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EFFECT OF PROTEIN CONCENTRATE SUPPLEMENT ON THE QUALITATIVE AND QUANTITATIVE PARAMETERS OF MILK FROM DAIRY COWS IN ORGANIC FARMING*

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Abstract

In our experiment, we studied the effects of protein concentrate addition on the qualitative and quantitative composition of milk in organically farmed dairy cows. A total number of 40 Holstein cows were divided into two groups. Live weight of dairy cows was around 625±25 kg. Average production efficiency of animals was 7600±50 kg milk for the lactation period. Animals of both groups received identical basal feed ration. The first experimental group of cows (n=20) received in the feed ration a protein concentrate from organic production (soybean cake 60%, sunflower cake 20%, linseed cake 20%) at 1 kg per head and day. The second group served as a control (n=20) without the addition of protein concentrate. The experiment lasted 30 days. The goal of the experiment was to investigate whether the addition of protein concentrate can affect individual milk components. The measured values show that the experimental group of dairy cows with the addition of protein concentrate exhibited increases in fat content by 7.4% (P≤0.05), in urea content by 83.1% (P≤0.001) and in citric acid content by 18.6% (P≤0.01), and decreases in free fatty acids by 54.9% (P≤0.001), in ECM by 5.5% (P≤0.05) and in FCM by 6.1% (P≤0.05). The control group of cows exhibited the citric acid content in milk increased by 20.0% (P≤0.05). The results indicate that the protein concentrate composed of soybean, sunflower and linseed cakes can affect the composition of milk from organically farmed dairy cows.

Key words: protein concentrate, milk, cows, organic farming

The importance of organic farming has been recently increasing – Figures 1 A and 1 B (Willer and Kilcher, 2009). The trend is also supported by the policy of the European Union largely promoting organic farming (Lelyon et al., 2012). Protein supplements represent an indispendable component of feed rations for high-yielding dairy cows. However, feeding of ruminants with animal proteins and fishmeal was

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banned in the European Union (Commission Decision 2001/25/EC) due to spreading the BSE (bovine spongiform encephalopathy) disease in beef cattle. Protein sources coming into consideration in the Czech Republic (and the EU) are soybean, oilseed rape, sunflower, legumes and possibly some products of oil industry – extracted meals (Homolka and Kudrna, 2006). With the growing efficiency of the production of dairy cows, which has been lately recorded, the nutrition and feeding of animals become ever more demanding. This primarily concerns the first stage of lactation when the need of basic nutrients (energy and N-substances) can be covered by common feeds only with difficulties (Tetens et al., 2013). Protein concentrate can affect milk components by which the farm economy can be improved (Danes et al., 2013). The use of protein concentrates (based on soybean) in organically farmed dairy cows can increase the content of individual components by up to 10% (Hoshide et al., 2011). The objective of this experiment was to verify whether a protein concentrate can increase the contents of individual milk components in organically farmed dairy cows.

