80.18** ± 3.11	**78.26** ± 2.64

D1 – peroral supplemented group, D2 – parenteral supplemented group on 21st and 12th day before parturition; D3 –parenteral supplemented group on 21st day before parturition; C – control group; *a. p.* – ante partum; *p.p* – post partum; [a,b,c] values in rows with different letters differ significantly P≤0.05

In table 2 there is described the activity of GPx (U/g of Hb) in blood of dairy cows during peripartal period. In D1 group was determined increased activity of GPx in compare to C group on 3rd and 21st (P≤0.05) day after calving. In D2 and D3 not showed increased the activity of GPx in comparison with the control group.

Table 2: Effect of peroral and parenteral supplementation of Selevit. inj. a.u.v. on the activity of GPx (U/g of Hb) in blood of dairy cows before and after calving

Period	Groups			
	C M±SD	D1 M±SD	D2 M±SD	D3 M±SD
21st day a. p.	**397** ± 25.3	**405** ± 26.7	**398** ± 30.2	**401** ± 28.6
3rd day p. p.	**386** ± 38.8[a]	**473** ± 31.2[b]	**431** ± 34.8	**443** ± 37.5
21st day p. p.	**415** ± 33.1[a]	**487** ± 40.1[b]	**453** ± 38.1	**460** ± 34.0

D1 – peroral supplemented group, D2 – parenteral supplemented group on 21st and 12th day before parturition; D3 –parenteral supplemented group on 21st day before parturition; C – control group; *a. p.* – ante partum; *p.p.* – post partum; [a,b] values in rows with different letters differ significantly P≤0.05

Pavlata et al. (2002) established reference values of Se in cattle, which are subdivided into three groups. Values Se concentrations below 30 μg/L are usually measured in the blood of animals with clinical signs of nutritional muscular dystrophy and they are referred to as a deficiently value. The values of 30 μg/L to 50 μg/L appear to be a threshold, however for dairy cows during dry period this value is higher (up to 75 g/L). Values above 75 μg/L are considered to be adequate for the proper function of the selenoproteins. At the beginning of the monitored period the measured values of the Se in blood plasma of dairy cows were in the range from 64.11 – 65.14 μg/L. These values can be considered as limiting the concentration of this element. Marginal values were also found on the 3rd (63.24 – 64.21 μg/L) after calving in the control and D3 groups. An adequate supply of Se in the post-natal period were found in the group D1 (78.13 – 89.8 μg/L) that was supplemented with 0.3 mg Se/kg of DM in form of Na_2SeO_3 and group D2 (77.32 – 80.18 μg/L) treated with parenteral supplementation on 21st and 12th day before calving.

In addition to determining Se in feed dose and in the blood of dairy cows, the authors recommend a diagnostic assay of activity GPx which ensures the removal of excess of peroxide radicals in the cytozole cells. Also determine the limits of the activity of GPx which

correspond to deficiency of Se, integral component of this enzyme. The activity of GPx 175-190 U/g of Hb rated as deficient and value in the range 190-225 U/g of Hb rated as a border for the supply of Se in organism. As a sufficient level of activity GPx for a body is value over 225 U/g of Hb. In our study the activity of GPx throughout the period under review in all groups was higher than 386U/g of Hb what we can be considered in terms of its activities as adequate. In the peroral supplemented group D1 we have seen increased activity on 3rd and 21st day ($P \leq 0.05$) after calving, but in D2 and D3 we didn't find changes in activity of GPx in comparison with the control group.

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References

1. Grešáková L., Čobanová K., Faix S. (2013). Selenium retention in lambs fed diets supplemented with selenium from inorganic sources. Small Ruminat Research. 111:76–82.
2. Kováč G., Petrovič V., Pisarčíková J., Zigo F., Vasiľ M. (2012). Effect of parenteral administration of selenium and vitamin E on chosen antioxidant indexes in blood of periparturient dairy cows. XXVII World buiatrics congress, Lisbon – Portugal. 106 – 107.
3. Pavlata L., Illek J., Pechova A., Matejíček M. (2002). Selenium Status of Cattle in the Czech Republic, Acta Vet Brno. 71: 3–8.
4. Petrovič V., Boldižarová K., Faix Š., Kowalczyk J., Czauderna M., Mellen M., Leng Ľ. (2005). Excretion routes and distribution of selenium in sheep tissues after selenite loading. Journal of Animal Feed Sci,. 14: 303 - 306.
5. Rodriguez E.M., Sanz M.T., Romero C.D. (1994). Critical study of fluorometric determination of selenium in urine. Talanta. 12: 2025–2031.
6. Slavik P., Illek J., Zeleny T. (2006). Zinc and Copper Status of Beef Cattle in the Šumava Region, Czech Republic. Acta Vet Brno 75, 4: 485-488.

PERIPARTAL PERIOD AND PREVENTION OF KETOSIS

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Abstract

Subclinical ketosis (SCK) is defined as elevated concentration of circulation ketone bodies (mainly β-hydroxybutyrate in blood 1.0 – 1.4 mmol.l^{-1}) in the absence of clinical signs (Duffield et al., 1998; Iwersen et al., 2009; Rollin et al., 2010). The present work was made under practical condition in *peripartal* phase of dairy cows on different lactation (first, second and third or higher lactation). The study was conducted to evaluate a degree of ketogenesis in dairy cows – measured β-hydroxybutyrate (BHB) concentration in blood by electronic BHB hand-held meter Precision Xtra® on 3, 5, 7, 9, 11, 15, 20, 25 day in milk (DIM). Together with evaluation of the degree of ketogenesis in dairy cows was performed an analysis of dynamics of BHB in *postpartum* period of dairy cows on third lactation. In this part of study was also evaluated the effect of potentiated nutrition (treatment of SCK with energy substances) on nutritional prevention of metabolic disorders, mainly subclinical or clinical ketosis. The average milk production was 36.4 ± *4.6* kg after calving until 25 DIM of the dairy cows on third lactation with lower ketogenesis – **group I,** *n=5* (BHB concentration between 1.0 – 1.2 mmol.l^{-1} diagnosed maximal 3 times in postpartum – *compensated ketogenesis*). The average milk production was 34.3 ± *3.6* kg after calving until 25 DIM of the dairy cows on third lactation with higher ketogenesis – **group II,** *n=7* (BHB concentration ≥ 1.2 mmol.l^{-1} diagnosed more than 4 times in postpartum – *uncompensated ketogenesis*).

Keywords: dairy cows, ketogenensis, peripartal period, farm diagnostic test

Circulating concentrations of non-esterified fatty acid (NEFA) and β-hydroxybutyrate (BHB) measure the success of adaptation to negative energy balance. NEFA reflects the magnitude of mobilization of fat from storage and mirrors dry matter intake (Adewuyi et al., 2005). BHB reflects the completeness of oxidization of fat in the liver. Ketone bodies (BHB, acetone and acetoacetate) are the intermediate metabolites of oxidation of fatty acids, specifically resulting from the incomplete oxidation of fatty acids. Programs to monitor management of the transition period may usefully include NEFA concentrations in the week before expected calving and BHB concentration in the first two weeks after calving. A nearly 5-fold increased risk of SCK was noted when the NEFA concentrations in the week before calving were greater than 0.7 mmol.l^{-1} (Oetzel, 2013). A routine ketone testing program should commence after calving. The first two weeks are the primary risk period for subclinical ketosis, defined by a serum concentration of 1.0 mmol.l^{-1} BHB or greater (Duffield, 2000; Oetzel, 2013). The gold standard diagnostic test for subclinical ketosis is the measurement of BHBA in serum or plasma (Duffield 2000). Serum BHBA measurement is useful for monitoring feeding management practices by examination of individual cows and evaluating herd health.

Material and method

1. The cows in farm (Holstein-Friesian breed), were high-yielding with a milk production over 11,500 kg of milk during 305-day lactation. The diagnosis of ketogenesis and dynamics of BHB in postpartum period was performed by analysis of concentration of BHB in blood obtained by Precision Xtra®. The blood was collected in peripartal period:

    a. 5 – 3 day *antepartum*,

b. *postpartum* on 3., 5., 7., 9., 11., 15., 20. a 25. day in milk (DIM); the cows with BHB blood level ≥ 1.0 mmol.l^{-1} was treatment with energy substances - the combination of propylene glycol, glycerol and high concentration of vitamin B_{12} in doses 0.5 L/treated cow,
c. The direct electrochemical hand-held BHBA meter (Precision Xtra®, Abbott Diabetes Care, Abingdon, UK) ketone monitoring system is a simple and direct electrochemical test. The ketone test strip contains the enzyme β-hydroxybutyrate dehydrogenase, which oxidizes BHBA to acetoacetate. This reduces nicotinamide adenine dinucleotide (NAD^+) to NADH. The NADH is then reoxidized to NAD^+ by an electron transfer mediator molecule. The electrical current generated by this conversion is measured by the electronic hand-held BHBA meter and is directly proportional to the BHBA concentration.

2. The conception of nutrition and animal breeding of experimental cows in the farm:
   a. dairy cows before calving (close up period) were kept in a free-stall barn and fed a total mixed ration (TMR) formulated for dry cows, based on maize silage, grass haylage and straw supplemented with minerals in the dry period (anionic salts),
   b. dairy cows after calving were housed in a free-stall system and the animals were fed a TMR based on maize silage, and grass haylage. Healthy dairy cows were subsequently relocated to main lactation group on 25 DIM. In the complete diet, the concentrate was supplemented with protein, minerals, vitamins and milk production enhancers (protein and rumen-protected fat). Cows after calving were fed a TMR formulated to meet or exceed NRC (2001) recommendations and to produce 40 - 45 kg of milk, 7.0 MJ.kg^{-1} net energy for lactation and 175 g.kg^{-1} crude proteins. Feed was offered once daily using mixer wagons, and it was gathered up several times per day to ensure ad libitum access. Cows were milked twice daily around 4 a.m. and 4 p.m.

Result and discussion

Diagnosis of ketogenesis in dairy cows was performed by analysis concentration of BHB in blood. Blood samples of 10 ml of blood were collected from the coccygeal vessels of each of 23 cows 2–3 hour after morning feeding using a tube without anticoagulant for in farm BHBA testing with Precision Xtra® meter. BHBA testing in farm was completed according to Precision Xtra® meter instructions and performed immediately after blood collection. Selected animals for this experiment consisted of antepartum and early postpartum cows until 25 days of lactation during 3 month period. In this time period was in calving 23 dairy cows: - 7 animals on first lactation, - 4 cows on second lactation and - 12 on third or higher lactation. The concentrations of BHB on different lactation in individual control day in milk (DIM) postpartum are in table 1. The mean concentrations of BHB were 0.4 ± *0.1* mmol.l^{-1} in the antepartum period (3 – 4 days before calving) and individual maximal values were 0.6 mmol.l^{-1} in this period. The concentration of BHB ≥ 1.0 mmol.l^{-1} were observed at least one time on designed control days postpartum in 100%, 50% and 14% of dairy cows on third or higher lactation, on the second lactation and on the first lactation, respectively. The highest mean concentrations of BHB were reported between 7 and 15 DIM.

The prophylactic treatment with energy substances - the combination of propylene glycol, glycerol and high concentration of vitamin B_{12} was administered to dairy cows dependent on the concentration of BHB in blood measured by hand-held meter Precision Xtra®. Those energy substances were administered to dairy cows with concentration of BHB ≥1.0 mmol.l^{-1}. This method of prophylactic nutrition was repeated every time for each dairy cow when was reported concentration of BHB ≥ 1.0 mmol.l^{-1} on individual control day until drop of BHB under threshold 1.0 mmol.l^{-1}.

Table 1. ***The concentrations of BHB (mmol.l^{-1}) on different lactation in individual control day in milk (DIM) postpartum***

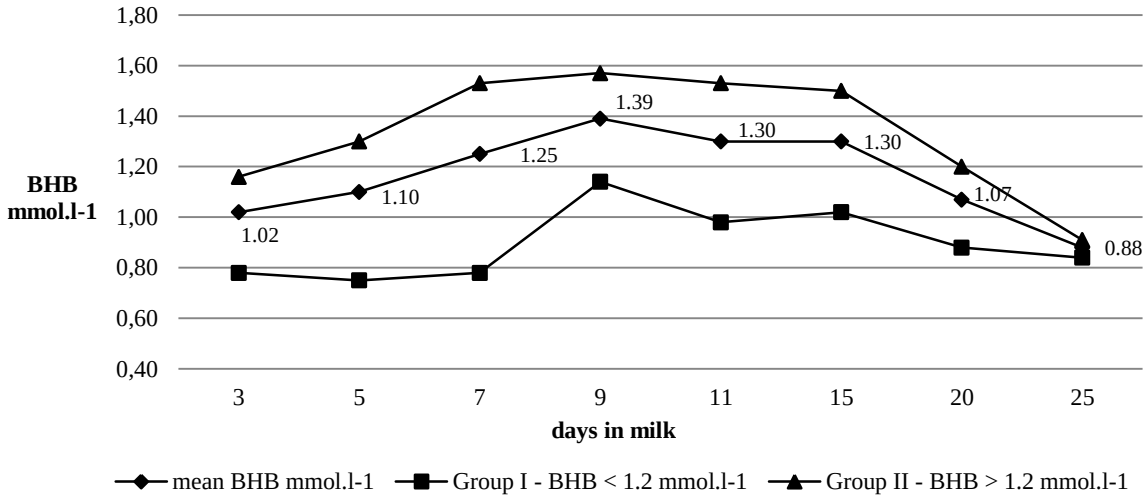
period and DIM BHB mmol.l^{-1}	n	ANTE PARTUM	POST PARTUM							
			3.	**5.**	**7.**	**9.**	**11.**	**15.**	**20.**	**25.**
mean of all dairy cows	2	**0,4**	**0,74**	**0,84**	**0,94**	**1,00**	**0,96**	**0,98**	**0,87**	**0,72**
minimal value	3	0,3	0,3	0,3	0,4	0,4	0,4	0,3	0,3	0,3
maximal value		0,6	2,2	2,2	2,8	2,8	3,3	2,8	1,7	1,3
cows on 1.and 2. lactation mean BHB mmol.l^{-1}	11	**0,3**	**0,49**	**0,63**	**0,67**	**0,61**	**0,65**	**0,70**	**0,70**	**0,58**
cows on ≥ 3. lactation mean BHB mmol.l^{-1}	12	**0,5**	**1,02**	**1,10**	**1,25**	**1,39**	**1,30**	**1,30**	**1,07**	**0,88**
mean group I - BHB < 1,2 mmol.l^{-1}	5	0,4	0,78	0,75	0,78	1,14	0,98	1,02	0,88	0,84
mean group II - BHB > 1,2 mmol.l^{-1}	7	0,5	1,16	1,30	1,53	1,57	1,53	1,50	1,20	0,91

BHB – β-hydroxybutyrate, clinical ketosis = BHB ≥ 2,5 mmol.l^{-1}, sub clinical ketosis = BHB ≥1,0mmol.l^{-1}

The objectives of this management are: - *herd or group level*: to monitor the success of current management with the goal of early detection of problems or deviation from the management program; - *individual level*: to identify cows at high risk for disease with the goal of intervention for these individuals to prevent or mitigate clinical disease. Only 2 dairy cows were diagnosed uncompensated ketogenesis with clinical sign of ketosis and concentration of BHB ≥ 2.5 mmol.l^{-1} during time of study. Those dairy cows have also suffered dislocation of abomasum and the proactive management helped identify and diagnose this complicated health disorders and provide adequate urgent therapy with quick restart of high lactation.

The dairy cows on third or higher lactation with calving were divided to two group depended on degree of ketogenesis. The **group I (***n=5*) consist of cows with BHB concentration between 1.0 – 1.2 mmol.l^{-1} diagnosed maximal 3 different times in designed control days in postpartum – *compensated ketogenesis*. The **group II (***n=7*) consist of cows with BHB concentration ≥ 1.2 mmol.l^{-1} diagnosed more than 4 times in designed control days in postpartum – *uncompensated ketogenesis*. The prophylactic therapy was administered to dairy cow after each control measure with result of BHB above the threshold 1.0 mmol.l^{-1}. In group I the average milk production was 36.4 ± *4.6* kg after calving until 25 DIM of the dairy cows on third or higher lactation with lower ketogenesis. In the group II the average milk production was 34.3 ± *3.6* kg after calving until 25 DIM of the dairy cows on third lactation with higher ketogenesis, which means the difference 2.1 kg of milk per cow per day. Very interesting is fact, that the highest average concentration of BHB was recorded between 9. and 15. days postpartum with drop of average concentration of BHB under threshold 1.0 mmol.l^{-1} on 25. DIM.

Graph 1. ***Dynamics of BHB (mmol.l^{-1})^{1} in individual lactating days of dairy cows on third or higher lactation (n=12) with compensated ketogenesis – group I (BHB <1.2 mmol.l^{-1}) (n=5) and uncompensated ketogenesis – group II (BHB ≥1.2 mmol.l^{-1}) (n=7)***



BHB – β-hydroxybutyrate

In group I the average concentration of BHB was 0.90 mmol.l^{-1} and for this group were detected the highest BHB levels in critical period for SCK between 9. – 15. DIM postpartum with average concentration of BHB 1.05 mmol.l^{-1}. In group II the average concentration of BHB was 1.34 mmol.l^{-1} and for this group were detected the highest BHB levels in critical period for SCK between 7. – 15. DIM postpartum with average concentration of BHB 1.53 mmol.l^{-1}.

The dry matter intake of all dairy cows on the farm was very precisely controlled and recorded every day. The average dry matter intake of dairy cows in postpartum group was 20.5 kg per cow per day. The dairy cows were relocated to main lactation group in average on 25 DIM depends on absence of any health disorders, high and balanced milk production and controlled high dry matter intake.

Routine daily management and control of postpartum dairy health consisted of: evaluation of body condition and decline, dry matter intake by weight of residues and rumen fill, auscultate control of rumination, control of rectal temperature, palpate examination of udder and per rectum palpation and massage of reproductive organs. Described control of health help minimalize the culling rate and maximize the number of reached peak of lactation of cows.

Conclusion

Ketosis is a prevalent and important disease, which is associated with increased risk of left displaced abomasum, decreased milk production, and decreased reproductive performance. Measurement of the prevalence of subclinical ketosis is useful for investigation of herd problems of transition cow health and performance, and for routine monitoring. Early detection of SCK by cow-side blood test followed by adequate treatment and management reduces the negative impacts of SCK. Our results show that monitoring change in the β-hydroxybutyric acid blood level in high-yielding cows in the early *post partum* period by electronic hand-held meter Precision Xtra® may be effective in reducing the incidence of ketosis and health problems associated with ketosis in dairy cattle herds.

