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The conference was held on the occasion of the 25th anniversary of the Faculty of Veterinary Hygiene and Ecology establishment and the 40th anniversary of the university education of food hygiene at the University of Veterinary and Pharmaceutical Sciences Brno.

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Content

ASSESSMENT OF MINERALS CONCENTRATIONS IN MAIZE SILAGES ANALYZED FROM YEAR 2011 TO 2013

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ABSTRACT

Maize silage is an important component of daily diets in cattle nutrition characterized by good digestibility, palatability and storage ability. In comparison with alfalfa silage, maize silage is lower in a content of minerals. In our study we determined and compared the average content of mineral elements in maize silages from 2011 to 2013. Totally 343 samples were collected by rod sampler from 9 parts of silage clamp to tights opaque plastic bags and analyzed for concentration of calcium (Ca), phosphorus (P), sodium (Na), magnesium (Mg), potassium (K), iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) by absorption spectrophotometry (device ContrAA®700, Analytikjena, Germany). Mineral elements in corn silages were found in the following ranges: Ca: 2.83 – 3.67 $g.kg^{-1}$, P: 1.86 – 2.80 $g.kg^{-1}$, Mg: 1.57 – 1.76 $g.kg^{-1}$, K 9.57 – 11.06 $g.kg^{-1}$, Na: 0.52 – 0.57 $g.kg^{-1}$, Zn 21.29 – 34.52 $mg.kg^{-1}$, Mn 30.08 – 33.10 $mg.kg^{-1}$, and Cu 5.49 – 6.49 $mg.kg^{-1}$.

Keywords: corn; silage; macroelements; microelements; evaluation

INTRODUCTION

Corn silage is popular worldwide since maximum yield of nutrients per land unit can be harvested from it and as a popular forage for ruminant animals due to its high yield, digestibility, palatability and storage ability (Hafez et al., 2012; Pishgar Komleh et al., 2011). Silages are preserved through a fermentation process that produces acids to inhibit spoilage (Chahine et al., 2009). Besides the increased proportion of forages being fed to lactating dairy cows, a current trend has been to

include higher proportions of corn silage in those diets. Reasons for this practice include higher DM (dry matter) yields from corn and easier ensiling of corn silage, because of its lower buffering capacity compared with alfalfa (Lim et al., 2015). Compared to alfalfa, corn silage is low in protein, ash, and lignin. In addition, the biological value of the protein in corn silage is low because it is low in lysine. Corn silage is also much lower in many trace minerals than alfalfa (Martin et al., 2008). Concentration of minerals in plants depends on four factors: genotype, soil enviroment, climate and stage of plant maturity. All nutrients, including minerals, have important role and may be the limiting factor of successful breeding (Skalicka and Maskalova, 2013). Dairy cows may suffer from events of hypocalcemia and hypomagnesemia, commonly known as milk fever and tetany. About 50% of cows in their second lactation or later one have blood Ca concentrations that fall below the threshold for subclinical hypocalcemia after calving (Reinhardt et al., 2011). Trace minerals are important for immune function, oxidative metabolism, nutrient and energy metabolism, and reproductive function in dairy cows (Spears and Weiss, 2008).

MATERIAL AND METHODS

A total of 343 samples were analyzed from years 2011 to 2013 for minerals content in maize silages collected from Slovak dairy cattle farms. In 2011, 176 samples were analysed, in 2012 - 122 samples and in 2013 - 45 samples. Samples were collected by rod sampler from 9 parts of silage clamp to tights opaque plastic bags. After samples transporting to the Laboratory of Quality and Nutritional Value of Feeds of the Department of Animal Nutrition at the Slovak University of Agriculture in Nitra, the samples were prepared for nutrient analysis in accordance with the law MPSR 2145/2004-100. Minerals concentrations (Ca, P, Na, K, Mg, Zn, Mn, Fe and Cu) were determined by the device ContrAA®700 (Analytikjena, Germany). Results were compared and analysed using One-Way ANOVA and Scheffe test (IBM SPSS v. 22).

RESULTS AND DISCUSSION

Table 1. Concentrations of minerals in maize silages in 2011

	Unit	Mean	Std. deviation	Minimum	Maximum
Dry matter	%	30.03	2.24	23.23	35.38
Ca	g.kg^{-1}	2.83	0.91	0.03	10.50
P		2.79	0.34	2.04	4.52
Mg		1.57	0.52	0.22	4.17
Na		0.57	0.55	0.18	3.71
K		9.62	1.34	1.60	13.49
Fe		0.15	0.06	0.08	0.42
Zn	mg.kg^{-1}	34.52	84.02	10.26	1097.00
Mn		30.08	23.14	10.73	317.70
Cu		6.49	10.69	2.52	140.03

Table 2. Concentrations of minerals in maize silages in 2012

	Unit	Mean	Std. deviation	Minimum	Maximum
Dry matter	%	34.85	5.23	28.76	64.80
Ca	g.kg^{-1}	3.67	2.13	2.05	18.17
P		2.80	0.96	2.15	9.96
Mg		1.59	0.56	0.93	3.59
Na		0.52	0.70	0.18	5.86
K		11.06	1.78	8.03	16.59
Fe		0.15	0.18	0.07	1.49
Zn	mg.kg^{-1}	23.63	19.63	13.31	167.20
Mn		32.29	9.78	13.14	65.21
Cu		5.49	1.43	2.87	12.43

Table 3. Concentrations of minerals in maize silages in 2013

	Unit	Mean	Std. deviation	Minimum	Maximum
Dry matter	%	32.23	1.97	27.85	35.12
Ca	g.kg^{-1}	2.34	0.37	1.49	3.16
P		1.86	0.20	1.49	2.27
Mg		1.76	0.22	1.35	2.12
Na		0.52	0.16	0.23	0.97
K		9.57	1.42	7.08	13.76
Fe		0.26	0.15	0.13	0.73
Zn	mg.kg^{-1}	21.29	2.88	17.25	32.12
Mn		33.10	4.36	22.28	40.38
Cu		5.59	1.04	3.91	8.74

Table 4. Statistical significance of differences of dry matter (DM) and minerals concentrations between observed years ($P < 0.05$ = *; $P > 0.05$ = -)

	DM	Ca	P	Mg	Na	K	Fe	Zn	Mn	Cu
2011 - 2012	+	+	-	-	-	+	-	-	-	-
2011 - 2013	+	-	+	-	-	-	+	-	-	-
2012 - 2013	+	+	+	-	-	+	+	-	-	-

Our results of average concentrations of dry matter and minerals for observed years are summarized in Tables 1 – 3. In Table 4 statistical significances of differences of dry matter and minerals concentrations between studied years are listed. We found a significant difference between years 2011-2012 and 2012-2013 in concentration of Ca, which was 2.83 to 3.67 g.kg^{-1}. These results are similar to findings reported by Ferraretto et al. (2015) 2.60 g.kg^{-1}, Hafez et al. (2012) 2.50 g.kg^{-1} and Blackwood (2007) 3.00 g.kg^{-1}. Significant difference in concentration of P was found between years 2011-2013 and 2012-2013, where average amounts of P were 1.86 to 2.80 g.kg^{-1}. Ferraretto et al. (2015) found values of P 2.60 g.kg^{-1}, Hafez et al. (2012), Kung et al. (2015), Suttle (2010) 2.3 g.kg^{-1}, and Blackwood (2007) 2.0 g.kg^{-1}. The range of Mg content was from 1.57 to 1.76 g.kg^{-1} without significant difference. Hafez et al. (2012) and Kung et al. (2015) found content of Mg 1.6 g.kg^{-1}. The average concentration of Na was found between all years without significant difference too.

Our results of Na content (0.52 – 0.57 g.kg^{-1}) were higher than those of Blackwood's (2007), Kung et al. (2015) 0.10 g.kg^{-1} and Hafez et al. (2012) 0.20 g.kg^{-1}.

The most concentrated element in maize silage was K (9.57 – 11.06 $g.kg^{-1}$) with a significant difference between years 2011-2012 and 2012-2013. In comparison with findings by Ferraretto et al. (2015; 11.5 $g.kg^{-1}$), Hafez et al. (2012; 10.6 $g.kg^{-1}$), and Blackwood (2007; 10.5 $g.kg^{-1}$) our results were similar to their. The average concentration of Fe in our study was from 0.15 to 0.26 $g.kg^{-1}$ (2011-2013; 2012-2013 = $P < 0.05$). Kung et al. (2015) found similar results (0.28 $g.kg^{-1}$), but another sources claim that the concentrations of iron in silage maize could be higher (Blackwood, 2006; 0.64 $g.kg^{-1}$). There were not significant differences between years 2011-2012, 2011-2013 and 2012-2013 in comparison of Zn, Mn and Cu concentrations. Finally, we found the average concentration of Zn 21.29 – 34.52 $mg.kg^{-1}$, Mn 30.08 – 33.10 $mg.kg^{-1}$, and Cu 5.49 – 6.49 $mg.kg^{-1}$. Hafez et al. (2012) found concentration of Zn 21.82 $mg.kg^{-1}$, Skalicka and Maskalova (2013) from 51.77 to 55.99 $mg.kg^{-1}$, and Kung et al. (2015) 24 $mg.kg^{-1}$. Similar results compared to our results are known for concentrations of Mn in maize silage: 34 $mg.kg^{-1}$ (Blackwood, 2007), 24 $mg.kg^{-1}$ (Kung et al., 2015). Skalicka and Maskalova (2013) state that the concentration of Cu was from 9.80 to 11.02 $mg.kg^{-1}$. Suttle (2010) found the average value of Cu content 4.4 $mg.kg^{-1}$ and Kung et al. (2015) 6 $mg.kg^{-1}$.

CONCLUSION

Maize silage is an important component of daily diets for dairy cows. Its mineral composition is characterized by high concentrations of K (9.57 – 11.06 $g.kg^{-1}$). Another macroelements were found in the dry matter in the following ranges: Ca: 2.83 – 3.67 g.kg-1, P: 1.86 – 2.80 $g.kg^{-1}$, Mg: 1.57 – 1.76 $g.kg^{-1}$, Na: 0.52 – 0.57 $g.kg^{-1}$. From trace elements the most abundant element was Fe (0.15 – 0.26 $g.kg^{-1}$). The average concentrations of another trace elements were: Zn 21.29 – 34.52 $mg.kg^{-1}$, Mn 30.08 – 33.10 $mg.kg^{-1}$, and Cu 5.49 – 6.49 $mg.kg^{-1}$.

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THE EFFECT OF PUMPKIN AND FLAXSEED OILS ON THE CONTENT OF CRUDE FAT AND CHOLESTEROL IN EGG YOLK

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ABSTRACT

The aim of the study was to analyze the effect of pumpkin and flaxseed oils on the content of fat and cholesterol in table eggs. Lohmann Brown Lite hens were randomly divided to three groups. A total of 18 hens (6 per group) were monitored. Hens in the control group (C) were fed a standard diet. The first experimental group (E1) was fed a diet supplemented with pumpkin oil, and the second experimental group (E2) with addition of flaxseed oil. A total of 12 eggs from each dietary treatment were gained. In content of fat no significant ($P > 0.05$) differences were found. There were found significant differences ($P < 0.05$) between the control group and both experimental groups in cholesterol content (12.53 $mg.g^{-1}$ yolk in C, 15.01 $mg.g^{-1}$ yolk in E1, 14.42 $mg.g^{-1}$ yolk in E2).

Keywords: laying hens; nutrition; additives; plant oils

INTRODUCTION

In recent years global consumers are concerned about the livestock and poultry product due to the addition of additives into the feedstuff (European Commission, 2007). The egg is one of the most complete foods from a nutritional point of view. However, consumers refrain from egg consumption due to the relatively high cholesterol content of eggs and the perception that cholesterol rich foods lead to coronary heart disease (CHD) and atheriosclerosis (Zeidler, 1998).

The hypocholesterolemic effect of essential oils has been reported for chickens (Bozkurt et al., 2012). It was reported that the pure components of essential oils inhibit hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMGCoA) reductase activity, which is a key regulatory enzyme in cholesterol synthesis (Crowell, 1999). Dietary fat type and fatty acid composition of consumed fats, are more important than the consumed amount of dietary cholesterol (Simopoulos, 2000). Lipids contribute to about 65% of the dry matter content of egg yolk (Anton, 2007). Pumpkin seeds contain L-tryptophan, n-6 and n-3 fatty acids and a very high concentration of vitamin E (Hashemi, 2013). Pumpkin seed oil has become a recognized source of phenolic compounds (Andjelkovic et al., 2010). Flaxseed oil contains high levels of polyunsaturated fatty acid and these compounds have been reported to reduce the incidence of heart disease and cancer in humans (Zhao et al., 2007). The objective of this work was to analyse and compare content of fat and cholesterol in egg yolk between dietary treatments of layers.

MATERIAL AND METHODS

Experiment was realized in cooperation with the Department of Poultry Science and Small Animal 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 dietary groups (C-control, E1-pumpkin oil (3%), E2-flaxseed oil (3%). There were housed six hens in one cage. In the control group, hens were fed the standard complete feed for laying hens, while in experimental groups, there were fed mixtures supplemented with pumpkin or flaxseed oils. Oils were added into mixtures before the start of feeding. These oils were obtained from business network in Slovakia. Analyzed oils have been declared by the producer as pure and virgin oils derived from the the cold-pressing technology. Feeding mixture was composed of 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 were: min. 160 $g.kg^{-1}$ of crude protein, min. 20 $g.kg^{-1}$ of crude fiber, max. 90 $g.kg^{-1}$ of crude fat, min. 110 $g.kg^{-1}$ of ash, min. 6.5 $g.kg^{-1}$ of lysine, min. 3.3 $g.kg^{-1}$ of methionine, min. 27 $g.kg^{-1}$ of Ca. Nutrient composition of feed after addition of oils is presented in Table 1. Laying hens in all groups received drinking water and feed *ad libitum*. During experiment, the light regime was 16 hours. The experiment lasted 52 days. During the whole period, the eggs were collected for analysis of laying intensity and egg′s weight. The last

week, the eggs were collected for chemical analysis. Twelve eggs from each dietary treatment were randomly selected and analyzed. Nutrients composition of the diets and eggs were determine by standard laboratory methods and procedures (AOAC, 2000). The fat content was determined by extraction and gravimetric method according to the Soxhlet principle. The cholesterol content was determined on the basis of the Liebermann-Buchard reaction. Laboratory analysis of essential oils was carried out in the Laboratory of Quality and Nutritional Value of Feed at the Department of Animal Nutrition in Slovak University of Agriculture in Nitra, Slovakia. 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. Values with different superscripts within a column are significant at $P < 0.05$.

Table 1. Nutrient composition of feed mixtures for experimental groups of layers

Nutrients	Unit	E1	E2
DM	%	90.88	91.4
Crude protein	%	17.81	17.45
Fat	%	7.61	7.59
Fiber	%	3.71	3.78
Ash	%	11.49	11.26
NFE	%	50.26	51.32
Organic matter	%	79.39	80.14
Starch	%	35.62	35.45
Sugar	%	3.84	3.94

NFE: nitrogen free extract, DM: dry matter

RESULTS AND DISCUSSION

Results of fat content and cholesterol in egg yolk are shown in Table 2. There were found significant differences ($P < 0.05$) between the control group and both experimental groups in cholesterol content. Tendency ($P > 0.05$) of higher amount of cholesterol was found after pumpkin oil supplementation. In content of fat, there were not found significant differences. In a study by Ansari et al. (2006), linseed supplementation (0, 5, 10 and 15% of the diet) did not affect the total fat content of the yolks at any level. Ansari et al. (2006) also found that yolk cholesterol decreased linearly with the increase in the level of linseed in the diet

and the highest yolk cholesterol content was seen in the control group. A significantly lower content of cholesterol was at the dose of 3.3, 6.6, and 10% pumkin meal adddition into feed mixture for poultry, compared to the control group (Martinez et al., 2012). Ebeid et al. (2008) established that different levels of dietary n-3 PUFA had no effect on the cholesterol contents of egg yolks in the laying hens when compared with the control. In contrast, feeding of linseed and linseed oil led to an increase in egg cholesterol contents up to 291mg per egg, while the egg cholesterol content was 283 mg per egg in the control group (Murata et al., 2003). Cholesterol and triglycerides concentrations of yolk were not affected by dietary treatments (Kaya et al., 2013). After addition of flaxseed (4.32%), content of cholesterol was 13.93 $mg.g^{-1}$ in egg yolk and at concentration of 8.64% it was 12.79 $mg.g^{-1}$ in egg yolk (Yalcyn et al., 2007). With increasing doses of linoleic acid in feed for laying hens lower cholesterol content was found in egg yolks (Szymczyk et al., 2003).

Table 2. The comparison of fat and cholesterol content in egg yolk amoung groups of layers

Group	Statistical parameter	Fat ($g.kg^{-1}$ DM)	Cholesterol ($mg.g^{-1}$ of egg yolk)
C	Mean	595.35	12.53^{a}
	Std. deviation	7.65	1.01
	Minimum	586.7	11.71
	Maximum	605.1	13.82
E1	Mean	598.25	15.01^{b}
	Std. deviation	5.22	1.5
	Minimum	594.1	13.95
	Maximum	605.1	16.94
E2	Mean	600.63	14.42^{b}
	Std. deviation	5.92	1.09
	Minimum	595.6	13.62
	Maximum	608.2	15.83

CONCLUSION

Pumpkin and flaxseed oil supplementations in feed mixtures of laying hens lead to an increase of fat and cholesterol content in egg yolk. Significant ($P < 0.05$) differences were found only in the content of cholesterol. The highest cholesterol content was found after pumkin oil supplementation.

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THE INFLUENCE OF UTILIZATION OF CHEMICAL PRESERVATIVE ON THE QUALITY OF THE SILAGE MADE FROM DIFFERENT SPECIES OF CLOVER

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ABSTRACT

The aim of this study was the evaluation of the quality of silage made from different species of clovers. The experimental silages were prepared from the biomass with low dry matter content. In this experiment *Trifolium alexandrinum* L., variety Faraon and *Trifolium pratense* L., variety Amos were used. Experimental stands were established in the locations Troubsko and Vatín. The experimental silages were treated with a mixture of organic acids (formic acid and propionic acid). There was evaluated a quality of silage liquors (pH, lactic acid, propionic acid, acetic acid, butyric acid, ethanol and ammonia). The results showed the difference in quality of silage among the species ($P < 0.05$). There was observed the influence ($P < 0.05$) of a treatment on the content of ethanol, acetic acid, propionic acid and ammonia. The preservative that was used in this experiment had a positive impact on the final quality of the silage.

Keywords: red clover; berseem clover; silage; low dry matter; chemical preparation; aqueous extract

INTRODUCTION

To make high quality silage it is necessary to use forage of high quality. The better quality of roughage can reduce the cost of feeding day. By using of silage additives we can improve quality and there is also a favorable effect on the palatability of the resulting silage. In contrast, silages with lower quality may have a negative impact not only on palatability, but mainly on the health and productivity of animals (Doležal a kol., 2012; Peymanfar et al., 2012).

The principle is that the higher moisture in the silage means more intensive and spontaneously fermentation. It also causes the production of higher amounts of fermentation products. Fermentation products are a result of microbial activity (Rajčáková et al., 2005).
The fermentation process is evaluated by the degree of proteolysis, which is of great importance in terms of health, performance and reproduction. In our agricultural practice over the last 7 years has become customary to begin harvesting alfalfa and clover in the early formation of flower buds. The result is the silage which has a high content of nitrogen compounds. In this phenological growth the vegetation has a low viscosity and wilting is very slow. In these silages with low viscosity can occur the buffering of silage leading to increase of pH above 4.6. This causes a high content of nitrogen compounds and potassium and at low osmotic pressure rapid proteolysis process is started and supported by the activaties of Clostridia (Pozdíšek et al., 2008).
Red clover is one of the basic fodder rich in proteins and vitamins. This perennial herb with a deep taproot is, in contrast to alfalfa, characterized by slower intensity of lignification. Despite the relatively short persistence in the stand (2-4 years) red clover is one of the most important forage. Red clover ensures the production of quality forage and improves soil quality (Pelikán and Hýbl, 2012).