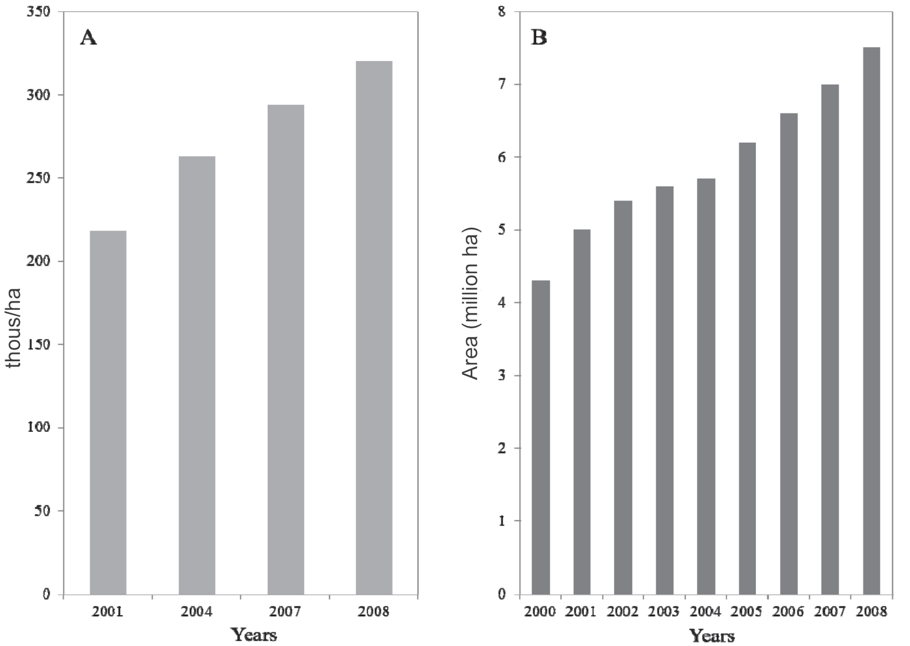

Figure 1. Organic area in the Czech Republic in 2001, 2004, 2007 and 2008 ('000 ha) – (A); Evolution of the area involved in the organic sector in the EU-25 (million ha) – (B) – (Anonymous, 2010)

Material and methods

The experiment was conducted on the organic farm of dairy cattle in Lesoňovice (Czech Republic) and included 40 Holstein dairy cows divided into two equal groups at the same stage of lactation. The experimental cows were in the third and fourth lactation. Live weight of dairy cows was around 625±25 kg. Average production efficiency of animals was 7600±50 kg milk for the lactation period. All animals were fed a basic feed ration in the form of TMR (Table 1) and had *ad libitum* access to water. The basic composition of nutrients in the respective feeds is presented in Table 3. The first group of dairy cows had the basal feed ration supplemented with a protein concentrate (Table 2) at 1 kg per head and day.

Table 1. Composition of the feed ration for dairy cows

Fodder	Amount (kg)	Dry matter (kg)
Maize silage	22.00	6.38
Clover-grass haylage – first cut	17.00	5.95
Grass haylage – first cut	10.00	4.00
Winter wheat	3.00	2.64
Spring barley	2.00	1.76
Protein concentrate*	1.00	0.88
Detamin GA Spezial	0.15	0.13

Detamin GA Spezial – mineral supplement for ruminants in organic farming (Germany).
*Protein concentrate was not fed to the control group of dairy cows.

Table 2. Composition of the protein concentrate

Component	Amount (%)
Soybean cake*	60.00
Sunflower cake*	20.00
Linseed cake*	20.00

* Raw materials originating from organic farming.
Concentration of crude protein = 36.60%.
Concentration of fat = 10.00%.

Table 3. Nutrient contents in the respective feeds – 100% dry matter (Zeman et al., 1995)

Fodder	Crude protein (g)	PDIN (g)	PDIE (g)	NEL (MJ)
Maize silage	93.6	42.4	63.0	6.9
Clover-grass haylage – first cut	171.0	98.2	72.1	5.3
Grass haylage – first cut	125.7	19.2	71.5	6.3
Winter wheat	130.0	88.7	111.2	9.0
Spring barley	135.0	88.9	104.6	8.2
Soybean cake	479.1	336.0	209.5	8.4
Sunflower cake	358.0	231.3	110.9	5.8
Linseed cake	373.2	257.6	174.9	7.5

PDIN = PDIA + PDIMN; PDIE = PDIA + PDIME.
PDIA – non-degraded fodder protein actually digestible in the small intestine.
PDIMN – amount of microbial protein, which can be synthesized in the rumen from the degraded fodder protein if the content of available energy and other nutrients is not limiting.
PDIME – amount of microbial protein, which can be synthesized in the rumen from available energy of the content if degraded fodder protein and other nutrients is not limiting (Zeman et al., 2006).

The protein concentrate was mixed of raw materials originating from organic farming. The second group of dairy cows served as a control and did not receive any additional protein concentrate in the feed ration. Values of nutrients contained in the feed ration for the two groups are presented in Table 4. The experiment started with the experimental group of dairy cows on day 52 of lactation on average (stage of lactation ranged from 41 to 65 days). The average lactation period in the control group of cows at the beginning of the experiment was 56 days (stage of lactation ranged from 38 to 60 days). The cows were kept in loose housing. They were fed twice a day (morning and evening). The experimental period was set to 30 days. Milk samples were taken before the experiment and at its end (after 30 days). The milk samples were collected before the early feeding and then analysed.