Acknowledgement

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References

1. Adewuyi A, Gruys E, Van Eerdenburg F 2005: Non esterified fatty acids (NEFA) in dairy cattle. A review. Vet Q 27: 117-126
2. Chapinal N, Carson M, Duffield T, Capel M, Godden S, Overton M, Santos J, Le Blanc S 2011: The association of serum metabolites with clinical disease during the transition period. J Dairy Sci 94: 4897-4903
3. Duffield T, Sandals F, Leslie K, Lissemore K, McBride B, Lumsden J, Dick P, Bagg R 1998: Efficacy of monensin for the prevention of subclinical ketosis in lactating dairy cows. J Dairy Sci 81: 2866-2873
4. Duffield T 2000: Subclinical ketosis in lactating dairy cattle. Vet Clin North Am Food AnimPract 16: 231-253
5. Duffield T, Lissemore K, McBride B, Leslie K 2009: Impact of hyperketonemia in early lactation dairy cows on health and production. J Dairy Sci 92: 571-580
6. Iwersen M, Falkenberg U, Voigtsberger R, Forderung D, Heuwieser W 2009: Evaluation of an electronic cowside test to detect subclinical ketosis in dairy cows. J Dairy Sci 92: 2618-2624
7. Oetzel G 2013: Understanding the impact of Subclinical ketosis. 24th ruminant nutrition symposium. Available at: http://dairy.ifas.ufl.edu/rns/2013/2_oetzel.pdf. Last modified February 2013. Accessed February, 2013.
8. Rollin E, Berghaus R, Rapnicki P, Godden S, Overton M 2010: The effect of injectable butaphosphan and cyano-cobalamin on *post partum* serum β-hydroxybutyrate, calcium, and phosphorus concentrations in dairy cattle. J Dairy Sci 93: 978-987

EFFECTS OF SELECTED ESSENTIAL OILS ON RUMEN FERMENTATION AND METHANE PRODUCTION *IN VITRO*

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Abstract

The effects of five essential oils on rumen fermentation and methane production were assessed under *in vitro* conditions. Single dose (250 µl/l) of essential oils was evaluated using *in vitro* 24 h batch culture of rumen fluid with a substrate (alfalfa silage:maize silage:concentrate 350:350:300 dry matter). Treatments were eugenol, carvacrol, citral, cineole, limonene and control (no additive). Treatments were incubated in triplicate in 300 ml serum bottles containing 1 g of substrate, 50 ml of the strained ruminal fluid, and 100 ml of phosphate-bicarbonate buffer. After 24 h, the pH and the methane production were determined and samples were collected to analyze volatile fatty acids (VFA). Essential oils did not modified total methane and VFA production. Acetate, propionate and butyrate proportion and acetate:propionate ratio and pH remained also unaffected. It can be concluded that the supplementation of eugenol, carvacrol, citral, cineole, and limonene at relatively low concentration 250 µl/l was not a potent manipulator of rumen fermentation *in vitro.*

Keywords: Essential oils, in vitro rumen fermentation, Methane, Volatile fatty acids

Materials and methods

The effects of five essential oils on rumen fermentation and methane production were evaluated using *in vitro* batch incubation system. The chemical analysis and *in vitro* experiments were done in the Institute of Animal Science in Prague.

The essential oils tested were eugenol [EUG, 2-Methoxy-4-(2-propenyl)phenol], carvacrol [CAR, 2-Methyl-5-(1-methylethyl)-phenol], citral [CIT, 7-dimethyl-2, 6-octadienal], cineole [CIN, 4-methyl-1-propan-2-yl-7-oxabicyclo(2.2.1)heptanes] and limonene [LIM, 1-Methyl-4-(1-methylethenyl)-cyclohexene]. The essential oils were obtained from Sigma-Aldrich Chemical. The intent of this experiment was to screen several essential oils to select those with positive effects for more in depth study. The dose used in this study (250 µl/l) was selected based on previous *in vitro* studies showing positive effect of essential oils on methane mitigation and moderate effect on VFA production (Macheboeuf et al., 2008; Soltan et al., 2011; Lin et al., 2013).

The experimental substrate consisted of three feeds (alfalfa silage, maize silage, concentrate) in proportion of 350:350:300 mg on a dry matter basis.

The rumen fluid was collected 2 hours after the morning feeding from three rumen fistulated Holstein cow. The cow was fed a total mixed ration (TMR) based on alfalfa silage, maize silage, and concentrate mix. Rumen content was brought to the laboratory in vacuum flasks, strained through layer of cheesecloth, and used within 20 min.

In vitro fermentations were conducted in serum bottles. Briefly, 1 g of dried substrate with additives was incubated in 300 ml capacity gas-tight serum bottles containing 100 ml of phosphate-bicarbonate buffer according to McDougall (1948) with modifications (per liter of distilled water: 9.8 g $NaHCO_3$; 7 g $Na_2HPO_4 \cdot 12\ H_2O$; 0.6 g urea; 0.6 g KCl; 0.03 $CaCl_2$; 0.06 $MgSO_4$) and 50 ml rumen fluid. Fermentation bottles without additives, but containing 1 g of

the substrate, were used as a control. Bottles with substrate, additives and incubation medium were stored for 24 h at 39 °C after sealing with a butyl rubber stopper and aluminium caps.

After 24 h of incubation, the gas in headspace of bottles was analyzed for CH_4 using a gas chromatography (Labio GC 82F) equipped with a flame ionization detector and capillary column. A sample of 0.1 ml of gas was injected using a 1 ml Sample-Lock® syringe (Hamilton, Nevada, USA).

After opening the incubation flask, pH was measured (pH 700, Eutech Instruments, Singapore), and 2 ml of incubation medium were collected and centrifuged 1 min at 13,000 RPM. 64 µl of supernatant was mixed together with 736 µl of H_2O, 30 µl of internal standard and 100 µl of formic acid and then centrifuged again 1 min at 13,000 RPM. Samples were stored at 8 °C until VFA analysis using gas chromatography on a Labio GC 82F equipped with a flame ionization detector and capillary column. Briefly, 1 µl was injected, the injector temperature was 200 °C and the inlet pressure 50 kPa. The temperature program was 75 °C at the start of the injection, increasing 5 °C/min until 80 °C (kept for 1 min), increasing 6 °C/min until 128 °C (kept for 10 min). The detection temperature was 200 °C.

Samples of alfalfa and maize silage were dried in forced air oven at 65 °C. Subsequently, silages and concentrate samples were ground to pass a 1 mm sieve. Ground samples were stored for chemical analyses. The chemical composition of substrates was determined according to AOAC (2005) as for crude protein (CP as 6.25 x N); starch, and ash, according to AOAC (1995) as for ether extracts. The neutral detergent fiber (NDF) and, acid detergent fiber (ADF) were measured according to Mertens (2002). The ADF and NDF were assayed with a heat stable amylase and expressed exclusive of residual ash.

Data were subjected to analysis of variance (ANOVA), using the General Linear Model procedure (GLM) of SAS software package (2002). Significance was declared at $P < 0.05$.

Results and discussion

The overall chemical composition of experimental substrate and chemical composition of individual feeds is presented in Table 1. The substrate content of crude protein and NDF was in accordance with the findings of Klevenhusen et al. (2012), who showed in meta-analysis of 20 studies, that the content of crude protein and the amount of NDF of the incubated diets ranged from 139 to 189 g/kg and 160 to 420 g/kg, respectively. It appears that effects of some metabolites in essential oils could depend on type of diet used for *in vitro* incubation (Calsamiglia et al., 2007). Dietary NDF potentiated the effect of bioactive compound of essential oils on total VFA, whereas it counteracted effects of bioactive compounds on acetate and propionate proportion and acetate:propionate ratio (Klevenhusen et al., 2012).

The effects of the essential oils supplementation after 24 h incubation on pH, CH_4 and VFA production, acetate, propionate and butyrate proportion and acetate:propionate ratio are presented in Table 2. Methane production and VFA production at dose of essential oils 250 µl/l remained unaffected in all supplemented groups, and was found similar in control and treated groups. Eugenol and cineole only tended to decrease methane production. These results are inconsistent with other findings. According to Lin et al. (2013) the optimal combination for methane production inhibition is when a mixture of eugenol, carvacrol, citral and cinnamaldehyde at an equal weight ratio is added at 200 µl/l. However, these authors used in their study mixtures of essential oils. A combination of different types of essential oils components that exert their activities through varying mechanism together would lead to the production of new essential oils with different bioactivity (Newbold et al., 2004). The minimal concentration of carvacrol-based essential oils that caused inhibition of rumen fermentation is between 150 and 300 µl/l (Macheboeuf et al., 2008). Conversely Calsamiglia

et al. (2007) found that the suitable level of essential oils was approximately 500 µl/l and Klevenhusen et al. (2012) reported that doses of bioactive compounds including essential oils above 100 mg/g DM (in our study 37.5 mg/g DM) do not necessarily modify *in vitro* ruminal fermentation in dose-response manner.

In present study the essential oils did not significantly influence pH value. It can be attributed to no changes in production of VFA. These results confirmed that none of the additives at examined dose had dramatic impact on rumen fermentation.

As the main mechanism of essential oils for manipulating rumen fermentation has been reported and was regarded selective inhibition by essential oils of specific microbes, such as methanogens, protozoa and hyper-ammonia-producing bacteria (Calsamiglia et al., 2007). Generally, it is assumed that gram-positive bacteria are more inhibited by essential oils than gram-negative due to their simple cell membrane compared to the more complex cell wall of gram-negative bacteria (Cimanga et al., 2002). Thus shifts in bacterial composition may overall reduce bacterial fermentative activity and selectively favour less essential oil-sensible gram-negative bacteria (Klevenhusen et al., 2012). In the rumen, gram-positive bacteria are generally acetate- and butyrate-producing bacteria, while Gram-negative bacteria are generally propionate-producing bacteria (Hobson & Stewart, 1997). However rumen microbial system is relatively stable and to some extent resistant to manipulation thus concentration in this experiment was too low to influence fermentation.

Eugenol is one of the main compound of clove and cinnamon oils (Davidson & Naidu, 2000), and it has been shown to have antimicrobial activity against gram-positive and gram-negative bacteria (Dorman & Deans, 2000). Treatments 5 mg/l, 50 mg/l, and 500 mg/l modified VFA proportions without affecting total VFA concentration (Castillejos et al., 2006).

Carvacrol is one of the major components of oregano and thyme oils. Dorman & Deans (2000) demonstrated the antimicrobial activity of carvacrol against gram-positive and gram-negative bacteria. The addition of carvacrol 400 mg/l did not affect total VFA concentration, but changed molar proportions of VFA compared with the control (Benchaar et al., 2007). Phenolic compounds such as thymol, carvacrol, and eugenol have been shown to possess high antimicrobial activity due to the presence of a hydroxyl group in the phenolic structure (Dorman & Deans, 2000).

Citral, a monoterpenoid, is the major constituent of lemon grass that gives off a lemony scent and is postulated to be responsible for most of its actions (Devi et al., 2011). Citral displayed moderate activity against gram-positive and gram-negative bacteria (Dorman & Deans, 2000). No studies determining effects of alone citral on rumen fermentation *in vitro* were found. Treatments 5 mg/l, 200 mg/l, and 500 mg/l of mixture with higher in two aldehyde-based essential oils (including citral) showed higher inhibitory effect on total VFA than phenolic-based mixture (Lin et al., 2013).

Limonene is most abundant monocyclic monoterpene in lemons, oranges, grapefruit, peppermint, spearmint, and other oils (Turner, 1999). Dorman & Deans (2000) demonstrated the antimicrobial activity of limonene, mainly against gram-negative bacteria. Castillejos et al. (2006) reported that limonene at 50 and 500 mg/l had reduced total VFA concentration by -4.5% and -5.6%, respectively, authors concluded that there appears to be no benefit of using limonene as an additive to modify rumen fermentation.

Table 1: Chemical composition of alfalfa silage (AS), maize silage (MS), concentrate (CON) and experimental substrate (SUB) (g/kg DM, except DM: g/kg)

	AS	MS	CON	SUB
Dry matter	410,8	299,0	890,9	1000,0
Crude protein	181,3	78,3	235,5	161,5
Ether extract	16,5	39,0	79,4	43,2
Starch	-	376,0	337,6	232,9
NDF[1]	472,6	419,2	178,4	365,6
ADF[2]	399,9	224,1	77,9	241,8
Ash	104,9	41,6	113,2	85,3

[1]NDF = Neutral detergent fiber
[2]ADF = Acid detergent fiber

Table 2: Effect of active components from essential oils on 24 h of in vitro rumen fermentation

Items	Active components						SEM[1]	P Value
	Control	Eugenol	Carvacrol	Citral	Cineole	Limonene		
pH	6,40	6,40	6,43	6,40	6,43	6,43	0,01	NS[2]
CH_4 (mmol)	4,10	3,88	4,33	4,18	3,61	4,44	0,17	NS
VFA[3] (mmol)	15,71	14,90	15,11	16,22	15,73	14,69	0,32	NS
mol/100 mol VFA								
Acetate	51,26	50,85	50,33	51,18	51,22	51,21	0,60	NS
Propionate	28,83	28,87	28,33	28,50	28,75	28,80	0,51	NS
Butyrate	11,25	11,54	12,30	11,44	11,32	11,24	0,16	NS
Acetate:propionate	1,79	1,78	1,79	1,81	1,80	1,79	0,05	NS

[1]SEM = Standard error of the means
[2] NS = No significant
[3]VFA = Volatile fatty acids

Conclusion

Based on the present findings, it can be concluded that the supplementation of eugenol, carvacrol, citral, cineole, and limonene at relatively low concentration 250 µl/l was not a potent manipulator of rumen fermentation *in vitro.* This may have implications for in vivo conditions, because supplementation of high doses of essential oils reported *in vitro* may not have physiological relevance. Hence, only low to moderate essential oils doses are feasible *in vivo.*

Acknowledgment

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Reference

1. Benchaar, C. et al., 2007. Effects of essential oils and their components on in vitro rumen microbial fermentation. *Canadian Journal of Animal Science*, 87(3), pp.413–419.
2. Calsamiglia, S. et al., 2007. Invited review: Essential oils as modifiers of rumen microbial fermentation. *Journal of dairy science*, 90(6), pp.2580–95.
3. Castillejos, L., Calsamiglia, S. & Ferret, A., 2006. Effect of essential oil active compounds on rumen microbial fermentation and nutrient flow in in vitro systems. *Journal of Dairy science*, 89(7), pp.2649–58.

4. Cimanga, K. et al., 2002. Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. *Journal of Ethnopharmacology*, 79(2), pp.213–220.
5. Davidson, P.M. & Naidu, A.S., 2000. Phyto-phenols. In *Natural Food Antimicrobial Systems*. pp. 265–293.
6. Devi, R.C., Sim, S.M. & Ismail, R., 2011. Spasmolytic effect of citral and extracts of Cymbopogon citratus on isolated rabbit ileum. *Journal of Smooth Muscle Research = Nihon Heikatsukin Gakkai Kikanshi*, 47(5), pp.143–56.
7. Dorman, H.J.D. & Deans, S.G., 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, 88(2), pp.308–316.
8. Hobson, P.N. & Stewart, C.S. eds., 1997. The rumen bacteria. In *The Rumen Microbial Ecosystem*. Dordrecht: Springer Netherlands, pp. 10–72.
9. Klevenhusen, F. et al., 2012. A meta-analysis of effects of chemical composition of incubated diet and bioactive compounds on in vitro ruminal fermentation. *Animal Feed Science and Technology*, 176(1-4), pp.61–69.
10. Lin, B. et al., 2013. In vitro rumen fermentation and methane production are influenced by active components of essential oils combined with fumarate. *Journal of Animal Physiology and Animal Nutrition*, 97(1), pp.1–9.
11. Macheboeuf, D. et al., 2008. Dose–response effects of essential oils on in vitro fermentation activity of the rumen microbial population. *Animal Feed Science and Technology*, 145(1-4), pp.335–350.
12. McDougall, E.I., 1948. Studies on ruminant saliva. 1. The composition and output of sheep's saliva. *The Biochemical Journal*, 43(1), pp.99–109.
13. Mertens, D., 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. *Journal of AOAC International*.
14. Newbold, C.. et al., 2004. Effects of a specific blend of essential oil compounds on rumen fermentation. *Animal Feed Science and Technology*, 114(1-4), pp.105–112.
15. Soltan, Y.A. et al., 2011. Carvacrol and eugenol as modifiers of rumen microbial fermentation, and methane production in vitro. In *Proceedings of the 4th Scientific Conference of Animal Wealth Research in the Middle East and North Africa, Foreign Agricultural Relations (FAR), Egypt, 3-5 October 2011*. Massive Conferences and Trade Fairs, pp. 354–364.
16. Turner, G., 1999. Limonene Synthase, the Enzyme Responsible for Monoterpene Biosynthesis in Peppermint, Is Localized to Leucoplasts of Oil Gland Secretory Cells. *Plant Physiology*, 120(3), pp.879–886.

EFFECT OF LOW-TEMPERATURE PLASMA DISCHARGE ON THE PROTEIN C OMPOSITION OF GRAINS.

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Abstract

One of the strategic and objectives goals of the nutrition policy of the Czech Republic is to ensure hygienic quality parameters of agricultural products that enter to the food chain. A significant risk is the contamination of feed by fungi or mycotoxins, which may have significa nt negative effects on animal health, which the contaminated feeds eat. How to fight with fung i and mycotoxins is difficult and not all ways are effective. One option how to protect product s is to use the low-temperature plasma.

Till today there has not been in detail examined the effect of this method, the protein c omposition of the treated grain. Any negative impact on the composition of essential amino ac ids in the future meant a significant limitation of use of this promising method. For treated gra ins plasma discharge has not been demonstrated statistically significant change in the content of essential amino acids.

Keywords: low-temperature plasma, grains, nutritional value

Material and methods

The plasma discharge is generated by supplying energy (mainly electric fields) in a ne utral gas, which creates a charge. The electrons, ions and free radicals are produced in the gas phase when sufficient energy electrons collide with neutral atoms and molecules in the feed g as (Conrads, Schmidt, 2000). After application of the plasma discharge occurs to disinfect the surface of the products (Perni et al. 2008) and inhibition of fungi which are on the surface and beneath the surface (Avramidis et al. 2010).

One advantage of using the plasma discharge is inhibition of microorganisms and the decomposition of some substances. This property is already used in medical practice, when th e low-temperature plasma is extremely effective to sterilize heat-sensitive substrates compare d sparing methods (Friedmann 2008). Use of plasma discharge potentially offers a good altern ative to conventional sterilization procedures, which are effective in killing microorganisms (Purevdorj et al, 2003).

As the experimental grain was selected triticale, samples were obtained from one ecolo gical farm near the town Český Krumlov. It was created 33 samples, 32 of which were treated by plasma discharge.

For the treatment of grain was used existing installations of low-temperature plasma di scharge type Gliding Arc consisting of high voltage power supplies, plasma head operating at atmospheric pressure (the nozzle), equipment for mixing of grains during the process, compre ssor and the flowmeter Omega regulating the amount of working gas. As a working gas was u sed compressed air. It was gradually modified the time for processing surface of the seeds. Th ere were subsequently detected any changes in the composition of proteins especially essentia l amino acids grain treated with low-temperature plasma discharge in different time intervals. The content of amino acids was determined by the machine AAA 400. There were monitored basic essential amino acids (arginine, lysine, histidine, isoleucine, leucine, methionine and ph

enylalanine, threonine), and if plasma discharge can change their content in each time interval s.

Results and discussion

At this experiment were evaluated basic essential amino acids, which were treated by plasma discharge. In table 1 is the comparison of the detected contents of lysine in triticale sa mples that have been treated by plasma discharge of varying duration. Lysine content does not show statistically significant differences.

