OPEN ACCESS
Potravinarstvo Slovak Journal of Food Sciences
Crossref Similarity Check
potravinárstvo®
Slovak Journal of Food Sciences
Potravinarstvo Slovak Journal of Food Sciences
vol. 11, 2017, no. 1, p. 88-95
doi: https://dx.doi.org/10.5219/707
Received: 1 January 2017. Accepted: 6 March 2017.
Available online: 22 March 2017 at www.potravinarstvo.com
© 2017 *Potravinarstvo Slovak Journal of Food Sciences*, License: CC BY 3.0
ISSN 1337-0960 (online)

EFFECT OF PROTEIN CONCENTRATE SUPPLEMENTATION ON THE COMPOSITION OF AMINO ACIDS IN MILK FROM DAIRY COWS IN AN ORGANIC FARMING SYSTEM

Pavel Horký, Jiří Skládanka, Pavel Nevrkla, Daniel Falta, Iva Čáslavová, Pavel Knot

ABSTRACT

Our experiment examined the effect of feeding a protein concentrate supplement on the composition of amino acids in milk from dairy cows managed in an organic farming system. The experiment included two groups of cows. Animals in both groups received an identical basic feed ration composed of maize silage, clover-grass haylage from the first cutting, grass haylage from the first cutting, winter wheat and spring barley. The first group of dairy cows (n = 10) served as a control without the addition of protein concentrate to the feed ration. The second experimental group (n = 10) received in addition to the basic feed ration a protein concentrate composed of soybean, sunflower and linseed cakes at rate 1 kg per head per day. The experiment lasted 30 days. Milk analysed for amino acid content was sampled at 10-day intervals. Addition of the protein concentrate significantly increased milk contents of aspartic acid, proline, threonine, glycine, alanine and glutamic acid. A significant decrease of valine also was recorded in milk from the experimental group. The results of our experiment show that a protein concentrate supplement can affect concentrations of some amino acids in milk from dairy cows.

Keywords: cow; protein concentrate; milk; amino acid; organic farming

INTRODUCTION

Organic farming currently creates a significant part of livestock and is receiving more and more attention in all its sectors **(Horký et al., 2012; Jančíková et al., 2012; Herbut, 2013; Horký et al., 2013)**.

Milk is classified as a basic raw material in the nutrition of animals and humans. Dairy cow nutrition can affect its composition: the amount of protein, fat, fatty acids, spectrum of amino acids **(Nevrkla et al., 2013; Gustavsson et al., 2014; Horký, 2014)**. The use of a protein concentrate based on sunflower and soybean cakes affects the composition of unsaturated amino acids in milk **(Maxin et al., 2013; Křížová et al., 2013)**. Linseed cake contains alpha-linolenic acid, which may pass into the meat and milk of farm animals **(Matthaus, 2004)**. Vegetable oil industry by-products can increase not only the content of unsaturated fatty acids in milk but also some milk components, particularly protein and fat content **(Vargas-Bello-Perez et al., 2013)**. Feeding a protein concentrate composed of faba bean and sunflower cakes can also boost milk production **(Avondo et al., 2013; Lipinski et al., 2013)**. Individual protein feeds are characterized by different degradation rates in the rumens of ruminants. Due to these differences in degradation, various protein feeds can influence milk composition in different ways **(Maxin et al., 2013; Svoboda et al., 2016)**. When feeding soybean protein concentrate to dairy cows, it should be taken into account that this feedstuff is digested in particular by the enzymes chymotrypsin, trypsin, pepsin and cysteine protease **(Banach et al., 2013)**. A range of inhibitors can cause problems in the digestion of these protein concentrates **(Espejo-Carpio et al., 2013; Belakova et al., 2015)**. Methionine is limiting essential amino acid in the milk. Lower content is in alanine (57.8%), arginine (52%), aspartic acid (87%), cysteine (59.4%), glycine (35.3%), histidine (96.8%), and serine (83.8%) after the conversion (to the amount of methionine) from non-essential amino acids. Other amino acids are in excess of the required amount **(Čermáková et al., 2012)**. The amount of protein can be increased by-pass protein and in this way to rise the amount of histidine, lysine, and methionine. Glucose plays a central role **(Maxin et al., 2013)**. The additive of abductive amino acids in the diet of dairy cows (methionine, phenylalanine, histidine) may also increase the concentration of these amino acids in milk **(Sizova and Zelenina, 2010)**. Mammary gland itself can regulate the synthesis of certain amino acids as needed **(Choi et al., 2002)**. Amino acids in cow's milk are distinguished by high lysine content and, in contrast, the lowest level of tryptophan **(Siciliano et al., 2013)**. Degradable and undegradable nitrogen is separated in the rumen. Microbial protein is formed from the

degradable protein and then continues on to the duodenum. In the duodenum, microbial protein is broken down into individual amino acids which pass into the blood **(Masoero et al., 2011)**.

The objective of our experiment was to ascertain the effect of a protein concentrate composed of soybean, sunflower and linseed cakes on amino acid content in milk. The partial aim of the study was to assess the relationship between individual amino acids in milk of dairy cows. The impact of the protein concentrate on milk yield in dairy cows managed in an organic farming system was also measured. Protein concentrate can be expected to increase both protein content in milk generally and the concentrations of specific amino acids.