Table 4. Total intake of nutrients in the feed ration

Fodder component	Experimental group	Control group
Starch (g)	3758.0	3721.0
Crude protein (g)	3313.0	2947.0
Fat (g)	613.0	513.0
PDIN (g)	2037.0	1764.0
PDIE (g)	1972.0	1838.0
NEL (MJ)	173.2	135.5
ADF (g)	6146.0	5948.0
NDF (g)	9467.0	9185.0

The values of nutrients were acquired by calculation according to tabular values.
Crude protein – N * 6.25.

Assessment of milk components

The milk was conserved by 2-bromo-2-nitropropane-1,3-diol and cooled to 4–6°C until analysed. The analysis was carried out within hours from the sampling. Milk components were analysed in a commercial laboratory on the MilkoScan FT 2 instrument (Foss Electric, Hillerod, Denmark). Fat was established acidobutyrometrically according to CSN ISO 2446. Crude protein was established by the Kjeldahl method according to CSN 57 0530 and lactose was ascertained on the commercial Lactose/D-Galactose Assay Kit MEGAZYME according to IDF 79B:1991. Urea in milk was determined enzymatically on the commercial Urea/Ammonia Assay Kit Megazyme. Free fatty acids were established by extraction-titration method according to CSN 57 0533. Somatic cells and citric acid were analysed by means of FTIR (Fourier Transform InfraRed) technology (MilkoScan, FT 6000) according to CSN EN ISO 13366-2.

Statistics

The data were processed using Microsoft Excel® and Statistica.Cz Version 10.0 (Czech Republic). Results are expressed as a least squares means ± standard deviation (SD) (Excel®). Values of statistical significance of differences among milk production, fat, protein, lactose, somatic cells, free fatty acids, urea and citric acid were ascertained using Statistica.Cz. This model was employed due to irregular calving of the cows. Differences with P≤0.05 were considered significant and were determined by Student's paired t-test, which was applied for the comparison of means. These values were detected in the respective monitored milk components based on differences between the first and the second milk sampling. Statistical model:

$$Y = groups + day\ of\ lactation + \varepsilon$$

Results

No health problems were observed in the experimental animals during the trial. Milk production, which ranged from 22.9 to 28.4 l/day (Figure 2 A), did not show significant differences in any of the groups of dairy cows. In assessing milk fat, we found a significant increase by 7.4% (P≤0.05) in the control group of animals as compared with the first sampling. The control group of cows exhibited a higher amount of milk fat, too (by 2.9%), but without statistical significance (Figure 2 B). The content of milk fat ranged from 3.7 to 5.9%. The content of milk proteins was practically at the same level in the experimental and control groups of dairy cows as it was before launching the experimental observation. The values of milk proteins ranged from 3.2 to 4.3% (Figure 2 C). The content of lactose did not show any significant changes in the two groups of animals (Figure 2 D) and ranged from 4.2 to 5.2%.