Table 1: The lysin content in the triticale samples treated with plasma discharge. Tukey HSD test

number	time (min)	{1} 3,9968	{2} 4,0720	{3} 4,1970	{4} 4,1636
1	1		0,902482	0,285226	0,441539
2	2	0,902482		0,670393	0,838510
3	3	0,285226	0,670393		0,990095
4	4	0,441539	0,838510	0,990095	

Graph 1: The lysin content in samples treated with plasma discharge in different time period.

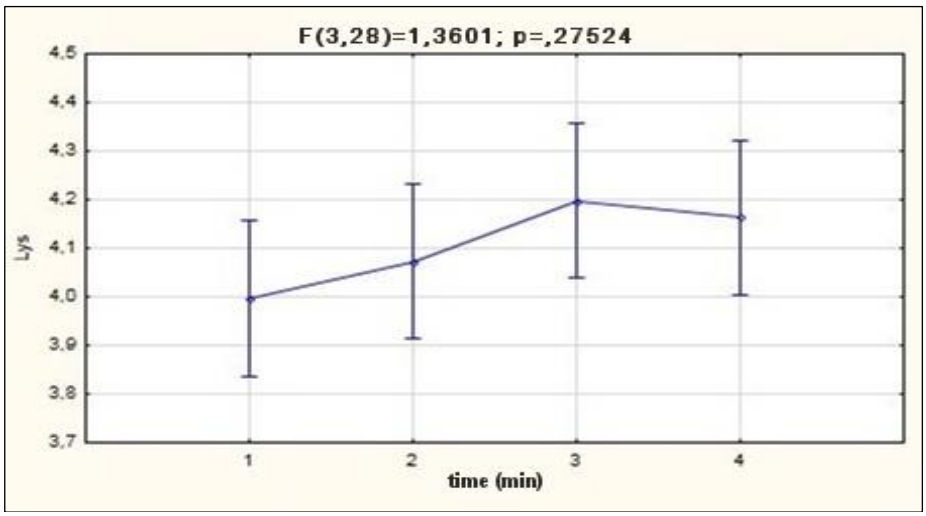


Table 2 compares the detected contents of methionine in triticale samples that have be en treated by plasma discharge in a different time period. The content of methionine does not show statistically significant differences.

Table 2: The methionine content in the triticale samples treated with plasma discharge . Tukey HSD test

number	time (min)	{1} 7,6036	{2} 7,8814	{3} 8,0750	{4} 8,1732
1	1		0,570665	0,146392	0,057681
2	2	0,570665		0,801846	0,530556
3	3	0,146392	0,801846		0,967197
4	4	0,057681	0,530556	0,967197	

Graph 2: The methionine content in samples treated with plasma discharge in differen t time period.

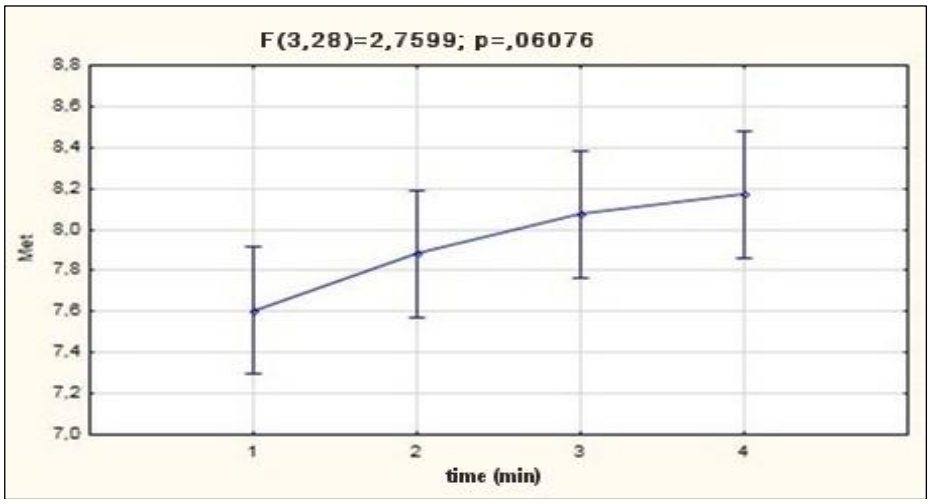
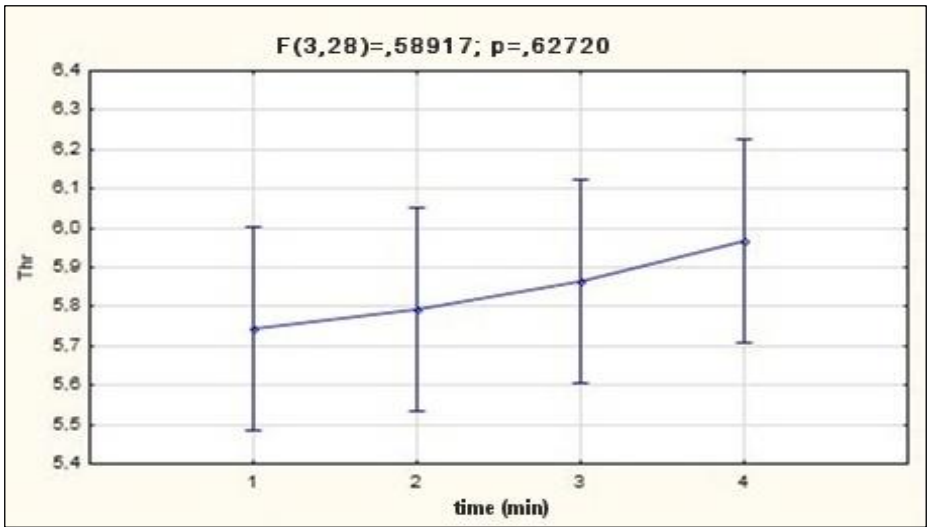


Table 3 compares the detected contents of threonine in triticale samples that have been treated by plasma discharge in a different time period. Threonine content does not show statist ically significant differences.

Table 3: The threonine content in the triticale samples treated with plasma discharge. Tukey HSD test

number	time (min)	{1} 5,7438	{2} 5,7916	{3} 5,8627	{4} 5,9671
1	1		0,993241	0,909450	0,602141
2	2	0,993241		0,978343	0,761061
3	3	0,909450	0,978343		0,936192
4	4	0,602141	0,761061	0,936192	

Graph 3: The threonine content in samples treated with plasma discharge in different time period.



The others evaluated essential amino acids showed the same results. None of the amino acid does not show statistically significant differences. This method does not change the value of basic amino acids treated by plasma discharge in grains.

Conclusion

The content of essential amino acids does not show statistically significant variations i n the treatment of plasma discharge in different time period. There is no change in the biologi cal value of the proteins contained in the treated grain. From this perspective it appears that lo w-temperature plasma can be used as a promising method to combat fungi and their residues. These mentioned results are only one part of whole experiment work. At this part were showe d basic essential amino acids and if there are some changes in different time. At his experimen t will be evaluate also the distance, temperature and the gas flow which are used to treat the gr ains.

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References

1. Avramidis G.et.al.. (2010): Fungicidal effects of an atmospheric pressure gas discharge a nd degradation mechanisms. Surface and Coating Technology 205, 405..
2. Conrads, H., Schmidt, M. (2000)**:** Plasma generation and plasma source. Plasma Sources Sci. Technol., 9, 441–454.
3. Friedmann G. et al. (2008)**:** Applied Plasma Mecine. Plasma Process. Polym., 5, 503.
4. Perni S. et. al**.** (2008): Cold atmospheric plasma decontamination of the pericarps of fruit. Journal of Food Protection, 71, 302-308.
5. Purevdorj D., Igura N., Ariyada O., Hayakawa I. (2003)**:** Effect of feed gas composition o f gas discharge plasmas on Bacillus pumilus spore mortality. Lett Appl Microbiol, 37, 31 –34.

LACTOBACILLUS FERMENTUM AND *ENTEROCOCCUS FAECIUM* IN THERAPY AND PREVENTION OF DIGESTIVE DISORDERS IN DOMESTIC RABBIT (*ORYCTOLAGUS CUNICULUS*)

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Abstract

The aim of this study was the monitoring of the weight gain and health status of three groups of rabbits. The first group was the control group (CG), the second was receiving *Lactobacillus fermentum* CCM 7158 (LP) and the last group was receiving probiotic strains *Lactobacillus fermentum* CCM 7158 and *Enterococcus faecium* EF 2019 (LEP). Very intensive growth as well as the best health state conditions and the lowest mortality were observed in the group of LEP.

Keywords: rabbit, probiotics, digestive, prevention

This study is aimed at the effect of probiotic cultures of *Lactobacillus fermentum* and *Enterococcus faecium* on rabbits digestive system. The rabbits digestive system is very sensitive system that answers rapidly not only to any imbalance in feed ration, breeding system, climatic changes, but also to administration of therapy or transport stress. Therefore, it is very important to ensure more effective protection of digestive system, especially in some critical periods as the weaning. First two weeks after the weaning, during breeding or tattooing and transportation. Coccidia and clostridia belong to the most important pathogenic agents in rabbits that are causing the severe clinical problems which are often fatal. So far, the general opinion of vets and breeders is that the therapy utilizing chemical anti-coccidia drugs is the most effective for treatment of coccidiosis and clostridiosis.

Protecttion period for slaughter rabbit meat, depression of immune system or degradation of favourable digestive microflora are the main disadvantages of the preparations which are mentioned above. Because of the elimination of favourable microflora, the digestive disorders appeared after 1 - 2 weeks after the application of therapy. The disorders are characterized by bloat, diarrhoea, innapetence, decrease of body weight gains and mortality. The study and work experiences disconfirmed these long-term used treatment methods with chemical agents. The aim of our study was to observe the effect of probiotic cultures *Lactobacillus* and *Enterococcus* on the digestive system of domestic rabbit *Oryctolagus cuniculus*. The probiotic cultures have local and systematic biomedical effect. They are effectively and successfully utilizable in nutrition, prevention and therapy of rabbits in small breedings as well as in farm breeding systems.

Material and methods

In this study, the combination of *Lactobacillus* and *Enterococcus* with maltodextrin and prebiotic (fructooligosaccharide) was used. The animals were divided into three groups. Only chemical anticoccidial drugs (sulfonamide base) were administered to the first group – the control group (CG) with 69 animals. The combination of *Lactobacillus* and maltodextrin and prebiotics (fructooligosaccharide) was administered to the second group (LP) with 74 rabbits .112 rabbits were included into third group (LEP). This group received both probiotic cultures (*Lactobacillus* and *Enterococcus*) with maltodextrin and prebiotics (fructooligosaccharide).In the each phase of breeding, the body weight, health status and the number of coccidia were determined.

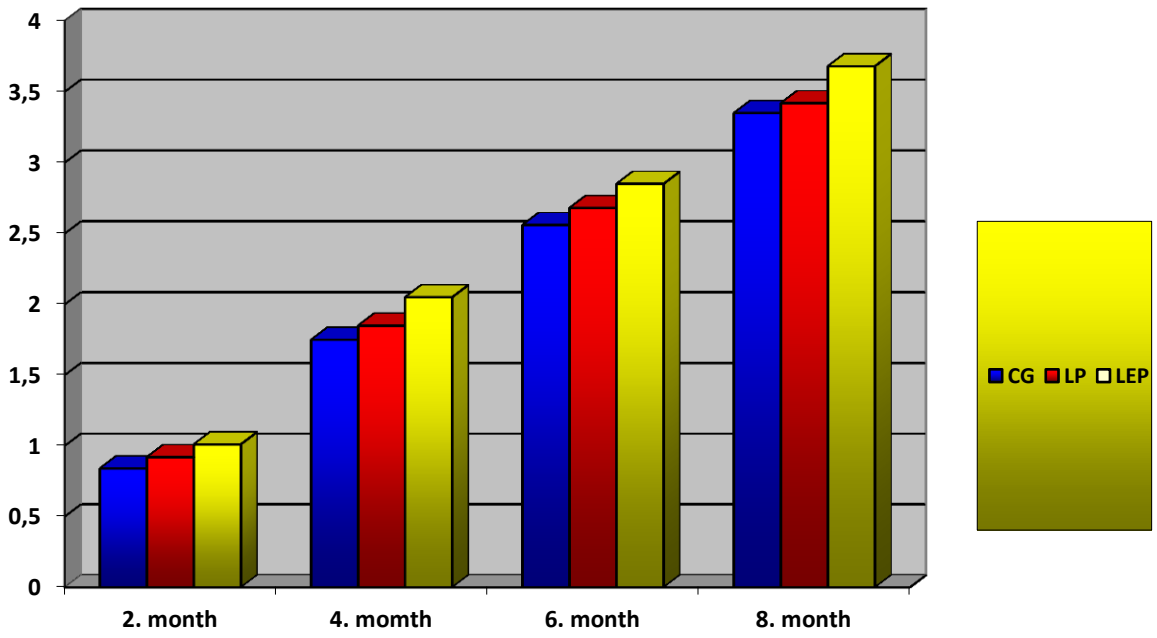
Results and discussion

Daily weight gains in the LEP group were 17% higher than in the LE group, and 24% higher than in the CG group. The number of animals with digestive disorders in the groups were 24% (CG), 14% (LE) and 5% (LEP).

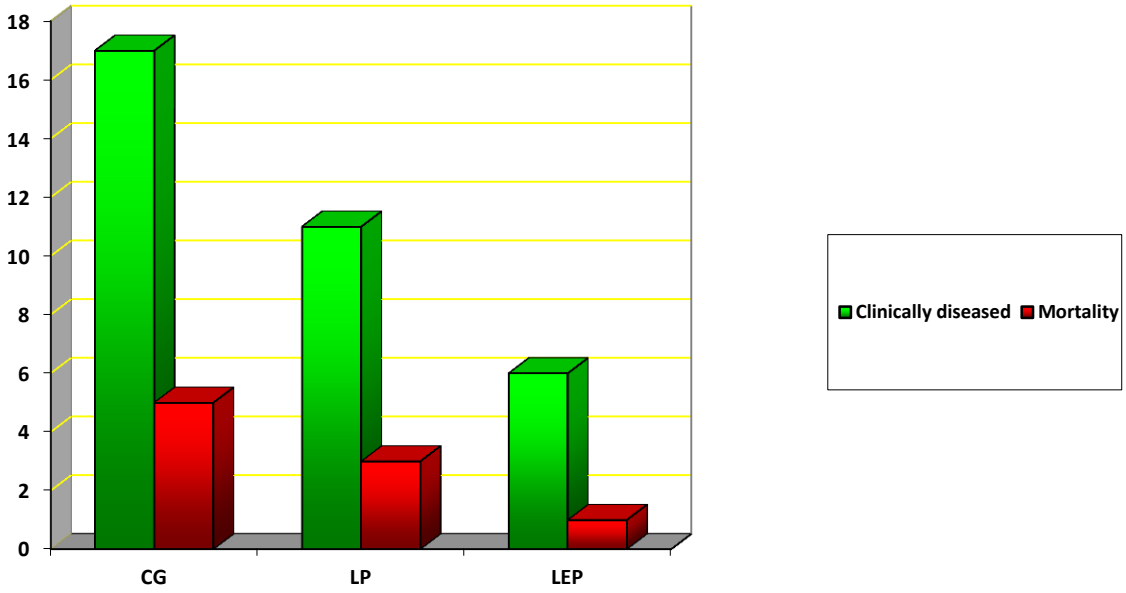
Table 1: Weight gains

	2. Month (0,9 kg)	**4. Month (1,9 kg)**	**6. Month (2,8 kg)**	**8. Month (3,5 kg)**
CG n=69	0,84	1,75	2,56	3,35
LP n= 74	0,92	1,85	2,68	3,42
LEP n=112	1,01	2,05	2,85	3,68

Graph 1: Weigth gains in the CG, LP and LEP groups compared to standard



Graph 2: Clinically diseased rabbits and mortality in the particular groups



Conclusion

During the experiment the most positive results were reported in the LEP group of rabbits that were fed with both probiotic cultures combined with maltodextrin and prebiotic (fructooligosaccharide). The best preventive effect was observed after the application of the mentioned combination of preparations.

References

1. Árvayová, M., Pospíšilová, D., Supuka, P.: Vplyv laktobacilov a humínových látok na mikrobiálne zloženie obsahu čreva u prepelíc a králikov. In: *Spravodajca* , 2012, 1, 10-13. ISSN 1337-6691.
2. Bomba, A., Nemcová, R., Gancarčíková, S., Mudroňová, D., Jonecová, Z., Koščová, J., Sciranková, Ľ., Buleca, V., Švalec, J. (2005): Uplatnenie probiotík vo výžive, prevencii a terapii chorôb hospodárskych a domácich zvierat. Slovenský veterinársky časopis XXX(1), p. 31-32.
3. Bomba, A., Strojný, L., Chmelárová, A., Hijová, E., Bertková, I., Nemcová, R. (2010): Synbiotics and Potentiated Probiotics in Modulation of the Gastrointestinal Ecosystem. International scientific conference probiotics and prebiotics, ISBN 978-80-970168-4-5, p. 18.
4. Pospíšilová, D.: Možnosti prevencie a liečby ochorení v chovoch hospodárskych zvierat bez použitia antibiotík a chemických látok. In: *Spravodajca*, 2012, 3, s. 6-7. ISBN 1337-6691.

THE EFFECT OF PUMPKIN AND FLAXSEED OILS ON SELECTED PARAMETERS OF LAYING HENS PERFORMANCE

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Abstract

The aim of the study was to analyze the dietary effect of pumpkin and flaxseed oils on performance parameters of laying hens. Lohmann Brown Lite hens were randomly divided to three groups. Total 18 hens (6 per group) were monitored. Hens in control group (C) were fed by standard diet. First group (E1) was fed by complete feed mixture supplemented with pumpkin oil, and second group (E2) with flaxseed oil. Dosage of oils was 3 % in feed mixture. Twelve eggs from each group were evaluated. Average weight of eggs was 60.87 g in C group, 65.28 g in E1 group and 65.68 g in E2 group. Average albumen´s weight was 38.24 g in group with standard diet, 41.47 g after pumpkin and 40.65 g after flaxseed oil supplementation. In control group was average shell´s weight 4.86 g, in E1 group 6.70 g and in E2 group 6.66 g. After oil supplementation, there were found significant ($P<0.05$) differences in average egg´s weight, albumen´s weight and shell´s weight. In yolk´s weight wasn´t found significant differences. Average value of colour of yolk was 5.78 in C, 6 in E1 and 5.25 in E2 group.

Keywords: additives, laying hens, eggs, flaxseed oil, pumpkin oil

Material and methods

Experiment was realized in cooperation with the Department of Poultry Science and Small Husbandry. At 38 weeks of age, Lohmann Brown Lite hens were housed in three-floor cages (943.2 cm^2 per hen), divided into three diets of groups (C-control, E1-pumpkin oil (3%), E2-flaxseed oil (3%)). There were housed six hens in one cage. Feed mixture was composed from wheat, corn, soybean meal, rapeseed meal, sunflower meal, animal fat, soybean oil, calcium carbonate, feed additives, sodium bicarbonate, monocalcium phosphate, sodium chloride and enzyme complex of phytase. The amount of nutrients is shown in Table 1. Laying hens in all groups received feed and drinking water *ad libitum*. During experiment, the light regime was 16 hours. The experiment lasted 52 days and during the whole period, the eggs were collected for analysis. Following parameters were egg´s weight, yolk´s weight, albumen´s weight, shell´s weight and colour of yolk. Whole egg, yolk, albumen and shell weight were measured in grams using an electronic scale. Colour of yolk was evaluated by Hoffman La Roche of colour scale.