MATERIAL AND METHODOLOGY

The experiment was conducted on an organic farm (the farm was registered in accordance with the Czech Republic's Act No. 242/2000 Coll. under registration number 42318335) keeping dairy cattle at Lesoňovice, Czech Republic. It included 20 Holstein dairy cows divided into two equal groups by weight (the experimental group had an average weight of 622 ±15 kg; the average weight of the control group was 630 ±11 kg) and at the same stage of lactation. Dairy cows in the experimental group had completed an average of 3.4 ±0.1 lactations prior to the experiment while cows in the control group had completed an average of 3.6 ±0.1 lactations. Average milk yield of the animals was 7,600 ±50 kg/lactation. All animals were fed a basic feed ration in the form of a total mixed ration (Table 1) and were allowed ad libitum access to water. The quantity of feed provided was recorded by the mixer-wagon (Luclar, Czech Republic). Uneaten amounts were estimated and not in any way analysed. The first experimental group of dairy cows (n = 10) had its basic feed ration supplemented with a protein concentrate (soybean cake 60%, sunflower cake 20%, linseed cake 20%) at the rate of 1 kg of dry matter per head per day. The crude protein content of the supplement was 36.6%. Individual amino acid contents in the basic feed ration and protein concentrate are shown in Table 2. The components of the protein concentrate all had originated from organically grown materials.

The second group of cows (n = 10) served as a control and received no supplemental protein concentrate in the feed ration. The nutrient contents of the feed rations for the two groups are presented in Table 3. At the start of the experiment, the experimental group of dairy cows was on average at day 52 of lactation (ranging from day 41 to 65). The average stage of lactation period in the control group of cows at the beginning of the experiment was 56 days (ranging from day 38 to 60). The cows were kept in loose housing and fed twice daily (morning and evening). The experimental period was 30 days. Milk samples were taken before the experiment and then every tenth day (10th, 20th and 30th days). Milk samples were collected prior to the early feeding and then analysed (Figure 1).

Analysis of feed mixture

Standard AOAC International procedures (2005) were used to determine the contents of crude protein, crude fat and starch in the ration. Crude protein content (6.25 × N) was determined using a Kjeltec 1030 Auto Analyser, and crude fat content was determined with a Soxtec 1043 (FOSS Tecator AB, Höganäs, Sweden). Neutral detergent fibre (NDF) was measured following a protocol described elsewhere **(Mertens et al., 2002)**, and acid detergent fibre (ADF) was determined according to procedure 973.18 of the AOAC (2000). Determination of PDIN (protein digested in the small intestine when rumen-fermentable nitrogen is limiting), PDIE (protein digested in the small intestine when rumen-fermentable energy is limiting) and NEL (net energy for lactation) was made in accordance with procedures described elsewhere **(Zeman et al., 2006)**.

Determination of amino acids

Amino acids were determined by ion-exchange liquid chromatography (Model AAA-400 amino acid analyser, Ingos, Czech Republic) using post-column derivatization with ninhydrin and a VIS detector. A glass column (inner diameter 3.7 mm, length 350 mm) was filled manually with a strong cation exchanger in the LG ANB sodium cycle (Laboratory of Spolchemie) with average particles size 12 µM and 8% porosity. The column was tempered within the range 35 to 95 °C. Elution of the studied amino acids took place at a column temperature set to 74 °C. A double-channel VIS detector with inner cell volume of 5 µL was set to two wavelengths: 440 and 570 nm. A solution of ninhydrin (Ingos, Czech Republic) was prepared in 75% v/v methyl cellosolve (Ingos, Czech Republic) and in 2% v/v 4 M acetic buffer (pH 5.5). Tin chloride ($SnCl_2$) was used as a reducing agent. The prepared solution of ninhydrin was stored in an inert atmosphere (N2) in darkness at 4 °C. The flow rate was 0.25 $mL.min^{-1}$. and the reactor temperature was 120 °C.

Table 1 Composition of the feed ration and dairy cows' average daily intake per head (Dry $matter.kg^{-1}$)

Feedstuff	Control group	Experimental group
Maize silage	6.38	6.38
Clover–grass haylage	5.95	5.95
Grass haylage	4.00	4.00
Winter wheat	2.64	2.64
Spring barley	1.76	1.76
Protein concentrate[a]	-	1.00
Detamin GA Spezial[b]	0.13	0.13
Total intake	20.86	21.86

Note: [b]Detamin GA Spezial is a mineral supplement for ruminants intended to be used in organic farming systems (H. Wilhelm Schaumann Gmbh, Pinneberg, Germany).

Table 2 Intake of amino acids by control and experimental groups of dairy cows (g.day dry matter^{-1}).

Amino acid	Control group	Experimental group
Lysine	105.6	130.7
Methionine	44.2	52.0
Threonine	104.2	122.5
Tryptophan	24.0	30.5
Arginine	123.4	160.5
Histidine	48.2	60.1
Isoleucine	115.3	137.5
Leucine	200.2	234.2
Phenylalanine	115.0	137.8
Valine	123.4	146.7
Tyrosine	79.4	95.0

Note: [b]Detamin GA Spezial is a mineral supplement for ruminants intended to be used in organic farming systems (H. Wilhelm Schaumann Gmbh, Pinneberg, Germany).

Table 3 Nutrient content of feed rations for experimental and control groups of dairy cows (per kg of dry matter).

Component	Control group	Experimental group
Starch (g)	79.6	78.7
Crude protein (g)	132.0	145.9
Fat (g)	27.0	28.9
PDIN (g)	80.1	90.2
PDIE (g)	79.5	84.2
NEL (MJ)	6.4	6.5
ADF (g)	263.9	269.8
NDF (g)	403.2	411.2

Note: PDIN = protein digested in the small intestine when rumen-fermentable nitrogen is limiting, PDIE = protein digested in the small intestine when rumen-fermentable energy is limiting, NEL = net energy for lactation, ADF = acid detergent fibre, NDF = neutral detergent fibre.