Berseem clover (*Trifolium alexandrinum* L.)
It is an annual species from the Fabaceae family with a deep taproot. The stems are upright to procumbent and hollow. The leaves are quite large and elliptical. The plant has similar habitus to alfalfa (*Medicago sativa* L.). The flower cones are spherical egg-shaped and grow on a long stems. The flowers are white to yellowish-white (Zohary et Heller, 1984).
The aim of this study was the evaluation of the quality of silage made from different species of clovers. The experimental silages were prepared from the biomass with low dry matter content.

MATERIAL AND METHODS

Small-plot experiments were based on two sites. The Research Foraging station Vatín Moravian Uplands (Czech Republic), altitude 560 m above sea level and at the Research Institute for Fodder Troubsko u Brna (Czech Republic), altitude 270 m above sea level. In this experiment two kinds of clover were observed - Alexandria clover

variety FARAON and tetraploid variety of red clover AMOS. Prior to founding experiments soil analyzes by Mehlich III were carried out. According to the contents of P and K fertilization was done. An attempt is based on 4 reps on parcels of 1 x 25 8 m. The seed rate was determinated by purity, germination and thousand seed weight (TSW). The experimental silages were prepared in containers with a diameter of 150 mm. Preparation of experimental micro silages described in their work Vyskočil et al. (2011). The silage additive treatments were without KemiSile 2000 – formic acid 55%, ammonium tetra formate 24%, propionic acid 5%, benzoic acid 1%, esters of benzoic acid 1%, water 14% - added at rate 5 ml·kg^{-1}. Silage samples were taken 60 days after ensiling.
The quality of the extracts (pH, lactic acid, acetic acid content, the content of the butyric acid, ammonia and ethanol) was evaluated. Analytical procedures including preparation of aqueous extract were described in work by Doležal (2002). The results were evaluated in the original dry matter. Results were evaluated by analysis of variance (ANOVA) followed by Tukey's test. The evaluation was carried at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

When we compared the individual variants of treatment (Table 1) a significant difference was revealed. The silages, that were treated with a mixture of organic acids showed a reduction of ammonia content and a significant reduction of acetic acid content. The samples of berseem clover silages contained even propionic acid and butyric acid. Those are indicators of decomposition of silage and proteins.

Table 1. Effect of treatment on the quality of silage liquors [g·kg $^{-1}$]

Varieties	Treatment	Dry matter	Ammonia	pH	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Ethanol
Berseem clover	Control	183^{a}	0.67^{b}	4.78^{a}	19.16^{a}	6.15^{b}	0.4^{b}	5.63^{a}	1.77^{a}
	Kemisile	193^{a}	0.37^{a}	4.43^{a}	15.22^{a}	4.32^{a}	0^{a}	1.55^{a}	1.22^{a}
Red clover	Control	165^{a}	0.39^{b}	4.24^{a}	22.3^{b}	7.08^{b}	0^{a}	0^{a}	2.36^{b}
	Kemisile	170^{a}	0.3^{a}	4.24^{a}	15.05^{a}	3.14^{a}	0^{a}	0^{a}	0.88^{a}

Different letters in the columns indicate significant differences at a level of $P < 0.05$

When we used the chemical preservative on berseem clover, the pH value decreased from 4.78 to 4.43. This decrease was not statistically significant. The ideal pH of silage should be 4 – 4. 2 (Wilkinson, 2005). Doležal et al. (2012) state a pH value of 3.7 – 5. The decrease in pH to 4 – 4.5 is also suitable measures against listeria in silage (Dvořáčková, 2010). The samples of berseem clover reached the limit values after the addition of a chemical preservative. The low mass acidification could cause a higher content of butyric acid and propionic acid in the control variant

Table 2. The differences in the quality of silages liquors according to treatment [g·kg^{-1}]

Varieties	Treatment	Dry matter	Ammonia	pH	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Ethanol
Red clover	**Control**	165^{a}	0.39^{a}	4.24^{a}	22.3^{a}	7.08^{b}	0^{a}	0^{a}	2.36^{c}
Berseem clover	**Control**	183^{a}	0.67^{b}	4.78^{b}	19.16^{a}	6.15^{b}	0.4^{b}	5.63^{b}	1.77^{bc}
Red clover	**Kemisile**	170^{a}	0.3^{a}	4.24^{a}	15.05^{a}	3.14^{a}	0^{a}	0^{a}	0.88^{a}
Berseem clover	**Kemisile**	193^{a}	0.37^{a}	4.43^{ab}	15.22^{a}	4.32^{a}	0^{a}	1.54^{ab}	1.2^{ab}

Different letters in the columns indicate significant differences at a level of $P < 0.05$

The content of lactic acid was decreased after application of the chemical additive (Table 2). This decrease was observed in silage of red clover (from 22.3 g·kg-1 to 15.05 g·kg^{-1}). Lactic acid is an indicator of the quality and stability of the silage so decrease of lactic acid is not so desirable. Kotal (1962) states that the lactic acid content in the silage should be 2/3 of the total amount of acids. This is also indicated by Zeman et al. (2006) in tables for evaluating the quality of silage, which specify the minimum content of lactic acid as 70% of the total acids. The content of lactic acid was lower in the chemically treated silages in both species (15.05 a 15.22 g·kg^{-1}). These results were not significant. The chemical treatment had a positive effect on acetic acid content. Its content was lower compared to the control variant. The optimal acetic acid content of the total content of acids in silages should be 20 to 30% of dry matter (Wilkinson, 2005). Drevjany et al. (2004) indicate the

proportion of acetic acid 4 - 9 g.kg^{-1} in the dry state from 35 to 35%. The acetic acid content is dependent on the number of cuts. On the second and each later mowing, less acetic acid than in the first cuts is formed (Santos et al., 2011). In both variants of silages there were desired values of acids after chemical treatment. The decrease of ethanol content was in silage of red clover (2.36 to 0.88 g.kg^{-1}) and berseem clover (1.77 to 1.2 g.kg-1) after chemical treatment. Wilkinson (2005) reported the ethanol content <10 g.kg^{-1} of dry matter. Mitrík (2013) presents the content of ethanol 4.06 g.kg^{-1}. The corresponding values of ethanol reached only variants treated by organic acids (Table 2). Higher content of ethanol may be a manifestation of the presence of yeast which fermented residual sugars, or lactic acid. Optimum ethanol content in silages should be according to Doležal et al. (2012) 8-10 g.kg^{-1}. Such content has a positive effect on palatability and cows preferred such silage. The high content of ethanol may negatively affect aerobic stability of silage and the rumen microflora (Rada et al., 2010).

Table 3. The differences in dry matter for each species of clover

Varieties	Station	Treatment	Dry matter	Dry mater silage
Red clover	Vatín	Control	120^{a}	130^{c}
		Kemisile	120^{a}	130^{c}
	Troubsko	Control	220^{b}	200^{a}
		Kemisile	220^{b}	210^{ab}
Berseem clover	Vatín	Control	158^{a}	165^{d}
		Kemisile	155^{a}	175^{d}
	Troubsko	Control	250^{b}	220^{ab}
		Kemisile	260^{b}	230^{b}

Different letters in the columns indicate significant differences at a level of $P < 0.05$

The stand had an effect on content of dry matter. On stand Troubsko was significantly higher dry matter of harvest forage compared to the stand Vatín for both species. The treatment of silage did not have significantly effect on dry matter content. The effect of species and stand were observed. When we produce silage from clovers it is desirable a higher content of dry matter (35 – 45%). Therefore, it is

necessary to let plants wither, this increase an osmotic pressure (Skládanka et al., 2014). The content of dry matter in our samples was up to half lower.

CONCLUSION

The experimental microsilages of red clover and berseem clover reached comparable values of lactic acid and dry matter. This effect was observed for both species. The observed species differed in all other parameters, especially in content of ammonium, acetic acid, butyric acid and ethanol. A silage quality can be influenced by using a chemical preservative. This had the significant impact on lower content of acetic acid, propionic acid and ammonium in silages of berseem clover and on lower content of ammonium, lactic acid and ethanol in silages of red clover. Based on the results it can be recommend that for a production of silage red clover can be used. The red clover may be used for a production of silage despite low dry matter. The resultant silage has adequate representation of fermentation acids and pH compared with berseem clover.

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THE USE OF CAPRYLIC ACID IN BROILER CHICKENS: EFFECT ON *CAMPYLOBACTER JEJUNI*

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ABSTRACT

The effect of caprylic acid on *Campylobacter* in chickens was evaluated using dietary caprylic acid. Individually housed broilers (n = 48) were divided into 4 groups (positive control, negative control, treatment I: 2.5 g/kg and treatment II: 5 g/kg of caprylic acid in the feed) and artificially infected. Dietary caprylic acid significantly decreased *C. jejuni* shedding ($P < 0.05$). However, the effect only lasted for 3 – 7 days after infection. The numbers of *Campylobacter* in the positive control birds reached its maximum on the 37th day of life. On that same day, both treatment I and treatment II groups shed significantly lower numbers of *Campylobacter* (by 0.8 and 1.8 orders of magnitude, respectively). After euthanasia, no differences in *Campylobacter* counts in the crop, gizzard, ileum and cecum were found between the positive control and the treated groups.

Keywords: broiler chickens; *Campylobacter jejuni*; contamination; caprylic acid; inhibition

INTRODUCTION

Campylobacteriosis has been reported as the most common zoonosis in many developed countries, with *Campylobacter jejuni* known as the pathogen responsible for the largest number of infections (Hermans et al., 2012). Chicken and turkey meat represents the main source (EFSA-ECDC 2014).
Caprylic acid (CA) and 1-monocaprin showed effectiveness against *C. jejuni* in *in vitro* (Thormar and Bergsson, 2001; Molatova et al., 2010)

and in *in vivo* conditions (Hilmarsson et al., 2006, Molatova et al., 2011).
The aim of this study was to determine the effect of CA on counts of *Campylobacter* in chickens intentionally infected with *C. jejuni*.

MATERIAL AND METHODS

Bacterium and culture conditions
Clinical isolates of *C. jejuni* (CAMP/VFU 612/21) was kindly provided by Dr. Steinhauserova (VFU Brno, CZ) and maintained in medium (Nutrient Broth No. 2, Oxoid, UK) with supplements. The inoculated culture was incubated at 42 °C for 48 h under microaerophilic conditions. Prior to the experiments, the bacterial culture was made rifampicin-resistant (described in detail in Skrivanova et al., 2015).

The effect of CA in chickens experimentally infected with C. jejuni
One-day-old male Ross 308 chickens (n = 48) (XAVERGEN a.s., Habry, CZ) were randomly divided into 4 groups of 12 animals (positive control, negative control, treated group I (2.5 g/kg CA in feed), treated group II (5 g/kg CA in feed), and housed in four floor pens until 14 days of life. At 14 days, chickens were moved to individual cages. Animals of both control groups were fed with a wheat and corn-based, granulated diet (Biopharm, Jilove u Prahy, CZ). Animals of treated groups received the same diet supplemented with CA (Sigma Aldrich, CZ).
At 21 and 35 days of age, chickens from the positive control and both treatment groups were orally challenged with rifampicin-resistant *C. jejuni* (0.5 ml of 10^6 CFU/ml). At regular time intervals post-inoculation, chickens were tested for the occurrence and counts of *C. jejuni*. At 42 days of age, chickens were euthanized with isofluranum (Torrex Chiesi, CZ), followed by cervical dislocation. The contents of the crop, stomach, ileum and cecum were taken immediately for further bacteriological analyses. The number of viable bacteria was determined on selective agar plates containing 20 µg/mL of rifampicin after 48 h incubation at 42 °C in microaerophilic conditions. Typical colonies were confirmed by Gram staining and microscopy. In some cases, the agglutination of the colony was performed (Dryspot Campylobacter Test Kit, Oxoid, UK).
Differences in bacterial counts among groups were compared using an analysis of variance, followed by Scheffe´s test (SAS Institute, 2001).

RESULTS AND DISCUSSION

The dynamics in *C. jejuni* shedding in all experimental groups and the reduction in counts of *C. jejuni* is shown in Table 1. The *Campylobacter* that was first detected occurred earlier in the excreta of the chickens from the positive control group. After the first experimental infection, the highest numbers of *Campylobacter* in the excreta were 5.59 $\log_{10}$ CFU/g and occurred in a positive control group (3rd day post-infection). The second infection was followed by the achievement of 7.12 $\log_{10}$ CFU/g of faeces in the positive control group. After the slaughtering, statistically significant changes in the number of *C. jejuni* associated with application of CA were observed (Table 2). *Campylobacter* (rifampicin-resistant) was not detected in the negative control.

Table 1. Mean numbers ($\log_{10}$ CFU/g) of *Campylobacter jejuni* in faeces of male chickens

Days after inoculation I	Treatment group			
	Negative control	Treatment I	Treatment II	Positive control
1	<DL*	<DL	<DL	<DL
2	<DL	<DL	<DL	4.16 ± 0.69
3	<DL	3.67 ± 0.82^{a}	3.09 ± 0.74^{a}	5.59 ± 0.80^{b}
4	<DL	3.28 ± 0.78^{a}	3.47 ± 0.75^{a}	4.74 ± 0.38^{b}
7	<DL	3.75 ± 0.88^{ab}	3.44 ± 0.73^{b}	4.56 ± 0.75^{a}
9	<DL	3.96 ± 0.62^{a}	4.14 ± 0.77^{a}	4.30 ± 0.98^{a}
11	<DL	4.08 ± 0.62^{a}	3.95 ± 0.48^{a}	4.12 ± 0.67^{a}
14	<DL	4.06 ± 0.57^{a}	4.19 ± 0.28^{a}	4.23 ± 0.56^{a}
II				
15	<DL	4.58 ± 0.35^{a}	4.25 ± 0.39^{a}	5.78 ± 0.79^{b}
16	<DL	6.32 ± 0.36^{a}	5.32 ± 0.42^{a}	7.12 ± 0.54^{b}
17	<DL	6.92 ± 0.69^{a}	5.55 ± 0.53^{b}	6.94 ± 0.95^{a}
18	<DL	6.71 ± 0.93^{a}	6.28 ± 0.98^{a}	6.76 ± 0.24^{a}

* Detection limit, 2 Log_{10} CFU/g; $^{a, b}$ Values in the same row with the same superscript are not significantly different ($P > 0.05$)

Table 2. Mean numbers (log_{10} CFU/g) of *C. jejuni* in specific sections of the chicken GIT at the day of slaughter

	Treatment group			
	Negative control	Treatment I	Treatment II	Positive control
Crop	<DL*	3.46 ± 0.34[a]	3.23 ± 0.52[a]	3.91 ± 1.16[a]
Gizzard	<DL	3.55 ± 0.33[a]	4.08 ± 0.44[a]	4.67 ± 1.42[a]
Ileum	<DL	3.92 ± 0.28[a]	4.13 ± 1.20[a]	4.33 ± 0.38[a]
Caecum	<DL	6.81 ± 0.64[a]	7.24 ± 0.53[a]	7.58 ± 0.68[a]

* Detection limit, 2 Log_{10} CFU/g; [a] Values in the same row with the same superscript are not significantly different ($P > 0.05$)

In a previous study, Solis de los Santos (2008) demonstrated a statistically significant reduction of *Campylobacter* in caecum in 15-day-old chickens fed with a feed mixture containing 0.35%, 0.7%, 1.4% and 2.8% of CA. Our results also demonstrate the effect of CA on reducing the number of *Campylobacter* spp. The concept of our study was slightly different in concentrations used and observing the dynamics of excretion of *Campylobacter*. Thompson and Hinton (1997) demonstrate an antibacterial effect of fatty acid supplement with CA in the crop, but not in other parts of the gastrointestinal tract (GIT). In our case, the absence of effect was observed throughout the entire GIT, probably because at the time of slaughter, the number of *Campylobacter* spp. was already too low, in order the effect to be observed.

CONCLUSION

Caprylic acid can be used as a dietary supplement for broiler chickens in order to reduce the *C. jejuni* occurrence.

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EVALUATION OF THE NUTRITIONAL VALUE OF PASTURES IN RELATION TO THEIR BOTANICAL COMPOSITION

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ABSTRACT

The aim of our study was to evaluate chemical composition of pasture vegetation and on the basis of analysis to assess its nutritional value with regard to species diversity. To ensure coverage of the diversity of vegetation the samplings were taken on 2 representative locations from each pasture. Mixed samples were analysed by Weenden's chemical analysis. On the basis of obtained results we can conclude that pastures contained unbalanced ratio of nutrients at the time of samplings. As an immediate solution of these nutritive deficiencies it seems to be necessary to feed also concentrates and mineral licks for beef cattle. Whereas, as a long-term solution it seems to be necessary to change the botanical composition of these pastures, mainly to improve their percentage representation of specific species of grasses and clovers.

Keywords: cattle, pasture, nutrient composition, species composition

INTRODUCTION

Grazing is one of the most natural way of herbivores' nutrition and has a positive effect on the animals' health. On well-managed pastures animals have enough pasture vegetation, which is rich in easily digestible nutrients, especially proteins, minerals and vitamins (Dušek

et al., 2007). Chemical composition of pasture vegetation (PV) is crucial (Tufarelli et al., 2010). Pond et al. (2005) state that the basic nutritional components of bulk feed include crude proteins, lipids, carbohydrates, and ash. Crude proteins are esencial in animal nutrition (Swaab et al., 2003). This group of nutrients belongs to building nutrients, although some of them may be utilized as an energy source in the body. Lipids are a group of energetic nutrients, while the most important compounds are fats (Zeman et al., 2006). Further compound of feed are carbohydrates, which are the major source of energy for rumen microbiota (Ishler and Varga, 2007). Furthermore, these nutrients ensure the normal function of the rumen, stimulates salivation and mastication and contribute to the buffering capacity of the rumen (Mika et al., 1997). Besides the nutrient composition of the pasture, there is also important to maintain plant biodiversity (Tallowin et al., 2005). Paine and Cates (2013) state that a good pasture should contain a high-diversity of perennial plants (grasses, clovers) and species that contribute to prolongation of grazing.
The aim of our study was to evaluate chemical composition of PV and on the basis of analysis to assess its nutritional value with regard to species diversity.

MATERIAL AND METHODS

Monitoring was conducted on an organic farm "Moulisových", located in the village Milínov (Plzeň district). A total area of land on farm is 100 hectares, of which is 95 hectares of grassland and 5 hectares of arable land. The farm production is mainly focused on the production of beef of Simmental cattle. The basic herd consists of 50 cows of this breed. The management of cattle rearing is realized by the traditional way: the first group (category) consists of cows and calves, the second group consists of heifers and the third group consists of fattening bulls.
Feed used in feed rations for all cattle categories originates exclusively from the organic farming system, while roughages (hay and silage) are produced on this farm and grain (barley, oat, wheat) is purchased from other organic farms. The PV is basic feed for cows with calves and heifers in the vegetative growth period of pasture.
To ensure coverage of the diversity of vegetation the samplings were taken on 2 representative locations from each pasture. In the study we evaluated 2 specific pastures. Samples of pasture forage were collected from surface of 0.5 x 0.5 m, with length of stubble approximately 3 cm. Thereafter, samples were dried at 60 °C to constant weight and homogenized. From the obtained samples the mixed sample was

prepared for each specific pasture. Mixed samples were analysed by Weenden's chemical analysis. The dry matter (DM), crude protein (CP), crude fat, and crude fiber (CF) were determined in samples. Also there were determined elementary mineral macroelements, such as Ca, P, and Mg.
To evaluate a botanical composition of pastures, the method of reduced projective dominance was used. Two representative surfaces of about 2 x 2 m were selected in each pasture area, within these surfaces present plant species were identified and subsequently their percentage representation in PV was appraised. The evaluation was conducted in mid-July 2014.

RESULTS AND DISCUSSION

Comparison of the nutritional value of two pastures is shown in Table 1. These values were determined by chemical analysis and compared with the values (Table 2) published by Sommer et al. (1994).

Table 1. Average content of evaluated nutrients in pastures in 100% dry matter

Nutrients	**Pasture 1**	**Pasture 2**
Dry matter in the original matter (g/kg)	79.9	77.7
Crude protein (g/kg)	129.5	95.6
Crude fat (g/kg)	31.0	30.6
Crude fiber (g/kg)	240.6	266.2
Ca (g/kg)	7.7	4.3
P (g/kg)	4.3	3.8
Mg (g/kg)	2.4	2.8

A fundamental problem for meeting preconditions of cattle nutrition is based on high variability of the quality and content of nutrients, not only among individual species or varieties of fodder crops, but also within a single fodder crop. Besides the required nutrient concentrations in DM of fodder crops, there is, for efficient use of nutrients in feed rations received by animals, a very significant relationship between energy and CP levels.
The rate of rumen microbial synthesis is closely related to the ratio between content of carbohydrates and CP in feed.