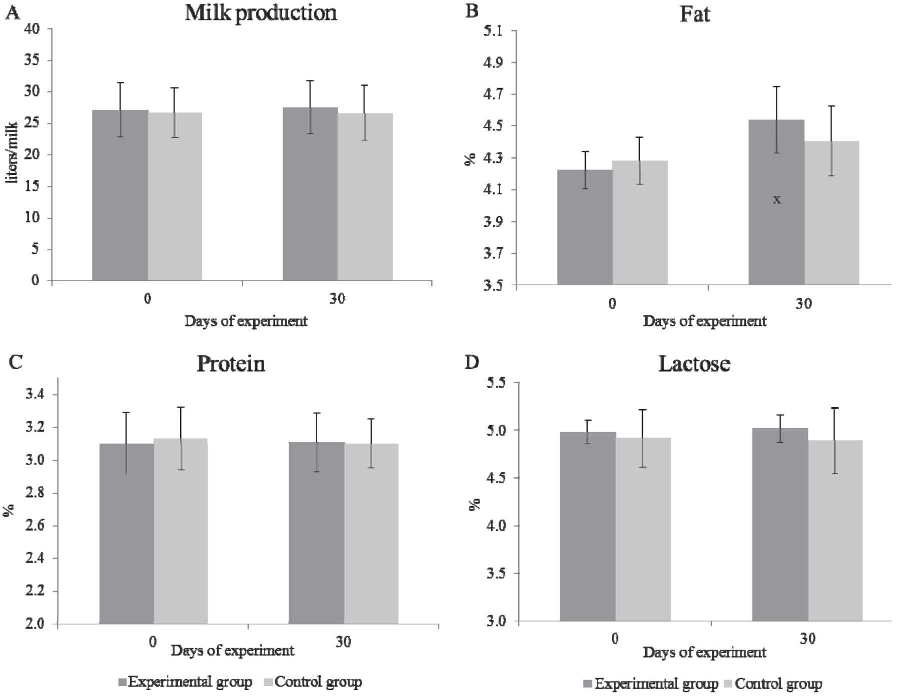

Figure 2. The effect of protein concentrate supplement on the production of milk (A), fat content (B), protein content (C) and lactose content (D).
x = statistical significance P≤0.05 between day 0 and day 30 of the experiment

As compared with the first sampling, somatic cells slightly increased in the experimental and control groups by 10.6% and 12.0%, respectively. This increase of somatic cells was, however, statistically non-significant (Figure 3 A) and ranged from 40.0 to 629.0 thousand/ml. As to free fatty acids, the experimental group of animals with the protein concentrate added in the feed ration showed a decrease by 54.9% ($P \leq 0.001$). A similar decrease was recorded also in the control group (by 26.8%) but was statistically non-significant (Figure 3 B). The values of free fatty acids ranged from 0.25 to 1.63 mmol/100 g of fat. Urea increased in the experimental group of cows by 83.1% ($P \leq 0.001$) as compared with the control sampling at the beginning of the trial. Compared with the control milk sampling, the control group of dairy cows exhibited a higher content of urea in milk (by 7.1%), too, which was, however, statistically non-significant (Figure 3 C). The concentrations of urea ranged from 16.7 to 36.4 mg/100 ml. Citric acid increased during the experimental period by 18.6% ($P \leq 0.01$) and the same pattern was recorded in the control group, in which the concentration of citric acid in milk increased by 20.0% ($P \leq 0.05$). The concentrations of citric acid ranged from 0.1 to 0.22 mmol/l (Figure 3 D). The assessment of ECM (energy corrected milk) showed an increase by 5.5% ($P \leq 0.05$) in the experimental group of dairy cows. The FCM (fat corrected milk) evaluation revealed a similar significant increase by 6.1% ($P \leq 0.05$) in the experimental group of cows (Table 5).