Sample preparation for determination of amino acids in feed ration

Before the amino acids analysis, acid and oxidative hydrolyses were performed on the samples. For the acid hydrolysis, 150 mg of the sample was weighed directly into the hydrolysis container. Then, 15 mL hydrochloric acid (6 mol.L^{-1}) was added to the weighed sample. To remove air, argon was subsequently bubbled through the content of the hydrolysis container for 30 seconds. After the completion of hydrolysis, the content of the container was quantitatively transferred by hydrochloric acid (0.1 mol.L^{-1}) through filtration paper into an evaporation flask. The filtrate was evaporated three times in a vacuum evaporator in a water bath set at 50 °C until it had a syrupy consistency. It was rinsed three times with 0.1 mol.L^{-1} hydrochloric acid. The remainder after evaporation was quantitatively transferred by buffer solution (pH 2.2) into a 25 mL volumetric flask. The remaining sample solution was then filtered through a microfilter into an Eppendorf microcentrifuge tube and used for amino acid analysis.

For oxidation of the sample for oxidative hydrolysis, 1 g of the freeze-dried sample was weighed out into an Erlenmeyer flask. An oxidation mixture of 90 mL formic acid and 10 mL hydrogen peroxide was prepared. This mixture was left for 2 hours at 22 °C, then cooled for 15 minutes at 10 °C. Next, 15 mL of the oxidation mixture was added to the sample. After careful blending of the mixture with the sample, the flask was left for 24 hours at 10 °C. Then, 1 mL of concentrated hydrochloric acid was added to the oxidized sample followed by 50 mL of hydrochloric acid (6 mol.L^{-1}). A reflux condenser was placed on the Erlenmeyer flask, and the flask was placed in an oil bath where it underwent oxygen hydrolysis for 24 hours at 118 °C. After hydrolysis, the contents of the flask were quantitatively transferred through filtration paper into a 250 mL volumetric flask, which was filled with 0.1 mol.L^{-1} hydrochloric acid. Then, 25 mL of the sample was pipetted into an evaporation flask. The subsequent filtration process was identical as in the case of the acid hydrolysis. Samples prepared in microcentrifuge tubes were analysed using the AAA 400 amino acid analyser. Amino acid separation proceeded in an ion-exchange column through increasing pH, regulated using sodium citrate buffers (buffer range: pH 2.6 – 7.9). After leaving the column, individual amino acids entered into a reaction with ninhydrin.

The subsequent process was the same as for determination of the milk samples.

Assessment of milk components

The milk was preserved using 2-bromo-2-nitropropane-1.3-diol and cooled to 4 – 6 °C until analysed. The analysis was carried out within hours of the sampling. Milk components were analysed in a commercial laboratory using a MilkoScan FT2 (Foss Electric, Hillerod, Denmark). Fat was established acidobutyrometrically according to standard CSN ISO 2446. Crude protein was established by the Kjeldahl method according to CSN 57 0530. Urea in milk was determined enzymatically using a commercially available urea/ammonia assay kit (Megazyme, Wicklow, Ireland) according to CSN 57 0533.

Statistics

The data were processed statistically using STATISTICA.CZ, version 10.0 (Czech Republic). Results are expressed as mean ±standard deviation (SD). Statistical significance was determined by examining the basic differences between among individual samples using ANOVA and Scheffé's test (two-way analysis, level of protein concentrates, day of sampling) for the parameters aspartic acid, proline, valine, tyrosine, arginine, threonine, glycine, methionine, phenylalanine, serine, alanine, isoleucine, histidine, glutamic acid, leucine and lysine. Differences with $p < 0.05$ were considered significant. Dependence between individual amino acids was expressed by means of a linear equation and correlation coefficient R.

RESULTS AND DISCUSSION

The study focused on evaluating the influence of this protein mixture on changes in the composition of amino acids in milk from the experimental animals. The values obtained for amino acid content were averaged within each of the two groups (control and experimental) and subsequently converted to percentages with average values at the beginning of the experiment representing 100%. The results are illustrated in continuous charts (at all times for four samplings).

In dairy cows, being fed with the protein concentrate, milk content of aspartic acid and proline increased with 22.3% ($p < 0.001$) and 9.5% ($p < 0.05$), respectively, while no changes were observed in milk from cows fed the control diet (Figure 2A and B). The cows receiving the protein supplement exhibited a decreasing trend for valine level in milk from the beginning of the experiment. At the end of the experiment, the overall decrease of this amino acid was 22.1% ($p < 0.05$), (Figure 2C). No significant changes were observed in amino acids such as tyrosine and arginine in the experimental and control groups of dairy cows (Figure 2D and E). The amount of threonine and glycine (Figure 2F and G) increased by 49.1% ($p < 0.001$) respectively by 34.9% ($p < 0.001$) in the experimental group. There were no significant differences found in the evaluation of methionine, phenylalanine and serine in the experimental and control groups of cows (Figure 2H, I and J). Alanine (Figure 2K) showed no significant differences in the control group. The experimental group exhibited increasing amounts of this amino acid from the beginning of the experiment, and the amount of alanine at the fourth sampling was higher by 30% ($p < 0.01$). In milk of dairy cows, no significant changes of histidine and isoleucine amount were reported in the experimental and control groups (Figure 2L and M). The increase of glutamic acid (Figure 2N) was observed of 16.5% ($p < 0.001$) in the test group of dairy cows with the addition of protein concentrate. Amino acids such as leucine and lysine were not significantly changed in the experimental and control group of cows (Figure 2O and P).

Inter-relationships among the individual amino acids were ascertained by calculating Pearson correlation coefficients. As shown in Table 4, the highest correlation coefficients were found between histidine and phenylalanine ($R^2 = 0.951$), phenylalanine and leucine ($R = 0.925$), and histidine and leucine ($R = 0.921$).