Differences between groups were analyzed with one-way analysis of variance (ANOVA) by using the statistical programme SPSS 20.0. Results were evaluated using Tukey test.

Table 1: **Nutrient composition of feed mixture (declared by feed manufacturer)**

Nutritive	Amount
Crude protein (g/kg)	min. 160
Crude fibre (g/kg)	min. 20
Crude fat (g/kg)	max. 90
Ash (g/kg)	min. 110
Lysine (g/kg)	min. 6.5
Methionine (g/kg)	min. 3.3
Ca (g/kg)	min. 27
P (g/kg)	min. 6
Na (g/kg)	min. 1
Fe (mg/kg)	75
Cu (mg/kg)	6
Zn (mg/kg)	25
Vitamin A (m.j./kg)	10000
Vitamin D 3 (m.j./kg)	2200
Phytase	290
Endox (mg/kg)	115

Results and discussion

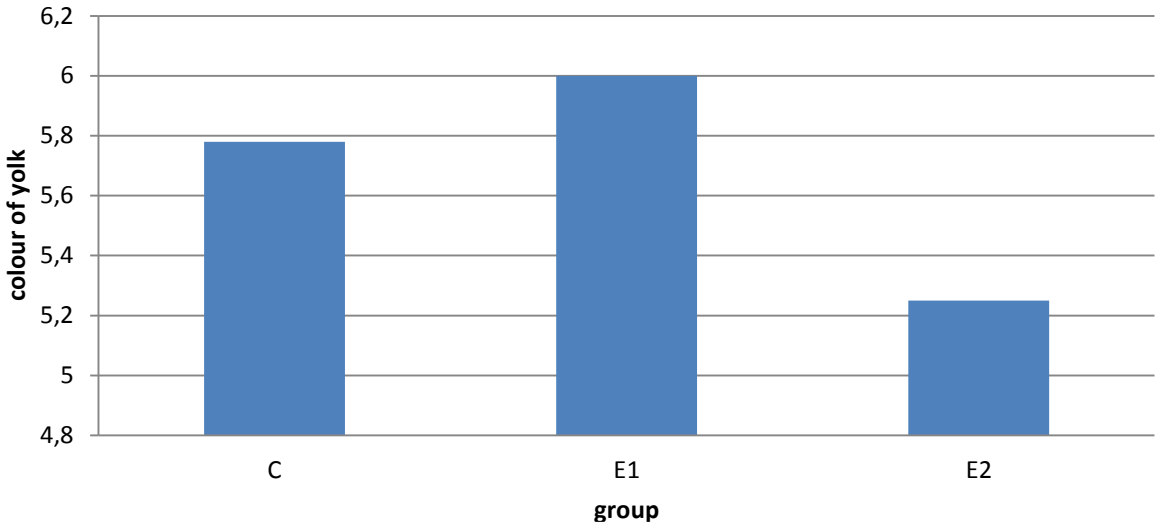
Egg´s weight, yolk´s weight, albumen´s weight, shell´s weight from control and experimental groups of hens are presented in Table 2 and colour of yolk in Figure 1. There were significant differences ($P<0.05$) between control group and both experimental groups in weight of eggs and shell´s weight. In yolk´s weight wasn´t significant ($P>0.05$) differences. There was found a tendency ($P>0.05$) of higher average yolk´s weight after flaxseed oil supplementation. In weight of albumen, there were significant differences found between control group and first experimental group (E1). It was reported that essential oils have a stimulating effect on animal digestive system (Ramakrishna et al., 2003). Fat is a generic term and is commonly included in poultry diets to increase the energy (Pinchasov et al., 1992). Digestibility of dietary fats is affected by the fatty acid profile, and several studies have shown better utilization of unsaturated fats, leading to higher metabolizable energy than saturated fats (Crespo et al., 2001). The lipid profile of eggs can be modified by the inclusion of specific oils such as flaxseed oil in the diet of laying hens (Shapira et al., 2008). Some researchers reported that supplementation of the diet with essential oils has improved egg´s weight (Akhtar et al., 2003; Denli et al., 2004). In accordance with the present findings, Bolukbasi et al. (2009) showed that supplementation of dietary essential oil had no effect on egg´s weight. Florou-Paneri et al. (2005) added oregano essential oil into feed mixture with any impact on rise of egg weight. Bean and Leeson (2003) found that egg´s weight, shell´s weight and albumen´s weight were not significantly different for hens consuming 0 and 10% flaxseed; however, yolk weight was reduced in hens fed flaxseed. Addition of flaxseed oil in a laying hen ration can reduce egg´s weight and yolk´s weight (Dunn-Horrocks et al., 2011). Denli et al. (2004) reported that addition of 1 g/kg *Nigella Sativa* extracts in diets of laying quails increased weight of yolk in eggs. Costa et al. (2008) did not observe worse performance in heavy layers fed a diet containing 2% linseed oil. Kaya et al. (2013) published that essential oil combination and vitamin E increased shell´s weight. Throughout the experimental period, yolk´s colour was greater after olive oil supplementation (Zhang et al., 2014). Average value of colour of yolk was 5.78 in C group, 6 in E1 group and 5.25 in E2 group.

Table 2: **Effect of oils addition on the selected parameters of eggs**

Group		Egg	Yolk	Albumen	Shell
		weight (g)			
C	Mean	60.87[a]	17.77	38.24[a]	4.86[a]
	S.D.	3.14	0.93	3.06	0.28
	CV (%)	5.16	5.23	8.00	5.76
E1	Mean	65.28[b]	17.29	41.47[b]	6.70[b]
	S.D.	4.64	1.43	3.55	0.55
	CV (%)	7.11	8.27	8.56	8.21
E2	Mean	65.68[b]	18.37	40.65[ab]	6.66[b]
	S.D.	2.53	1.39	2.13	0.45
	CV (%)	3.85	7.57	5.23	6.76

SD: standard deviation, CV: coefficient of variance. Values with different superscripts in a column are different at the 0.05 level.

Figure 1: **Comparison of yolk´s colour after oils supplementation**



C - control group, E1 - group with pumpkin oil supplementation, E2 - group with flaxseed oil

Conclusion

After oils supplementation in feed mixtures for laying hens was found positive effect on the egg´s weight, albumen´s weight and shell´s weight. Average weight of egg and shell was significantly ($P<0.05$) higher after oils supplementation and in albumen´s weight was found significant ($P<0.05$) differences only between first experimental group and control group. Tendency ($P>0.05$) of the highest value of yolk´s colour was found after pumpkin oil supplementation.

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References

1. Akhtar, M. S. – Nasir, Z. – Abid, A. R. 2003. Effect of feeding powdered Nigella sativa L. seeds on poultry egg production and their suitability for human consumption. In Veterinarski-Arhiv. vol. 73, pp. 181-190. DOI:10.2141/jpsa.009072.
2. Bean, L. D. - Leeson, S. 2003. Long-term effects of feeding flaxseed on performance and egg fatty acid composition of brown and white hens. In *Poultry Science*, vol. 82, pp. 388-394.
3. Bolukbasi, S. C. – Kaynar, O. – Erhan, M. K. – Urusan, H. 2009. The effect of feeding Nigella Sativa Oil on Laying Hen Performance, Cholesterol and Some Proteins Ratio of Egg Yolk an Escherichia Coli Count in Feces. Archives fur Geflugelkunde, vol.73, pp. 167-172. DOI:10.2141/jpsa.009072.
4. Costa, F. G. P. – Souza, J.G. – Silva, J. H. V. - Rabello, C. B. - Goulart C. C. - Lima, N. R. C. 2008. Influência do óleo de linhaça sobre o desempenho e a qualidade dos ovos de poedeiras semipesadas. In *Revista Brasileira de Zootecnia*, vol. 37(5), pp. 861-868.
5. Crespo, N. – Esteve-Garcia, E. 2001. Dietary fatty acid profile modifies abdominal fat deposition in broiler chicken. In Poultry Science, vol. 80, pp. 71-78. DOI: 10.1002/jsfa.3360.
6. Denli, M. – Okan, F. – Uluocak, A. N. 2004. Effect of dietary black seed (*Nigella Sativa* L.) extract supplementation on laying performance and egg quality of quail (*Coturnix coturnix japonica*). In *Journal of Applied Animal Research*, vol. 26, pp. 73-76. DOI:10.2141/jpsa.009072.
7. Dunn-Horrocks, S. – Pichardo-Fuchs, M. – Lee, J. – Ruiz-Feria, C. – Creger, C. – Hyatt, D. – Stringfellow, K. – Sanchez, M. – Farnell, M. 2011. Effect of omega-3 enriched layer rations on egg quality. In *International Journal of Poultry Science*, vol. 10 (1), pp. 8-11.
8. Florou-Paneri, P. – Nikolakakis, I. – Giannenas, I. – Koidis, A. – Botsoglou, E. – Dotas, V. – Mitsopoulos, I. 2005. Hen performance and egg quality as affected by dietary oregano essential oil and tocopheryl acetate supplementation. In *International Journal of Poultry Science*, vol. 4, no. 7, pp. 449-454.
9. Kaya, H. – Kaya, A. – Celebi, S. – Macit, M. 2013. Effect of dietary supplementation of essential oils and vitamin E on performance, egg quality and Escherichia coli count in excreta. In *Indian Journal of Animal Research*, vol. 47, pp. 515-520. DOI: 10.5958/j.0976-0555.
10. Pinchasov, Y. – Nir, I. 1992. Effect of dietary polyunsaturated fatty acid concentration on performance, fat deposition and carcass fatty acid composition in broiler chickens. In Poultry Science, vol. 71. pp. 1504-1512. DOI: 10.1002/jsfa.3360.
11. Ramakrishna, R. R. – Platel, K. – Srinivasan, K. 2003. In vitro influence of species and spice-active principles on digestive enzymes of rat pancreas and small intenstine. In *Nahrung*, vol. 47, pp. 408-412. DOI: 10.2141/jpsa.009072.
12. Shapira, N. – Weill, P. – Loewenbach, R. 2008. Egg fortification with n-3 polyunsaturated fatty acids (PUFA): Nutritional benefits versus high n-6 PUFA western diets, and consumer acceptance. In *Israel Medical Association Journal*, vol. 10, pp. 262-265.
13. Zhang, Z. F. – Kim, I. H. 2014. Effects of dietary olive oil on egg quality, serum cholesterol characteristics, and yolk fatty acid concentrations in laying hens. In *Journal of Applied Animal Research*, vol. 42, pp. 233-237. DOI: 10.1080/09712119.2013.822815.

EFFECT OF CHEMICAL ADDITIVE ON MYCOTOXIN CONTAMINATION OF MAIZE SILAGES

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Abstract

Contamination of mycotoxins in maize silages conserved by chemical additive was investigated. Whole maize plants (*Zea mais*, L.) were ensiled without additive (variant C) and with chemical additive (variant E: sodium chloride in a dose 1.5 $kg.t^{-1}$). The samples were examined for mycotoxins content by direct competitive enzyme-linked immunosorbent assays (ELISA). The highest mean values of mycotoxins were DON 786.60; ZON 437.97; FUM 225.88; T-2 223.33; and AFL 6.97 $\mu g.kg^{-1}$ in E and OTA 20.87 $\mu g.kg^{-1}$ in variant C. The negative tendency to reduction monitored mycotoxins (besides OTA) after application chloride sodium was recorded with statistically significant differences in total aflatoxins ($P \leq 0.05$).

Material and methods

Crop and ensiling: The aim of the study was to determine the mycotoxins contamination of maize silage after application of chemical additive. The experiment was carried out at the Department of Animal Nutrition, Slovak University of Agriculture in Nitra, Slovak Republic. Whole maize plants (*Zea mais*, L. FAO 450) were harvested in vegetation stage of the milk-wax maturity of grain and cut to 20 mm length. The experiment consisted of 2 variants: maize ensiled without additive (variant C) and with chemical additive (variant E) consisted of sodium chloride (powder form, homogeneously applied at the rate of 1.5 $kg.t^{-1}$). Three replicates per variant were ensiled. Silage matter was packed into laboratory silos with volume 4 dm^3 (n=3), hermetically sealed and stored in an air conditioned laboratory at 20±2°C. All silos were opened after 4 weeks of ensiling.

Mycotoxins analysis: Mycotoxin content of the maize silage was determined by direct competitive enzyme-linked immunosorbent assays (ELISA) (AOAC, 2000). Samples of maize silage were analyzed for 6 mycotoxins, including total aflatoxin (AFL) total fumonisins (FUM), total ochratoxins (OTA), zearalenone (ZON), deoxynivalenol (DON) and T-2 toxin (T-2). Samples of maize silage for determination of mycotoxins were collected randomly from laboratory silos ($4dm^3$). After that, samples were dried at 50^oC (20 hours) and grounded to a fine powder. Extraction of samples was carried out in distilled water (DON), in methanol: water: (70:30 v/v) for FUM, AFL, ZON and 50:50 (v/v) for T-2 and OTA. The veratox quantitative test kits (Neogen, USA) were used and the ELISA procedure performed following the manufacturer's recommendations. The basis of the test is the antigen-antibody reaction. The wells in the microtiter plates were coated with antibodies to each mycotoxins. By adding standards of each mycotoxin or the sample solution the antibody binding sites were occupied in proportion to the concentration of each mycotoxin. Any remaining free binding sites were occupied in the next stage by enzyme labeled toxin (enzyme conjugate). Any unbound enzyme conjugate was then removed in a washing step. Enzyme substrate and chromogen were added to the wells and incubated. Bound enzyme conjugate converted the colourless chromogen into a blue product. The addition of the stop reagent resulted in a colour change from blue to yellow. Absorbance was determined using the microwell strip reader (Neogen, USA) at 650 nm. A calibration curve for standards for each toxin dilution was plotted using a standard concentration against the percentage inhibition of the standard. For determination, each mycotoxins concentration was calculated by correlation coefficient from the following calibration curve: AFL 0.995 (r^2 = 0.990), FUM 0.988 (r^2 = 0,998), OTA

0.997 (r^2 = 0.995), T-2 0.998 (r^2 = 0.997), DON 0.997 (r^2 = 0.999), ZON 0.992 (r^2 = 0.996). Through the use of a microwell reader, the test provided sample results in $\mu g.kg^{-1}$ for all mycotoxins.

Statistical analysis: Concentrations of mycotoxins were found in 3 repetitions for each sample of maize silage at the same time. The analyses were carried out in statistical package SPSS (version 20.0). Student´s *t*-test was used to detect significant differences among samples.

Results and discussion

The analytical results for mycotoxins (Table 1) indicate that samples of maize silages were contaminated with all the determined mycotoxins. The most prevalent mycotoxin was DON, followed by ZON and FUM. DON is associated with decreasing body weight, weight gain, food intake disorders and haematological effects were also observed (Pestka, 2007). DON was detected at concentrations: 753±20.67 (C) and 786.60±90.46 (E) $\mu g.kg^{-1}$. Differences in DON concentrations observed between variants of maize silages were not significant ($P \geq 0.05$). Eckhard et al. (2011) and Driehuis et al. (2008) reported higher DON values in maize silage (1360±630 and 850± 90 $\mu g.kg^{-1}$, respectively), but Richard et al. (2009) recorded lowers values of DON (160±10 $mg.kg^{-1}$).

Zearalenon is classified as an estrogenic mycotoxin because it frequently uses hyperestrogenic syndrome in animals. ZON contaminated feed or grain, when consumed by livestock, can contribute to a wide variety of reproductive problems (Dong et al., 2014). In the present study were reported ZON values: 402.47±52.10 (C) and 437.97±22.29 (E) $\mu g.kg^{-1}$, that was similar to the study Škrinjar et al. (2011). Driehuis et al. (2008) also Tangni et al. (2013) observed lower ZEA concentration in maize silage.

Fumonisins induce equine leucoencephalomalacia in horses and porcine pulmonary edema in swine (Voss et al., 2007). Experimentally, fumonisin is hepatoxic and nephrotoxic to calves whether given orally or i.v. (Mathur et al., 2001). FUM in our study range from 225.81±3.67 (C) to 225.88±8.10 (E) $\mu g.kg^{-1}$, than is higher values of FUM when reported Teller et al. (2012).

T-2 toxin is associated with reduced feed consumption, loss in yield, gastroenteritis, intestinal hemorrhage, reduce reproductive performance and death (Wannemacher et al., 1991). T-2 toxin was detected at concentrations: 218.03±4.87 (C) to 223.33±22.40 $\mu g.kg^{-1}$ (E). The results of our observation resulted in ascertaining that contamination by T-2 toxin (range from 218.03±4.87 (C) to 223.33±22.40 $\mu g.kg^{-1}$ (E)) were similar to the study by Bíro et al. (2009). In our study, data showed that concentrations of T-2 in treated maize silages were not significantly different in comparison with control ($P \geq 0.05$).

Ochratoxins contamination has been linked to outbreaks of nephropathy in pigs and poultry. It is furthermore associated with immunosuppression, reduced growth rate and increased mortality (Duarte et al., 2011). I the present study, the samples of control maize silages had highest mean level of OTA (20.87±1.76 $\mu g.kg^{-1}$). Škrinjar et al. (2011) reported higher OTA content, but did not exceed the 250 $\mu g/kg^{-1}$, set as maximum acceptable limit (EC, 2006).

Aflatoxins are toxic and highly carcinogenic secondary metabolites (Brown et al., 1999) associated with inappetance, ataxia, enlarged fatty livers, and reduced feed efficiency and milk production (Nibbelink, 1986). The maize silage of variant E had significantly ($P \leq 0.05$) highest content of AFL 6.97±0.72 $\mu g.kg^{-1}$ than in control (3.97±0.50 $\mu g.kg^{-1}$). Richard et al. (2009) reported higher AFL value of maize silages (15.68±5.12 $\mu g.kg^{-1}$).

In none of the maize silages analysed in the study was the EC guidance values (EC, 2006) for observed mycotoxins exceeded.

Table 1: Concentration of mycotoxins in maize silages

$\mu g.kg^{-1}$	C			E		
	x	sd	v	x	sd	v
AFL	3.97^{a}	±0.50	12.59	6.97^{b}	±0.72	10.33
DON	753.33	±20.67	2.66	786.60	±90.46	11.39
FUM	225.81	±3.67	1.63	225.88	±8.10	3.59
OTA	20.87	±1.76	8.43	18.37	±1.65	8.98
T-2	218.03	±4.87	2.23	223.33	±22.40	10.03
ZON	402.47	±52.10	12.95	437.97	±22.29	5.09

AFL- total aflatoxins FUM: total fumonisins; ZON: zearalenone; DON: deoxynivalenol ; T-2: T-2 toxin; OTA: total ochratoxins; C - control variant (without additive), E - chemical additive consisting of sodium chloride; ^{a,b} Values indicated with different superscripts in a row are significantly different at $P \leq 0.05$.

Conclusion

Mycotoxins are natural toxic products synthetized by certain filamentous fungi on many agricultural commodities. Mycotoxin contamination of feedstuffs is a significant worldwide problem. Occurrence of all observed mycotoxins was detected in all samples of maize silages. The results show that deoxynivalenol was the secondary metabolite with the highest concentration. Our results indicate that sodium chloride as silage additive was ineffective in reduce the concentration of mycotoxins in maize silages.