Table 2. Average content of selected nutrients according to Sommer et al. (1994)

Nutrients	**Pasture – high quality**	**Pasture – good quality**	**Pasture – poor quality**
Dry matter (g)	1000.0	1000.0	1000.0
Crude protein (g)	214.6	151.5	140.7
Crude fat (g)	28.7	25.3	26.2
Crude fiber (g)	208.6	207.8	246.4
Ca (g)	8.4	7.6	6.9
P (g)	3.5	3.8	2.0
Mg (g)	2.1	2.3	1.8

A state of equilibrium in rumen synthesis and degradation is achieved with the content of 130 g CP and 5.9 MJ NEF per 1 kg of DM of the feed ration (Pozdíšek et al. 2008). According to Sommer et al. (1994) a high quality pasture should provide at least 215 g CP per 1 kg of DM, however, the contents of CP of both pastures did not achieve this amount. The recorded lower values of CP could have been affected by many factors, such as climate conditions, plant age, or also by plant species diversity and their mutual ratios.

Fiala (2007) states that a good quality pasture composition consists of 50-60% desirable grasses, 20-30% clovers, and 10-30% herbs. Neither of our pasture compositions achieved these ratios (Table 3), and plant species of a lower nutritional value were also present.

Table 3. Plant species composition of pastures (%)

Plant species composition of pastures		Pasture 1	Total content (%)	Pasture 2	Total content (%)
Grasses - higher nutritional value	*Dactylis glomerata*	11	**23**	9	**90**
	Lolium perenne	4		12	
	Festuca pratensis	-		19	
	MRH – Hybrids of grasses	8		50	
Grasses - lower nutritional value	*Festuca rubra*	10	**11**	-	**0**
	Galium mollugo	1		-	
Clovers	*Trifolium repens*	52	**52**	1	**1**
Herbs	*Taraxacum officinale*	6	**12**	6	**6**
	Achillea millefolium	3		-	
	Plantago lanceolata	2		-	
	Alchemilla vulgaris	1		-	
Weeds	*Convolvulus arvensis*	-	**2**	3	**3**
	Rumex obtusifolius	1		-	
	Cirsium arvense	1		-	

However, *Festuca rubra*, as a bentgrass type of grass, is in a certain content desirable due to its higher endurance in grassland in comparison to *Festuca pratensis* or *Lolium perenne*. Higher CP content of the pasture 1 can be achieved e.g by seeding of *Lolium multiflorum* (Skládanka et al., 2014) or *Festuca pratensis* (Holúbek et al., 2007), which is considerably adaptable to ecological conditions (Pavlů et al., 2001). In case of the pasture 2, there can be recommended seeding of some clover species rich in CP, such as *Lotus corniculatus* (Fulkerson et al., 2007) or *Trifolium repens* (Kalač and Mika, 1997).

With exception of Ca, both pastures in our study contained higher amounts of the CF, fat and minerals as compared to general recommendations of Sommer et al. (1994). Skládanka et al. (2009) state

that the fiber content is the lowest in stage of stem elongation, whereas, in further stages of plants´ development the fiber content increases. The fiber is an energetic nutrient and it is also important for the digestion and proper function of the gastrointestinal tract, however, its large amount in the feed ration has a negative effect on digestibility of other nutrients (Suchý et al., 2011). The fiber content can be effectively reduced e.g. by increasing the number of pasture cycles (Skládanka et al., 2009).

The fat content should not exceed 4-5% of DM in a feed ration, because its higher doses can cause the reduction of DM intake and utilization of fiber and proteins. Higher doses of fats can be recommended only in animals exposed to heat stress, such as energy compensation for a lower DM intake (Suchý et al., 2011), however, it can be generally mentioned that the amount of lipids is rather lower in grassland.

The mineral content is considerably different between species and also between their varieties (Míka et al., 1997). While higher contents of Ca and Mg tend to have clovers. *Dactylis glomerata* tends to have the highest mineral content of grass species. Deficiency and also an excess amount of minerals, or their unbalanced ratio predispose animals to a lot of diseases. A proper balance of minerals for grazing animals is accomplished mainly by using of specific mineral licks under pasture management.

CONCLUSION

On the basis of obtained results we can conclude that pastures contained unbalanced ratio of nutrients at the time of samplings. As an immediate solution of these nutritive deficiencies it seems to be necessary to feed also concentrates and mineral licks for beef cattle. Whereas, as a long-term solution it seems to be necessary to change the botanical composition of these pastures, mainly to improve their percentage representation of specific species of grasses and clovers.

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IN VITRO SCREENING OF SELECTED ACTIVE COMPOUNDS OF ESSENTIAL OILS FOR MANIPULATION OF RUMEN FERMENTATION

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ABSTRACT

The experiment was conducted to determine the effects of eleven essential oil active compounds (1000 µl/l) on *in vitro* rumen fermentation and methane production. Treatments were evaluated using an *in vitro* 24-h batch culture of rumen fluid with a 70:30 forage: concentrate diet (16% crude protein; 37% neutral detergent fiber). The study showed that carvacrol, citral, bornyl acetate can significantly ($P < 0.05$) decrease methane production. But carvacrol and citral may be nutritionally detrimental to ruminants because carvacrol decreases ($P < 0.05$) total volatile fatty acids (VFA) and citral decreases ($P < 0.05$) proportion of propionate. The study identified plant compound (bornyl acetate) that can assist in developing novel feed additives for methane mitigation from the rumen.

Keywords: essential oils active compounds; volatile fatty acids production; methane production

INTRODUCTION

Major goal in ruminant nutrition is improving the efficiency of energy and protein utilization (Klevenhusen et al., 2012). Antibiotic ionophores have been very successful in reducing energy and protein losses in the rumen (Van Nevel and Demeyer, 1988). However, the use of antibiotics has been banned in the European Union since January 2006 (Directive 1831/2003/CEE, European Commission, 2003). The use of plant secondary metabolites as rumen modifiers seems to be a better approach since these are natural products that might be

environment friendly and have better acceptance with the consumers (Agarwal et al., 2009). Previous studies showed that some essential oils and its compounds have potential to enhance rumen fermentation (Castillejos et al., 2007; Benchaar et al., 2007; Durmic et al., 2014). However, the effects reported in the literature are variable and contradictory. Essential oils are complex mixtures of several individual compounds (Dung et al., 2008), which makes difficult to elucidate the precise mechanism of action on rumen microbial fermentation and its use as a feed additive (Busquet et al., 2005).
The objective of presented study was to determine the effects of eleven active compounds of essential oils (ACEO) on rumen fermentation and methane production.

MATERIAL AND METHODS

The active compounds of essential oils tested were eugenol, carvacrol, citral, limonene, 1,4-cineole, γ-terpinene, *p*-cymene, linalool, bornyl acetate, α-pinene, and β-pinene. Active compounds were provided by Sigma-Aldrich Chemical (Sigma-Aldrich, St. Louis, MO). The concentration of active compounds used (1000 µl/l) in this study was selected based on its effects on rumen microbial fermentation in batch culture incubations conducted in our laboratory (unpublished data). The compounds were added directly to the incubation bottles, no solvent was used. 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. 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 g $CaCl_2$; 0.06 g $MgSO_4$) 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 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. 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. Sixty-four 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. The chemical composition of substrates was determined according to standard AOAC (1995; 2005) methods. All data were analysed using SAS software and the treatment response in CH_4, total VFA, individual VFA production and pH were examined in separate models with treatment as a factor. Each factor had nine observations and data were analysed performing one-way analysis of variance and treatment as a fixed effect. Significant differences were declared at $P < 0.05$.

RESULTS AND DISCUSSION

The chemical composition of experimental substrate (in kg of dry matter: crude protein 161.5 g; ether extract 43.2 g; starch 232.9 g; neutral detergent fibre 365.6; acid detergent fibre 241.8 g; ash 85.3 g) was similar to that found in other studies (Castillejos et al., 2007; Benchaar et al., 2007).

Results of the effects of ACEO on *in vitro* rumen fermentation characteristics and methane production are presented in Table 1 and Table 2. Among the ACEO investigated only carvacrol addition resulted in an increase ($P < 0.05$) in pH compared with control. Carvacrol, citral, and bornyl acetate decreased ($P < 0.05$) methane production by −86%, −44%, and −38%, respectively (compared with control). Total VFA concentration was decreased ($P < 0.05$) only by carvacrol.

Eugenol is phenylpropanoid, and is one of the main compounds of clove (85 %) and cinnamon oils (Davidson and Naidu, 2000). Castillejos et al., (2006) reported that eugenol (500 mg/l) modified proportions of VFA (decrease propionate) without affecting total VFA. These results are consistent with our findings. **Carvacrol**, a monoterpenic phenol, is one of the major components of oregano and thyme oils. Benchaar et al. (2007) observed that when used at the concentration of 400 mg/l, carvacrol did not change total production of VFA but changed molar proportion of VFA. In contrast, our findings were opposite, carvacrol (1000 µl/l) strongly decreased total VFA and CH_4 production but did not change molar proportion of VFA. It suggests that antimicrobial activity of carvacrol may be too strong and nonspecific to improve rumen fermentation.

Table 1. Effect of active components from essential oils on pH, CH_4, total VFA concentrations in *in vitro* rumen fermentation

Treatment	Amount per bottle (µl/l)	pH	Fermentation product		
			CH_4 (mmol/l)	**VFA[1]** (mmol/l)	**CH_4** (mmol/mol VFA)
Control	0	6.45	17.34	102.07	175.10
Eugenol	150	6.55	13.10	78.10	171.70
Carvacrol	150	*6.78	*2.35	*51.36	*47.01
Citral	150	6.57	*9.67	77.34	125.99
Limonene	150	6.53	13.38	90.00	151.92
1,4-Cineole	150	6.50	13.61	94.31	149.58
γ-Terpinen	150	6.52	14.22	89.27	162.80
p-Cymene	150	6.54	14.00	86.12	167.93
Linalool	150	6.56	13.20	86.01	157.04
Bornyl acetate	150	6.53	*10.79	91.09	122.42
α-Pinen	150	6.51	12.27	89.45	141.36
β-Pinen	150	6.55	12.41	86.46	148.74
SEM[2]		0.02	4.49	1.79	5.13

[1]VFA = volatile fatty acids; [2]SEM = standard error of the means; *Means within a column differ from control ($P < 0.05$)

Citral is a lemon scented acyclic monoterpene aldehyde (Jäger, 2010). Treatments 5 mg/l, 200 mg/l, and 500 mg/l of mixture four ACEO (eugenol, carvacrol, citral and cinnamaldehyde) with higher in two aldehyde-based essential oils (including citral) showed higher inhibitory effect on total VFA than phenolic-based mixture, all combination also decreased CH_4 production (Lin et al., 2013). **Limonene** is monocyclic monoterpene (Turner, 1999). 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. In our study, limonene had no effect on rumen fermentation. In accordance with Castillejos et al. (2006), we can conclude that there appears to be no benefit of using limonene as an additive to modify rumen fermentation.

Table 2. Effect of active components from essential oils on individual VFA concentrations and molar proportion of acetate and propionate

Látka	Amount per bottle (µl/l)	Fermentation product			
		Acetate (mol/100 mol)	**Propionate** (mol/100 mol)	**Butyrate** (mol/100 mol)	**A:P**[1]
Control	0	52.76	27.14	11.39	2.13
Eugenol	150	53.66	*18.40	*17.68	*3.04
Carvacrol	150	53.88	24.62	11.14	2.20
Citral	150	55.22	*16.41	*17.43	*3.54
Limonene	150	52.88	24.97	12.50	2.14
1,4-Cineole	150	52.43	27.31	11.40	1.94
γ-Terpinene	150	52.82	24.39	13.04	2.18
p-Cymene	150	54.09	*21.40	*14.49	2.56
Linalool	150	51.18	23.08	*15.17	2.27
Bornyl acetate	150	51.51	26.60	11.83	1.95
α-Pinen	150	51.84	27.65	11.69	1.89
β-Pinen	150	51.81	26.41	12.50	1.98
SEM[2]		0.30	0.41	0.24	0.06

[1]A:P = acetate/propionate; [2]SEM = standard error of the means; *Means within a column differ from control ($P < 0.05$)

1,4-Cineole, a monoterpene cyclic ether, is known to be a major flavour constituent of lime (*Citrus aurantiifolia*) and *Eucalyptus polybractea.* According to Patra and Yu (2012) Eucalyptus oil (1 g/l) tended ($P < 0.1$) to increase total VFA concentrations, and significantly decreased ($P < 0.01$) molar proportion of acetate and propionate and increased molar proportion of butyrate. Eucalyptus oil also decreased CH_4 production. In our study 1,4-Cineole did not affect VFA and CH_4 production. Differences may be due to synergistic effects of cineole and other ACEO in Eucalyptus oil. **γ-Terpinene** is a monoterpene and a major component of essential oils made from citrus fruits (Suzuki et al., 2004). There are no reports in the literature on the effects of γ-terpinene on rumen fermentation. Our results indicate that γ-terpinene is not a potential rumen fermentation modifier. **p-Cymene** is a monoterpene. Chaves et al. (2008) observed that p-cymene at relatively low dose (20 mg/l) did not affect total VFA production and proportion of VFA, but reduced CH_4 production by 30%. In contrast, higher dose (1000 µl/l) in

our study did not affect CH_4 production, but decreased propionate production and increased butyrate production, implying dependence of the outcome on the dose used and other conditions such as the substrate. **Linalool** is a terpene alcohol. The far highest concentration of linalool is found in the essential oil of *Ocimum basilicum* (up to 75%) (Jäger, 2010). Hristov et al. (2008) showed that *Ocimum basilicum* essential oil (10 mg/l and 100 mg/l) did not affect total VFA and pH in *in vitro* incubation. The higher dose of linalool (1000 µl/l) in our study also did not affect total VFA, pH and CH_4 but increased butyrate production. **Bornyl acetate** is a monoterpenic ester. Rich in bornyl acetate is *Valeriana officinalis* root oil (up to 34 %) (Raal et al., 2007). García-González et al., (2008) in screening assay presented that *Valeriana officinalis* caused only negligible changes in ruminal fermentation *in vitro*. In contrast, bornyl acetate was most promising modifier in present study. Bornyl acetate did not affect total VFA and molar proportion of VFA but CH_4 production was significantly decreased (-38%). **Pinene** is bicyclic monoterpene. There are two structural isoforms found in nature: α- and β-pinene. The α-pinene is found in the essential oil of rosemary (*Rosmarinus officinalis*) (Jäger, 2010). Gunal et al. (2013) reported that rosemary essential oil (125 mg/l, 250 mg/l and 500 mg/l) affected molar proportion of VFA. In our study neither α-pinene nor β-pinene affected rumen fermentation and CH_4 prodcution, suggesting less inhibitory effect of single ACEO than mixture of ACEO in essential oils probably due to synergistic effects in essential oils.

CONCLUSION

In summary, this study showed that some ACEO (carvacrol, citral, bornyl acetate) can significantly decrease methane production. But carvacrol and citral may be nutritionally detrimental to ruminants because carvacrol decreases total VFA and citral decreases proportion of propionate. Most promising ACEO in this study was bornyl acetate that decreased CH_4 without negative effects on VFA production. Whether the reduction in methane observed *in vitro* persist over longer period and is effective *in vivo* requires further research.

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THE USE OF HULL-LESS BARLEY IN THE DIET OF BROILER CHICKEN

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ABSTRACT

The aim of this study was to determine the effect of hull-less barley addition to the feed ration for fattening male broilers of hybrid Ross 308. The effect on feed consumption, weight gain and carcass yield were evaluated. The content of hull-less barley in the experimental diets was 30 or 60%. The control diet contains 30 or 60% of common wheat. Differences between groups in performance parameters were not significant ($P > 0.05$). Our results suggest that the difference between feeding barley and wheat was not significant. The hull-less barley can be considered as a potential feed for broilers, respectively, as a possible replacement for a certain amount of wheat in feed for broilers.

Keywords: hull-less barley; carcass yield; poultry nutrition

INTRODUCTION

In most European countries, wheat and barley are the most commonly used cereal grains in poultry and pig feeds (Inborr et al., 1993). Young poultry is extremely sensitive to the antinutritional effect of non-starch polysaccharides (NSP). These carbohydrates are not degraded by endogenous enzymes of the gastrointestinal tract, but may be fermented microbiologically in caeca and colon to short chain fatty acids (SCFA), which may be absorbed and utilised in the body (Kirchgessner et al., 1999). Cereals containing a high proportion of partly soluble dietary fibre polysaccharide residues, such as barley, depress growth in broiler

chickens (Wang et al., 1992). It has been shown that the soluble fibre fraction of barley consists predominantly of mixed-linked (1→3), (1→4)-β-D-glucan (henceforth referred to as β-glucan) and that this component is responsible for the observed growth depression and poorer nutrient utilisation of barley-fed broilers (White et al., 1981). Since water soluble barley β-glucan forms viscous solutions and, furthermore, cannot be completely hydrolysed in the gastrointestinal tract of the broiler chicken, it has been shown that feeding of barley diets to chickens will increase gastrointestinal viscosity. This increase in gastrointestinal viscosity may help to explain reductions in growth rate, nutrient absorption and plasma cholesterol concentrations observed among barley-fed chickens (Almirall et al., 1995). Supplementation of broiler chicken diets with cell wall degrading enzymes has been shown to improve growth (Graham and Pettersson, 1992), nutrient utilisation (Guenter, 1993) and reduce the incidence of sticky droppings (Elwinger and Saéterby, 1987). In recent years, hull-less barley has attracted attention as a food grain, due to its high soluble dietary β- glucan and arabinoxylan content (Li et al., 2014; Zheng et al., 2012). The aim of this study was to investigate possibility of including new varieties of hull-less barley to feed mixtures of fattened broiler chickens.

MATERIAL AND METHODS

An experiment was performed with cockerels of Ross 308 hybrid (n = 96) which were fattened in cage batteries from day 20 to day 43 of age. Cockerels were divided into 4 groups. Two experimental groups of chickens received feed containing 30% or 60% of hull-less spring barley, line KM 1057-1906 (group JB30 and group JB60, respectively) and two control groups received 30% or 60% of common wheat (groups C30 and C60, respectively). The spring barley line KM 1057-1906 with hull-less grain was obtained from the company Agrotest fyto, s.r.o. The content of beta glucans was 3.9%, dry matter 93.0%, crude ash 2.3%, crude protein 15.2%, crude fat 2.8%, crude fibre 3.5% and brutto energy 16.8 MJ/kg. The crumbly feed mixture was supplied *ad-libitum* and its consumption was recorded. The feed mixtures for every group were isonitrogenous. Composition and nutrient content of the feed mixtures is shown in Tables 1 and 2. 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.
Room temperature and humidity were controlled. Feed mixture was prepared by Recommended nutrient content in poultry diets and

nutritive value of feeds for poultry (Zelenka et al., 2007). At the age of 43 days broilers were weighted and slaughtered. The percentage of eviscerated carcase, breast meat and leg meat (meat from thigh and drumstick without skin and bone) were calculated as a percentage of live weight.

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

Component	**JB60**	**C60**	**JB30**	**C30**
Corn 9% of crude protein	78	56	354.5	348
Corn starch	21.5	12	-	-
Soybean meal < 3.5% of fibre	210	242	256	273
Sunflower oil	50	50	50	40
Limestone 37.5 % of Ca	3	3	3	3
Monocalcium phosphate 24% P	6	6	6	6
Lysine 78%	1.5	1	0.5	-
Premix	30	30	30	30
Hull-less barley	600	-	300	-
Wheat CONTROL	-	600	-	300

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

Table 2. Nutrient content of the diet in individual groups.