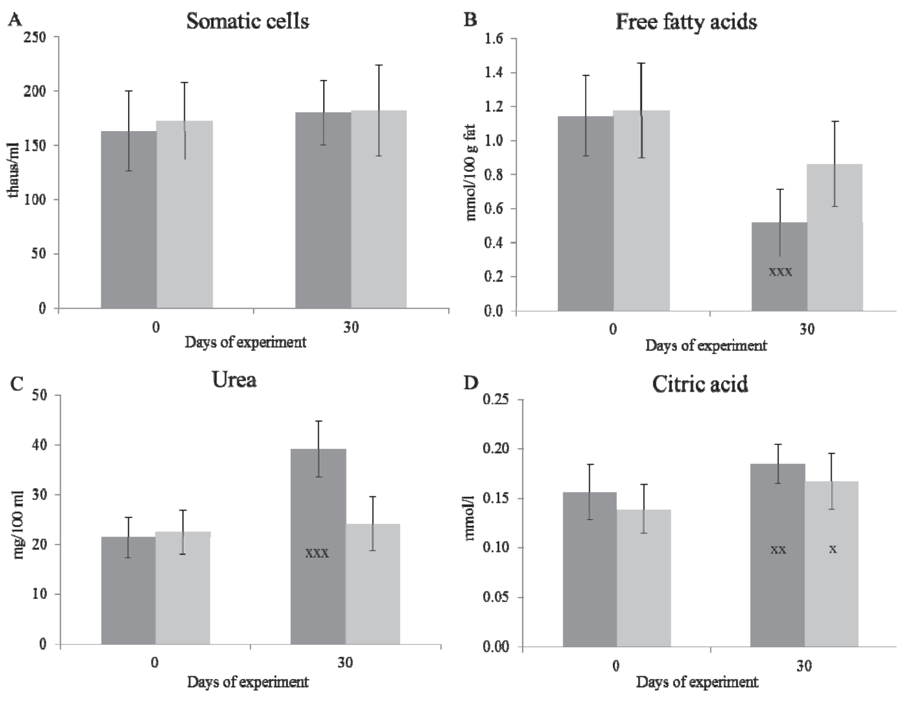

Figure 3. The effect of protein concentrate supplement on the concentration of somatic cells (A), free fatty acids (B), urea (C) and citric acid (D)
Statistical significance between day 0 and day 30 of the experiment = $P \leq 0.05$x, $P \leq 0.01$xx, $P \leq 0.001$xxx

Table 5. Milk quality indicators (daily milk production, ECM, FCM) in the individual dairy cows

Experimental group	Milk production (kg)		ECM		FCM	
Dairy cows	day 0	day 30	day 0	day 30	day 0	day 30
1	2	3	4	5	6	7
1_e	28.2	30.0	33.3	33.5	33.0	31.7
2_e	24.2	25.2	26.2	28.9	24.8	28.0
3_e	24.4	24.0	25.0	27.4	23.7	26.6
4_e	30.0	28.4	31.6	32.4	29.8	30.4
5_e	31.2	39.2	31.6	40.7	30.4	39.2
6_e	27.2	26.6	28.6	29.3	27.3	28.4
7_e	26.4	27.6	25.7	30.3	24.0	28.5
8_e	26.4	26.4	27.9	29.3	26.6	28.2
9_e	30.0	30.0	36.0	34.1	35.2	33.1
10_e	25.8	28.0	29.4	31.3	28.2	29.8
11_e	27.6	29.4	29.6	30.8	28.3	29.3
12_e	26.4	26.0	26.2	26.9	24.5	25.6
13_e	28.0	26.8	29.6	30.8	28.0	29.7
14_e	28.8	26.2	34.5	35.0	33.8	34.9
15_e	26.8	25.2	33.4	32.6	32.6	32.7
16_e	25.2	27.2	24.2	32.1	22.4	31.3
17_e	28.8	26.8	32.1	29.0	30.7	27.7
18_e	26.2	25.2	25.4	25.8	23.5	24.0
19_e	26.8	27.2	27.7	28.9	25.9	26.9
20_e	23.4	24.8	28.0	28.7	26.0	26.3
Average	27.1±2.0	27.5±3.2	29.3±3.4	30.9±3.3^{x}	27.9±3.8	29.6±3.5x
Control group	Milk production (kg)		ECM		FCM	
Dairy cows	day 0	day 30	day 0	day 30	day 0	day 30
1_c	38.0	38.0	43.3	43.0	40.9	40.2
2_c	30.0	30.0	35.4	35.6	33.6	33.6
3_c	22.8	21.4	29.1	29.1	27.4	27.5
4_c	34.0	34.0	42.1	41.0	38.7	36.6
5_c	30.0	30.0	28.5	27.9	26.2	25.5
6_c	23.2	20.8	28.5	24.9	26.4	24.4
7_c	22.0	21.2	23.2	22.7	22.0	21.2
8_c	23.2	24.4	24.1	25.8	22.3	23.5
9_c	32.0	32.0	36.5	36.3	33.2	33.2
10_c	22.8	24.4	25.2	30.0	23.0	28.3
11_c	25.8	24.4	30.3	30.6	27.8	28.2
12_c	28.0	30.0	31.8	34.2	30.4	32.3

Table 3 – contd.