In evaluating milk yield and the amounts of protein and fat, no significant difference was found between cows of the control and experimental groups. Urea concentration in the milk of the experimental group was significantly different starting from the second sampling. By the end of the experiment, the urea in the milk had increased by 83.2% for the experimental group ($p < 0.001$) in comparison with the first sampling, as can be seen in Table 5.

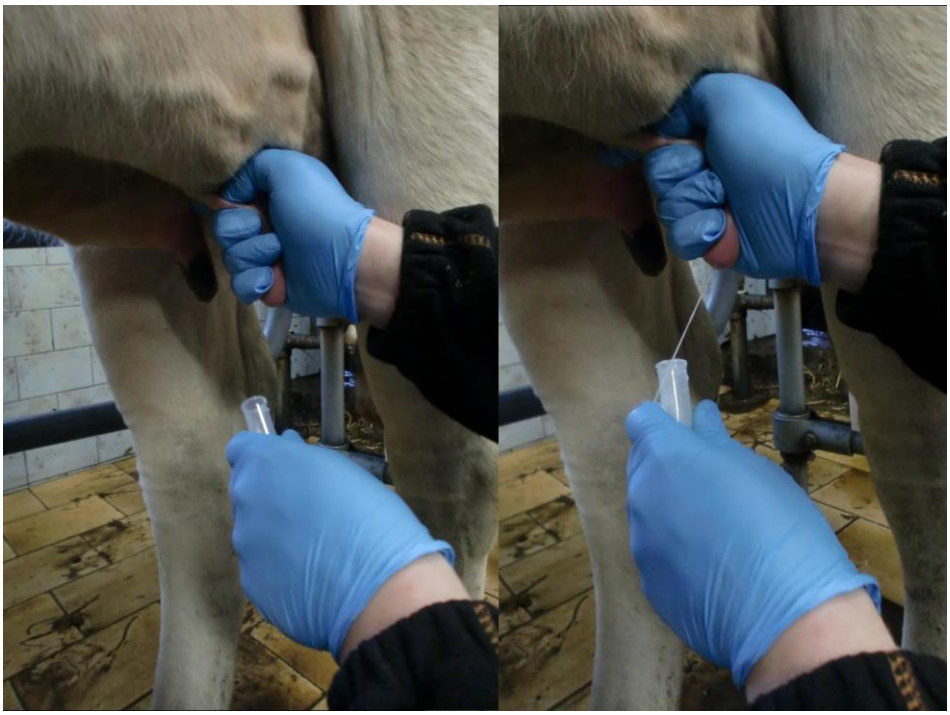

Figure 1 The sampling of milk.

In our experiment, increases in the content of the amino acids aspartic acid, threonine, glycine, serine, and alanine were observed following protein concentrate supplementation. The protein supplement was also associated with increased urea level in milk while milk yield as well as fat and protein content were not affected.

The direct addition of amino acids (methionine, histidine, phenylalanine) in the diet of dairy cows increased the concentrations of these amino acids not only in the blood and in milk as well. Protein concentrate has not brought this effect in our monitoring **(Sizova and Zelenina, 2010)**. It proves to be more effective to add abductive methionine and lysine directly into the diet of dairy cows in comparison with protein concentrate. Abductive methionine and lysine increase directly the content of these amino acids in milk. Soya, linen and sunflower cake did not prove to be so effective. In an experiment conducted in Poland, a basic feed ration (maize silage, alfalfa silage and sugar beet pulp) was supplemented with linseed cake at 10% of the total ration. In that case, cows fed the supplemental linseed cake had higher milk production. The milk produced had lower fat and protein content compared to a control group of dairy cows without linseed cake supplementation **(Osieglowski et al., 2007)**. In contrast, no significant differences were found out in performance of dairy cows in our monitoring. The significant increase of