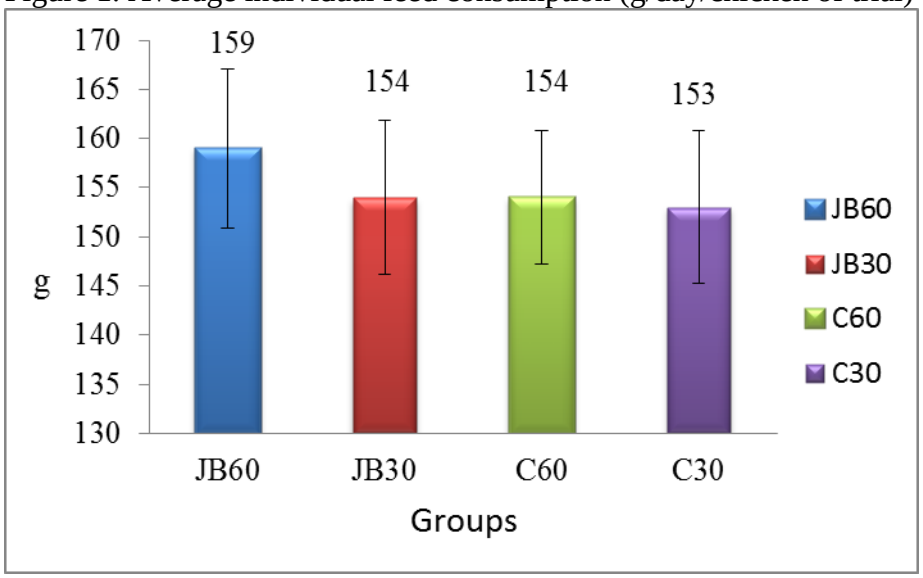
Nutrient	**JB60**	**JB30**	**C60**	**C30**
GE (MJ/kg)	17.16	17.24	16.90	17.07
Dry matter (%)	91.94	91.59	91.91	92.40
Crude protein (%)	20.73	21.22	21.12	21.78
Crude Fat (%)	7.52	7.02	6.08	6.62
Crude Fiber (%)	3.24	3.85	2.94	3.34
Crude Ash (%)	6.07	6.01	5.44	5.43

GE – gross energy

RESULTS AND DISCUSSION

The highest total feed consumption during the experiment (from 20th to 43rd days of age) was found in the experimental group JB60, namely 3.82 kg per chicken. The lowest total feed consumption for this period was achieved in the group C30, namely 3.67 kg. In JB30 and C60 groups total feed consumption was 3.70 kg and 3.69 kg, respectively. The differences were not significant ($P > 0.05$). Tabeidian and Sadeghi (2006) found no significant difference in feed intake between chicks fed with hulled or hull-less barley and the control group in all ages. Figure 1 shows average feed consumption per chicken and day. We observed the highest average feed intake of 159 g per chicken and day in the group JB60. Onderci et al. (2008) reported an average feed consumption of 124 g per chicken from day 21 to 42 of trial with the addition of 63.25% hull barley to feed mixture. Jamroz et al. (2002) found average feed consumption of 141 ± 4 g per chicken from 21 to 42 days of their age when including 40% of hull barley to feed.

Figure 1. Average individual feed consumption (g/day/chicken of trial)



The highest average live weight at the end of fattening was achieved in the experimental group JB30 with value 2,914.41 ± 80.48 grams, while the lowest weight was observed in the experimental group JB60 2,685.33 ± 148.09 grams (Figure 2). The differences were not significant ($P > 0.05$). According to the technological procedure for ROSS 308, the average body weight of cockerels would be 3 129 g at

43 days of age (Anonymous, 2014). Jamroz et al. (2002) found at 42nd day of age live weight of chickens 1.71 ± 0.10 kg when including 40% of hull barley to feed mixture. The values of carcass composition are shown in Table 3.

The highest carcass yield was found in the control group C60 (71.22 ± 0.51%). The lowest value (69.39 ± 0.74%) was found in the experimental group JB60. The differences amoung groups were not statistically significant. Carcass yield stated in the technological procedure for ROSS 308 (Anonymous, 2014) is higher than values found in our work.

Figure 2. Average live weight at the end of the fattening

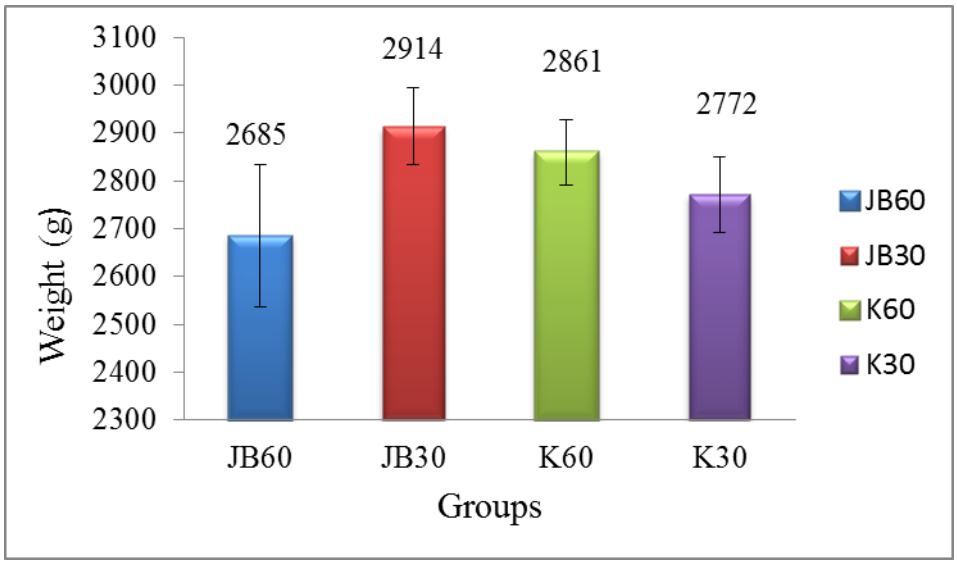


Table 3. Carcass traits

Group	Carcass yield	Breast meat yield	Leg meat yield
	Mean (%) ± standard deviation		
JB60	69.39 ± 0.74	21.01 ± 0.63	14.46 ± 0.15
JB30	70.38 ± 0.76	22.16 ± 0.57	15.42 ± 0.28
C60	71.22 ± 0.51	23.08 ± 0.47	16.21 ± 0.24
C30	70.60 ± 0.22	22.06 ± 0.47	14.45 ± 0.87

Percentages of breast muscle of body weight were highest for the control group C60 (23.08 ± 0.47%), while the lowest value was observed in the experimental group JB60 (21.01 ± 0.63%). In the manual of hybrid Ross 308 (Anonymous, 2014) is stated similar percentage of breast muscle of body weight to our results
Percentages of leg meat of body weight was attempted highest for the control group C60 (16.21 ± 0.24%), while the lowest value was observed in the control group C30 (14.45 ± 0.87%). The manual for the hybrid Ross 308 (Anonymous, 2014) indicates a lower yield of leg meat from 12.91 to 13.13%.

CONCLUSION

Our results suggest that the difference between feeding barley with hull-less grain and wheat was not significant in any parameters. Nevertheless, the experimental groups fed hull-less barley in feed mixtures resulted in convenient growth performance and carcass traits. In our experiment it was found that the addition of higher amounts of hull-less barley in feed mixtures caused the worse carcass yield only by 1 - 2%. The hull-less barley can be considered as a potential feed for broilers, respectively, as a possible replacement for a certain amount of wheat in feed for broilers.

ACKNOWLEDGEMENT

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INTERACTIONS OF CALCIUM LEVEL, HOUSING AND GENOTYPE ON EGG SHELL MEASUREMENTS

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ABSTRACT

This experiment was conducted to evaluate the effect of different feed calcium levels (3.0 % vs. 3.5 %) on eggshell quality in Lohmann White and Czech Hen housed in cages and on litter system. Significant interaction ($P \leq 0.001$) between genotype, housing system and Ca level was detected for egg weight. Lohmann White produced eggs with significantly ($P \leq 0.001$) heavier eggs (61.83 g) than Czech Hen (47.34 g). Eggshell strength was significantly ($P \leq 0.003$) affected by the interaction of all evaluated factors. The highest values (4480 g/cm^2) were revealed to Czech Hen housed in cages with 3.0 % calcium, whereas, the lowest values were detected also in Czech Hen in cages with 3.5 % calcium content (3665 g/cm^2). Significant effect of calcium level on egg weight ($P \leq 0.001$); shell percentage ($P \leq 0.001$); shell thickness ($v \leq 0.001$) and eggshell density ($P \leq 0.001$) were observed in our study. Eggshells from Lohmann White were significantly thicker ($P \leq 0.001$) and stronger ($v \leq 0.002$) in comparison with Czech Hen. Litter housing system significantly ($P \leq 0.003$) affected eggshell thickness, with higher values (0.339 mm) than (0.330 mm) in cages. Higher eggshell density (8.01 g/100 cm^2) was detected on litter in comparison with cages (7.81 g/100 cm^2).

Keywords: eggshell quality; calcium level; housing system; genotype

INTRODUCTION

Optimizing the eggshell quality requires applying efficient genetic improvement and nutrition programs as well as, suitable housing system for laying hens which directly influence the feed intake to meet hens daily needs. Mineralized eggshell is about (96%) calcium carbonate; the remaining components include the organic matrix (2%)

as well as, magnesium, phosphorus and variety of trace elements (Nys et al., 2004). However, for eggshell formation, the hen needs 2.5–3.5 g of calcium from the feed in the calcite form and for this reason it is necessary to provide 3.4–3.8% calcium carbonate in feed composition (Tullet, 1987). Several studies investigated the effect of housing systems on eggshell quality parameters, to indicate the most efficient housing system for better eggshell quality. Singh et al. (2009) detected heavier eggs on litter, whereas, Tůmová et al. (2011) found heavier eggs in cages. Hidalgo et al. (2008); Tůmova et al. (2011); Ledvinka et al. (2012) obtained that, shell thickness was the lowest in eggs produced in cages than on litter housing system. The effect of genotype on eggshell quality was further discussed by (Campo et al., 2007; Tůmová et al., 2011; Ledvinka et al., 2012) who detected better eggshell quality in tinted and darker eggs than the white one. Lichovníková and Zeman (2008) studied the effect of housing system on calcium requirements and reported that, the amount of calcium deposited in the eggshells was higher in the cage system than on litter system. Moreover, the same calcium intake of the hens housed in cages and on litter showed higher eggshell thickness and eggshell strength cage system.

The present study aimed on determining the eggshell quality parameters in Lohmann White and Czech Hen housed in cages and on litter with different calcium intake.

MATERIAL AND METHODS

In the experiment, 132 laying hens of Lohmann White and pure breed Czech Hen were housed in conventional cages Eurovent (72 hens, 550 cm^2 / hen) and on six littered pens (60 hens, 7 hens / m^2, 10 hens / pen). Laying hens in both housing systems were fed identical feed mixtures during laying cycle. In each phase, there were two feed mixtures K and P which differed in Ca content, K – 3.48 % and P – 3.0 %. Composition of feed mixtures N1 (20–40 weeks of age) and N2 (41–60 weeks of age) is given in Table 1.

Table 1. Nutrient content in feed mixtures

Feed mixture	20-40 weeks of age		41-60 weeks of age	
	N1 K	N1 P	N2 K	N2 P
Crude protein (%)	16.66	16.70	15.37	15.52
Metabolizable energy (MJ)	11.4	11.5	11.48	11.54
MET (%)	0.32	0.32	0.27	0.27
LYS (%)	0.8	0.8	0.77	0.78
Ca (%)	3.48	2.95	3.48	3.03
P total (%)	0.56	0.57	0.56	0.56

The daily photoperiod consisted of 15 h light and 9 h darkness, and the environmental conditions were kept in accordance to laying hens requirements.
Eggs for the egg shell quality assessment were collected in four week interval, two days in row, all eggs laid from each cage or litter pen. Egg weight was recorded each day during the experimental period; egg shell proportion was calculated by dividing eggshell weight after drying on egg weight multiplied by 100. Eggshell strength was measured by the shell-breaking method using a QC-SPA device (TSS York, UK). Egg shell thickness was evaluated by QCT shell thickness micrometer (TSS York, UK) as the average of both ends and in the middle.
Egg shell quality data were evaluated by three-way analysis of variance using the GLM procedure (housing, genotype, calcium interactions) of SAS (SAS Institute Inc., Cary, Nc, 2003).

RESULTS AND DISCUSSION

Significant interaction ($P \leq 0.001$) of calcium level, housing system and genotype was detected for egg weight (Table 2). The heaviest eggs (61.83 g) were observed in Lohmann White housed on litter housing system with 3.5 % of calcium, and the lightest were found in Czech Hen housed in cages with 3.0 % of calcium (47.34 g). However, in literature there is lack of data about the interactions of housing system, genotype and calcium level. Egg weight was significantly ($P \leq 0.001$) affected by laying hen genotype; Lohmann White produced heavier eggs (61.83 g) than Czech Hen (47.34 g). Our results are in agreement with Campo et al. (2007), Singh et al. (2009) and Tůmová et al. (2011)

who reported heavier eggs in brown and tinted egg genotype. Significant effect of calcium level ($P \leq 0.001$) was observed for egg weight with heavier eggs at 3.5% of calcium feed content than 3.0%, which corresponds with the results of Chowdhury and Smith (2002); Narvaez-Solarte et al. (2006) who found an increasing linear effect of Ca level on egg weight.

Table 2. Results of eggshell characteristics

Genotype	Housing	Ca	Ew (g)	Esp (%)	Est (%)	Ess (g/cm^2)	Esd (g/100cm^2)
Czech Hen	Cage	3.0	47.34^{e}	11.62	0.318	4480^{a}	7.45
		3.5	51.63^{d}	10.87	0.299	3665^{c}	7.18
	Litter	3.0	50.91^{d}	11.85	0.331	4254^{ab}	7.82
		3.5	48.20^{e}	11.38	0.317	4147^{b}	7.54
Lohmann White	Cage	3.0	60.45^{b}	12.17	0.350	4341^{ab}	8.27
		3.5	60.86^{b}	12.25	0.356	4378^{a}	8.34
	Litter	3.0	58.44^{c}	12.05	0.346	4262^{ab}	8.17
		3.5	61.83^{a}	12.45	0.363	4445^{a}	8.53
	RMSE		60.04	0.890	0.027	896.70	0.559
Genotype			0.001	0.001	0.001	0.002	0.001
Housing			0.601	0.096	0.003	0.694	0.002
Calcium			0.001	0.002	0.001	0.603	0.001
Genotype × housing× Ca			0.001	0.190	0.341	0.033	0.071

$^{a, b, c, d,e}$ statistically significant differences ($P \leq 0.05$) within columns are indicated by different superscripts
Ew= Egg weight; Esp= Eggshell percentage; Est= Eggshell thickness; Ess= Eggshell strength; Esd= Eggshell density

Eggshell strength was significantly ($P \leq 0.03$) affected by the interaction of calcium level, housing system and genotype, with stronger shells (4480 g/cm^2) observed in Czech Hen housed in cages with 3.0 % of calcium content, and the lowest values were detected also in Czech Hen in the same housing system with 3.5 % calcium content (3665 g/cm^2). There are no data of interactions of genotype, housing and calcium to compare it with literature. Eggshell percentage was significantly ($P \leq 0.003$) affected by genotype with the highest value (12.24 %) in eggs from Lohmann White and the lowest in Czech Hen eggs (10.87 %). Eggshell thickness was significantly ($P \leq 0.003$) affected by housing system, litter housing system produced thicker

eggshell (0.339 mm) than cages (0.330 mm). Similarly, Hidalgo et al. (2008) indicated that, shell thickness was the lowest in eggs produced in cages, while free-range and barn eggs presented the highest values. In addition, the effect of genotype on eggshell thickness was also detected. Lohmann White produced eggs with significantly ($P \leq 0.001$) thicker eggshell (0.353 mm) than Czech Hen (0.316 mm) which in agreement with our previous study (Ketta and Tůmová 2014) who found thicker eggshells in Lohmann White than Czech Hen.

CONCLUSION

The results of this study have demonstrated significant interaction of calcium level, housing system and genotype on egg weight and eggshell strength. Egg weight has been significantly increased with growing calcium level from 3.0 % to 3.5 %. Eggshells from Lohmann White genotype were significantly thicker and stronger in comparison with Czech Hen. Better eggshells characteristics were observed on litter housing system than cages.

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APPLICATION OF CORNELL SYSTEM IN RUMINANT NUTRITION AT THE ROUGHAGES

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ABSTRACT

One of the systems evaluation of nitrogenous substances fractions is the Cornell Net Carbohydrate and Protein System (CNCPS), which uses a chemical fractionation. Data set of the roughages (n = 8) of four silages (clover, corn, rye grass and grass), alpine dock and meadow foxtail was used for this study for evaluation of nitrogen fractions in ruminant nutrition. In this case, an important correlation dependence between content of crude protein and B2 and C nitrogen fractions, nitrogen insoluble in acid detergent, and nitrogen insoluble in neutral detergent was determined. This correlation was determined at significance level of $P < 0.05$. Other important statistically significant differences were found for individual nitrogen fractions A, B1, B2, B3 and C.

Keywords: ruminants; nutrients; crude protein; non-protein nitrogen; soluble protein

INTRODUCTION

Performance and the resulting quality of livestock production can be affected by many factors. One of them is the delivery of feed ration, which is important for their life. Essential nutrients in animal nutrition are nitrogen compounds, which in the body of animals constitute 80-90% of organic substances that are particularly important for the growth, repair, and metabolism of animal cells (Jelínek et al., 2003). Feeds contain a variety of proteins and several types of non-protein nitrogen. Proteins are large molecules of various sizes, shapes, functions, solubility and the amino acid composition and can be classified based on their solubility and structure into three levels (A, B,

C) (NRC, 2001). Non-protein nitrogen compounds (NPN) are smaller molecules comprising peptides, free amino acids, nucleic acids, amides, amines, nitrates and ammonia (Swaab et al., 2003).
One of the systems for the evaluation of nitrogen compounds is the Cornell system (CNCPS) (Sniffen et al., 1992), which is based on the chemical fractionation by Licitra et al. (1996). Cornell system estimates the requirements of ruminants and the needs for nutrients based on a number of factors (type of animal, environmental and feed composition information in diverse production situations) (Merchen and Bourquin, 1994; Jung and Allen, 1995). The requirements of animals correspond to different physiological states, such as lactation, pregnancy, growth, body stores and environmental impact. Cornell system uses carbohydrate and protein degradation rate of passage of feed and for estimating the degradation of rumen microbial protein production, post-ruminal absorption and total metabolisable energy supply and animal proteins (Fox et al., 2004; Lanzas et al., 2008).
Fractions of nitrogenous substances are divided into fraction A (NPN = non-protein nitrogen), B1 (rapidly degradable protein), B2 (intermediately degradable protein), B3 (slowly degradable protein) and C (bound, indigestible protein) and are calculated using the soluble protein (SOLP), non-protein nitrogen (NPN in % SOLP), nitrogen insoluble in neutral detergent (NDIP) and nitrogen insoluble in acid detergent (ADIP = fraction C) (Polat et al., 2014). Fractions A and B1 represents nitrogen soluble in buffer and fractions B1 (rapidly degradable in the rumen of the animal) is determined as a fraction precipitated with trichloroacetic acid and soluble in buffer. True, intermediately degradable protein (fraction B2) is a residual nitrogen which is estimated as the difference between the nitrogen which is insoluble in the nitrogen buffer and insoluble in neutral detergent. Rapidly undegradable protein (fraction B3) is detected as the difference between nitrogen insoluble in neutral detergent and nitrogen in acid detergent insoluble and the last fraction C is a protein associated with the lignin, tannin-protein complex and the Maillard reaction and is insoluble in acid detergent (Gupta et al., 2011).

MATERIAL AND METHODS

The experiment samples (n = 8) of roughages were selected. Four samples were originated from farm of Netluky (silages of clover, rye grass, corn and grass) and four forage samples of meadow foxtail (*Alopecurus pratensis*) and alpine dock (*Rumex alpinus*) harvested at

the Krkonoše Mountains National Park, collected in two different cut during the same year.
Samples were analysed for chemical composition. Nitrogen fractions of feeds were determined according to Licitra et al. (1996), namely (1) the determination of non-protein nitrogen (NPN) using trichloroacetic acid, (2) the determination of soluble protein (SOLP), (3) determination of nitrogen insoluble in acid detergent (ADIP) using apparatus Fibertec and (4) determination of nitrogen insoluble in a neutral detergent (NDIP) using apparatus Fibertec. The individual fraction of nitrogen compounds were calculated according to equations of Ghoorchi and Arbabi (2010).