1	2	3	4	5	6	7
13c	24.4	23.8	28.9	28.9	26.6	26.9
14c	14.4	15.2	15.2	17.1	14.0	16.1
15c	20.4	20.8	22.3	22.8	20.3	20.8
16c	25.4	30.0	26.1	31.1	24.4	29.1
17c	30.0	30.0	31.4	31.4	29.5	29.6
18c	32.0	32.0	35.9	34.7	33.2	32.0
19c	27.6	27.2	31.0	30.2	28.9	28.4
20c	26.2	25.2	27.9	28.0	26.4	26.3
Average	26.6±5.4	26.7±5.5	29.8±6.7	30.3±6.2	27.8±6.3	28.2±5.6

Statistical significance between day 0 and day 30 of the experiment = $P \leq 0.05^x$.

Model for ECM and FCM calculation:
*ECM (Energy corrected milk) = (0.3246 * kg milk) + (12.86 * kg fat) + (7.04 * kg protein)*
*FCM (Fat corrected milk – 4 %) = 0.4*kg milk+15.0*kg fat (Chen et al., 2008)*

Discussion

In an experiment in which dairy cows (conventional breeding) received three protein concentrates – soybean meal (407 g/day), rapeseed meal (510 g/day) and rapeseed cake (618 g/day), milk production efficiency was not affected by the addition of protein feeds and the difference between the groups was 0.8 l/day (Brzóska, 2008). Neither was affected the content of lactose, crude protein and urea in milk. Similar results were observed in our experiment, too. By contrast, the amount of whey significantly increased (P≤0.01) in rapeseed cake as compared with rapeseed meal. The group of dairy cows with the supplement of rapeseed cake exhibited the highest milk fat content, which was, however, without statistical significance. The cows were in the first stage of lactation (Brzóska, 2008). Marston et al. (2011) fed dairy cows in organic farming a standard ration based on maize (control group). The second (experimental) group received additional protein concentrate in the form of pellets. Contents of fats, proteins and lactose remained unchanged in the two groups of animals. The experimental group of dairy cows exhibited significantly increased intake of dry matter, and the authors observed, similarly as we did in our experiment, a higher amount of urea in milk. Similar results were observed in our experiment, too. In our experiment, we recorded a significantly higher milk fat content (P≤0.05), which can be explained by a higher dose of the protein concentrate in our trial. In another experiment, a basic feed ration (maize silage, alfalfa silage, sugar-beet pulp) was supplemented with 10% of linseed cake. The conclusions indicate that cows fed with the addition of linseed cake exhibited higher milk production. The produced milk had lower contents of fat, crude protein and lactose than the control group of dairy cows without the addition of linseed cake (Osięgłowski et al., 2007). After having been fed cotton cake at 0% (control group), 5%, 10% and 15%, the control group showed the highest daily milk yield of 12.1 kg/day while the lowest milk yield was recorded in dairy cows with the addition of 15% of cotton cake (7.5 kg/day). Cows in the control group had also the highest amount of milk fat ($P \leq 0.05$) as compared with the experimental groups of dairy cows. The experimental group of dairy cows with 15% of cotton cake in the feeding ration exhibited a significantly higher percentage of crude protein ($P \leq 0.05$) as compared with the control group. The content of lactose was identical in all groups (Madzimure et al., 2011). In our experiment, we recorded no increase of milk proteins but rather a significant increase of milk fat content. In an experiment with Holstein cows, a protein concentrate with protected methionine was added into the diet. In cows fed with the addition of methionine, the amount of milk proteins increased ($P \leq 0.05$) as compared with dairy cows fed without the addition of this amino acid (Trinacty et al., 2009). In an experiment in which two sources of protein were compared – rapeseed cake and soybean cake (similar components were used also in our experiment), higher production of milk was recorded when feeding rapeseed cakes, but without statistical significance. Both groups showed a significant increase of milk proteins ($P \leq 0.05$) as compared with the period before the beginning of the experiment (Shingfield et al., 2003). The addition of soybean protein at 800 g increased the intake of dry matter in dairy cows, which positively affected milk yield (Faverdin et al., 2003). In our experiment, no difference was found in the production of milk between the control and experimental groups of animals. Since it is not clear from both our and other authors' research whether the addition of protein concentrate affects production efficiency in dairy cows, this fact should be verified in follow-up studies. Goats that were fed with the protected soybean protein, exhibited a significantly higher