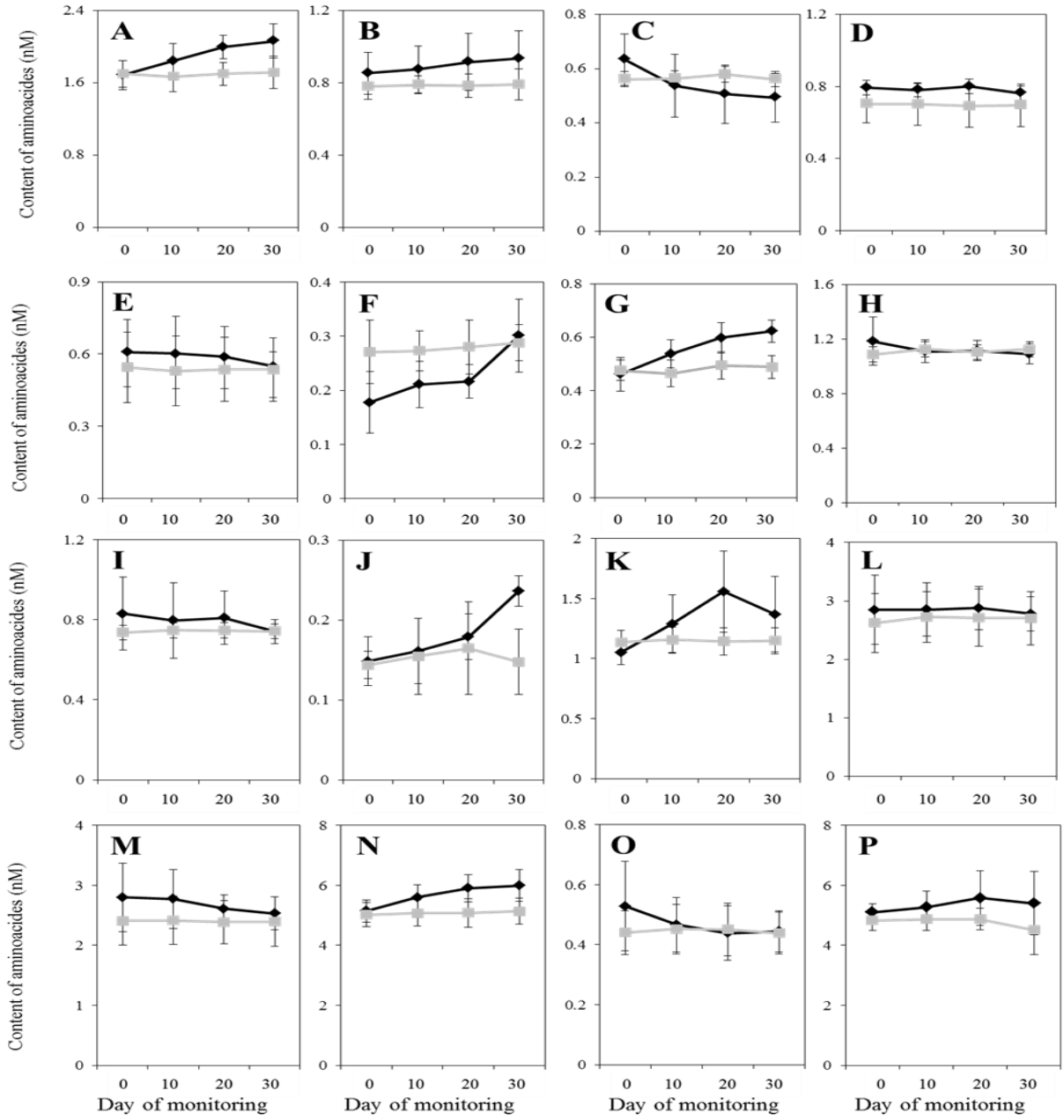

Figure 2 Effect of protein concentrate on amino acid contents in milk during 30 days of experimentation. Note: A = aspartic acid, B = proline, C = valine, D = tyrosine, E = arginine, F = threonine, G = glycine, H = methionine, I = phenylalanine, J = serine, K = alanine, L = isoleucine, M = histidine, N = glutamic acid, O = leucine, P = lysine. Black connecting line illustrates development in the control group of cows (n=10). Grey connecting line illustrates development in the experimental group of cows (n = 10).

Table 4 Correlations among the studied amino acids occurring in milk of experimental group of cows.

	Asp	Pro	Val	Tyr	Arg	Thr	Gly	Met	Phe	Ser	Ala	Ile	His	Glu	Cys	Leu	Lys
Asp		0.7	-0.1	-0.2	0.0	-0.3	-0.2	-0.1	-0.5	-0.6	0.7	-0.5	-0.3	0.5	0.1	-0.4	0.5
Pro	0.7		-0.3	-0.2	-0.4	-0.4	-0.3	-0.2	-0.5	-0.3	0.6	-0.4	-0.4	0.5	0.0	-0.4	0.3
Val	-0.1	-0.3		0.8	0.9	0.1	-0.4	0.9	0.7	0.8	-0.7	0.6	0.8	-0.7	0.7	0.9	-0.8
Tyr	-0.2	-0.2	0.8		0.7	-0.1	-0.4	0.7	0.6	0.8	-0.6	0.9	0.6	-0.5	0.4	0.8	-0.8
Arg	0.0	-0.4	0.9	0.7		0.1	-0.1	0.9	0.8	0.5	-0.6	0.7	0.9	-0.7	0.8	0.9	-0.6
Thr	-0.3	-0.3	0.1	-0.1	0.1		0.3	-0.1	0.2	0.1	-0.2	0.0	0.3	0.0	0.1	0.1	-0.2
Gly	-0.2	-0.3	-0.4	-0.4	-0.1	0.3		-0.3	0.0	-0.3	0.2	0.0	0.1	0.1	0.0	-0.2	0.3
Met	-0.1	-0.2	0.9	0.7	0.9	-0.1	-0.3		0.8	0.7	-0.7	0.6	0.8	-0.9	0.9	0.9	-0.8
Phe	-0.5	-0.5	0.7	0.6	0.8	0.2	0.0	0.8		0.6	-0.9	0.8	0.9	-0.9	0.7	0.9	-0.8
Ser	-0.6	-0.3	-0.7	0.8	0.5	0.1	-0.3	0.7	0.6		-0.8	0.7	0.6	-0.7	0.5	0.8	-0.9
Ala	0.7	0.6	0.6	-0.6	-0.6	-0.2	0.2	-0.7	-0.9	-0.8		-0.8	-0.8	0.9	-0.4	-0.9	0.9
Ile	-0.5	-0.4	0.8	0.9	0.7	0.0	0.0	0.6	0.8	0.7	-0.8		0.7	-0.6	0.4	0.8	-0.8
His	-0.3	-0.4	0.8	0.6	0.9	0.3	0.1	0.8	0.9	0.6	-0.8	0.7		-0.8	0.8	0.9	-0.8
Glu	0.5	0.5	-0.7	-0.5	-0.7	0.0	0.1	-0.9	-0.9	-0.7	0.9	-0.6	-0.8		-0.7	-0.9	0.8
Cys	0.1	0.0	0.7	0.4	0.8	0.1	0.0	0.9	0.7	0.5	-0.4	0.4	0.8	-0.7		0.8	-0.9
Leu	-0.4	-0.4	0.9	0.8	0.9	0.1	-0.2	0.9	0.9	0.8	-0.9	0.8	0.9	-0.9	0.8		0.9
Lys	0.5	0.3	-0.8	-0.8	-0.6	-0.2	0.3	-0.8	-0.8	-0.9	0.9	-0.8	-0.8	0.8	-0.6	-0.9	