The equation for calculation of nitrogen fractions (% CP) (Ghoorchi and Arbabi, 2010):
Fraction A = NPN (% SOLP) × 0.01 × SOLP (% CP)
Fraction B1 = SOLP (% CP) - fraction A (% CP)
Fraction B2 = 100 - A (% CP) - B1 (% CP) - B3 (% CP) - C (% CP)
Fraction B3 = NDIP (% CP) - ADIP (% CP)
Fraction C = ADIP (% CP)
Where: fraction A = non-protein nitrogen; ADIP = nitrogen insoluble in acid detergent; fraction B1 = rapidly degradable protein; fraction B2 = intermediately degradable protein; fraction B3 = slowly degradable protein; fraction C = bound (indigestible) protein; CP = crude protein; NDIP = nitrogen insoluble in neutral detergent; SOLP = soluble protein.

The results were statistically evaluated in the SAS 9.4 GLM procedure (PROC GLM) and using the PROC CORR procedures correlation coefficients between the observed variables were evaluated. Test statistically significant differences were estimated by Scheffe analysis.

RESULTS AND DISCUSSION

Chemical composition of estimated feeds is in Table 1. Crude protein (CP) ranged from 69.5 to 280.1 $g.kg^{-1}$ of dry matter (DM). Crude protein content of alpine dock and meadow foxtail shows higher values taken during the first miter. The second miter has a lower value of CP, which corresponds to the trend published by Rinne and Nykanen (2000) and Arthington and Brown (2005). Crude fiber (CF) ranged from 130.7 to 355.4 $g.kg^{-1}$ of DM. Crude fiber content of alpine dock and meadow foxtail shows a clear difference between various miters.

Values of CF are higher with the second miter. This trend is confirmed by many authors (Coblentz et al., 1998; Elizalde et al., 1999).

Table 1. The content of individual nutrients (g.kg^{-1} of DM) of estimated samples.

Sample		CP	EE	CF	NFE	OM
1	Silage – clover	179.8	17.5	268.9	439.4	905.6
2	Silage – corn	69.5	28.1	210.3	647.4	955.2
3	Silage – rye grass	192.8	26.4	255.8	332.9	807.8
4	Silage – grass	161.3	24.4	265.9	474.5	926.1
5	Alpine Dock Ist miter 2007	280.1	18.8	130.7	500.3	929.9
6	Alpine Dock IInd miter 2007	120.6	16.7	293.0	515.1	945.4
7	Meadow foxtail Ist miter 2007	205.2	34.1	256.7	427.2	923.3
8	Meadow foxtail IInd miter 2007	120.5	17.0	355.4	458.5	951.4

CF = crude fiber, CP = crude protein, EE = ether extract, NFE = nitrogen-free extract, OM = organic matter.

The values of nitrogen fractions are expressed in % of CP. The resulting values of individual nitrogen fractions express potential degradability in the rumen. In the experiment the highest degradability (fraction A) for silage of rye grass (mean value 59.5 % of CP) was found. In contrast to silage of rye grass the fraction A of alpine dock was 9.0 % of CP. For all estimated samples similar values of fraction B1 (about 3.6 % of CP) were obtained. Fraction B2 varied for alpine dock (from 1.2 to 3.0 % of CP), and meadow foxtail (from 3.9 to 5.0 % of CP), silages had different values (from 17.8 to 45.5 % of CP). Fraction B3 was from 0.7 % of CP (grass silage) to 37.7 % of CP (meadow foxtail of second miter). Non-degradable fraction C was much lower for meadow foxtail (first and second miter), which value were 7.7 % of CP and 6.9 % of CP, respectively. Alpine dock had much higher C values for both miters (32.8 % of CP and 26.9 % of CP, recpetively). Compared with other authors (Shannak et al., 2000) there is especially noticeable difference in grass silage, especially between fraction B2, B3 and C. In this case, fraction A corresponds to the trend that represents the largest proportion and fraction B1 corresponds to the trend that represents the lowest proportion (Shannak et al., 2000).
The high positive correlation dependence ($P < 0.05$) was between CP and ADIP and NDIP, fraction B2 and fraction C. High negative correlation dependence was between CP, CF and NFE. Further high positive correlation dependence was between ADIP and NDIP and fraction C and between NDIP and fraction B3 and fraction C. High

correlation by individual fraction was just between fraction A and fraction B1.
Multiple comparisons (Scheffe test) method was conducted by the test of significant differences ($P < 0.05$). Between estimated feed samples were found significant differences (Table 2) for individual nitrogen fractions ($P < 0.05$).

Table 2. Determination of nitrogen fractions (% CP) of estimated samples.

Sample		A	B1	B2	B3	C
		% CP				
1	Silage – clover	38.5^{b}	$6.6^{a,b}$	18.3^{c}	22.4^{b}	$14.1^{c,d}$
2	Silage – corn	32.7^{e}	$1.7^{b,c}$	45.5^{c}	12.7^{f}	7.3^{d}
3	Silage – rye grass	59.5^{a}	6.9^{a}	21.5^{c}	2.0^{g}	$10.0^{c,d}$
4	Silage – grass	42.2^{b}	0.1^{c}	17.8^{c}	0.7^{g}	39.2^{b}
5	Alpine Dock Ist miter 2007	18.6^{c}	$1.2^{a,b,c}$	36.7^{a}	10.6^{d}	32.8^{a}
6	Alpine Dock IInd miter 2007	9.0^{f}	$3.0^{a,b,c}$	49.2^{b}	11.8^{e}	26.9^{c}
7	Meadow foxtail Ist miter 2007	16.6^{d}	$5.0^{a,b,c}$	53.8^{a}	16.9^{c}	$7.7^{c,d}$
8	Meadow foxtail IInd miter 2007	18.2^{e}	$3.9^{a,b,c}$	33.2^{c}	37.7^{a}	6.9^{d}

A = non-protein nitrogen, B1 = rapidly degradable protein, B2 = intermediately degradable protein, B3 = slowly degradable protein, C = bound (indigestible) protein, CP = crude protein.
^{a,b,c,d,e,f,g} different letters indicate significant difference between the feeds ($P < 0.05$; Scheffe test).

CONCLUSION

Correct nutrition and feeding can largely affect the production potential of ruminants, which is closely connected with good functioning of rumen microflora. To ensure correct protein needs it is important to accurately predict the input and utilization of the individual protein fractions into the digestive tract of ruminants.

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NUTRITIONAL VALUE OF SEA BUCKTHORN PELLETS AND POSSIBILITY OF THEIR USE IN ANIMAL NUTRITION

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ABSTRACT

The aim of the study was to find out the effect of sea buckthorn pellets, which were added to the feed for laying hens. It was observed, how the supplement of sea buckthorn affects colour intensity of egg yolk. One kg of sea buckthorn pellets contained: 208.4 g of crude protein, 169.2 g of crude fat, 171.3 g of crude fiber, 430.1 g of nitrogen-free extract substances, 21.1 g of ash, and gross energy was 23.9 MJ/kg.
Laying hens (Isa Brown) were placed in 4 groups, 1 control group (without addition of sea buckthorn pellets) and 3 experimental groups (feed with addition of 2%, 5% and 10% supplement of sea buckthorn pellets). Length of experiment was 4 weeks. Every week 40 eggs of each group were taken. The results show that the supplement of sea buckthorn pellets in feed for laying hens significantly increased ($P < 0.05$) colour intensity of egg yolk in the experimental groups. Intensity of egg yolk colour was measured by using a scale La Roche. We have chosen as an optimal 2% addition of sea buckthorn pellets in feed for laying hens.

Keywords: *Hippophae rhamnoides*; fruit pellets; chemical composition; feed mixture hens

INTRODUCTION

Sea buckthorn (*Hippophae rhamnoides*) belongs to the family *Elaegnaceae* (Christaki 2012). Its name has origin in Ancient Greek. "*Hippo*" means a horse and "*phoas*" can be translated as a glow or light. These two words together signify "glittering horse"(Suryakumar et Gupta 2011). It is dioecious, deciduous shrub or tree with rigid thorns (Kumar and Sagar 2007; Reznicek and Plsek 2008). Its natural

habitat is in areas of Central Asia and northwest Europe (Yang and Kallio 2001). The roots of sea buckthorn are able to fix atmospheric nitrogen. The massive system of roots is used as a protection against soil erosion and for restoration of nature (Kumar and Sagar 2007). Sea buckthorn trees typically reach a height of 3 to 4 m. The green coloured leaves have trichomes on their surface, which give them a silver tint. The leaves are narrow and lanceolate and have alternating arrangement (Li and Beveridge 2003; Suryakumar and Gupta 2011). Numerous fruits grow up to size of 6 to 9 mm, have oval shape and are juicy and rich in oil content. Ripe berries have a colour from dark yellow to reddish (Suryakumar and Gupta 2011). Sea buckthorn very well manages extreme temperatures (from – 43 to + 45 °C), can tolerate drought and thrives salty and acidic (pH 5.5 to 8.3) soils (Kumar and Sagar 2007; Khan et al. 2010). The most common sites of occurrence are on the river banks and on the sunny sides of the steep slopes at an altitude of 2,000 to 3,600 meters above mean sea level (Dhyani et al. 2007). At present, the topic of sea buckthorn is increasingly discussed. All its parts contain many bioactive compounds, but the highest amount is in its fruits. Sea buckthorn is very good source of natural antioxidants (ascorbic acid, tocopherols, carotenoids and flavonoids). It is also an important source of proteins, lipids (mainly unsaturated fatty acid – oleic acid, linoleic acid and linolenic acid), vitamins (especially vitamin C), minerals, carbohydrates, organic acids and phytosterols (Christaki 2012). Great interest has gained thanks to its numerous positive effects, primarily for its beneficial influence on the health of the body. Sea buckthorn is appreciated for its antioxidant, cardioprotective, hepatoprotective, immunomodulatory, anticancer, anti-diabetic, antiviral, antibacterial and anti-inflammatory effects. Sea buckthorn also reduces the incidence of gastric ulcers, promotes wound healing and relieves pain (Suryakumar and Gupta 2011; Christaki 2012). For these reasons sea buckthorn is used in human and animal nutrition.

MATERIAL AND METHODS

This work was based on requirements of sea buckthorn growers, who cultivate sea buckthorn for obtaining juice of its fruits. During obtaining of juice from sea buckthorn berries waste products are formed and we are trying to find a suitable use for them, especially in the field of animal nutrition.
Growers farm is on 4 ha area, where on 1 ha is cultivated around 1,000

plants. Every year is harvested only one half of plantation. During harvesting of 1 ha area 1,500 l of sea buckthorn juice is obtained and around 570 kg of sea buckthorn waste products.
By basic chemical analysis of sea buckthorn a content of crude protein and nitrogen amount were determinated. The content of crude protein was defined by the Kjeldahl method and multiplied by the factor of 6.25. Nitrogen was determined by the analyzer Buchi. Fat was determined by the device ANKOM^{XT10} Fat Analyzer. Crude fiber was detected by the device ANKOM^{220} Fiber Analyzer. The ash content was evaluated gravimetrically after incineration at 550 °C under prescribed conditions. Gross energy was determined by the device AC 500.
The aim of the study was to evaluate on the basis of chemical analysis nutrition value of sea buckthorn pellets and assess their potential use in animal nutrition especially in feed for laying hens and their effect on colour of egg yolk.
The experiment was carried out on laying hens Isa Brown. Laying hens were placed in 4 groups (1 control and 3 experimental groups - feed enriched by 2%, 5% and 10% of sea buckhorn pellets). Each group contained 8 laying hens. Laying hens were kept in enriched cages in accredited stable of Department of Animal Nutrition VFU Brno. Duration of experiment was 4 weeks. Every week 40 eggs from each group were collected. Over the course of the experiment 160 eggs of each group were obtained.

RESULTS AND DISCUSSION

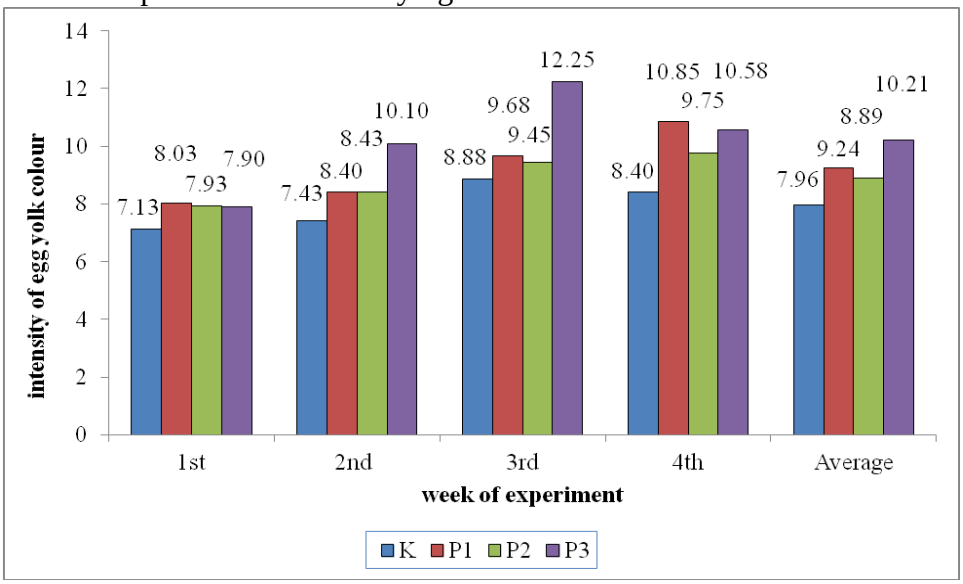
During chemical analysis of sea buckthorn was found that the sea buckthorn pellets contain very valuable nutrients and that these pellets can be used in animal nutrition as enrichment of feed for animals. The obtained results of sea buckthorn chemical analysis (fruits and pellets) are summarized in Table 1. In Table 1 is shown that sea buckthorn pellets are a very good source of crude protein (208.4 g/kg pellets) and also have a very high amount of fat (169.2 g/kg pellets) and crude fiber (171.3 g/kg pellets). Sea buckthorn pellets have also a big amount of minerals. Sea buckthorn pellets are rich, from macroelements, only in potassium (6.3 g/kg pellets), but they are very rich in the whole range of microelements.
This study shows that sea buckthorn pellets added to feed for laying hens have a positive effect on colour intensity of egg yolk. The results of the study are based on 4 monitored groups of laying hens.

Group C was the control group without addition of sea buckthorn pellets. Group P1 was fed feed with 2% addition of sea buckthorn pellets. Group P2 received feed with 5% addition of sea buckthorn pellets and group P3 was fed feed with 10% addition of sea buckthorn pellets. The results presented in Figure 1 show that feed enriched by sea buckthorn pellets significantly increased ($P < 0.05$) the colour intensity of egg yolk in the experimental groups compared to the control group. Over the course of the experiment a trend of increasing of egg yolk colour in the experimental groups was detected. We have chosen as an optimum 2% addition of sea buckthorn pellets in feed for laying hens.

Table 1. Chemical analysis of sea buckthorn fruits and pellets, results are presented in 100% dry matter.

Nutrient content		**Units**	**Sea buckthorn fruits**	**Sea buckthorn pellets**
Crude protein		g/kg	140.5	208.4
Crude fat		g/kg	284.5	169.2
Crude fiber		g/kg	76.9	171.3
Acidodetergent fiber		g/kg	139.9	269.0
Neutral-detergent fiber		g/kg	183.4	372.7
Acidodetergent lignin		g/kg	84.7	131.8
Nitrogen-free extract substances		g/kg	456.8	430.1
Organic matter		g/kg	958.8	979.0
Ash		g/kg	41.3	21.1
Gross energy		g/kg	24.7	23.9
Calcium		g/kg	1.2	1.2
Phosphorus		g/kg	1.9	2.7
Magnesium		MJ/kg	0.4	0.6
Sodium		g/kg	0.2	0.1
Potassium		g/kg	9.8	6.3
Elemental analysis	**Nitrogen**	g/kg	22.5	33.4
	Carbon	g/kg	604.3	592.8
	Hydrogen	g/kg	78.1	73.1
	Sulphur	g/kg	1.2	1.8

Figure 1. The intensity of egg yolk colour after addition of sea buckthorn pellets to feed for laying hens.



K: control group, P1: experimental group with 2% supplement of pellets, P2: experimental group with 5% supplement of pellets, P3: experimental group with 10% supplement of pellets

CONCLUSION

The results of this study show that sea buckthorn pellets are suitable component of feed for laying hens. Pellets of sea buckthorn are appropriate for the enrichment of feed primarily due to their properties: high content of crude protein, high content of fat, as a good source of minerals and for its considerable effect on the egg yolk colour.

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ASSESSMENT OF DIGESTIBILITY OF MEADOW HAY FOR HORSES

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ABSTRACT

The aim of this study was to compare the nutrient content in meadow hay, fed to horses, and then to compare dry matter (DM) and neutral detergent fiber (NDF) digestibility, obtained from *in vivo* experiment. The next task was to determine whether differed DM and NDF digestibility between the given days. Tested feed was produced in the National Stud Kladruby nad Labem, there was purchased and transported to the location of the experiment. Eight adult mares were used in experiments to determine *in vivo* digestibility of meadow hay. Horses were individually housed and fed. The diet, refusals and faecal samples were collected for chemical analyses and calculation of *in vivo* digestibility. In the test feed (meadow hay) a low digestibility of DM (40.12 %) and NDF (34.40 %) was found. These results indicate that the meadow hay was harvested at a later stage of maturity. There was no significant difference ($P > 0.05$) in digestibility of DM and NDF among days of the experiment.

Keywords: horses, meadow hay, feed nutrients, digestibility

INTRODUCTION

Good quality of forage is the basis for feeding of horses (Hintz, 2005; Bergero and Peiretti, 2011). Equine nutrition, and the importance of implementing correct diets for horses, is becoming increasingly significant to ensure good health and welfare (Pagan, 2009; Bergero and Peiretti, 2011; Murray et al., 2015). There are a number of equine ailments that are commonly seen that could be prevented if dietary

rations were better understood by those who administer them. However, despite the growing recognition and evidence of the impact of poor nutrition on equine health, widespread inappropriate feeding management still exists (Hoffman et al., 2009; Murray et al., 2015). There is evidence to suggest that many horse owners have a poor understanding of equine nutrition and decisions regarding nutritional management are often based on tradition, folklore and misinformation (Murray et al., 2015).

Knowledge of diet digestibility is a basic requirement for the development of well-balanced diets and for the evaluation of diet quality (Lowman et al., 1999; Särkijärvi et al., 2012). Highly digestible feed provides more nutrients compared to a feed with lower digestibility. High quality forages are recommended for horse diets because of the high rate of passage and low digestibility of fibre compared to ruminants (Särkijärvi et al., 2012).

The horse is a non-ruminant herbivore with a significant microbial fermentation in the colon. This allows it to utilize fibrous feed. The flow of feed with an optimum fiber is essential for the proper function of the digestive tract of horses (Hintz, 2005; Pagan, 2009). Hay is the most common type of forage when pasture is unavailable. Hay production depends to a great extent on weather conditions. Dry storage during harvesting is crucial to achieve and maintain quality and to avoid mold growth. The quality problems concerning a dry hay are usually connected to dust. In general, a moldy 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 (Bergero and Peiretti, 2011). 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 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 fiber and hence the low digestibility and utilization of energy and nutrients (Müller, 2005).

MATERIAL AND METHODS

Tested feed

In experiment the nutrient content and the digestibility of DM and NDF in ration were monitored. In the experiment the feeding of field-dried meadow hay was examined. Test feed was produced in the National Stud Kladruby nad Labem, there was purchased and transported to the location of the experiment.

In vivo experiment

The experiment was performed between the months of October and November (2014) in accredited barn in Netluky owned by Institute of Animal Science, Praha - Uhříněves. Eight adult Czech Warmblooded mares of 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 experiment was divided into two periods, to the preparation and the trial. In preparation period 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 similar feed intake. Before the beginning of each experiment and at the end, the live weights of mares were recorded.