percentage of milk fat (3.54%, $P \leq 0.001$) as compared with the control group of animals without the addition of soybean meal (3.14%). A similar increase was observed in the concentration of urea (which is an indicator of the intake of N-substances) and crude protein in the experimental group ($P \leq 0.05$); the results of this trial indicate that the addition of soybean meal can increase the intake of dry matter, too (Chowdhury et al., 2002). A similar increase of milk fat was recorded in our experiment. Mogensen and Kristensen (2002) fed organically farmed dairy cows a standard feeding ration based on maize (control group). The second (experimental) group was fed barley and rapeseed cake. The experimental group exhibited higher percentages of milk proteins and fat by 0.06% and 0.16%, respectively, without statistical significance. Milk yield and ECM did not significantly differ between the experimental group and the control group either. A similar experiment on Holstein dairy cows was conducted by Mogensen et al. (2004), who added 1 kg of rapeseed cake to the diet of cows (the same supplement dose was used in our study). The basal ration consisted of clover-grass silage. The addition of rapeseed cake affected neither production efficiency nor the levels of individual milk components. Velik et al. (2008) compared two concentrates in feeding organically farmed dairy cows. The first one was based on corn grains and the second one consisted of horse bean and pea. Milk yield was not affected in either of the two groups. Cows receiving the corn concentrate showed a significantly higher percent of milk proteins and a lower amount of urea in milk. In their experiment with feeding soybean, Chen et al. (2008) did not find significant differences in monitoring ECM and FCM between the experimental group and the control group of cows without the soybean supplement in the ration. In both groups, the levels of citric acid were low, likely due to energy deficit. Higher antioxidant potential of milk was measured in an experiment in which 9.8% of linseed cake was added to the feed ration for dairy cows (Cortes et al., 2012). The addition of linseed cake into the feeding ration of dairy cows at 0, 50, 100, 150 or 200 g/kg DM did not result in any difference among the experimental groups of animals during a period of 20 weeks. Monitored parameters were milk production, concentration of proteins, fat, lactose and somatic cells in milk. The amount of unsaturated fatty acids in the milk of dairy cows started to rise already from the dose of 50 g/kg DM (Petit and Gagnon, 2011). The results suggest that in spite of the fact that no increase was observed either in the production of milk or in the amount of milk components, the introduction of linseed cake into the feeding ration of dairy cows can improve the health potential of their milk. The increased production of urea indicates a body burden of crude protein with a potential damage to liver and kidneys. In our experiment, the amount of urea in the experimental group of dairy cows averaged 39.2 mg/100 ml. According to Aguilar et al. (2012), the level of urea in milk above 30 mg/100 ml indicates a surplus of crude protein and energy deficiency. An increased level of urea in milk (above 19 mg/100 ml) reduces the percentage of pregnancy by up to 20% (Butler et al., 1996). According to another study, the increased level of urea in milk (above 30 mg/100 ml) had no negative impact on the reproduction performance of cows (Gath et al., 2012).

In conclusion, our experiment studied the effect of protein concentrate supplement on the qualitative and quantitative composition of milk in organically farmed dairy cows. The measured values show that in the experimental group of dairy cows with the addition of protein concentrate the fat content increased by 7.4% ($P \leq 0.05$), urea increased by 83.1% ($P \leq 0.001$), citric acid increased by 18.6% ($P \leq 0.01$) and free fatty acids decreased by 54.9% ($P \leq 0.001$), ECM decreased by 5.5% ($P \leq 0.05$) and FCM decreased by 6.1% ($P \leq 0.05$). The control group of dairy cows exhibited the content of citric acid in milk increased by 20.0% ($P \leq 0.05$). Other parameters (crude protein, lactose and somatic cells) were not affected. These results demonstrate that a protein concentrate composed of soybean cake, sunflower cake and linseed cake can influence the composition of milk from dairy cows. Nevertheless, a potentially higher health risk has to be taken into account, as indicated by the higher production of urea in milk.

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