Note: lys = lysine, leu = leucine, cys = cysteine, glu = glutamic acid, his = histidine, ile = isoleucine, ala = alanine, ser = serine, phe = phenylalanine, met = methionine, gly = glycine, thr = threonine, arg = arginine, tyr = tyrosine, val = valine, pro = proline, asp = aspartic acid.

urea was observed in milk. At the same time, another group of authors confirm that supplementation by protein feed increases milk yield and milk protein **(Choi et al., 2002)**. None of these effects were observed in our case. In another reported experiment, cottonseed cake was fed at 0% (control group), 5%, 10% and 15% of the total ration **(Madzimure et al., 2011)**. In that case, the control group showed the highest daily milk yield (12.1 kg.day^{-1}) while the lowest milk yield was recorded in dairy cows fed with the ration containing 15% cottonseed cake (7.5 kg.day^{-1}). In comparison with cows in the experimental groups, those in the control group had the highest amount of fat in their milk (p <0.05). The experimental group of cows receiving 15% cottonseed cake exhibited a significantly higher percentage of milk protein (p <0.05) as compared with the control group. It was not possible to confirm an increase in milk's protein content in our case. In our view, the dose of the protein concentrate would be necessary to increase to investigate the effect on milk production. In another experiment with Holstein cows, a protein concentrate with rumen-protected methionine was added to the cows' diet. The milk of cows receiving the methionine-enriched diet exhibited increased protein (p <0.05) as compared with the milk of cows without the supplement **(Třináctý et al., 2009)**. In an experiment involving goats, those receiving rumen-protected soybean protein showed a significantly higher percentage (3.54%) of fat in their milk (p <0.001) as compared with the milk fat percentage for the control group of animals without the supplement (3.14%). That experimental group had exhibited a similar increase in the concentration of urea (p <0.05), **(Chowdhury et al., 2002)**.

The influence of additions of protein concentrates and additions of mineral compounds were tested on dairy cow yield in the ecological farming. Although, mineral compounds (selenium) did not increase the yield of dairy cow, the animal health was increased. From this reason, the combination of protein concentrates is recommended in combination with antioxidants leads to improve the quality of milk and health state of animals **(Horký 2015)**. According to several authors, the mycotoxins could decrease the protein bioavailability in the dairy cow diets **(Horký and Cerkal, 2014)**. Not only mycotoxins have an impact on protein digestibility, but the content of phytoestrogens could decrease the utilization of nitrogenous compounds. For further monitoring, it would be beneficial to study these factors **(Hloucalová et al., 2016)**. According to these results, it can be suggested that the addition of abductive methionine and soya protein has a higher efficiency to increase proteins in milk than protein concentrate used in our monitoring. The abduction of cake could be the part of further research with the aim to rise up by-pass protein. When feeding soybeans to dairy cows, researchers have used two different treatments. In the first method, feeding roasted soybeans yielded no significant differences in the concentrations of fat and protein in milk.

It also did not affect organic matter digestibility. In our experiment, the experimental group of dairy cows

Table 5 Effect of supplemental protein concentrate on the components of milk as measured by both quantitative and qualitative indicators.

	Control group				Experimental group			
Days of experiment	Milk yield (kg)	Protein (g.100mL^{-1})	Fat (g.100mL^{-1})	Urea (g.100mL^{-1})	Milk yield (kg)	Protein (g.100mL^{-1})	Fat (g.100mL^{-1})	Urea (mg.100mL^{-1})
0	26.9 ±1.9	3.4 ±0.2	4.2 ±0.3	26.2 ±4.5	27.1 ±2.9	3.2 ±0.2	4.2 ±0.1	21.4 ±3.2
10	28.2 ±2.3	3.5 ±0.3	4.3 ±0.6	25.1 ±3.2	28.9 ±1.6	3.3 ±0.3	4.3 ±0.3	37.4 ±3.1***
20	27.8 ±1.8	3.3 ±0.1	4.1 ±0.5	22.2 ±3.2	28.3 ±1.6	3.3 ±0.2	4.1 ±0.3	37.9 ±5.2***
30	27.6 ±2.0	3.4 ±0.2	4.4 ±0.4	25.3 ±3.6	27.5 ±4.2	3.4 ±0.2	4.5 ±0.4	39.2 ±4.0***

Note: ***Statistically significant difference in comparison with the first sampling (p <0.001).

exhibited a significant decrease of valine in their milk, which could point to a decreased rate of catabolism in these animals.

CONCLUSION

In our experiment, organically managed dairy cows were fed a protein concentrate based on linseed, sunflower and soybean cakes. The protein concentrate supplementation significantly increased the amounts in milk of aspartic acid (+22.3%; p <0.001), proline (+9.5%; p <0.05), threonine (+49.1%; p <0.001), glycine (+34.9%; p <0.001), alanine (+30.0%; p <0.01) and glutamic acid (+16.5%; p <0.001). The control group of cows exhibited stable amino acids content in their milk throughout the experiment. The results demonstrate a favourable influence of feeding the protein concentrate on the contents of selected amino acids in the milk of cows. Differences in the aforementioned amino acids can increase the nutritive value of the milk produced, and such product therefore becomes more valuable for final consumers.