In the experiment the feeding of meadow hay was examined. The ration contained 14 kg of meadow hay and was divided into two dosages per day. The 7 day preparation period preceded the 6 day trial period. The preparation period was limited to a short period of time because 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.

During the experiment, the mares carried out light work (walk, trot) for one hour. All feed, residues and faeces were weighed with the accuracy of 0.01 kg. Feces were collected immediately after excretion and stored, daily determination of the total weight and then 10% of representative samples of individual animals were taken. All the diets, refusals and faecal samples were preserved in polyethylene 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 6 h at 105 °C. Ash was determined after 6 h at 550 °C, and then the organic matter (OM) was calculated. 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. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the methods of Van Soest et al. (1991) using an ANKOM^{220} Fiber Analyzer (ANKOM Technology Corporation, NY, USA). The DM and the CF digestibility were calculated then. The *in vivo* digestibility coefficients of horses were calculated as follows:

$$\% \, D = \frac{\text{Nutrient in feed} - \text{Nutrient in faeces}}{\text{Nutrient in feed}} \times 100$$

Statistical analysis

Statistical analyses were performed by Friedman ANOVA (level of significance set at $P < 0.05$) with interactions in STATISTICA CZ version 12.0 software (StatSoft Inc., Czech Republic).

RESULTS AND DISCUSSION

Content of nutrients in feed is affected by many factors, such as feed species, soil conditions, fertilization, manurity status and management factors (Pagan, 2009). 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).

The aim of the experiment was to study nutrient content in meadow hay, fed to horses, and then compare DM and NDF digestibility, obtained from *in vivo* experiment. The next task was to determine whether differed DM and NDF digestibility between the given days. The chemical composition of the meadow hay is shown in Table 1.

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

	DM	OM	NDF	ADF	CP	EE	A
Meadow hay	87.84	95.21	66.01	38.45	7.72	1.13	4.85

DM – dry matter; OM – organic matter, NDF – neutral detergent fibre, ADF – acid detergent fibre, CP – crude protein; EE – ether extract; A – ash

Digestibility coefficients of meadow hay are shown in Table 2. The digestibility coefficients of hay we measured were not in agreement with other recorded data, in studies where similar forages were given to horses (Drogoul et al., 2001; Bergero and Peiretti, 2011). The hay DM digestibility (40.12%) was lower compared to the study by Bergero and Peiretti (2011), in their study DM digestibility was 57.8 %. The hay NDF digestibility (34.40%) was lower compared to the study by Drogoul et al. (2001), in their study DM digestibility was 46.1 %.

Tab.2. Digestibility of DM (dDM, %) and NDF (dNDF, %)

	Meadow hay
dDM (%)	40.12
dNDF (%)	34.40

We conclude that the DM and NDF digestibility of meadow hay was low. According to the low digestibility of the DM and NDF of hay it is suggested that this type of feed was harvested at a later stage of maturity.
Also was observed difference among days of the experiment. But we did not find a significant difference ($P > 0.05$) in digestibility of DM and NDF among days of the experiment.

Tab. 3. Statistical evaluation of *in vitro* digestibility of DM and NDF among the days of the experiment

Variable	Friedman ANOVA **(dDM)** ANOVA chi-sq. (N = 8, sv = 5) = 2.071429 **p = .83918**			
	Average order	Sum order	Average	Standard deviation
1	3.75	30.00	40.41	9.72
2	3.50	28.00	39.65	5.16
3	3.75	30.00	41.48	7.51
4	4.00	32.00	42.53	9.76
5	2.87	23.00	39.70	9.36
6	3.12	25.00	36.94	7.66
Variable	Friedman ANOVA **(dNDF)** ANOVA chi-sq. (N = 8, sv = 5) = 8.214286 **p = .14482**			
	Average order	Sum order	Average	Standard deviation
1	4.37	35.00	38.71	10.43
2	3.75	30.00	36.60	5.58
3	4.00	32.00	36.24	11.30
4	4.00	32.00	36.69	16.53
5	2.37	19.00	30.52	12.50
6	2.50	20.00	27.60	10.22

CONCLUSION

Good quality forage is the basis of horse feeding. In our study we found that there was no significant difference in digestibility of DM and NDF of meadow hay among days of the experiment. The meadow hay was harvested at a later stage of maturity. Feed of this quality is not suitable for horse nutrition. We recommend meadow hay harvest at optimal maturity stage (early flowering).

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IS THE JERUSALEM ARTICHOKE ACTUALLY A PLANT OF THE 21ST CENTURY?

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ABSTRACT

The aim of the experiment was to ascertain the possibility of effective use of dried leaves of artichoke in fattening rabbits. Experiment included 30 rabbits after weaning, they were divided into three groups according to the used feed mixture. The control group was fed a commercial feed for fattening rabbits. In two experimental groups Jerusalem artichoke leaves were added. In the control mixture artichokes replaced LAPITEST mixture that contained pomaces of apples and grapes, sunflower husks, and dried sugar beet pulp. The nutritional and energy value of mixtures which were used for the control group and experimental groups were approximately same. Addition of 10% Topinambour improved the weight gains and also rabbit meat quality.

Keywords: rabbits; foods for rabbits; Jerusalem artichoce

INTRODUCTION

Chinese scientists from the University of Dalian (Fengwu, 2011) started to call Jerusalem artichoke, for its multi-exposure effect, as a potential human food, animal feed, and for its dietary, preventive and stimulative effect, as the plant of the 21st century. About the extraordinary effects of artichokes informs Cherbut (2002). In our experiment, we investigated Jerusalem artichoke leaves addition on nutritional and production effect in rabbits. Such experiments are part of a larger complex research of the possibility of using Jerusalem artichoke as feed. Jerusalen artichoke as feed was used in fresh or dry form. Tubers and haulm were used, again as fresh, dried or conservated. The possibility of usage of Jerusalem artichoke as silage is explored too (Bouma et al., 1995).

The aim of the experiment was to determine the actual production efficiency of Jerusalem artichoke leaves in fattening rabbits.

MATERIAL AND METHODS

In our study artichoke leaves were added to the commercial mixtures for fattening rabbits and we compared the production efficacy with an original commercial mixture for fattening rabbits. In the experiment 30 rabbits were used, regardless of gender, the only concern was the weight of rabbits of individual groups. Each group contained 10 animals. The animals were housed and fed under the same conditions. The only difference was in used feed ration (Table 1 and 2). The difference was the use of dried leaves of Jerusalem artichoke. The control group was fed with commercial mixture. The experimental group P1 was supplemented with 10% dried Jerusalem artichoke leaves. The experimental group P2 was supplemented with 20% dried artichoke leaves. Water for drinking was available from the automatic drinkers for each animal separately. During the fattening period of rabbits all groups were weighed every week. At the end of fattening, 6th week, rabbits were slaughtered. Carcass yield was determined. During the experiment, 2nd, 4th and 6th week, intake of feed mixtures was controlled. On the basis of the average weight gain and feed consumption a feed conversion rate was calculated. Rabbits were kept under the same conditions in an accredited stables and accredited cages. After certain adjustments it was possible to control an individual feed intake of animals and their output of feces.

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

	GROUP OF RABBITS		
COMPONENTS	CONTROL	P 1	P 2
LAPILEST	20	15	10
Alfalfa	24	24	24
Wheat bran	10	10	10
Apple pomaces	15	13	10
Sugar beet pulp	15	12	10
Barley	5	5	5
Soybean meal	3	3	3
Oats	7	7	7
Aminovitan DB - KC	1	1	1
Artichoke leaves	0	10	20
Total	100	100	100

LAPILEST - a special mixture for fattening rabbits, composed from grape pomace, pomace from seeds of Vine grapes, apple pomace, dry sugar beet pulp, sunflowers husks, mineral and vitamin additives.

Table 2. Nutritional value of rabbits diets

NUTRITIONAL VALUE	DIETS FOR GROUPS OF RABBITS		
	CONTROL	P 1	P 2
Digestible crude protein - %	12.8	13.5	12.8
Fat - %	3.38	3.96	4.2
Crude fibre - %	20.8	19.3	20.8
NDF - %	40.8	39.7	40.7
ADF - %	27.6	26.5	28.7
Starch - %	12.0	10.9	8.3
Digestible energy - MJ.kg^{-1}	11.73	11.79	9.07

RESULTS AND DISCUSSION

The results of our experiment are presented in Tables 3 and 4 and Figure 1.
When we compare the nutrient contents of the mixtures there are no big differences. Crude protein content was in the C group 12.8%, in the experimental group P1 - 13.5% and in the group P2 - 12.8%. It is true that the group P2 had a higher daily feed consumption of about 150 g compared to 128 g of the C group. Overall the rabbits received daily - in case of the group P1 about 20.4 g of CP and the group C about 16.38 g of CP. Higher food consumption was probably caused by a lower amount of neutral detergent fiber (NDF) in the mixtures, the rabbits of the control group received less NDF (53 g vs. 59.5 g for the group P1).
In the daily dose of the group P1 the content of digestible energy (DE) was 177 kJ and in the daily dose of the C group 152 kJ. Energy content is important for the growth intensity, which was in the group P1 higher. Different intensity of growth at the C group and experimental groups of rabbits is shown in Table 3.

Table 3. Average daily weight gains in rabbits over the course of experiment

Animal groups	Daily weight gains in weeks of fattening					
	1.	2.	3.	4.	5.	6.
Control	24.83	36.80	39.09	41.09	36.99	37.89
P 1	29.25	41.21	48.36	53.58	43.71	41.12
P 2	26.60	35.30	36.50	41.50	31.40	36.90

The daily weight gains in the P1 group, with 10% addition of Jerusalem artichoke leaves, were higher compared to the P2 group (20% addition of Jerusalem artichoke leaves). Differences in the intensity of growth can be cause by different content of main nutrients and energy in the feed. Differences in weight gains could be caused by better dietary effects of Jerusalem artichoke, mainly due to the composition of carbohydrate, primarily inulin content. However, a different content of fibre fractions (NDF and ADF) and energy should be also considered. Feed mixture with 10% addition of dried leaves of artichoke showed better results.

Figure 1. Average daily weight (g) in rabbits in the weeks of fattening

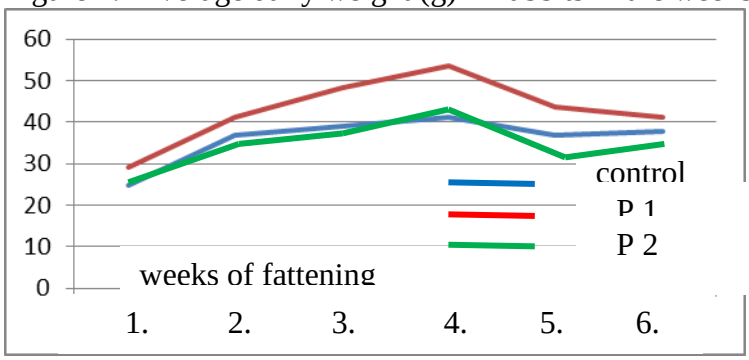

Figure 1 shows average daily weight gains in rabbits over the course of the experiment. Higher growth intensity was observed in the group P1 from the beginning of fattening, in this case higher energy content of the feed ration should be considered. Increase in daily weight gains was observed till the 4th week in all the groups, after that weight gains were decreased, as can be seen in Table 3. Differences in the intensity of the growth among compared groups can be influenced by different nutritional and energy values of feed mixtures (Table 1). These values can be compared with a recommendation for fattening rabbits. Nutritional and energy valuable feed for fattening rabbits, according to Zeman and Klapil (2006), contains 13-17% CP, at least 12.5% digestible CP and 11 MJ of digestible energy. Our feed mixtures contained 12.8% (C group) and 13.5% (P1 group) of digestible CP and energy level was 11.73 MJ (C group) and 11.79 MJ (P1 group).
Achieved slaughter weights of rabbits in the C group (2,901 kg) and the P1 group (3,028 kg) are comparable and can be considered as good.

Table 4. Carcass evaluation of rabbits

Groups of animal	Slaughter weight	Carcase weight	Indigestible viscera	Carcass yield
Control	2901.44	1348.88	566.66	57.03
P 1	3028.60	1479.00	553.00	58.83
P 2	2744.33	1318.77	577.77	57.61

The obtained results clearly showed that 10% addition of dried leaves of Jerusalem artichoke can be used in mixtures for fattening rabbits and may show positive effects compared to the conventional commercial mixtures. In scientific literature there is very little information about using of the Jerusalem artichoke, especially usage as a prebiotic has been studied (Xu et al., 2009; Yang et al., 2009).

CONCLUSION

The Jerusalem artichoke deserves attention, there is the possibility of its application at non-ruminant herbivores. Results of our work allowed us to state some general recommendations:
a) 10% addition of dried leaves of artichoke can be used in mixtures for fattening rabbits;
b) 10% content of artichoke in feed has same or better effect on the production efficiency of fattening rabbits compared to the conventional commercial feed;
c) composition (nutritional and energy content) of feed with artichoke addition should meet the actual requirements of fattening rabbits.
The final decision of using the artichoke as forage for rabbits is influenced by the economy and technology of Jerusalem artichoke growing. In case of achieving good economic outcomes, using of the Jerusalem artichoke for feeding rabbits can be strongly redommended.
The results of the experiment are part of a comprehensive research processed under the PhD study.

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OCCURRENCE OF MYCOTOXINS IN MAIZE SILAGE

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ABSTRACT

The occurrence of mycotoxins in maize silage conserved by chemical additive was investigated. Whole maize plants (*Zea mais*, L.) were ensiled without additive (variant C) and with chemical additive (variant E: acid salts of sodium chloride, calcium propionate, calcium formate, sodium carbonate in a dose of 1.75 $kg.t^{-1}$). The samples were examined for mycotoxins content by direct competitive enzyme-linked immunosorbent assays (ELISA). Incidence of all monitored mycotoxins were found in both variant of maize silage. The highest mean values of mycotoxins were: zearalenone (ZEA) 451.30; T-2 toxin (T-2) 231.40 $\mu g.kg^{-1}$ in variant E and deoxynivaleno (DON) 757.63; fumonisins (FUM) 225.8; total ochratoxins (OTA) 20.87; total aflatoxins (AFL) 3.93 $\mu g.kg^{-1}$ in variant C. The positive tendency of reduction of monitored mycotoxins (besides ZEA and DON) after application of acid salts was recorded with significant differences in total aflatoxins ($P \leq 0.05$).

Keywords: maize; silage; mycotoxins; ELISA

INTRODUCTION

Mycotoxins are secondary toxic metabolites produced by filamentous fungi with harmful effects on animals (Kačániová et al., 2010; Skládanka et al., 2013). When contaminated feed is fed to the animals, toxic responses referred to as mycotoxicoses may occur (Binder, 2007). In the European Union, the maximum levels of some mycotoxins in feedstuffs have been established. The obligatory regulation applies for aflatoxin B1 (Directive 2002/32 EC) and the recommended guidance values are available for deoxynivalenol, zearalenone, fumonisins, and

ochratoxin A (Commission recommendation 2006/576 EC). The aim of the presented study was to determine the occurrence and the effect of chemical additive on mycotoxin in maize silage.

MATERIAL AND METHODS

Silage preparation and treatments

The trial was carried out in 2014 at the Department of Animal Nutrition, Slovak University of Agriculture in Nitra, Slovak Republic. The whole-crop maize was harvested at the half-milk line stage and chopped with a forage harvester with a theoretical cut length of 10 mm. Treatments included the addition of no additive (control) and the addition of chemical additive (acid salts: sodium chloride, calcium propionate, calcium formate, sodium carbonate) applied in a powder form in a dose of 1.75 $kg.t^{-1}$. Forage samples (n=3) were collected before additive application. Following treatment application, the forage was packed to 4 dm^3 laboratory silos. Three replicates were performed for both treatments. Following packing, the silos were sealed with appropriate plastic lids and maintained at room temperature (20±2 °C) for 4 weeks. Next, the silos were opened and approximately 5 cm of silage from the surface of each silo was discarded and the remaining quantity was homogenized and subsampled. The mycotoxins concentrations were determined in triplicate for each subsample.

Sample preparation and analyses

The both samples were oven-dried at 60 °C for 24 hours and milled through a 1-mm sieve for mycotoxin analysis. Samples were analysed for the content of deoxynivalenol (DON), zearalenone (ZEA), total fumonisins (FUM), total aflatoxins (AFL), total ochratoxins (OTA) and T-2 toxin (T-2). ELISA (Enzyme linked immunosorbent assay) analyzes were performed with a commercially available test kit (Veratox® - Neogen, USA). Mycotoxins were extracted from a ground sample with a specific solvent. The extracted sample and enzyme-conjugated mycotoxin were mixed and added to the antibody-coated microwell. Mycotoxins in samples and control standards were allowed to compete with enzyme-conjugated mycotoxins for the antibody binding sites. After a washing step, an enzyme substrate was added and blue colour developed. The intensity of the color was inversely proportional to the concentration of mycotoxins in the sample or standard. A stop solution was then added which changed the color from blue to yellow. The microwells were measured optically by a microplate reader (Neogen, USA) at 650 nm.

The optical densities of the samples were compared to the optical densities of the standards and an interpretative result was determined in $\mu g.kg^{-1}$ for all monitored 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 mycotoxin levels in the maize silage samples are shown in Table 1. The presence of mycotoxins was detected in all the samples analysed. Aflatoxins (AFL) are most commonly known for causing acute or chronic liver disease, but they are also considered immunosuppressive, hepatotoxic, mutagenic, teratogenic, and carcinogenic (Fink-Gremmels, 2008). The levels of total aflatoxins ranged from 1.57±0.17 (E) to 3.97±0.50 $\mu g.kg^{-1}$ (C) ($P < 0.05$), which is lower than in the study by Reyes-Velázquez et al. (2008) (13.5 $\mu g.kg^{-1}$). Deoxynivalenol (DON) is one of trichothecene mycotoxins produced by some fusarium that frequently contaminate food and feed (Martins et al., 2008). Deoxynivalenol intake causes immune suppression (Sobrova et al., 2010), emesis and diarrhea (Pestka, 2007) in animals. Deoxynivalenol showed the highest levels detected 753.33±20.67 (C) and 753.30±29.32 $\mu g.kg^{-1}$ (E) which is lower value than reported Schollenberger et al. (2005) 1426 $\mu g.kg^{-1}$ and Rasmussen et al. (2010) 990 $\mu g.kg^{-1}$. Deoxynivalenol levels in analysed maize silage samples were lower than that provided by prescribed level (12 $mg.kg^{-1}$) by EU Commission Recommendation (2006/576/EC). Fumonisins (FUM) provoke pulmonary edema, hepatic fibrosis and leukoencephalomalacia or liver disease (Bräse et al., 2013). Fumonisins in our study did not exceed the guidance value of 20 $mg.kg^{-1}$ and ranged from 210.99±9.31 (E) to 225.81±3.67 (C) $\mu g.kg^{-1}$, that are higher values than reported by Teller et al. (2012). Ochratoxins (OTA) represents a potential threat to animal production due to its nephrotoxic, immunotoxic, mutagenic, teratogenic and carcinogenic effects (Duarte et al., 2011). Ochratoxins ranged from 20.87±1.76 (C) to 24.30±1.02 $\mu g.kg^{-1.}$ Reyes-Velázquez et al. (2008) reported lower mean value (5.1 $\mu g.kg^{-1}$). T-2 toxin (T-2) is a cytotoxic and immunosuppressive toxin, which can cause acute intoxication or chronic diseases in livestock (Zhou et al., 2008). T-2 occurred with mean values from 218.03±4.87 (C) to 231.40±7.33 $\mu g\ kg^{-1}$ (E). Similar

results were found in maize silage by Bíro et al. (2009). Our results are lower than 500 µg.kg^{-1} as recommended by EU (2013/165/EU) for products for feed and compound feed. Zearalenone (ZEA) is an estrogenic mycotoxin, which is often present in corn and other cereals and causes hyperestrogenism in livestock, especially in the pigs (Rashedi et al., 2012). Zearalenone was found with a maximum level of 451.30 µg.kg^{-1} (E), which is close to the maximal guidance values of 0.5 mg.kg^{-1} for dairy cows' feedstuffs. Similar results recorded Schollenberger, et al. (2006) 432 µg.kg^{-1}, but Driehuis et al. (2008) recorded lower ZEA contamination (90 µg.kg^{-1}).