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References

1. AOAC, 2000. Official methods of analysis of AOAC International, 17th Ed. Gaithersberg: Maryland, ISBN 09-3-558-4544.
2. Bíro, D. - Juráček, M. - Kačániová, M. - Šimko, M. - Gálik, B. - Michálková, J. - Gyöngyová, E. 2009. Occurrence of microscopic fungi and mycotoxins in conserved high moisture corn from Slovakia. In *Annals of Agricultural and Environmental Medicine*, vol.16, no. 2, pp. 227-232. ISSN 1898-2263
3. Brown, R.L. - Chen, Z.Y. - Cleveland, T.E. - Russin, J.S. 1999. Advances in the development of host resistance in corn to aflatoxin contamination by *Aspergillus flavus*. In *Phytopatology*, vol. 89, pp. 113-117. ISSN 0031-949X.
4. COMMISION REGULATION (EC). (2006/576/EC). of 17 August 2006 on the presence of deoxynivalenol, zearalenone, ochratoxin A, T-2 and HT-2 and fumonisins in products intended for animal feeding amending Regulation (EC) In *Official Journal of European Union*, vol. 229, pp. 7-9. ISSN 1977-0677.
5. Dong, H.K. - In, H.L - Woo, H.D. - Woo, S.N. - Hua, L. - Han, S.J. - Chan, L. 2014. Incidence and Levels of Deoxynivalenol, Fumonisins and Zearalenone Contaminants in Animal Feeds Used in Korea in 2012. In *Toxins*, vol. 6, no. 1, pp. 20-32. ISSN 2072-6651.
6. Driehuis, F. - Spanjer, M. C. - Scholten J. M. - Giffel, M. C. 2008. Occurrence of mycotoxins in maize, grass and wheat silage for dairy cattle in the Netherlands. In *Food Additives and Contaminats: Part B: Surveillance*, vol. 1, no. 1, pp. 41 – 50. ISSN 1939-3229.

7. Duarte, S.C. - Lino, C.M. - Pena, A. 2011. Ochratoxin A in feed of food-producing animals: An undesirable mycotoxin with health and performance effects. In *Vet. Microbiol.*, vol. 154, pp. 1–13. ISSN 0378-1135.
8. Eckard, S. - Wettstein, F.E. - Forrer, H.R. - Vogelsang, S. 2011. Incidence of *Fusarium* Species and Mycotoxins in Silage Maize. In *Toxins*, 3, 8, pp. 949 - 967
9. Mathur, S. - Constable, P. D. - Eppley, R. M. - Waggoner, A. L. - Tumbleson, M. E. - Haschek, W. M. 2001. Fumonisin B1 is hepatotoxic and nephrotoxic in milk-fed calves. In *Toxicological Sciences*, vol. 60, no. 2, pp. 385-396. ISSN 1096-0929.
10. Nibbelink, S.K. 1986. Aflatoxicosis in food animals: A Clinical Rev. In *Iowa State University Veterinarian*, vol. 48, no. 1, pp. 28-31. ISSN 0099-5851.
11. Pestka, J.J. 2007. Deoxynivalenol: Toxicity, mechanisms and animal health risks. In *Anim Feed Sci Technol*., vol. 137, pp. 283–298. ISSN 0377-8401.
12. Richard, E. - Heutte, N. - Bouchart, V. - Garon, D. 2009. Evaluation of fungal contamination and mycotoxin production in maize silage. In *Animal Feed Science and Technology*, vol. 148, no. pp. 309-320. ISSN 0377-8401.
13. Škrinjar, M. - Jakič, D - Blagojev, N. - Šošo, V. 2011. Results of one-year investigations of the contamination of dairy cattle feed and raw milk with moulds and mycotoxins. In *Biotechnology in Animal Husbandry*, vol. 27, no. 3, pp. 985-992. ISSN 1450-9156.
14. Tangni, E. K. - Pussemier, L. - Hove, F. 2013. Mycotoxin Contaminating Maize and Grass Silages for Dairy Cattle Feeding: Current State and Challenges. In *Journal of Animal Science Advances*, vol. 3, no. 10, pp. 492-511. ISSN 1993-601X.
15. Teller, R.S. - Schmidt, R. J. - Whitlow, L.W. - Kung, L. 2012. Effect of physical damage to ears of corn before harvest and treatment with varioum additives on the concentration of mycotoxins, silage fermantation, and aerobic stability of corn silage. In. *Journal of Dairy Science*, vol. 95, no. 3. pp. 1428 – 1436. ISSN 0022-0302.
16. Voss, K. A. - Smith, G. W. - Haschek, W. M. 2007. Fumonisins: Toxicokinetics, mechanism of action and toxicity. In *Animal Feed Science and Technology*, vol. 137, no. 3-4, pp. 299-325. ISSN 0377-8401.
17. Wannenmacher, R.W. - Bunner, D.L. - Neufeld, H.A. 1991. Toxicity of trichothecenes and other related mycotoxins in laboratory animals. In: J.E. Smith, R.S. - Henderson (Eds.). *Mycotoxins and Animal Foods.* CRC Press Inc., Boca Raton, FL., pp. 499-552. ISBN 978-0849349041.

EFFECT OF HYBRID ON QUALITY AND NUTRITIVE VALUE OF MAIZE SILAGE MATTER

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Abstract

The aim of this study was to determine and compare the nutritive value and quality indicators of maize silage with two maize hybrids**,** with the FAO number 350 (A) and 410 (B). Maize hybrids were grown in the same agro – ecological and soil conditions in PD Nižná. Dry matter of the maize silage mass was found 291.7 $g.kg^{-1}$(hybrid A) and 265.3 $g.kg^{-1}$(hybrid B). The content of nitrogen – free extract was higher in hybrid A, while hybrid B had a lower content of 15.15 $g.kg^{-1}$ in DM. In both test hybrids we investigate high content of a fibre, from 223.25 $g.kg^{-1}$(hybrid A) to 234,65 $g.kg^{-1}$(hybrid B) and a relatively low starch content 262.6 $g.kg^{-1}$(hybrid A) and 226.9 $g.kg^{-1}$ in DM (hybrid B), coinciding with the phenological stage of collection. When we were comparing two silage hybrids with different FAO number, grown in the same agro – ecological and soil conditions, we observed statistically significant differences in the content of starch.

Keywords maize hybrids, FAO number, quality, nutritive value

Material and methods

In experiment we tested 2 maize hybrids with different FAO numbers. They were grown on farm PD Nižná in Piešťany (West Slovakia, 183 m a.s.l). Both hybrids were in the same agroecological and soil conditions. A hybrid with FAO number 350 is evenly ripening hybrid with the dent type of grain and hybrid B with FAO number 410 is a stay-green hybrid also with the dent type of grain. Seedbed preparation and seeding was made on 07. 04. 2011. Seeding was realized with fertilizing by a 6 rows MONOSEM pneumatic seeder. Sowing depth was set to letter H, interred distance of 75 cm, seeds distance in a row was 18 cm. Maize plants were harvested by hand in the phonological stage at the beginning of the milky-wax ripeness of grain, height of stubble was 20 cm + / - 2 cm. Subsequently, the plants were cut into 2 cm theoretical cutting length by a POTTINGER MEX V cutting machine.

After collection, we found the following average weight parameters per plant (hybrid A) 957 g and (hybrid B) 1010 g. An abundance of both hybrids (A and B) at harvest time reached 69,500 individuals. Ha-1. In the average silage mass samples of maize we determined nutrient content under standard laboratory procedures and techniques according to Regulation of the Ministry of Agriculture and Rural Development of the Slovak republic no. 2145-2004-100. Basic statistical parameters were evaluated in the SAS (THE SAS SYSTEM V 9.2). We used parametric t - test (THE SAS SYSTEM V 9.2) for testing the statistical significance of differences between the variants. Mean differences were considered significant at $P<0.05$.

Results and discussion

According to Mitrík and Vajda (2006) about high quality forage often decide not days, but hours. Loučka and Jambor (2009) declare that currently are in our market maize hybrids that contain more than 50% of dry matter in grain as it is in the whole plant, thus maize silage cannot longer be considered as the typical forage, but a feed with high energy potential. Many authors have dealt with assessing and evaluating a quality of different silage maize hybrids (Forouzmand et al., 2005 Bíro et al., 2008, Der Bedrosian et al., 2012). Zimolka et al. (2008) reported that from the nutritionists point of view, the best time for harvesting of maize silage

is at the end of a dough-ripening grain (DM of whole plant 28-34%), when is terminating a synthesis of starch in the grain and whole plant achieves the highest concentration of energy. In the matter of maize silage we harvested, we found dry matter content of 291.7 $g.kg^{-1}$ (hybrid A) and 265.3 $g.kg^{-1}$ (hybrid B) at the beginning of milky-wax ripeness. Crude protein content was low in the whole maize plant (Bíro et al., 2005). It ranged from 8 to 9.5%. Millner et al. (2005) found crude protein content moving from 66 to 72.5 $g.kg^{-1}$ DM in varieties of silage maize hybrids in interval 330-379 $g.kg^{-1}$DM. The Hybrid A was containing 77.6 g and hybrid B 80.85 g of crude protein per kilogram of dry matter. Ballard et al. (2001) referred an average fat content 24 and 22 g per 1 kg of dry matter in maize silage hybrids with a dry matter content ranging between 273 and 278 $g.kg^{-1}$. In the hybrids we tested, we found the fat content 22.05 $g.kg^{-1}$ dry matter (hybrid A) and 18.75 $g.kg^{-1}$ of dry matter (hybrid B). A higher ash content (78.2 $g.kg^{-1}$ DM) was in maize hybrid with 225.7 $g.kg^{-1}$ DM content, acclaimed Forouzmand et al. (2005). We assessed the significantly lower ash content, in hybrid A 42.05 g and hybrid B 45.85 $g.kg^{-1}$ of dry matter. Maize silage has a high content of nitrogen free extract (NFE), which is a substrate for development of desirable microorganisms in the silage fermentation process. Petrikovič et al. (2000) reported average content of NFE 618 $g.kg^{-1}$ dry matter, in Tables of Nutritional Value of Feed - harvested at the same maturity stage. Bíro et al. (2005) ascertained higher content of NFE (673.4 $g.kg^{-1}$DM) in maize hybrid with FAO number 270 harvested at the stage of milky - waxy ripeness of grain. Hybrid A contained 635.05 g and hybrid B 619.9 g NFE in 1 kg of dry matter. We detected a higher fibre content in both forage hybrids. In the hybrid A was a fibre content 223.25 $g.kg^{-1}$ and hybrid B contained 11.4 $g.kg^{-1}$ DM more. Juráček et al. (2010) found out the fibre content 215.4 $g.kg^{-1}$ and 28.59% of dry matter in maize silage. Bal et al. (1997) indicated that the highest content of acid detergent and neutral detergent fibre is at the stage of milk ripeness of grains contra the lowest content in stage of 2/3 milk line of grains. Rumen microorganisms are able to transform fibre into volatile fatty acids (VFA), which are an essential energy source for ruminants. Balch (1958), cited by Vrzgula et al. (1990) reported that dairy cow produces daily 2 - 5 kg volatile fatty acids, what represents 2000 - 4300 kJ. After Vrzgula et al. (1990) production of VFA can satisfy energy need of macroorganism up to 82%. Petrikovič et al. (2000) recorded an average content of organic matter 948$g.kg^{-1}$ of dry matter of maize silage in early stage of milky - wax ripeness of grain. Forouzmand et al. (2005) discovered in tested hybrids of maize in different phenological stages that organic matter content ranged from 916 to 932.6 $g.kg^{-1}$ dry matter, and they confirmed that the organic matter content increased by progressing the phenological phases. Hybrid A contained 957.95 $g.kg^{-1}$ DM of organic matter, hybrid B had lower level: 954.15 $g.kg^{-1}$ of dry matter. Organic matter content was relatively equal. Hybrid A contained statistically significant ($P < 0.05$) more starch compared to hybrid B. The difference was 35.7 $g.kg^{-1}$ dry matter. Starch is the main source of energy for rumen microflora. The starch content of the whole maize plant is significantly lower than starch in grain (25-35%) (Mlyneková and Čerešňáková, 2008). Arriola et al. (2012) detected content of starch 147-206 $g.kg^{-1}$ DM in five maize hybrids with a DM content 220-267 $g.kg^{-1}$. Filya (2004) determined that maize harvested at full maturity stage (the appearance of black spots) has lower starch digestibility compared with maize harvested in phase 1/2 or 2/3 milk line of grain. Jurjanz and Monteils (2005) indicate that a storage length of maize silage increases moisture content of grain and thus the degree of starch gelatinization, what is expressing in a gradual increasing of ruminal starch digestibility (this phenomenon is associated with the so-called the Spring acidosis in several dairy cow herds) (Owens , 2008).

Table 1: Nutritive value of different maize hybrids

n=3		DM	CP	Fat	Fibre	Ash	NFE	OM	Starch
		g.kg^{-1}	g.kg^{-1} DM						
A	$\bar{x}$	291,7	77,6	22,05	223,25	42,05	635,05	957,95	262,6*
	S.D.	8,49	1,70	0,35	9,97	1,77	6,15	1,77	2,83
	C.V.	2,91	2,19	1,59	4,47	4,21	0,97	0,18	1,08
B	$\bar{x}$	265,3	80,85	18,75	234,65	45,85	619,9	954,15	226,9*
	S.D.	4,38	0,07	0,78	8,70	1,91	11,46	1,91	0,71
	C.V.	1,65	0,09	4,16	3,71	4,17	1,85	0,20	0,31

A: hybrid FAO 320, B: hybrid FAO 410, S.D.: standard deviation, C.V.: coefficient of variation, DM: dry mater, CP: crude protein, NFE: nitrogen-free extract, OM: organic mater, *: the values with identical superscript in bar are significantly different at P<0,05

Conclusion

In the experiment, we evaluated indicators of nutritional value of 2 maize hybrids with FAO number 350 (hybrid A) and with FAO 410 (hybrid B). Both were collected in the same phenological stage at the beginning of milky - wax ripeness of grain. In the matter of maize silage we found dry matter content 291.7 g.kg^{-1} in hybrid A and 265.3 g.kg^{-1} in hybrid B. The content of nitrogen-free extract was lower by 15.15 g.kg^{-1} DM in the hybrid B.

In both tested hybrids, we found a higher content of fibre: 223.25 g.kg^{-1} (hybrid A) and 234.65 g.kg^{-1} (hybrid B) and relatively low content of starch 226.9 g.kg^{-1} (hybrid B) and 262.6 g.kg^{-1} DM (hybrid A), which was associated with phenological stage of harvesting. When comparing two silage hybrids with different FAO numbers what were grown in the same agro-climatic and soil conditions, sampled at the same phenological stage, we found a statistically significant (P<0.05) differences in the content of starch. In the other studied nutrients we did not find statistically significant differences.

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References

1. Arriola, K., G. et al. 2012. Stay-green ranking and maturity of corn hybrids: 1. Effects on dry matter yield, nutritional value, fermentation characteristics, and aerobic stability of silage hybrids in Florida. In In Journal of Dairy Science, vol. 95, 2012, no. 2, p. 964–974
2. Bal, M.A. et al. 1997. Impact of the maturity of corn for use as silage in the diets of dairy cows on intake, digestion, and milk production. In Journal Dairy Science, vol. 80, p. 2497- 2503
3. Ballard, S.C. et al. 2001. Effect of corn silage hybrid on dry matter yield, nutrient composition, in vitro digestion, intake by dairy heifers, and milk production by dairy cows. In Journal of Dairy Science, vol. 84, 2001, no. 2, p. 442–452
4. Bíro, D. et al. 2005. Analýza výživnej hodnoty nových hybridov kukurice siatej. In Dni výživy zvierat. Nitra: SPU, 2005, s. 10-12
5. Bíro, D. et al. 2008. Fermentation process characteristics of different maize silage hybrids. In Journal of Central European Agriculture, vol. 9, 2008, no. 3, p. 463-468
6. Der Bedrosian, M.C. et al. 2012. The effects of hybrid, maturity, and length of storage on the composition and nutritive value of corn silage. In Journal of Dairy Science, vol. 95, no. 9, p. 5115-5126

7. Filya, I. 2004. Nutritive value and aerobic stability of whole crop maize silage harvested at four stages of maturity. In Animal Feed Science and Technology, vol. 116, p. 141–150
8. Forouzmand, M.A. et al. 2005. Influence of hybrid and maturity on the nutritional value of corn silage for lactating dairy cows 1: Intake, milk production and component yield. In Pakistan Journal of Nutrition, vol. 4, no. 6, p.435-441
9. Juráček, M. et al. 2010. Energetická hodnota siláží pre bioenergetické využitie. In Acta fytotechnica et zootechnica. roč. 13, č. 3, s. 76-78. ISSN 1336-9245
10. Jurjanz, S. – Monteils, V. 2005. Ruminal degradability of corn forages depending on the processing method employed. In Animal research, no. 54, pp. 3-15
11. Loučka, R. - Jambor, V. 2009. Jakou technologii úprav a konzervace kukuřice zvolit. In Kukuřice objemné krmivo, Limagrain, p. 25-27
12. Millner, J.P. et al. 2005. The yield and nutritive value of maize hybrids grown for silage. In New Zealand Journal of Agricultural Research, vol. 48, no. 1, p. 101-108
13. Mitrík, T. – Vajda, V. 2006. Objemové krmivá. Schaumann Slovensko s.r.o. 96 s. ISBN 978-80-969658-0-9
14. Mlyneková, Z. – Čerešňáková, Z. 2008. Nutritional value and degradability of nutrients in selected morphological parts of flint x dent maize. In: Zborník 13. medzinárodnej konferencie „Konzervovanie krmív“, poster, 3.-5.9. 2008 , ISBN 978-80-88872-78-8, s. 86-87
15. Petrikovič, P. et al. 2000. Výživná hodnota krmív 1. časť. 1. vyd. Nitra: VÚŽV, ISBN 80-88872-12-X
16. Vrzgula, L. et al. 1990. Poruchy látkového metabolizmu hospodárskych zvierat a ich prevencia. dopln. vyd. Príroda: Bratislava, 503 s. ISBN 80 – 07-00256-1
17. Výnos MPSR z 23. augusta 2004 č. 2145/2004-100, ktorým sa mení a dopĺňa výnos Ministerstva pôdohospodárstva Slovenskej republiky zo 7. októbra 1997 č. 1497/4/1997-100 o úradnom odbere vzoriek a o laboratórnom skúšaní a hodnotení krmív v znení výnosu
18. Ministerstva pôdohospodárstva Slovenskej republiky z 12. februára 2003 č. 149/2/2003-100
19. Zimolka, J. et al. 2008. Kukuřice – hlavní a alternativní užitkové směry. Praha : Profi Press. 200 s. ISBN 978-80-86726-31-1

THE USE OF PROBIOTIC IN HORSES

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Abstract

Horse breeding in Slovakia has an upward trend but increasingly we can observe various digestive disorder ethology. The possible prevention treatment is costly and the outcome is not always positive. In our study, we investigated the effect of probiotic cultures in the composition of microflora of the digestive system in selected horses. Group was formed by nine horses from the riding club Slavia SPU and probiotics *Lactobacillus* spp. and *Enterococcus faecium* were offered them.