REFERENCES

Avondo, M., Pagano, R. I., De Angelis, A., Pennisi, P. 2013. Diet choice by goats as effect of milk production level during late lactation. *Animal*, vol. 7, no. 7, p. 1113-1118. http://dx.doi.org/10.1017/S1751731113000244

Banach, J. C., Lin, Z., Lamsal, B. P. 2013. Enzymatic modification of milk protein concentrate and characterization of resulting functional properties. *Lwt-Food Science and Technology*, vol. 54, no. 2, p. 397-403. http://dx.doi.org/10.1016/j.lwt.2013.06.023

Běláková, S., Benešová, K., Mikulíková, H., Svoboda, M., The occurrence of ochratoxin A in beers, *Kvasný průmysl*, 2015, vol. 61, p. 34-37.

Čermáková, J., Kudrna, V., Illek, J., Blažková, K., Haman, J. 2012. Effects of a rumen-protected form of methionine and a methionine analogue on the lactation performance of dairy cows. *Czech Journal of Animal Science*, vol. 57, no. 9, p. 410-419.

Espejo-Carpio, F. J., De Gobba, C., Guadix, A., Guadix, E. M., Otte J. 2013. Angiotensin I-converting enzyme inhibitory activity of enzymatic hydrolysates of goat milk protein fractions. *International Dairy Journal*, vol. 32, no. 2, p. 175-183. http://dx.doi.org/10.1016/j.idairyj.2013.04.002

Gustavsson, F., Buitenhuis, A. J., Johansson, M., Bertelsen, H. P., Glantz, M., Poulsen, N. A., Mansson, H. L., Stalhammar, H., Larsen, L. B., Bendixen, C., Paulsson, M., Andren, A. 2014. Effects of breed and casein genetic variants on protein profile in milk from Swedish Red, Danish Holstein, and Danish Jersey cows. *Journal of Dairy Science*, vol. 97, no. 6, p. 3866-3877. http://dx.doi.org/10.3168/jds.2013-7312

Herbut, P. 2013. Temperature, humidity and air movement variations inside a free-stall barn during heavy frost. *Annals of Animal Science*, vol. 13, no. 3, p. 587-596. http://dx.doi.org/10.2478/aoas-2013-0025

Hloucalová, P., Skládanka, J., Horký, P., Klejdus, B., Pelikán, J., Knotová, D. 2016. Determination of phytoestrogen content in fresh-cut legume forage. *Animals*, vol. 46, no. 6, p. 1-15. https://doi.org/10.3390/ani6070043

Horký, P. 2014. Effect of protein concentrate supplement on the qualitative and quantitative parameters of milk from dairy cows in organic farming. *Annals of Animal Science*, vol. 14, no. 2, p. 341-352. http://dx.doi.org/10.2478/aoas-2014-0008

Horký, P. 2015. Effect of selenium on its content in milk and performance of dairy cows in ecological farming. *Potravinarstvo*, vol 9. no. 1, p. 219-224. https://doi.org/10.5219/492

Horký, P., Cerkal, R. 2014. Effect of Fungicidal Treatment on Digestibility of Mycotoxins In Vitro. *Potravinarstvo*, vol. 8, no. 1, p. 328-332. https://doi.org/10.5219/409

Horký, P., Jančíková, P., Sochor, J., Hynek, D., Chavis, G.J., Ruttkay-Nedecký, B., Cernei, N., Zítka, O., Zeman, L., Adam, V., Kizek, R. 2012. Effect of Organic and Inorganic Form of Selenium on Antioxidant Status of Breeding Boars Ejaculate Revealed by Electrochemistry. *International Journal of Electrochemical Science*, vol. 7, no. 10, p. 9643-9657.

Horký, P., Ruttkay-Nedecký, B., Kremplová, M., Kryštofová, O., Kensová, R., Hynek, D., Babula, P., Zítka, O., Zeman, L., Adam, V., Kizek, R. 2013. Effect of Different Doses of Organically Bound Selenium on Antioxidant Status and Levels of Metal Ions in Postpartum Sows. *International Journal of Electrochemical Science*, vol. 8, no. 5, p. 6162-6179.

Choi, C. W., Vanhatalo, A., Ahvenjarvi, S., Huhtanen, P. 2002. Effects of several protein supplements on flow of soluble non-ammonia nitrogen from the forestomach and milk production in dairy cows. *Animal Feed Science and Technology*, vol. 102, no. 1-4, p. 15-33. http://dx.doi.org/10.1016/S0377-8401(02)00251-1

Chowdhury, S. A., Rexroth, H., Kijora, C., Peters, K. J. 2002. Lactation performance of German Fawn goat in relation to feeding level and dietary protein protection. *Asian-Australasian Journal of Animal Sciences*, vol. 15, no. 2, p. 222-237. https://doi.org/10.5713/ajas.2002.222

Jančíková, P., Horký, P., Zeman, L. 2012. The effect of feed additive containing vitamins and trace elements on the elements profile and growth of skin derivatives in horses. *Annals of Animal Science*, vol. 12, no. 3, p. 381-391.

Křížová, L., Hanuš, O., Roubal, P., Kučera, J., Hadrová, S. 2013. The effect of cattle breed, season and type of diet on nitrogen fractions and aminu acid profile of raw milk. *Archiv Tierzucht*, vol. 56, no. 71, p. 709-718.