Table 1. Mycotoxins content of maize silage.

µg.kg^{-1}	C			E		
	x	sd	v	x	sd	v
AFL	3.97^{a}	±0.50	12.59	1.57^{b}	±0.17	10.85
DON	753.33	±20.67	2.66	753.30	±29.32	3.89
FUM	225.81	±3.67	1.63	210.99	±9.31	4.41
OTA	20.87	±1.76	8.43	24.30	±1.02	4.20
T-2	218.03	±4.87	2.23	231.40	±7.33	3.17
ZEA	402.47	±52.10	12.95	451.30	±32.73	7.25

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

CONCLUSION

Occurrence of observed mycotoxins was detected in all maize silages. We have demonstrated, that application of selected chemical additive may be effective in reducing of aflatoxins, which is group of mycotoxins with the highest toxicity to animals. In our work, the low concentration of mycotoxins detected in the silage cannot be considered as a risk for the animal health.

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EFFECT OF EVENING PRIMROSE OIL ON THE ORGANISM OF SPORT HORSES

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ABSTRACT

The effect of evening primrose oil (EPO) administration on the selected biochemical blood parameters of race horses during their regular training period was determined. The sixteen-week experiment was performed on ten clinically healthy Thoroughbred horses. All the horses were enrolled in a regular training program. Eight weeks before the experiment, the horses were fed a diet which remained the same for the following eight weeks, only supplemented with 150 ml. The blood samples were collected every four weeks during the experiment. There were analysed a selected biochemical parameters including glucose, triacylglycerol, lactate, cholesterol and insulin. Values of glucose and cholesterol were significantly higher ($P \leq 0.05$) after administration of EPO. All results can be interpreted within the reference values.

Keywords: horses; *Oenothera biennis*; performance; oxidative stress

INTRODUCTION

Physical exercise leads to enhanced production of reactive oxygen species (ROS) and free radicals (Sen and Packer, 2000). Overproduction of oxidants exceeding the cellular antioxidant capacity results in oxidative stress (Gomes et al., 2012). Oxidative stress may result to fatigue, tissue damage and causes metabolic changes that consequently influence performance (Kinnunen et al., 2005; Deaton and Marlin, 2003).

Effective antioxidant defence system protects the organism against overproduction of reactive oxygen species (ROS) (Sacheck and Blumberg, 2001). The defence system is made from enzyme and non-enzyme antioxidants, which remove ROS and thereby limit their destructive effect on tissue (Avellini et al., 1999; Sacheck and Blumberg, 2001; Deaton and Marlin, 2003). Animals do not have such a high concentration of non-enzymatic antioxidants in comparison with human (White et al., 2001; De Moffarts et al., 2005). Therefore intake of antioxidants in the feed, especially in horses, is necessary. Horses are often given supplements to reduce the purported harmful effects of oxidative stress caused by free radicals (Bean et al., 2010). The most common antioxidants added to horse diets are vitamin E and C or combination of both (De Moffarts et al., 2005; Williams et al., 2004). To increase the antioxidant capacity of the organism of humans and animals are currently used as vegetable oils with higher content of n-3 and n-6 fatty acids (Bergero et al., 2004). Addition of EPO with a higher content of gamma-linolenic acid and a number of antioxidants such as catechin, epicatechin, gallic acid and α-tocopherol (Wettasinghe et al., 2002; Lu and Foo, 1995) to the diet can change the situation in favor of increased antioxidant capacity of the race horses organism (Belch and Hill, 2000). The effect of EPO on oxidative stress is related not only to its inhibitory effect on lipid peroxidation, but also with the support of the glutathione synthesis (De La Cruz et al., 1997).
The aim of this study was to examinate the effect of EPO addition to the diet of Thoroughbred horses on selected blood biochemical parameters.

MATERIAL AND METHODS

The experiment was performed from June 2012 to October 2012 on 10 clinically healthy Thoroughbred horses (5 male, 5 female, age 3-5 years, weight 470 ± 30 kg). All the horses had been in a regular training program prior to the study.
First 8 weeks of the experiment all horses were fed with the diet without EPO (Solio KFT., Hungary) – sampling 1, 2, 3 (sampling 3 = day 0 – start of EPO addition). Next 8 weeks all horses were fed with this diet with 150 ml of EPO (Solio KFT., Hungary) – sampling 4, 5, 6. The exercise of horses in the experiment was maximal through the whole time of experiment and training protocol.
The diet of the horses was based on oats, meadow hay, and extruded supplemental feed for horses - Fitmin Horse House müsli (Dibaq a. s., Czech Republic), Fitmin Horse Opti (Dibaq a. s., Czech Republic),

Fitmin Horse Energy (Dibaq a. s., Czech Republic) and supplement of vitamins and minerals Equistro (Vetoquinol, s.r.o., Czech Republic). The examination of the diet compounds was performed by laboratory of the Czech University of Life Sciences Prague. The horses had free access to water. Feeding doses were related to the rate of maximal workload (Table 1).

The blood samples were collected into polyethylene tubes (Vacutainer system Sarstedt, s.r.o., Czech Republic) 8 weeks and 4 weeks before EPO application, on day 0 and 4 weeks, 6 weeks and 8 weeks after EPO application, from jugular vein at 6:30 a.m. (which is ½ hour after feeding) and were immediately processed in the laboratory. The biochemical parameters – glucose, triacylglycerols, lactate and cholesterol were determined by commercial kits (Erba Lachema, s.r.o., CZ) on an automatic analyser XL – 200 (Erba Lachema s.r.o., CZ). Insulin was determined by commercial kit ELISA kit Equine Insulin (Mercordia, UK).

The data were first subjected to repeated measures, split-plot design analysis of variance using the SAS statistical software package (SAS V91, SAS Institute Inc.) and treatment alignments were done using the least squares significant difference method (Duncans Multiple Range Test). For all the statistical analyses, the level of significance was set at $P < 0.05$, and the data were presented as means ± SE.

Table 1. Composition of feed ration for Thoroughbred horses 470 ± 30 kg, maximal workload.

Nutrients	Meadow hay	Oats	Horse Opti	Horse Energy	Horse House Müsli	∑
Dry matter (g)	6521.20	3569.20	468.85	744.56	905.00	12 208.81
Crude protein (g)	398.30	472.00	75.00	64.00	100.00	1 109.30
Ash (g)	364.00	128.00	40.65	10.80	59.20	602.65
Ether extract (g)	114.10	157.20	33.50	81.60	49.20	435.60
Fiber (g)	2 695.00	389.20	26.00	14.56	55.00	3 179.76

RESULTS AND DISCUSSION

The aim of this work was to confirm that the addition of EPO in the diet can increase the antioxidant capacity of the organism of racehorse at full load. The effect of EPO with a higher content of gamma - linolenic and many antioxidants such as catechin, epicatechin, gallic acid and α-tocopherol (Art and Lekeux, 2005; Kinnunen et al,. 2005; Lu and Foo, 1995; Wettasinghe et al., 2002) shown increase of total antioxidant activity a positive effect on blood parameters, including glucose, triacylglycerol, lactate, cholesterol and insulin. Overview of blood parameters is given in Table 2.

Many studies have already dealt with the possibility of filing enriched diet for supplements that would be able to naturally increase the levels of glucose, or insulin and affect the energy balance of the horse. Tholstrup et al. (2013) have considered the effects of medium-chain fatty acids and oleic acid on blood lipids, lipoproteins, glucose, insulin, and lipid transfer protein activities and published that glucose and insulin did not differ significantly between the diets. Treiber et al. (2013) examined the dietary energy source affects glucose kinetics in trained Arabian geldings at rest and during endurance exercise. There has been described all parameters of glucose transfer increased with exercise. Jose-Cunilleras et al. (2002) investigated the glycemic index of a meal fed before exercise alters substrate use and glucose flux in exercising horses and they published, that the feeding corn, compared with fasting, resulted in higher plasma glucose and serum insulin and lower serum nonesterified fatty acid concentrations before exercise ($P < 0.05$) and in lower plasma glucose, serum glycerol, and serum nonesterified fatty acid concentrations and higher skeletal muscle utilization of blood-borne glucose during exercise ($P < 0.05$). Pagan et al. (2002) examined the effects of fat adaptation on glucose kinetics and substrate oxidation during low-intensity exercise. In this study five mature Arabian horses were used. The study was conducted as a crossover design with 2 dietary periods, each of 10 week's duration: a) a control (CON) diet, and b) a fat-supplemented (FAT) diet. For determination of glucose kinetics, a stable isotope ([6-6-d2] glucose) technice was used. Compared to the CON diet, FAT diet consumption for 5–10 weeks was associated with an altered metabolit response to low-intensity exercise, as evidenced by a more than 30% reduction in the production and utilisation of glucose. The aim of our study was to evaluate the effect of EPO on blood parameters of sport horses.

The results of this experiment showed that the administration of the EPO had a significant effect on blood glucose. This resulted by the average blood glucose was significantly higher ($P \leq 0.05$) at the end of the experiment (six sampling), before the beginning of the experiment (0 sampling). This tendency was shown in the increase over the course of the experiment, when the administration of the EPO (consumption 4, 5, 6) occurred in the experimental group, glucose levels gradually increased. Unfortunately, these results were not significant. The level of insulin during the experimental period slowly decreased, but the changes were not significant. Values of cholesterol were significantly higher ($P \leq 0.05$) after administration of EPO too. This result corresponds with the conclusion of the study by Reily et al. (1998). Reilly et al. (1998) described significantly higher amounts of cholesterol ester ($P < 0.05$), triglycerides ($P < 0.001$) in horses with diets containing EPO. The other selected biochemical parameters (triacylglycerol and lactate) were not significant ($P > 0.05$).

Table 2. Blood parameters in racehorses (n = 10) at intense training before (week 8, 4, day 0) and after (week 4, 6, 8) application of 150 ml of evening primrose oil (EPO) (means ± SD) .

Measurements	n	**Sampling**					
		8 weeks before	4 weeks before	day 0	4 weeks EPO	6 weeks EPO	8 weeks EPO
Glucose ($mmol.l^{-1}$)	10	5.94± 0.30 ^{B,C}	6.14 ± 0.30 ^{B}	5.38± 0.46 ^{D}	6.05 ± 0.38 ^{B,C}	5.56 ± 0.29 ^{B,C}	7.14 ± 1.06 ^{A}
TAG ($mmol.l^{-1}$)	10	0.41±0.10 ^{A,B}	0.52±0.14 ^{A}	0.38±0.07 ^{B}	0.53±0.15 ^{A}	0.42±0.11 ^{A,B}	0.50±0.15 ^{A,B}
Lactate ($mmol.l^{-1}$)	10	0.87±0.19 ^{D}	1.40±0.24 ^{B,C}	1.81±0.08 ^{A}	1.32±0.45 ^{C}	1.74±0.38 ^{A}	1.12±0.08 ^{A,B}
Cholesterol ($mmol.l^{-1}$)	10	2.25±0.25 ^{A}	2.85±0.31 ^{A}	2.27±0.18 ^{A}	3.19±0.29 ^{B,C}	2.47±0.18 ^{A}	3.15±0.22 ^{B,C}
*Insulin ($\mu g.l^{-1}$)	10			0.21±0.08 ^{B}	0.14±0.07 ^{B}	0.14±0.03 ^{B}	0.17±0.11 ^{B}

* Values of insulin were measured from the 3rd blood sampling for economic reasons, A–D different superscripts within the lines indicate significant differences ($P \leq 0.05$)

CONCLUSION

In conclusion, the feeding of the EPO had an increased effect on blood glucose and cholesterol, while the level of insulin slightly decreased. The EPO in the diet of horses can be an interesting supplement to reduce the harmful effects of oxidative stress.

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THE EFFECT OF EXOGENOUS PHYTASE ON RETAINABLE PLANT ORIGIN PHOSPHORUS AND CALCIUM IN LAYING HENS

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ABSTRACT

The experiment was conducted to determine the effect of exogenous phytase on retainable phosphorus and calcium in laying hens fed diets with low level only plant original phosphorus. Twenty four Lohmann Brown hens in 31 weeks of age were housed in individual cages. The levels of total phosphorus and calcium in both diets were 3.72 g/kg and 36.94 g/kg. The control diet contained no exogenous phytase. The experimental diet contained exogenous phytase Natuphos 150 FTU. The excreta were collected daily during the experimental period (2x5 days). The results showed that the phytase improved retainable phosphorus and calcium ($P < 0.05$) compared with the control diet. The excreta of hens fed diet with exogenous phytase contained less phosphorus and calcium than excreta of hens fed control diet.

Keywords: phytase, phosphorus, calcium, laying hens

INTRODUCTION

Phosphorus (P) is one of the essential minerals for all animals. It plays critical role in cellular metabolism, as a part of the energy currency of the cell, in cellular regulatory mechanism, and in bone (Applegate, 2008). All animals need to absorb P mainly as phosphate (Rodehutscord, 2013). Marounek et al. (2008) reported that a large proportion of P in cereals, oil-seeds and grain legumes is in the form of phytate P (myo-inositol hexakisphosphate). Phosphorus contained in plants in phytate form is only utilizable by monogastric animals from 20 to 30% (Zobač et al., 1997). This unavailability is due to the very low phytase activity found in the digestive tract (Pallauf et al., 1994).

Phytase (myo-inositol hexakisphosphate phosphohydrolase, EC 3.1.3.8) catalyses the release of P from phytate (Pandey et al., 2001). Phytase can be derived from a number of sources including plants, animals and micro-organisms. Plant ingredients of poultry diets differ greatly in native phytase activity (Eeckhout and De Paepe, 1994), therefore phytase has been added to poultry diets as exogenous phytase (Yao et al., 2007). The effectiveness of phytase was evaluated on the basic of growth rate, feed intake, phosphorus retention and phosphorus content in tibia ash (Farrell et al., 1993). It is well documented fact that phytase increases not only bioavailability of phosphorus, but also bioavailability of calcium and amino acids (Singh, 2008). Gordon and Roland (1997) reported that hens consuming the low nonphytate P (NPP) diet with supplementary phytase performed as well as the hens fed diets containing higher levels of NPP without supplementary phytase. A high or low level of available P in a laying hen´s diet may adversely affect the bird´s performance and reduce the eggshell quality (Harms, 1982). However, the effects of phytase in layer diets are complicated by the intimate link between Ca and P metabolism (Scott et al., 1999).
This study was conducted to determine the effect of exogenous phytase on retainable plant origin phosphorus and calcium in laying hens.

MATERIAL AND METHODS

Birds and management

Twenty four Lohmann Brown laying hens, 31 weeks old, were housed in individual cages. All procedures were approved by the animal welfare committee. The cages provided 1806 cm^2 of floor area without the nest, 43 cm of feeder and 2 nipples. The average weight of hens was 1.7 kg at the beginning of the experiment. All hens were allowed *ad libitum* access to the feed and water. Feed intake was controlled daily and average daily intake was calculated. A 15-h photoperiod from 06.00 to 21.00 was used throughout the experiment. The room temperature was kept at 21 °C.
Laying hens were divided into two groups (n=12). The experimental diets were offered for 5 days of adjustment and for the subsequent 5 days, during which excreta were quantitatively collected from each individual hen. Excreta were collected and weighed daily and immediately dried on 65 °C. Feathers and spilled feed were removed from excreta before each collection. Dried excreta were ground and stored at room temperature until being analysed.

Experimental diets

Two diets (control and experimental) were used in this experiment. Composition of the diets and content of the nutrients are shown in Table 1. The control diet contained no exogenous phytase. Experimental diets contained exogenous phytase. There were used Natuphos 150 FTU. The experiment was arranged according to latin square (2 x 2 x 12).

Table 1: Ingredients of diets and nutrient composition.

Component	Content in the diet (%)
Wheat	29.22
Maize	38.86
Soybean meal	21
Rapeseed oil	1.5
Limestone	4.23
Grit	4.24
Salt	0.2
Lysine	0.1
Methionine	0.15
Indicator	0.3
Aminovitan[1]	0.5
Total	100

ME (MJ/kg)	11.82
CP (%)	17.6
Lysine (%)	0.9
Methionine (%)	0.42
met + cys (%)	0.71
Ca (%)	3.5
NPP[2] **(%)**	0.115

[1] premix provided in kg: vit. A, 3340000 MJ; vit. B12, 3300 mcg; vit. D3, 1000000 MJ; vit. E, 11000 MJ; vit. K3, 670 mg; niacinamid, 8350 mg; folic acid, 170 mg; vit. B1, 670 mg; biotin, 25000 mcg; vit. B2, 1700 mg; cholinchlorid, 80000 mg; vit. B6, 1350 mg; Cu, 2000 mg; Mn, 23350 mg; Fe, 13350 mg; Zn, 16670 mg; I, 340.0 mg; Se, 67.0 mg; butylhydroxytoluen, 400 mg; butylhydroxyanisol, 80 mg

[2]non-phytate phosphorus

Analyses of samples

The dry matter was determined by drying the samples at 103 ± 2 °C for four hours. The ash was determined at 550 ± 20 °C for six hours. The content of calcium and phosphorus in feed and excreta was determined by the spectrophotometric method.

Calculations and statistical analysis

Retention of P and Ca was the difference between quantitative intake and the amount contained in excreta.

Retention of nutrient = intake of nutrient – excretion of nutrient

Data obtained from this experiment were analysed using t-test at a significance level $P < 0.05$ using the software package Unistat 5.1 (UNISTAT Ltd, ENGLAND).

RESULTS AND DISCUSSION

Retention of phosphorus and calcium was determined in laying hens fed diet with and without exogenous phytase Natuphos 150 FTU. Level of total phosphorus and calcium in both diets was 3.72 g/kg and 36.94 g/kg, respectively. Table 2 presents the intake of feed during experiment. There was found no significant difference ($P > 0.05$) in feed intake between groups.

Table 2. Feed intake in the experiment per hen/day.

Group	Feed intake (g)	Standard error	Variation coefficient
Control	100.7[a]	3.96	0.19
Experimental	103.2[a]	3.03	0.14

Data of phosphorus intake, excretion and retention are shown in Table 3. Retention of phosphorus was significantly higher ($P < 0.05$) in hens fed diet with exogenous phytase more than 8%. Average daily retention of phosphorus was 0.08 g in the control group and 0.12 g in the experimental group.

Table 3. Retention of phosphorus.

Group	P intake (g/kg)	SE	P excretion (g/kg)	SE	Retention (%)	SE
Control	0.36[a]	0.01	0.28[a]	0.01	20.5[a]	2.21
Experimental	0.40[b]	0.01	0.28[a]	0.01	28.7[b]	2.66

[a, b] indicate statistical significant difference between groups ($P < 0.05$) for the same characteristics

Calcium intake, excretion and retention are shown in Table 4. There was found significant effect ($P < 0.05$) of exogenous phytase on retention of calcium. Retention of calcium was higher in the experimental group (58.4%) than in the control group (54.0%).

Table 4. Retention of calcium.

Group	Ca intake (g/kg)	SE	Ca excretion (g/kg)	SE	Retention (%)	SE
Control	3.62[a]	0.14	1.66[a]	0.07	54.0[a]	1.35
Experimental	3.89[a]	0.11	1.63[a]	0.08	58.4[b]	1.38

[a, b] indicate significant difference between groups ($P < 0.05$) for the same characteristics

Supplement of exogenous phytase Natuphos 150 FTU had a positive effect ($P < 0.05$) on retention of both, phosphorus and calcium. Content of these nutrients in excreta was lower in hens fed diet with exogenous phytase. Um and Paik (1999) reported that availability of phosphorus and calcium was significantly increased by supplementation of phytase and excretion of phosphorus was significantly reduced. Supplement of phytase Nartuphos 150 FTU in this short term experiment had no significant effect ($P > 0.05$) on eggshell quality (Musilová et al., 2014). Exogenous phytase is added to the diets not only to enhance phytate P utilization, but also to reduce potential environmental pollution by phytate P and also to reduce dietary costs (Waldroup, 1999). Van der Klis et al. (1994) reported that supplementation of 250 FTU of phytase/kg diet in laying hens was equivalent to 0.8 g of P from monocalcium phosphate (MCP).

CONCLUSION

Addition of exogenous phytase Natuphos at level 150 FTU to the diets with 0.115% NPP and with the total content of phosphorus 0.372% and calcium 3.69% had significant effect ($P < 0.05$) on retention of phosphorus and calcium.