Keywords: horse, probiotics

This study is aimed at the effect of probiotic cultures *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Lactobacillus paracasei* subs. *Paracasei* and *Enterococcus faecium* on horse's digestive system. These cultures can nonspecifically activate the immune system, suppress reproduction of pathogenic and conditionally pathogenic microorganisms and reduce the influence of the procarcinogenic substances which can be generated during certain digestive processes and enzymatic activities of the microorganisms in the colon.
The horses digestive system is very sensitive system that answers rapidly not only to any imbalance in feed ration, breeding system, climatic changes, but also to administration of therapy or transport stress.

Material and methods

The clinical trail of the veterinary medical product PROFOAL tabl. a.u.v. was aimed to check the effect and safety of its use and was done with target animals (9 horses from horse-rider club SLAVIA SPU Nitra). Before the first use of the medical product the individual faecal samples were taken and bacteriologically tested to count the actual amount of lactobacilli, clostridia and enterococci. The preparation was applied to the animals in the dose of 4 tables (12 g) daily during 14 days. The day after the last application, the repeated testing of the faecal samples was performed with the further bacteriological investigation. The detected amount of lactobacilli, clostridia and enterococci which was found before and after the use of the preparation was compared.

Results and discussion

The results of bacteriological investigations clearly showed that the use of probiotic product PROFOAL increased the amount of *Lactobacilli* in the faeces up to 2 logarithms and amount of enterococci up to 1 logarithm. It is very important, that in the horses with *Clostridium* in the faeces before taking the preparation the same bacteria were almost not found after the use of probiotics (they were under detectable level 1 – 3 logarithms). For example, before the use of probiotic preparation the highest level of *Clostridium* was detected

in the case of horse CHICAGO ($2.0x10^4$/g). After the use of preparation the amount of *Clostridium* fell under the detectable level which means 4 logarithms less.

Horses	Before the Theraphy	After the Theraphy
CAVALO	Lac.: $8,0 \times 10^6$/g Clos.: $4,2 \times 10^3$/g Enter.: $3,3 \times 10^5$/g	Lac.: $1,4 \times 10^8$/g Clos.: < 10 KTJ /g Enter.: $2,9 \times 10^6$/g
RAPOLLO	Lac.: $4,4 \times 10^6$/g Clos.: < 10 KTJ /g Enter.: $2,6 \times 10^5$/g	Lac.: $3,7 \times 10^8$/g Clos.: < 10 KTJ /g Enter.: $1,0 \times 10^7$/g
ROCKY	Lac.: $4,0 \times 10^6$/g Clos.: < 10 KTJ /g Enter.: $1,0 \times 10^5$/g	Lac.: $1,2 \times 10^8$/g Clos.: <10 KTJ /g Enter.: $7,7 \times 10^6$/g
CARIS	Lac. $6,8 \times 10^6$/g Clos.: $3,0 \times 10^2$/g Enter.: $3,0 \times 10^5$/g	Lac.: $5,6 \times 10^8$/g Clos.: < 10 KTJ /g Enter.: $1,1 \times 10^7$/g
CALINESTA	Lac.: $5,4 \times 10^6$/g Clos.: $2,1 \times 10^2$/g Enter.: $2,7 \times 10^5$/g	Lac.: $3,0 \times 10^8$/g Clos.: < 10 KTJ /g Enter.: $9,2 \times 10^6$/g
BALERINA	Lac.: $1,4 \times 10^7$/g Clos.: < 10 KTJ /g Enter.: $1,1 \times 10^5$/g	Lac.: $2,1 \times 10^8$/g Clos.: < 10 KTJ /g Enter.: $1,3 \times 10^7$/g
CORDOBA	Lac.: $2,8 \times 10^6$/g Clos.: $9,0 \times 10^3$/g Enter.: $5,3 \times 10^4$/g	Lac.: $3,0 \times 10^8$/g Clos.: < 10 KTJ /g Enter.: $1,1 \times 10^7$/g
SILVERSTONE	Lac.: $2,6 \times 10^7$/g Clos.: $3,0 \times 10^3$/g Enter.: $4,0 \times 10^5$/g	Lac.: $9,6 \times 10^8$/g Clos.: < 10 KTJ /g Enter.: $1,6 \times 10^7$/g
CHICAGO	Lac.: $2,2 \times 10^7$/g Clos.: $2,0 \times 10^4$/g Enter.: $3,9 \times 10^5$/g	Lac.: $3,6 \times 10^8$/g Clos.: < 10 KTJ /g Enter.: $9,6 \times 10^6$/g

These results support the assumption that the use of probiotics which contain *Lactobacillus* prevent the reproduction of *Clostridium* in the digestive tract of animals. *Lactobacilli* are antagonistic microorganisms against *Clostridia*. *Clostridia* are potentially pathogenic microorganisms which are normally present in the colon of the animals. In appropriate conditions, mainly in the case of raised pH of the digestive tract, they can overgrowth. *Lactobacilli* produce organic acids (lactic acid, propionic acid, butyric acid) which decrease pH in the gut and in such way prevent the overgrowth of opportunic microorganisms such as *Clostridia*, *E.coli*, *Staphylocoocci*, *Listeria*, and *Salmonella*.

Generally the use of preparation will improve the digestive process and it will lower the toxic influence on livers and kidneys. The general clinical condition of the horses was good before, during and after the finishing of the use of the preparation of new product.

References

1. Bomba, A., Nemcová, R., Gancarčíková, S., Mudroňová, D., Jonecová, Z., Koščová, J., Sciranková, Ľ., Buleca, V., Švalec, J. (2005): Uplatnenie probiotík vo výžive, prevencii a terapii chorôb hospodárskych a domácich zvierat. Slovenský veterinársky časopis XXX(1), p. 31-32.
2. Bomba, A., Strojný, L., Chmelárová, A., Hijová, E., Bertková, I., Nemcová, R**.** (2010): Synbiotics and Potentiated Probiotics in Modulation of the Gastrointestinal Ecosystem. International scientific conference probiotics and prebiotics, ISBN 978-80-970168-4-5, p. 18.
3. Nemcová R., Gancarčíková S., Mudroňová D., Koščová J., Pistl J. (2009): Probiotiká a naturálne látky v prevencii a liečbe infekčních ochorení. Odborný seminár MŠ SR 2009, 002UVL-8/2008 p. 59-61

EFFECTS OF SILAGE ADDITIVES ON QUALITY OF ALFALFA AND COCKSFOOT SILAGES

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Abstract

This paper deals with the evaluation of quality of silages made from cocksfoot (*Dactylis glomerata*) and alfalfa (*Medicago sativa*) forage. Crops were established in two locations - Troubsko and Vatín. Silages from first cuts, that took place in late May and the begining of June, in time of earing and before flowering, respectively. Experimental silages were treated with mixture of organic acids (formic acid and propionic acid). Leachates (pH, lactic acid, acetic acid, NH_3) and content of organic nutrients (crude protein, crude fiber) were evaluated. Results showed that the contents of fermentation acids, pH, ammonia, crude protein and crude fiber were influenced by the type of forage or the variety ($P < 0.05$) as well as the use of additive ($P < 0.05$), which had a positive impact on the quality of each silage.

Material and methods

Cocksfoot crops (Luxor and Husar variety) were based in Forage Research Station in Vatín, Bohemian-Moravian Highlands (Czech republic), 560 m of altitude. Alfalfa crops (Palava variety) were based at the Research Institute for Forage in Troubsko u Brna (Czech republic), 270 m of altitute. Crops were used as three-cuts. First cut, conducted in early stage of earing cocksfoot in the end of May and begining of June and in the stage of before flowering (alfalfa), was evaluated.

Experimental silages were prepared in containers of 150 mm diameter. Preparation of experimental microsilages is described in work of Vyskočil et al. (2011). Samples of silages were collected after 60 days. The quality of leachates (pH, content of lactic acid, acetic acid and ammonia) was evaluated. Organic nutrients were analyzed from silage samples dried at 60°C and homogenized to a particle size of 1mm. Organic nutrient (crude protein, crude fiber) content was also evaluated. Analytical procedures, including the preparation of water extract, are described in work of Doležal (2002). Results were converted to 100% dry matter. Results were evaluated by analysis of variance (ANOVA) and then by Turkey test. The evaluation was carried at a signifikance level of $P < 0.05$.

Results and discussion

Ammonia content in alfalfa silages was higher ($P < 0.05$) than in different varietes of cocksfoot. In cocksfoot silages ammonia contents were comparable ($P < 0.05$). The content of ammonia was decreasing ($P < 0.05$) after the application of organic acid on alfalfa forage and cocksfoot forage.

Our results (Table 1) show that the ammonia content in untreated silages was slightly higher than indicated in Doležal et al. (2010), (0.3 – 0.7 $g.kg^{-1}$). We have achieved the reduction of ammonia concentration from 1.65 to 0.76 $g.kg^{-1}$of dry matter by using a chemical preservative on silage. Jakobe et al. (1987) reported a value of ammonia in grass silage after 70 days as 0.68 $g.kg^{-1}$ of dry matter.

When comparing the ammonia content in silages from alfalfa and cocksfoot, we found significant differences. This may be influenced by higher protein content in alfalfa and therefore their greater decomposition. The total ammonia content in alfalfa silages in 2012 averaged 1.51 $g.kg^{-1}$ of dry matter (Mikyska, 2013). We achieved these levels in silages after

the application of organic acid on forage. Animals are able to tolerate ammonia in the range of 1.4 – 2 g.kg^{-1} of dry matter (Kalač, 2012).

The value of pH in silages of alfalfa and cocksfoot silages was affected ($P < 0.05$) by variety as well as by the used treatment.

The value of pH was higher in alfalfa silage ($P < 0.05$) in comparison with silages of two varietes of cocksfoot. When treated with organic acids, pH decreased ($P < 0.05$) in silage of cocksfoot (variety SW Luxor) and in alfalfa silage.

The ideal pH of silage should be according to Wilkinson (2005) in the range of 4 to 4.2; according to Doležal et al. (2012) between 3,7 and 5. These values were achieved in the experimental silages of cocksfoot (4.56 in untreated and after organic acid treatment the pH was lowered to 4.23). Adding a chemical preservative always resulted in reduction of pH in alfalfa and also cocksfoot silages. Especially the SW Luxor variety responded with significant drop of pH after the addition of organic acids.

The lactic acid content was higher ($P < 0.05$) in silage from alfalfa and from cocksfoot‘s Husar variety compared to SW LUXOR varienty. Addition of organic acids resulted in reduction of lactic acid content ($P < 0.05$).

Kotal (1962) states that the lactic acid content in silages of high quality should be 2/3 of the total amount of acids. This is also indicated by Zeman et al. (2006) in Tables for Assessing the Silage Quality, where the minimum content of lactic acid is specified as 70 % of the total amount of acids. Silages contained 107.76 g of lactic acid in 1 kg of dry matter and after the treatment with the preservative the content decreased to 68.21 g.kg^{-1} of dry matter. When compared with other acids contents, silages contain sufficient amount of lactic acid. If we follow the evaluation given by Zeman et al. (2006), silages should be considered as very succesful.

The acetic acid content was influenced by the variety and by the type of treatment. Addition of organic acids resulted in the decrease in acetic acid content ($P < 0.05$) for all observed silages.

The optimal acetic acid content from the total content of acids in silages should be 20 – 30 % of dry matter (Wilkinson, 2005). Rajčáková et al. (2009) presents acetic acid content of 9.15 g.kg^{-1} of dry matter in untreated grass silages with higher dry matter content. Also Kotal (1962) states the acetic acid content as 1/3 of total acids content. DREVJANY et al. (2004) indicates the proportion of acetic acid as 4 – 9 g.kg^{-1} with dry matter content of 30 – 35 %. In experimental silages of cocksfoot there was achieved higher content of acetic acid, but if we take into account the conversion to 100 % of dry matter, the content of acetic acid is equivalent to 21.10 g.kg^{-1} of dry matter without treatment and 9.17 g.kg^{-1} of dry matter after application of chemical preservative. Alfalfa silages reached values of 27.32 g.kg^{-1} of dry matter in untreated samples and 12.94 g.kg^{-1} of dry matter in those treated with organic acids.

Alfalfa silages were higher ($P<0.05$) in crude protein content compared to cocksfoot silages. Crude protein (CP) content in experimental silages of cocksfoot was approximately 90 g.kg^{-1} of dry matter; 91.31 g.kg^{-1} in the Husar variety and 87.26 g.kg^{-1} in the SW Luxor variety, which is approaching the value indicated by Zeman et al. (1995) in the average fresh crop of cocksfoot (93 g.kg^{-1}). Norm 2004 (Loučka et al., 2010) for silage evaluating states that high quality grass silage should contain at least 140 g.kg^{-1} CP of dry matter. In alfalfa silages the CP content was twice as much (214.49 g.kg^{-1} of dry matter). CP content in alfalfa silage was lower than in the green mass of alfalfa at the begining of before flowering 249 g.kg^{-1} (Zeman et al., 2006). Norm 2004 (Loučka et al., 2010) states that the CP content in alfalfa silages should be 200 g.kg^{-1} of dry matter.

The crude fiber content in alfalfa silages was lower than in cocksfoot silages. This difference was not statistically significant. The use of chemical additive in SW Luxor variety of cocksfoot resulted in decrease ($P < 0.05$) of crude fiber content. This was not evident in alfalfa silages, neither in silage of Husar variety of cocksfoot.

The crude fiber content in model silages was different for each variety and use of chemical preservatives (Table 1).

Mikyska (2013) states the crude fiber content in grass silages as 256.9 $g.kg^{-1}$, which is less than what was achieved in experimental silages. According to Zeman et al. (1995), the crude fiber content in the grassland should be 322.9 $g.kg^{-1}$. This value is higher than that of silage samples. Skládanka (2009) states the crude fiber content in green vegetation of Dactylis glomerata at the begining of earing 210 $g.kg^{-1}$. JAKOBE et al. (1987) reported in his work that the crude fiber of alfalfa in the early bloom is 305 $g.kg^{-1}$. This value is higher than that achieved in model alfalfa silages.

Table 1.: Influence of species and preservative on the pH, content of lactic acid (LA), acetic acid (AA), crude protein (CP), crude fiber (CF), and NH_3

Factor	pH	LA [$g.kg^{-1}$]	AA [$g.kg^{-1}$]	CP [$g.kg^{-1}$]	CF [$g.kg^{-1}$]	NH_3 [$g.kg^{-1}$]
Species						
Dactylis glomerata (SW Luxor)	$4,05^{a}$	$81,31^{a}$	$14,04^{a}$	$87,26^{a}$	$275,29^{a}$	$0,72^{a}$
Dactylis glomerata (Husar)	$4,35^{b}$	$94,69^{b}$	$9,19^{b}$	$91,31^{a}$	$275,06^{a}$	$0,84^{a}$
Medicago sativa (Pálava)	$4,69^{c}$	$103,55^{b}$	$20,79^{c}$	$214,49^{b}$	$269,57^{a}$	$1,87^{b}$
Preservative						
Control	$4,56^{a}$	$107,79^{a}$	$21,10^{a}$	$129,71^{a}$	$272,36^{a}$	$1,65^{a}$
Organic acidc	$4,23^{b}$	$68,21^{b}$	$9,17^{b}$	$132,33^{a}$	$274,25^{a}$	$0,76^{b}$

Average values in columns with different indexes (a, b) are on the statistically significant border of $P < 0.05$

Conclusion

Ammonia contents in cocksfoot silages were normal in both, treated and untreated samples. The ammonia content in the untreated alfalfa silage was on the border of values that animals are still able to tolerate. Dactylis glomerata silage with regard to the amount of ammonia would be possible to be fed even without preservative additives.

Optimal pH values were achieved in both types of evaluated silages. Even without silage additive they reached good pH values. Feeding these silages should not cause dietary disorders.

Evaluated silages reached the limit values of lactic acid content. From this we can assume that resulting silages were well preserved with the optimal content of lactic acid in order to provide the final quality followed by aerobic stability. They should not go bad. They would be rated as very good and suitable for animal feeding.

The acetic acid content met the requirements even in untreated silages. Evaluated silages didn’t contain excessive amount of acetic acid and they would be possible to feed untreated.

Crude protein content in evaluated silages was influenced by the addition of organic acids, which reduced CP content. In comparison with green vegetation, the values for untreated silage were very favourable because of a conservation almost the same content of CP as the original material.

The crude fiber content in silages was at a level required for high quality silages. Chemical additive has an impact on the reduction of crude fiber content in silages, therefore it is appropriate to use it particularly for the preservation of forages with a high crude fiber content or those harvested at later stages of vegetation or later cuts.

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References

1. Doležal P.: Vliv přídavku Lctobacillus plantarum DSM 12771 na kvalitu siláží silně zavadlé vojtěšky a trávy (Effect of supplements of Lactobacillus plantarum DSM 12771 on the quality of ensiled alfalfa and grass with a high kontent of dry metter). Acta univ. agric. et silvic Mend. Bruno, 5, 2002, s. 37 – 44.
2. Doležal P. (ed.): *Konzervace, skladování a úpravy objemných krmiv*: *(přednášky)*. 2. přeprac. vyd. Brno: Mendelova univerzita v Brně, 2010, 247 s. ISBN 978-80-7375-441-9.
3. Doležal P. (ed.): *Konzervace krmiv a jejich využití ve výživě zvířat*. Olomouc: Petr Baštan, 2012, 307 s. ISBN 978-80-87091-33-3.
4. Doležal P., Zeman L., Dvořáček J.: Posuzování hygienické kvality krmiv. *Zemědělec: Hygienická a zdravotní rizika krmiv* [online]. 2012, č. 43 [cit. 2013-04-17]. Dostupné z: http://www.agroweb.cz/Posuzovani-hygienicke-kvality-krmiv__s1708x61791.html
5. Drevjany L., Kozel V., Padrůněk S.: *Holštýnský svět.* ZEA Sedmihorky, 2004, 346 s.
6. Jakobe P. (ed.): *Konzervace krmiv*. Praha: Státní zemědělské vydavatelství, 1987, 264 s.
7. Kalač P.: Zemědělec: Hygienická a zdravotní rizika krmiv. *Chemická a mikrobiální rizika siláží* [online]. 2012, č. 43 [cit. 2013-04-10]. Dostupné z: http://www.agroweb.cz/Chemicka-a-mikrobialni-rizika-silazi__s1708x61793.html
8. Kotal V.: *Výživa a krmní hospodářských zvířat: Učební text pro střední zemědělské technické školy oboru pěstitelsko - chovatelského*. Druhé přepracované vydání. Praha: Státní zemědělské nakladatelství, 1962, 267 s.
9. Loučka R.,Šlosárková S., Pěnkava O.: (eds.): *Sborník referátů ze semináře Biominu o silážování: duben 2010, [ZD Pluhův Žďár u Jindřichova Hradce]*. Brno: Veterinární a farmaceutická univerzita, 2010, 44 s. ISBN 978-80-7305-100-6.
10. Mikyska F.: Kvalita siláží v období 1997 - 2012 - z databanky objemných krmiv. *Náš chov*. 2013, LXXIII, č. 3, 66 - 70.
11. Rajčáková L., Mlynár R.: *Zásady využívania potenciálu silážnych a konzervačných prípravkov pri výrobe kvalitných a hygienicky nezávadných konzervovaných krmív (Metodická príručka)* [online]. Nitra, 2009 [cit. 2013-04-17]. Dostupné z: http://www.cvzv.sk/pdf/Konzervacia-a-silazovanie-krmiv/Silazovanie-metodicka%20prirucka.pdf
12. Skládanka J.: Pastevní porosty, s. 129 – 143. In: Zahrádková R. (ed.), *Masný skot: od A do Z*. 1. vyd. Praha: Český svaz chovatelů masného skotu, 2009, 397 s. ISBN 978-80-254-4229-6.