Lipinski, K., Skorko-Sajko, H., Purwin, C., Antoszkiewicz, Z., Werpachowski, M. 2013. Effect of xylanase supplementation to cereal-based diets on apparent fecal digestibility and growth performance of pigs. *Annals of Animal Science*, vol. 13, no. 2, p. 303-311. http://dx.doi.org/10.2478/aoas-2013-0011

Madzimure, J., Musimurimwa, C., Chivandi, E., Gwiriri, L., Mamhare, E. 2011. Milk yield and quality in Guernsey cows fed cottonseed cake-based diets partially substituted with baobab (Adansonia digitata L.) seed cake. *Tropical Animal Health and Production*, vol. 43, no. 1, p. 77-82. http://dx.doi.org/10.1007/s11250-010-9656-5

Masoero, F., Gallo, A., Zanfi, C., Giuberti, G., Spanghero, M. 2011. Effect of nitrogen fertilization on chemical composition and rumen fermentation of different parts of plants of three corn hybrids. *Animal Feed Science and Technology*, vol. 164, no. 3-4, p. 207-216. http://dx.doi.org/10.1016/j.anifeedsci.2011.02.001

Matthaus, B. 2004. Camelina sativa - revival of an old vegetable oil? *Ernährungs-Umschau*, vol. 51, no. 1, p. 12-16.

Maxin, G., Ouellet, D. R., Lapierre, H. 2013. Effect of substitution of soybean meal by canola meal or distillers grains in dairy rations on amino acid and glucose availability. *Journal of Dairy Science*, vol. 96, no. 12, p. 7806-7817. http://dx.doi.org/10.3168/jds.2013-6976

Mertens, D. R., Allen, M., Carmany, J., Clegg, J., Davidowicz, A., Drouches, M., Frank, K., Gambin, D., Garkie, M., Gildemeister, B., Jeffress, D., Jeon, C.S., Jones, D., Kaplan, D., Kim, G. N., Kobata, S., Main, D., Moua, X., Paul, B., Robertson, J., Taysom, D., Thiex, N., Williams, J., Wolf, M. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. *Journal of AOAC Internatioanl*, vol. 85, no. 6, p. 1217-1240. PMid:12477183

Nevrkla, P., Čechová, M., Hadaš, Z. 2013. Evaluation of selected reproductive parametres in gilts and loss of piglets after repopulation. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, vol. 61, no. 5, p. 1357-1364. http://dx.doi.org/10.11118/actaun201361051357

Osieglowski, S., Strzetelski, J. A., Zymon, M., Kowalczyk, J. 2007. Effect of fish oil and high or low linolenic acid linseed or rapeseed on fatty acid composition of cow's milk. *Annals of Animal Science*, vol. 7, no. 1, p. 63-74.

Siciliano, R. A., Mazzeo, M. F., Arena, S., Renzone, G., Scaloni, A. 2013. Mass spectrometry for the analysis of protein lactosylation in milk products. *Food Research International*, vol. 54, no. 1, p. 988-1000. http://dx.doi.org/10.1016/j.foodres.2012.10.044

Sizova, Y. V., Zelenina, A. S. 2010. Productivity and technological properties of cow milk at various aminoacid composition of metabolic protein ration. *Sel'skokhozyaistvennaya Biologiya*, no. 6, p. 50-53.

Svoboda, Z., Mikulíková, H., Pluháčková, H., Běláková, S., Benešová, K. 2016. Analysis of Essential Oils Usable for Fortification of Food Products, *Kvasný průmysl*, no. 62, p. 157-160.

Třináctý, J., Křížová, L., Richter, M., Černý, V., Říha, J. 2009. Effect of rumen-protected methionine, lysine or both on milk production and plasma amino acids of high-yielding dairy cows. *Czech Journal of Animal Science*, vol. 54, no. 6, p. 239-248.

Vargas-Bello-Perez, E., Vera, R. R., Aguilar, C., Lira, R., Pena, I., Fernandez, J. 2013. Feeding olive cake to ewes improves fatty acid profile of milk and cheese. *Animal Feed Science and Technology*, vol. 184, no. 1-4, p. 94-99. http://dx.doi.org/10.1016/j.anifeedsci.2013.05.016

Zeman, L., Doležal, P., Mrkvicová, E., Prochazková, J., Ryant, P., Skládanka, J., Straková, E., Suchý, P., Veselý, P., Zelenka, J. 2006. *Výživa a krmení hospodářských zvířat*. 1st ed. PRAHA, Czech Republic : Profi Press. 360 p. ISBN 80-86726-17-7.

Acknowledgments:

This project was funded from grants IGA TP1/2016 New trend in the cultivation and use of milk thistle (*Silybum Marianum* L.) in agriculture.

Contact address:

Pavel Horky, Mendel University in Brno, Faculty of Agronomy, Department of Animal Nutrition and Forage Production, Zemedelska 1, 613 00 Brno, Czech Republic, E-mail: pavel.horky@mendelu.cz

Jiří Skládanka, Mendel University in Brno, Faculty of Agronomy, Department of Animal Nutrition and Forage Production, Zemedelska 1, 613 00 Brno, Czech Republic, E-mail: jiri.skladanka@mendelu.cz

Pavel Nevrkla, Mendel University in Brno, Faculty of Agronomy, Department of Animal Breeding, Zemedelska1, 613 00 Brno, Czech Republic, E-mail: pavel.nevrkla@mendelu.cz

Daniel Falta, Mendel University in Brno, Faculty of Agronomy, Department of Animal Breeding, Zemedelska1, 613 00 Brno, Czech Republic, E-mail: daniel.falta@mendelu.cz

Iva Čáslavová, Mendel University in Brno, Faculty of Agronomy, Department of Animal Nutrition and Forage Production, Zemedelska 1, 613 00 Brno, Czech Republic, E-mail: iva.caslavova@mendelu.cz

Pavel Knot, Mendel University in Brno, Faculty of Agronomy, Department of Animal Nutrition and Forage Production, Zemedelska 1, 613 00 Brno, Czech Republic, E-mail: pavel.knot@mendelu.cz