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EVALUATION OF THE BREED EFFECT ON HAEMATOLOGICAL INDICATORS IN SELECTED DWARF RABBIT BREEDS

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ABSTRACT

The aim of our study was to evaluate the breed effect on selected haematological indicators within three dwarf rabbit breeds. This study was conducted on well-known dwarf rabbit purebreds: Netherland Dwarf (n=8), Teddy Dwarf (n=8) and Dwarf Lop (n=8). Used rabbits came from the common pet stock. Female rabbits were approximately 6 to 7 months old and were housed, handled and fed under the same management conditions. In blood plasma, we determined these indicators: red blood cell count, white blood cell count, haemoglobin concentration and haematocrit value and we also calculated the erytrocytic parameters mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration. Obtained results showed significant differences within all of the indicators among evaluated purebreds. Results can serve more accurate assessment of the health status in dwarf breeds, also in relation to nutrition of pet rabbit.

Keywords: dwarf rabbit; haematology; physiological laboratory values

INTRODUCTION

Recently, the rabbit becomes a favourite pet animal and it has been used as suitable companion for human (Harcourt-Brown, 2002). Zadina (2011) states that this interest came also in the Czech Republic, but with certain delay as compared to abroad. The dwarf breeds are the most common rabbits within private pet husbandry (Snook et al., 2013).

As in foreign countries, this situation is related to the increasing breeders´ interest about the rabbit veterinary care. Moreover, this interest is also focused on specifics of dwarf rabbit nutrition, especially in growth and health respects. Nevertheless, the most of rabbit physiological reference ranges come from laboratory rearing, which can complicate a diagnosis procedure (Harcourt-Brown, 2002). Therefore, when laboratory results are evaluating, a breed effect should be taken into consideration too (Wesche, 2014). Some preliminary studies dealt with rabbit haematology reported useful data with respect to a breed effect (Burnett et al., 2006; Martinec et al., 2012). However, the current popular dwarf rabbit breeds have not been included in these studies. Knowledge of the appropriate reference ranges of rabbit physiology is very important for more accurate assessment of the health status and nutrition requirements.
The aim of this study was to evaluate the breed effect on selected haematological indicators within three dwarf rabbit purebreds.

MATERIAL AND METHODS

A total of 28 rabbits were used in our study. These rabbits belonged to three well-known dwarf breeds – the Dwarf Lop (n=8), the Netherland Dwarf (n=8) and the Teddy Dwarf (n=8). Only females (does), approximately 6 to 7 months old, were used in the study. Rabbits originated from the common pet stock that focusing on show activity under rules of the Czech Small Animal Breeder Association. Used individuals possessed the typical breed traits. All rabbits were housed, handled and fed under the same conditions. Commercial pellets (special formula for dwarf rabbits) were used to feed rabbits. The blood collection was taken by close method at the same time – from 8 to 9 a.m. General health status of investigated rabbits was evaluated before blood sampling. Blood was loaded into heparinized sample containers. These were unmistakably marked and immediately transported to the laboratories of the Department of Animal Nutrition and the Department of Animal Husbandry and Animal Hygiene, UVPS Brno. Haematology procedures were performed by manual way according to Doubek et al. (2003). In blood plasma we determined these indicators: red blood cells count (RBC), white blood cells count (WBC), haemoglobin concentration (HGB), and haematocrit value (HCT). Then, we also calculated the erytrocytic parameters: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

Obtained results were statistically analyzed using software STATISTICA CZ version 9. One-way variance analysis (ANOVA) was used to determine differences in the evaluated indicators. When ANOVA showed significant differences between the breeds, Fisher´s LSD test was used. The significant differences among the breeds are in the text and table marked as $P < 0.01$ (statistically highly significant; **) and $P < 0.05$ (statistically significant; *).

RESULTS AND DISCUSSION

Obtained results of haematological examination including statistical evaluation are presented in Table 1. Our findings showed that the breed affected all of seven haematological indicators.

Table 1. Mean values of haematological indicators within investigated dwarf rabbit breed.

Haematological indicator	Breed		
	Netherland Dwarf (x ± SEM)	Teddy Dwarf (x ± SEM)	Dwarf Lop (x ± SEM)
RBC ($T.l^{-1}$)	$4.02 \pm 0.28^{A,a,b}$	$4.39 \pm 0.45^{A,B,a}$	$5.95 \pm 0.46^{B,b}$
HGB ($g.l^{-1}$)	133.14 ± 7.38^{B}	93.07 ± 5.38^{A}	103.58 ± 4.35^{A}
HCT ($l.l^{-1}$)	$0.38 \pm 0.01^{B,b}$	0.31 ± 0.01^{A}	$0.34 \pm 0.01^{A,B,a}$
MCV (fl)	96.97 ± 7.15^{B}	$75.38 \pm 5.85^{A,B}$	59.05 ± 4.00^{A}
MCH (pg)	34.05 ± 2.53^{B}	21.98 ± 1.14^{A}	17.96 ± 1.17^{A}
MCHC ($g.l^{-1}$)	353.51 ± 17.30^{B}	295.67 ± 8.68^{A}	305.07 ± 8.95^{A}
WBC ($G.l^{-1}$)	5.65 ± 0.25^{b}	4.14 ± 0.33^{a}	$5.14 \pm 0.47^{a,b}$

(x –Arithmetic Mean, SEM – Standard Error of the Mean, ^{A,B}: $P < 0.01$, ^{a,b}: $P < 0.05$)

The highest value of RBC was recorded in the Dwarf Lop, whereas the lowest value was found in the Netherland Dwarf ($P < 0.01$). In addition, we also found significant differences between the Teddy Dwarf and Dwarf Lop breed ($P < 0.05$). In comparison with the most of literature reference sources, our values were slightly lower, however within physiological ranges. Jenkins (2006) states that the RBC reference range is very wide and varies among breeds too, which is in agreement with our results. In haemoglobin concentration, the statistical differences were found between the Netherland Dwarf and

Teddy Dwarf, and also between the Netherland Dwarf and Dwarf Lop ($P < 0.01$). The effect of a breed on haemoglobin concentration was also confirmed by Martinec et al. (2012), who evaluated blood indicators of the Czech national rabbit breeds. Haemoglobin concentration of 84 – 155 $g.l^{-1}$ is considered to be within the physiological reference range for a rabbit (Konrád, 1972). As for the haematocrit value, its higher level was found in the Netherland Dwarf as compared to the Teddy Dwarf breed ($P < 0.01$). In addition, significant differences in haematocrit value were found between the Netherland Dwarf and Dwarf Lop ($P < 0.05$). Our findings agree with those of Harcourt-Brown and Baker (2001), who described the typical haematocrit values 0.30 – 0.40 $l.l^{-1}$ in pet rabbits. Regarding a MCV value, the highest level was found in the Netherland Dwarf ($P < 0.01$). Values of MCV in the Dwarf Lop (59.05 fl) and Teddy Dwarf (75.38 fl) were in the physiological reference range reported by Vennen and Mitchel (2009). Whereas the MCV value of the Netherland Dwarf (96.97 fl) was quite higher. However, Martinec et al. (2012) also found higher MCV values in some breeds. The MCH indicator in the Netherland Dwarf was higher than in both of other breeds ($P < 0.01$). This might be a result of higher haemoglobin compensation because the lower RBC was found in this breed. As for MCHC values, we found their same range as for MCH values. Jenkins (2006) states that WBC can also be affected by a rabbit breed. This statement is in accordance with findings of our study.

CONCLUSION

It can be concluded that haematogical indicators were affected by dwarf rabbit breeds. Interestingly, we found four the lowest data of seven monitored haematological indicators in the Teddy Dwarf breed. The results of our study display more accurate details of physiological reference ranges in dwarf rabbit breeds. These findings may be utilized in the further experimental studies conducted on dwarf rabbits in relation to their nutrient requirements and health status.

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EFFECT OF VARIOUS LUPINE SPECIES ON MILK PRODUCTION OF RABBIT DOES AND PERFORMANCE OF THEIR LITTERS BEFORE WEANING

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ABSTRACT

The effect of various lupine seeds on milk production of rabbit does and performance of their litters before weaning was studied. Two isonitrogenous and isoenergetic lactation diets were formulated. The first lactation diet (WLS) contained white lupine seeds as the main crude protein source, whereas the second lactation diet (BLS) was based on blue lupine seeds. The WLS diet had higher ether extract content than the BLS diet. A total of 32 Hyplus does (16 per treatment; at the 3rd parturition) were fed one of these 2 diets. The litters were standardized to 9 kits on the day of birth. Feed intake, feed efficiency and milk production of does were not affected by dietary treatments, as well as performance of their litters. Live weight at weaning ($P = 0.005$), milk dry matter ($P = 0.028$) and fat contents ($P = 0.016$), as well as fat output per kg of metabolic weight ($P < 0.001$) were higher in does fed the WLS diet.

Keywords: rabbit; milk yield; milk composition; white lupine; blue lupine; protein source

INTRODUCTION

Traditionally used crude protein (CP) source in diets for broiler rabbits is soybean meal. It is known that this CP source has favourable effect on growth and feed conversion ratio, but in higher concentrations increases risk of digestive disorders in growing rabbits (Gutiérrez et al.,

2003). Therefore it is necessary to seek adequate alternatives. Recent experiments showed that white lupine seeds (*Lupinus albus* cv. Amiga) were a suitable alternative CP source for growing-fattening rabbits (Volek and Marounek, 2009) as well as for lactating rabbit does (Volek et al., 2014).

However, there are also another lupine species, e.g. blue lupine (*Lupinus angustifolius* L.), whose use in rabbit nutrition is not mentioned in literature so far. Therefore, the aim of this study was to compare white lupine seeds and blue lupine seeds as the main CP source for rabbit does and their effect on milk production and milk composition of rabbit does and performance of their litters before weaning.

MATERIAL AND METHODS

Two lactation diets were formulated (Table 1). The first lactation diet (control, WLS) contained white lupine seeds (cv. Amiga) as the CP source, whereas the second lactation diet (BLS) was based on blue lupine seeds (cv. Probor). The diets had similar CP, fiber fractions, amino acids, starch, and digestible energy contents. The WLS diet was higher in ether extract (EE), due to the higher EE content of WLS.

Table 1: Ingredients and chemical composition of experimental diets based on white lupine seeds (WLS) or blue lupine seeds (BLS).

Ingredients (%)	Diet		Chemical composition (g/kg as-fed basis)	Diet	
	WLS	BLS		WLS	BLS
Alfalfa meal	30	30	Dry matter	886	880
White lupin seeds	25	0	CP	180	175
Blue lupin seeds	0	28.5	EE	51	31
Wheat bran	5	1.5	NDF	329	337
Sugar beet pulp	2	2	ADF	194	198
Oats	13	10	ADL	45	39
Barley	22	25	Starch	197	195
Aminovitan[1]	1	1	Lysine	8.4	8.0
Dicalcium phosphate	0.7	0.7	SAA[2]	5.2	5.1
Limestone	1	1	Threonine	6.7	6.2
Salt	0.3	0.3	Arginine	11.8	11.5
			Calcium	12.4	11.9
			Phosphorus	5.8	5.8
			Sodium	1.6	1.5

[1] Aminovitan – vitamin, mineral and amino acid supplement, per 1 kg of Aminovitan (without coccidiostats), which is the same for both of

diets: Lysine 30 g, DL-methionine 100 g, L-threonine 50 g; [2]SAA – sulphur amino acids.

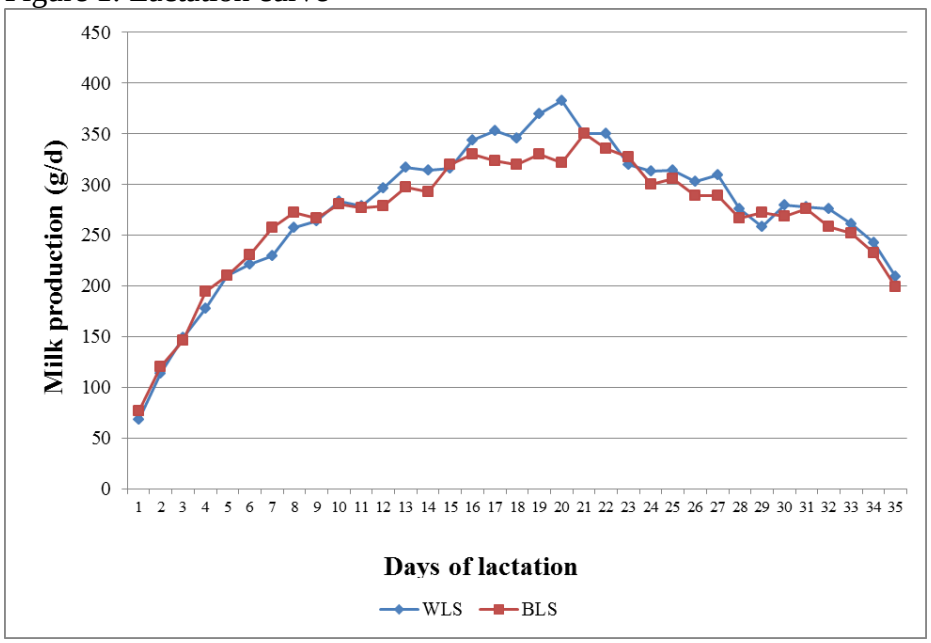
A total of 32 Hyplus rabbit does were used in the trial. All does were at the same physiological stage (3rd parturition) in order to eliminate the effects of non-dietary factors on milk production (Maertens et al., 2006). The does were housed in modified cages (97 x 75 x 45 cm) which allowed controlled suckling (once a day at 7 am) and separate access of does and their litters to feed. After the parturition (day 0) does were distributed in comparable groups (16 animals per treatment) based on live weight and were fed one of the 2 lactation diets (WLS or BLS). The litters were standardized to 9 kits immediately after the birth. The feed intake and live weight of does, milk production (difference between live weight of does immediately before and after suckling) and the growth of litters were recorded. Five does of each group were used to evaluate milk composition (detailed description of analytical methods is described by Volek et al., 2014). Milk was collected manually at d 21 of lactation after application of 1 IU oxytocin. Due to the influence of the milk sampling on the subsequent milk yield the sampled does were not considered in the statistical evaluation of milk production. Litters were offered weaning diets with the same CP source as in lactation diet of their mothers from d 17 of lactation. The solid feed intake of litters was recorded from d 21 of lactation. At d 25 of lactation does were inseminated. Weaning was conducted at d 36 of lactation.
Data were examined by one-way analysis of variance using the GLM procedure of the SAS programme.

RESULTS AND DISCUSSION

Growth performance and milk production of rabbit does are given in Table 2. Live weights of does at the beginning of the experiment (after parturition) as well as at the peak of lactation were in both groups without significant difference. However, difference was observed at the time of weaning (P = 0.005). Does fed the WLS diet had significantly higher live weight (5171 g) than does fed the diet containing blue lupine seeds (4748 g). The average daily feed intake during the entire lactation period was not affected by dietary treatments. Similarly, daily milk production did not vary significantly between both groups, although slightly higher values, regarding the entire lactation period and also individual phases, were recorded in does fed the WLS diet. The average daily milk production during the entire lactation is

illustrated in Figure 1. The course of the lactation curve in both groups of animals was consistent with the average milk production of multiparous hybrid rabbit does reported by other authors (Maertens et al., 2006). The evaluation of the milk, fat and protein output expressed per kg of metabolic weight revealed a significant difference in fat production between both groups. Higher values were recorded in the group fed the WLS diet (8.28 and 6.37 g/d/kg of metabolic weight for the WLS and BLS diet, respectively; $P < 0.001$).

Figure 1: Lactation curve



Significant difference found in live weight of does at weaning might be associated with the lower dietary EE content and intake in the BLS diet. Because daily milk production did not significantly vary between groups in our study, it can be assumed that a higher mobilisation of body fat reserves in the later phase of lactation in does fed the BLS diet occurred. In fact, fat is the main energy source for milk production (Pascual et al., 2003).

Table 2: Milk yield, milk, fat and protein outputs, and performance of rabbit does fed the diet containing white lupine seeds (WLS) or blue lupine seeds (BLS).

	Diet		RMSE	*P*-value
	WLS	BLS		
Live weight (g)				
at partum	4542	4370	414	0.249
d 21 of lactation	5061	5062	501	0.998
at weaning	5171[a]	4748[b]	396	0.005
Average daily feed intake (g/d)				
d 1 to 21 of lactation	404	402	47	0.891
d 22 to 35 of lactation	466	444	49	0.250
d 1 to 35 of lactation	429	419	41	0.538
Daily milk production (g)				
d 1 to 21 of lactation	270	258	31	0.352
d 22 to 35 of lactation	265	256	65	0.733
d 1 to 35 of lactation	268	257	35	0.443
Feed efficiency				
d 1 to 21 of lactation	0.39	0.38	0.03	0.771
d 28 to 35 of lactation	0.54	0.54	0.05	0.714
Output (g/d/kg of metabolic weight) d 1 to 21 of lactation				
milk	72.3	75.7	7.2	0.258
fat	8.3[a]	6.4[b]	0.7	< 0.001
protein	7.2	7.6	0.7	0.199

[a,b] $P \leq 0.05$

The growth performance of litters during the suckling period is presented in Table 3. It is obvious that live weight of litters was not significantly affected by CP source used in the lactation diets during the entire lactation period. Similarly, milk efficiency was not affected by dietary treatments.

Table 3: Performance of litters of rabbit does fed the diet containing white lupine seeds (WLS) or blue lupine seeds (BLS).

	Diet		RMSE	*P*-value
	WLS	BLS		
Live weight of litter (g)				
at birth	621	617	53	0.831
d 21 of lactation	3396	3246	387	0.281
at weaning	9896	9787	658	0.643
Average daily weight gain (g/d per rabbit)				
d 1 to 21 of lactation	17.9	17.2	2.0	0.289
d 22 to 36 of lactation	48.1	48.5	3.4	0.752
d 1 to 36 of lactation	30.5	30.2	1.0	0.630
Milk efficiency				
d 1 to 21 of lactation	0.59	0.59	0.02	0.763

Chemical composition of rabbit milk is presented in Table 4. A higher dry matter content was observed in milk of does fed the WLS diet than in does fed the BLS diet (25.50 and 22.34 g/100g for the WLS and BLS diets, respectively; $P = 0.028$). Milk dry matter content corresponds with milk fat content, which was higher in does fed the WLS diet (11.46 and 8.39 g/100 g for the WLS and BLS diets, respectively; $P = 0.016$). In fact, rabbit milk fat content at the peak of lactation should range between 10.0 to 16.6 g/100 g (Maertens et al., 2006). A lower milk fat content and thereby fat output expressed per kg of metabolic weight in milk of does fed the BLS diet was probably caused by the lower EE content and intake in the BLS diet. Milk amino acids and mineral contents did not significantly vary between dietary treatments.

Table 4: Milk composition of does fed the diet containing white lupine seeds (WLS) or blue lupine seeds (BLS) at d 21 of lactation.

	Diet		RMSE	*P*-value
	WLS	BLS		
Chemical composition (g/100 g)				
dry matter	25.50[a]	22.34[b]	1.56	0.028
fat	11.46[a]	8.39[b]	1.31	0.016
protein	9.93	10.00	0.68	0.869
Amino acids (g/100g)				
Lysine	0.64	0.62	0.06	0.588
Methionine	0.22	0.21	0.02	0.619
Cysteine	0.19	0.19	0.02	0.618
Threonine	0.42	0.41	0.04	0.628
Arginine	0.43	0.41	0.03	0.461
Minerals (g/kg)				
Calcium	5.55	6.09	2.22	0.709
Phosphorus	3.21	2.96	0.34	0.292
Magnesium	0.41	0.36	0.05	0.213
Sodium	1.23	1.34	0.29	0.539

[a,b] $P \leq 0.05$

CONCLUSION

It can be concluded that, from the perspective of milk production, growth and viability of kits before weaning, blue lupine seeds could be another suitable dietary CP source for lactating rabbit does. When using blue lupine seeds, however, dietary fat should be added in order to eliminate higher mobilisation of body reserves of does in the later phase of lactation. This experiment also included evaluating of performance and health status of growing rabbits after weaning. These data are in processing at the moment.

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