13. Vyskočil, I., Skládanka, J., Doležal, P., Havlíček, Z.: *Metodika výroby experimentálních mikrosiláží.* Brno: Mendelova univerzita v Brně, 2011, 23 s. ISBN 978-80-7375-543-0
14. Wilkinson J.M.: *Silage*. Lincoln: Chalcombe Publications, 2005. ISBN 09-486-1750-0.
15. Zeman L. (ed.): *Katalog krmiv*: *(tabulky výživné hodnoty krmiv)*. 1.vyd. Pohořelice: VÚVZ, 1995, 465 s. ISBN 80-901598-3-4.
16. Zeman L. (ed.): *Výživa a krmení hospodářských zvířat*. 1. vyd. Praha: Profi Press, c2006, 360 s. ISBN 80-867-2617-7.

EVALUATION OF QUALITY OF A FORAGE AND BOTANICAL COMPOSITION OF GRASSLAND USED FOR GRAZING OF HORSES

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Abstract

The study deals with evaluation of quality of a forage and botanical composition of grazing area which was used for grazing of horses in 2012. In the study the quality of grassland (E_{GQ}) was evaluated on the basis of quantity of plant species and also the forage value (FV) of each species. Furthermore, the content of organic nutrients in the forage (crude protein, fibre, WSC- water-soluble carbohydrates) was determined by the NIRS method.
According to the calculated quality of the grassland the grazing area can be categorized as valuable grassland. It is mainly because of the presence of valuable grass species as *Arrhenatherum elatius*, (FV=7) and *Medicago sativa* (FV=7). The quality of grassland reached the best value in May (70,6) and then was gradually decreasing, yet in the autumn it was still at a quite good value (64,5).

Marerial and methods

The research was conducted in 2012 on the northern outskirts of Brno, in Jihomoravský kraj, Česká republika. The grazing area is located at the altitude of 287 m and its slope was on average 7,4°. On the evaluated area the arable land was converted to the grassland 13 years prior to the research. The evaluated area had an area of 18 ha which was divided into three parts. The load of the pasture was 1.8 LU / ha (Arabian Horse and Czech Warmblood). The mowing was done regularly at the end of grazing period. There was used a system of yearlong grazing. Animals were grazed approximately 8 hours a day. In the stables the horses were fed with grains and at night with hay eventually with green forage. The box stalls in the stable were littered with straw and there were available mineral licking buckets. During the winter the horses in the pasture were fed with hay.
During the period of research (May-October) the forage samples were picked up every fifteenth day of the month from all the three parts in the area. Similarly were chosen the areas for the analysis of botanical composition of grassland. The botanical composition of grassland was evaluated on the area of 2 sq. m using the method of projective dominance. The evaluated characteristics was the grassland quality (evaluation of grassland quality, E_{GQ}), (Novák, 2004), which divides plants in grassland into groups according to their palatability, production, nutrient content and digestibility.

Table 1 Division of grassland into groups according to the calculated value E_{GQ} (Novák, 2004)

E_{GQ}	Grassland
90 – 100	highly valuable - most valuable
70 – 90	valuable - highly valuable
50 – 70	less valuable - valuable
25 – 50	least valuable - less valuable
15 – 25	worthless - least valuable
0 – 15	deleterious - worthless
< 0	toxic

For calculating of the quality of grassland (E_{GQ}) was used the formula:
$E_{GQ} = \Sigma (D * FV) / 8$
D – predominance of the species [%]
FV – forage value of species
According to the calculated value the grassland can be divided into 7 groups (Table 1).

The samples for analysing of the quality of pasture were taken from the area of 0,5 sq. m. The plants were cut at the height of 3 cm. The samples were subsequently dried at the temperature of 60 °C and homogenised. The size of particles was circa 1 mm. The NIRS method was used for the evaluation of the content of organic nutrients. It is one of the molecular spectroscopy methods, which uses the spectral region near infrared (Matějka, 2013). The characteristics which were analysed were the content of crude protein, fibre and water-soluble carbohydrates.
The results were furthermore statistically processed using the software STATISTICA 10. The multifactorial analysis of variance (ANOVA) followed by Tukey test testing were used for the data processing.

Results and discussion

According to the quality of grassland the grazing area was categorized as valuable grassland. It was mainly because of the occurrence of valuable species in the grassland as *Arrhenatherum elatius* and *Medicago sativa* (FV 7), (Novák, 2004), which were relatively abundantly represented in the vegetation. The quality of grassland reached the maximum value in May (70,6) and then was gradually decreasing, yet in the autumn it still reached quite good value (64,5).
There was also observed relatively high proportion of *Medicago sativa* in the grassland (up to 25 %). This was possibly caused by the rejuvenation of grassland using supplementary seeding in recent years. Yet its proportion in the grassland was quite surprising, because by Hakl and Šantrůček (2002) *Medicago sativa* is not very suitable clover for pasture utilization for its intolerance for depressing. In contrast Church and Pond (1988) state that *Medicago sativa* is the most common pasture clover in North America. Pelikán et al. (2012) do not recommend *Medicago sativa* for intensive grazing which was not our case (1.8 LU / ha), and according to Suchý et al. (2010) it is even suitable to have *Medicago sativa* in the grazing area for horses.
The high percentage of *Arrhenatherum elatius* could be caused by its good competitiveness and drought resistance as well as its good ability for shedding seeds (Hrabě et al., 2004). However it is also considered as a species which is not suitable for pasture utilisation (Straková et al., 2007). Another possible reason for its expansion in the crop could be the fact that, according to Cosyns et al. (2001) it is not tasty for horses. However this grass is commonly included into mixtures specially designed for grazing horses. Our results also indicate that horses do not prefer this species. Its share in the crop was relatively high (40 %) and by the end of the vegetation it was even higher (45 %). On the contrary, the share of *Medicago sativa* in the crop was gradually decreasing. It had 25 % share in May, which decreased to 7 % in September. This decrease could be caused either by the competition between grass species or by the selective feed intake by grazing animals.
The most widespread herb species were *Achillea millefolium* and *Pastinaca sativa* each of which was represented over 10 % in the crop, which reduced the general quality of the crop. Undesired was also a regular occurrence of deleterious and worthless species (*Artemisia vulgaris*, *Cirsium vulgare*, *Carduus acanthoides*).

The highest ($P < 0.05$) crude protein content was measured in June, the lowest ($P < 0.05$) in September, according to Zeman et al. (1995), the forage quality was similar to the quality of forage in an older pasture. The forage quality most likely reflected the unusual

weather course, which was very poor on rainfalls in winter and especially in spring. Under these conditions the crop with higher percentage of drought resistant species as *Medicago sativa* and *Arrhenatherum elatius* prospered better (Suchý et al., 2010). Legumes contain higher proportion of crude protein than grasses (Church a Pond, 1988), therefore the high share of *Medicago sativa* in the grassland meant higher proportion of crude protein in measured samples. The crude protein content decreased in following months. This could cause the natural senescence of vegetation, reduced proportion of *Medicago sativa* and increased proportion of ungrazed patches.

Table 2: The content of organic nutrients in the forage

Month	Crude protein [g.kg^{-1}]	Fibre [g.kg^{-1}]	WSC [g.kg^{-1}]
May	171,3	227,1	47,4
	169,3	227,1	45,7
	173,4	224,3	46,5
June	209,7	195,6	46,9
	197,0	211,9	48,1
	199,0	205,3	46,3
August	147,2	203,2	44,0
	157,8	194,1	43,3
	149,9	205,1	44,0
September	146,5	263,5	17,9
	150,4	263,0	16,8
	142,4	276,2	13,3
October	161,6	267,0	19,2
	158,4	276,2	18,4
	162,3	272,3	16,2

The lowest (P<0,05) fibre content in the forage was measured in August, the highest (P<0,05) in October. The increase of fibre content in the following months after August was quite rapid. In comparison of fibre content and crude protein the negative correlation is evident, which should correspond to the aging process of the crop (Fiala, 2001). The high fibre content in autumn could be due to senescence of the crop and higher proportion of ungrazed patches by the end of the growing season.

The highest (P <0.05) WSC content was in June, while the lowest (P <0.05) value was found in September. Fiala (2001) states that the maximum content of WSC in crops fertilized with nitrogen (90 kg N.ha^{-1}) is 68 g.kg^{-1} in dry matter and he points to the negative correlation with content of nitrogen. The highest content of WSC is in spring when forage generally tends to the highest nutrient content. Then the concentration decreases (Church a Pond, 1988), which proved to be true for the evaluated crop as well.

Conclusion

The evaluated crop had good quality of grassland and forage and provided adequate yield despite of the unfavourable weather development during the grazing period. This fact was mainly due to the occurrence of *Arrhenatherum elatius* and *Medicago sativa*, which can be characterised as the drought resistant species. These plant species stabilised production of forage and its quality in the year 2012. Although the species composition and related forage value in the pasture area could be find as convenient it would be suitable to decrease the share of *Arrhenatherum elatius*. The horses do not feed it as willingly as anticipated. Part of its share could be replaced with supplementary sowing, for example of *Festuca arundinacea*,

which could provide the forage for horses in winter season. Another problem with this crop was a regular occurrence of undesirable species such as *Cirsium vulgare* and *Carduus acanthoides*. For regulating the growing percentage of these undesirable species and *Arrhenatherum elatius*, it would be suitable to mow the ungrazed patches after each grazing cycle. Mowing would also support the growth of young, nutrient-rich forage. These grass species respond well on fertilization. Regular nitrogen fertilization (50 $kg.ha^{-1}$) would be therefore adequate for increasing yield and quality of the forage and for promoting the appropriate species in the crop.

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References

1. Cosyns E., Degezelle T., Demeulenaere E. et al., 2001: Feeding ecology of Konik horses and donkeys in Belgian coastal dunes and its implications for nature management. *Belgian journal of zoology*, 131 (2): 111 – 118.
2. Fiala J., 2001: Kvalita píce travních porostů. *Farmář*, 7 (3): 21 – 26.
3. Hakl J., Šantrůček J., 2002: *Pícninářská charakteristika a uplatnění českého novošlechtění vojtěšek typu falcata.* Databáze online [cit. 2013-3-18]. Dostupné na: http://www.agris.cz/zemedelstvi?id_a=116457
4. Hrabě F. et al., 2004: *Trávy a jetelovinotrávy v zemědělské praxi*. Vydavatelství ing. Petr Baštan, Olomouc, 121 s. ISBN 80-903275-1-6.
5. Church D. C. A Pond W. G., 1988: *Basic animal nutrition and feeding*. John Wiley & Sons, New York, 472 s. ISBN 0-471-85246-5.
6. Matějka P., 2013: *Spektrometrie v blízké infračervené oblasti.* Databáze online [cit. 2013-3-08]. Dostupné na: http://www.vscht.cz/anl/lach2/NIR.pdf
7. Novák J., 2004: Evaluation of grassland quality. *Ekológia (Bratislava)*, 23 (2): 127 – 143.
8. Pelikán J., Hýbl M., Hutyrová H., Knotová D., Minjaríková P., Nedělník J., Raab S., Vymyslický T., 2012: *Rostliny čeledi Fabaceae LINDL. (bobovité) České republiky*. Vydavatelství Petr Baštan, Olomouc, 230 s. ISBN 978-80-905080-2-6.
9. Straková M., Straka J., Michalíková L., Plevová K., 2007: *Kapesní atlas trav*. tiskárna BRKO, s. r. o., Rousínov, 46 s.
10. Suchý P., Lesák J., Straková E., Neumannová K., 2010: Racionální využití lučních a pastevních porostů pro výživu koní. *Veterinářství*, 60 (7): 423 – 426.
11. Zeman L. et al., 1995: *Katalog krmiv*. VÚVZ Pohořelice, Znojmo, 465 s. ISBN 80-901598-3-4.

EFFECT OF CHAMOMILE EXTRACT ON ANTIOXIDANT STATUS IN POULTRY

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Abstract

In this experiment, effects of three different concentrations of chamomile (*Matricaria chamomilla*) extract, (0.3 %; 0.6 % and 1.2 %) in feeding doses on the antioxidant status in broiler chickens were monitored. Chickens were weighted every week at the age of 17, 24, 31 and 38 days. The experiment lasted 39 days and involved 48 chicks that were slaughtered. Antioxidant status in blood were evaluated using the ABTS method. The highest antioxidant activity was found in the control group (562,6 mg GAE/l) and in the group of chickens fed with 0,3 % chamomile extract (560,6 mg GAE/l). The results may show possible beneficial effect of chamomile extract,on health status in broiler chickens.

Keywords: broiler, chicken, chamomile, antioxidant status

Material and methods

Experimental design

The experiment involved 48 male chicks of the hybrid combination Ross 308. All chicks were seven days old. There were altogether 4 groups of these birds, viz. control and three experimental groups receiving chamomile extract supplements in concentrations of 0.3 %, 0.6 % and 1.2 %.

Birds and experimental conditions

Prior to the beginning of the experimental period, chicks were weighed, identified with wing tags, assorted into four groups and placed into metabolic cages. All birds received water and feed mixture *ad libitum*. The feed mixture consisted of following components: wheat (25 %), maize (37 %), soybean meal (28 %), sunflower oil (6 %), mineral-vitamin mixture without anticoccidial drugs (3 %), monocalcium phosphate (0.8 %) and finely ground limestone (0.2 %). Chamomile extract was added into the feed mixture in concentrations of 0.3 %; 0.6 % and 1.2 %.

The light regime was 6 hours of darkness and 18 hours of light. On the 7th day of age chicks were kept at the ambient temperature of 29.9 °C and relative humidity of 50 %.

Preparation of samples

The experiment lasted 39 days and involved 48 chicks that were slaughtered. Blood sample was collected at slaughter chickens from the vena jugularis into plastic sample containers with coagulant heparin. Antioxidant status in blood were evaluated using the ABTS method (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)).

Determination of antioxidant activity by ABTS test

The ABTS radical method is one of the most used assays for the determination of the concentration of free radicals. It is based on the neutralization of a radical-cation arising from the one-electron oxidation of the synthetic chromophore 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS): ABTS• – e- ABTS•+. This reaction is monitored spectrophotometrically by the change of the absorption value. A 150 μL volume of reagent (7 mM ABTS• (2,2´-azinobis 3-ethylbenzothiazoline-6-sulfonic acid and 4.95 mM

potassium peroxodisulphate)) is poured with 3 µL of a sample. Absorbance is measured at 660 nm. For calculating of the antioxidant activity, difference between absorbance at the last (12th) minute and second minute of the assay procedure was used.

Statistical processing
Obtained results were analysed using the programme Microsoft Excel 2010 and the software Statistica 10 CZ.).

Results and discussion

The aim of this study was to assess effects of different concentrations of chamomile extracts on the antioxidant status of broiler chickens of hybrid combination Ross 308.
Chickens were weighted every week at the age of 17, 24, 31 and 38 days. Antioxidant status in blood were evaluated using the ABTS method (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)).

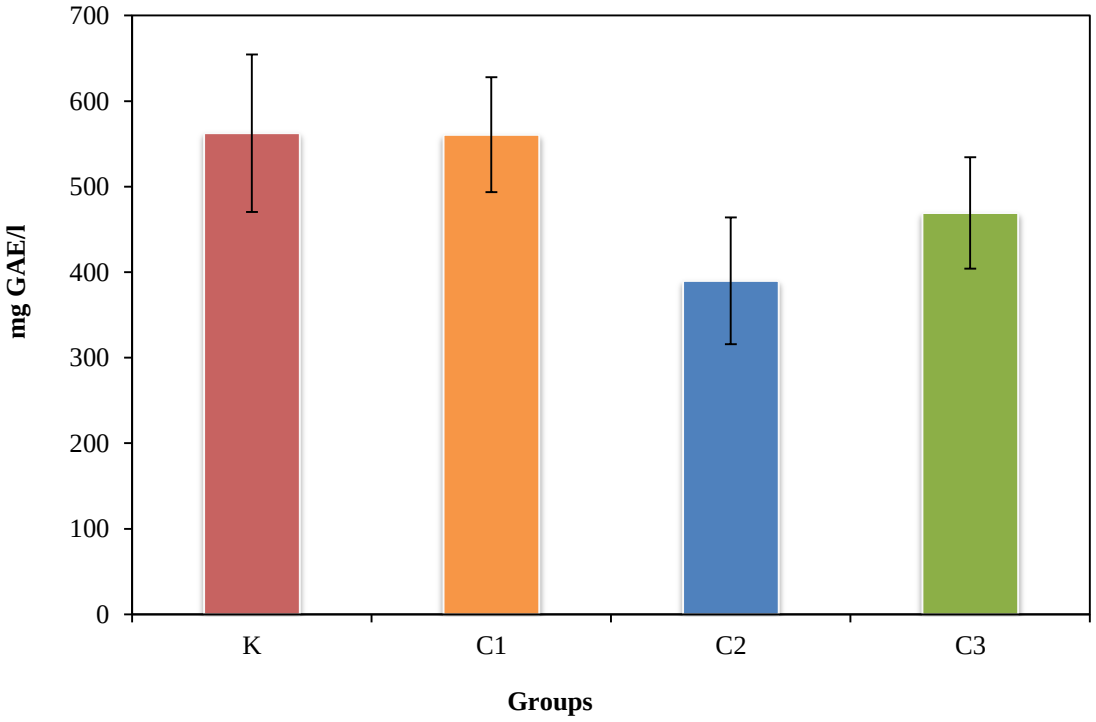

Figure 1 Antioxidant status evaluated by ABTS method (C 1 – concentration 0.3 % of chamomile extract, C 2 – concentration 0.6 % of chamomile extract, C 3 – concentration 1.2 % of chamomile extract, C 0 – concentration 0 % of chamomile extract)

The antioxidant activity and total phenolic content of selected herbs and apices using the DPPH method followed Roby et al., 2012. In their study chamomile and fennel showed highest total phenolic content, associated with the relatively higher antioxidant activities. Further demonstrated in their study that the essential oil of *M. chamomilla* exhibits antioxidant activity Abdoul-Latif, et al., (2011).

Conclusion

In our experiment, which was based on supplementation of chamomile extract, we monitored its effect on the antioxidant status of broiler chickens. According to our results, the

doses of liquid chamomile 0.6 % of feed mixture significantly increased the activity measured by ABTS methods.

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References

1. Abdoul-Latif, F. M., Mohamed, N., Edou, P., Ali, A. A., Djama, S. O., Obame, L. C., Bassole, I. H. N.,Dicko, M. H. Antimicrobial and antioxidant activities of essential oil and methanol extract of Matricaria chamomilla L. from Djibouti. *Journal of Medicinal Plants Research*, 2011, 5 (9), p. 1512 – 1517.
2. Pohanka, M., Sochor, J., Ruttkay-Nedecky, B., Cernei, N., Adam, V., Hubalek, J., Stiborova, M., Eckschlager, T., Kizek, R. Automated assay of the potency of natural antioxidants using pipetting robot and spectrophotometry. *Journal of Applied Biomeicine*, 2012, 10, p. 155 – 167. ISSN 1214-0287.
3. Roby, M. H. H., Sarhan, M. A., Selim, K., A., Khalel Ibrahim Khalel, K. I. Antioxidant and antimicrobial activities of essential oil and extracts of fennel (Foeniculum vulgare L.) and chamomile (Matricaria chamomilla L.). *Industrial Crops and Products*, 2013, 44, p. 437 – 445.

THE USE OF GARLIC EXTRACT IN FEED RATION OF BROILER CHICKENS

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Abstract

Aim of this study was to detect effect of the garlic extract (*Allium sativum*) addition to the diet Ross 308 cockerels on blood antioxidant activity expressed by FR method, nitrogen retention and body weight gain. Statistically significant ($P < 0,05$) the highest antioxidant activity levels were achieved in the control group as measured by FR method. Statistically significant ($P < 0,05$) effect on nitrogen retention was found in the control group compared to groups containing 10 and 15 g of garlic extract per kilogram of feed mixture. Feeding garlic extract had no statistically significant effect on body weight gain and feed conversion ratio.

Keywords: garlic extract, nitrogen retention, antioxidant activity, poultry nutrition

Material and methods

102 Ross 308 cockerels were divided into 3 groups at the age of 7 days. Chickens were kept in cages and received diet of the composition view at Table 1. The first group obtained feed mixture with 10 g (G10) and the second group obtained in feed mixture 15 g (G15) of liquid garlic extract per 1 kg. Control group (C) of cockerels obtained feed mixture without garlic extract. Diets were fed *ad-libitum* for 26 days. Water was available continuously. Body weight was measured on arrival and then every week. Lighting regime was set to 16 hour light and 8 hour dark. Excreta were collected daily into Petri dishes for follow determination of nitrogen retention. At the age of 39 days broilers were weighted and slaughtered and blood from the carotid artery was taken. From the blood samples was determined antioxidant status.

Determination of antioxidant activity by Free Radicals method

This method is based on ability of chlorophyllin (the sodium-copper salt of chlorophyll) to accept and donate electrons with a stable change of maximum absorption. This effect is conditioned by an alkaline environment and the addition of catalyst (VOTRUBA et al., 1999). Data has been processed by Microsoft Excel (USA) and Statistica version 10.0 (CZ).

Table 1: *Composition (g/kg) of the diet used in our experiment*

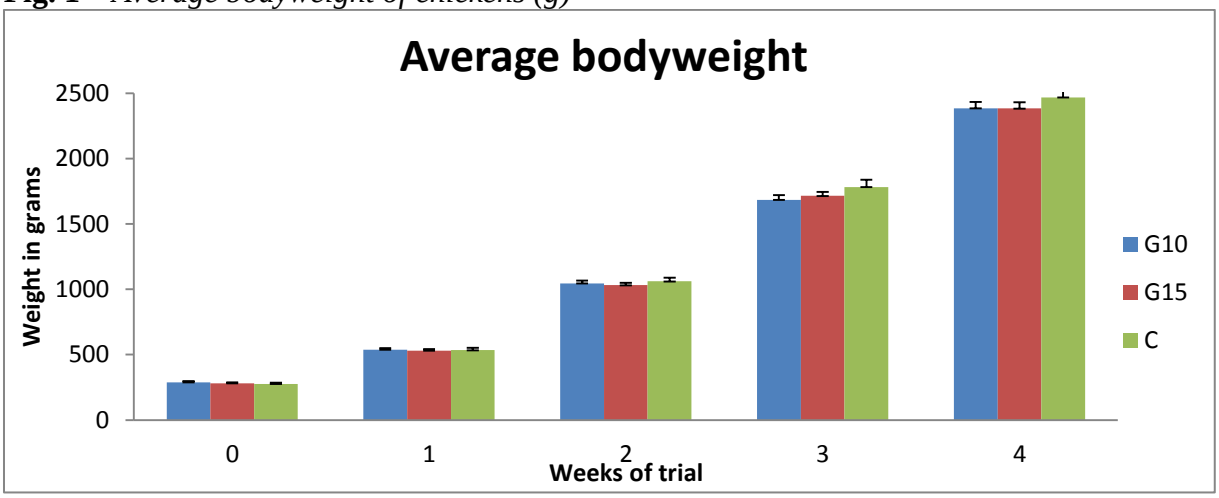
COMPONENT	VALUE
MAIZE MEAL	370
SOYBEAN MEAL	280
WHEAT MEAL	246
SUNFLOWER OIL	60
PREMIX VBR3*	30
MONOCALCIUM PHOSPHATE	8
CHROMIUM OXIDE	3
LIMESTONE	2,5

* lysine 60,0 g; methionine 75,0 g; methionine + cysteine 75,0 g; calcium 195,0 g; phosphorus 55,0 g per; sodium 46,0 g per kg; copper 4,0 mg; zinc 3,70 mg; tocopherol 1,50 mg; biotin 6,0 mg per kg and retinol 450 IU; calciferol 166,70 IU

Results and discussion

At the end of trial we observed non-significant (P > 0,05) higher weight (2515 ± 132,72 g SD) in group of chickens fed without garlic extract. Average bodyweight of our cockerels are shown in Figure 1.

Fig. 1 – *Average bodyweight of chickens (g)*



The addition of garlic extract to the diet of broiler chickens had no significant influence on bodyweight gain even on feed conversion ratio. Significantly higher (P < 0,05) nitrogen retention was observed in the control group as shown in Table 2. Equally significantly higher (P < 0,05) antioxidant activity was measured in the control group. ***A***ntioxidant activity values measured by the Free radical method are shown in Table 3.

Fayed et al. (2011) in their trial observed then raw garlic powder at dose of 0,5 kg/ton gained the highest live weight (P < 0,05). Even then Onibi *et al*. (2009) observed then raw garlic powder at dose 5,000 mg/kg diet had non-significantly (P > 0,05) influence to average weight gain and feed conversion ratio.

Table. 2: *Nitrogen retention*

Group	N	Average (%) ± standard deviation
G10	7	50 ± 1,83 [a]
G15	7	54 ± 1,59 [a]
C	7	64 ± 1,76 [b]

[a,b] – different letters mean statistically significant differences (P < 0,05)

Table. 3: *Antioxidant status by Free radical's method*

Group	N	FR (GAE µg/ml)* Average (µg/ml) ± standard deviation
G10	18	77 ± 1,36 [a]
G15	20	78 ± 1,97 [a]
C	16	90 ± 2,96 [b]

[a,b] – different letters mean statistically significant differences (P < 0,05)

*GAE – Gallic acid equivalent

Jakubcová et al. (2013) in their experiment found statistically significant difference ($P < 0,05$) in antioxidant status by the FR method in favour of group with 10 ml of liquid garlic extract compared with the control group.

Conclusion

Statistically significant ($P < 0,05$) highest antioxidant activity levels were achieved in the control group as measured by FR method. Statistically significant ($P < 0,05$) effect on nitrogen retention was found in the control group compared to groups containing 10 and 15 g of garlic extract per kilogram of feed mixture. Feeding garlic extract had no statistically significant effect on body weight gain and feed conversion ratio.

Botsoglou *et al.* (2004) observed that well-fed and healthy chickens in clean animal hygiene conditions at a moderate stocking density may not respond positively to grow promoting supplements.

The differences in the results when fed garlic supplementation by different authors can have several reasons: a) differences in the use of the product - garlic powder, garlic meal or derivatives; b) the use of additives and the concentration of active ingredients, which vary considerably between experiments; c) a complicated chemical composition of garlic (Amagase *et al*., 2001) and d) using different commercial preparations of garlic. Commercial garlic products can be divided, according to the active substances they contain, to products with raw garlic, which are rich in allicin and preparations are processed garlic, which are poor to allicin (Khan *et al*., 2008).

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References

1. Amagase, H.; Petesch B. L.; Matsuura H.; Kasuga S.; Itakura Y.: *Intake of garlic and its bioactive components. J. Nutr.*, 2001. 131:955S – 962S.
2. Botsoglou, N. A.; Christaki E.; Florou-Paneri P.; Giannenas L.; Pa-Pageorgiou G.; Spais A. B.: *The effect of a mixture of herbal essential oils or α-tocopheryl acetate on performance parameters and oxidation of body lipid in broilers.* South African Journal of Animal Science, 2004, 34, 52-61.
3. Fayed R. H.; Razek A. H. A.; Ouf M.: *Effect of dietary garlic supplementation on performance, carcass traits, and meat quality in broiler chickens. ISAH Congress, Vol. XV, 2011.*
4. Jakubcová, Z.; Mrkvicová E.; Horký P., Mrázková E.: *Effect of addition garlic extract in broiler feed ration.* Nutrinet, 2013, p. 43 – 47.
5. Khan, Q. S. H; Hasan, S.; Sardar, R. Anjum, M. A.: *Effects of dietary garlic powder on cholesterol concentration in native Desi laying hens.* American journal of Food Technology, 2008, 3:207-213.
6. Onibi G. E.; Adebisi O. E.; Fajemisin A. N.; Adetunji A. V.: *Response of broiler chickens in terms of performance and meat quality to garlic (Allium sativum) supplementation. African journal of agricultural research,* 2009, 4:511-517.
7. Votruba, M.; Stopka, P.; Hroudova, J.; Vesely, K.; Nejedlova, L. *A simple method for quantitative estimation of free radicals in serum.* Klin. Biochem. Met. 1999, 7, 96-101.

THE EFFECT OF COLORED WHEAT FEEDING ON BROILER CHICKENS PERFORMANCE PARAMETERS AND ANTIOXIDANT ACTIVITY MEASURED IN THE LIVER TISSUE

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Abstract

The aim of this study was to determine effect of yellow wheat Citrus with a higher content of lutein (0.34 mg/100 g) in grain included in feed ratio for fattening male broilers of hybrid combinations Ross 308. The effect on feed consumption, weight gain, carcass yield and antioxidant activity measured in liver tissue of cockerels were evaluated. The content of yellow wheat Citrus in experimental diet was 30% and 60%. Control diet contain 30% and 60% of common wheat. Effect on performance parameters was not significant ($P > 0.05$). Significantly higher ($P < 0.05$) antioxidant activity measured in liver tissue by method of free radicals (FR) was observed in experimental group with broilers fattened by 100% of wheat Citrus.

Keywords: yellow wheat Citrus, nutrition of chickens, broiler Ross 308, antioxidant activity

Material and methods

An experiment was performed with 156 cockerels of Ross 308 hybrid combination which were fattened in cage batteries from Day 15 to Day 42 of age. Cockerels were divided into 4 groups. Two experimental groups of chicken received feed mixtures containing 30 and 60% of yellow wheat Citrus (EXP30 and EXP60) and two control groups received 30 and 60% of common wheat (CONT30 and CONT60). The crumbly feed mixture was supplied *ad libitum* and its consumption was recorded. The feed mixtures for every groups were isonitrogenous. Composition of the feed mixtures is shown in Table 1.

The lighting regime during our experiment was set to 16 hours light and 8 hours dark. The live weight of chickens was estimated in the three-day intervals.

Twelve chickens from each group were slaughtered at the age of 42 days. Breast and thigh muscles without skin were separated from carcasses after cooling. Breast and thigh meat was then weighed and their percentages of body weight were calculated.

At the same time 80 cockerels were chosen for the 2nd experiment to determine effect of feeding wheat Citrus on antioxidant activity in broilers, which was measured in the liver tissue by DPPH, FRAP and FR methods (vide infra). The 2nd experiment continue from Day 43 to Day 57 of age. Cockerels were divided into two groups of 40 chickens. From 43rd to 57th days of age the experimental group received only crumbly wheat Citrus - 100% of Citrus (EXP100) and control group only crumbly common control wheat - 100% of control wheat (CON100). The control wheat were mixed from two wheat with different content of crude protein (CP) so that results the same content of CP like wheat Citrus. Cockerels were fed *ad libitum*. Nutrient composition of wheat is shown in Table 2.

Chickens from the 2nd trial were slaughtered at the age of 57 days. Chickens were eviscerated and liver were taken for antioxidant analyses.

Determination of antioxidant activity by the test using 0.95 mmol/l solution of radical 2,2-diphenyl-1-picrylhydrazyl (DPPH)**,** Free Radicals method (FR) and Ferric Reducing

Antioxidant Power method (FRAP) is described in SOCHOR et al. (2010), DOBEŠ (2012) and JAKUBCOVÁ et al. (2013).

Table 1: *Composition of the diet BR2* and BR3** (g/kg)*

Component	EXP60	CON60	EXP30	CON30
Corn 9 % of crude protein	56	56	348	348
Cornstarch	12	12	-	-
Soybean meal < 3,5 % of fiber	242	242	273	273
Sunflower oil	50	50	40	40
Limestone 35 % of Ca	3	3	3	3
Monocalcium phosphate 24 % P	6	6	6	6
Lysine 78 %	1	1	-	-
Premix VBR2* and VBR3**	30	30	30	30
Wheat CITRUS	600	-	300	-
Wheat CONTROL	-	300	-	300

*lysine 60 g; methionine 75 g; threonine 34 g; calcium 200 g; phosphorus 65 g; sodium 42 g; copper 500 mg; iron 2500 mg; zinc 3400 mg; manganese 4000 mg; cobalt 7 mg; iodine 30 mg; selenium 6 mg; tocopherol 450000 mg; calciferol 166700 m.j.; tocoferol 1500 mg; vit K 350 mg; B1 140 mg; B2 230 mg; B6 200 mg; B12 1000 mg; biotin 7 mg; niaciamid 1200 mg; folic acid 57 mg, calcium pantothenate 450 mg; choline chloride 6000 mg; salinomycin sodium 2333 mg

**lysine 60 g; methionine 75 g; threonine 34g; calcium 185 g; phosphorus 55 g; sodium 46 g; copper 500 mg; iron 2500 mg; zinc 3700 mg; manganese 4000 mg; cobalt 7 mg; iodium 30 mg; selenium 12mg; tocoferol 450000 mg; calciferol 166700 m.j.; tocoferol 1500 mg; vit K 350 mg; B1 140 mg; B2 230 mg; B6 200 mg; B12 960 mg; biotin 6 mg; niaciamid 1200 mg; folic acid 57 mg, calcium pantothenate 4450 mg; choline chlorid 6000 mg

Table 2: *Nutrient content of wheat (g/kg)**

Structure	Citrus	Control
Dry matter	907	903
Brutto energy	15.7	15.8
Crude protein	128	127
Fat	9.4	12.4
Fiber	36.3	27
Ash	16.7	16.6

*Initial dry matter

Data has been processed by Microsoft Excel (USA) and Statistica version 10.0 (CZ).

Results and discussion

The highest total feed consumption during the experiment (from 15th to 42nd days of age) was found in the experimental group EXP60 namely 4.14 kg. The lowest total feed consumption for this period was achieved in the group EXP30 namely 3.94 kg. The values were not significant. The manual of hybrid Ross 308 are similar to the average values of feed consumption as the experimental group, while the control group have lower feed consumption compared to manual for hybrid Ross 308 (ANONYM, 2002).

The highest average weight at the end of fattening was achieved in the experimental group EXP60 with value 2 953 ± 49.8 grams, while the lowest weight was observed in the control group CON30 2 797 ± 63.6 grams. The differences was not significant ($P > 0.05$). In the experiment of SUN et al. (2013) the average weight at the end of the fattening was 2884 grams. In ANONYM (2002) is the body weight at the end of fattening lower.

The highest carcass yield was found in the experimental group EXP60, the average carcass was 71.49%. The lowest values 70.39% were found in the control group CON30. The results are not statistically significant. In ANONYM (2002) and in the experiment of SUN et al. (2013) was the carcass yield higher to compare with our results.

Percentages of breast muscle of body weight were highest for experimental group EXP60 (23.15 ± 0.29 %), while the lowest was observed in the control group CON30 (22.77 ± 0.38%). In SUN et al. (2013) and in the manual of hybrid Ross 308 (ANONYM, 2002) are lower percentage of breast muscle of body weight to compared with our experiment.

Percentages of thigh muscle of body weight was attempted highest for experimental group EXP60 (15.88 ± 0.25%), while the lowest value was observed in control group CON30 (14.80 ± 0.47%). The manual for the hybrid Ross 308 (ANONYM, 2002) indicates a higher proportion of thigh muscle 16.13%.

The values of antioxidant activity measured in the liver tissue of chickens fed by Control (CON100) and Citrus wheat (EXP100) measured by DPPH, FRAP and FR methods are listed in Table 3. Significantly higher values ($P < 0.05$) were found in measuring by FR method in chickens fed wheat Citrus (EXP100; 983.12 GAE * mg/l), compared to the control (833.29 GAE * mg/l).

Table 3 *Methods to determine the antioxidant activity*

Methods	Units	Citrus (EXP100)				Control wheat (CON100)			
DPPH	% inhibition	20.06	±	1.139	[a]	19.12	±	1.002	[a]
FR	GAE* mg/l	983.12	±	69.073	[a]	833.29	±	44.033	[b]
FRAP	GAE* mg/l	57.61	±	2.165	[a]	60.95	±	1.113	[a]

[1]) The average ± standard error of the mean; n = 40
Averages in the same tissue labeled with different superscripts: [ab] differ significantly ($P < 0.05$)
* GAE - gallic acid equivalents

Conclusion

In the experiment has been found that feeding of wheat Citrus does not significant effect ($P > 0.05$) on feed consumption, weight gain and carcass yield compared to feeding control wheat. Significant effect ($P < 0.05$) was detected on the antioxidant activity of liver tissues of fattened chickens measured by FR method. In the experiment it was found that even with the inclusion of 60% of color wheat Citrus in the diet, was achieved a good results during of fattening and good carcass yield. Also, in an attempt of RUCKSCHLOSSE et al. (2010) inclusion of 60% of the color wheat in the diet for laying hens give good performances and egg quality. Wheat Citrus can also be used for its high content of lutein in the food industry in order to enhance the color of baked products, or for the production of functional food.

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References

1. Anonym, 2002: Broiler management manual. AviagenRoss, 2002. *Aviagen Group*., s. 111
2. Dobeš, J., 2012: *Stanovení biologicky aktivních substancí v rostlinném materiálu*, Dizertační práce, Brno: MENDELU v Brně, 106 s.
3. Jakubcová, Z. - Mrkvicová, E. - Horký, P. - Mrázková, E. 2013: Effect of addition garlic extract in broiler feed mixture. In: Bíro, D. - Šimko, M. - Zelinková, G. *Proceedings of the NutriNET 2013.* Nitra: Slovak University of Agriculture in Nitra, s. 43-48. ISBN 978-80-552-1065-0.
4. Ruckschloss, L. – Matúšková, K. – Hanková, A. – Jančík, D. 2010: Vplyv pšenice s purpurovou farbou zrna na parametre úžitkovosti nosníc a kvalitu vajec. Potravinárstvo, s. 231 – 235.
5. Sochor, J., et al., 2010: *Fully Automated Spectrometric Protocols for Determination of Antioxidant Activity: Advantages and Disadvantages*, Molecules, 2010, 15, p. 8618-8640, ISSN: 1420-3049.
6. Sun, Z. G. - Lv, M. B. - Yan, Z. W. - Wang, S. An. - Lv, Z. Z., 2013: Effects of feeding age and inclusion level of whole wheat on performance, carcass characteristics and economic benefits of broiler chickens. Aust. Poult. Sci. Symp. 2013.

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