# THE INFLUENCE OF COW FEED ENRICHED WITH CARROTS ON MILK QUALITY AND NUTRITIONAL VALUE

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## Abstract

β-Carotene (BC) and α-tocopherol in milk fat have positive implications in human nutrition, besides the specifically protecting polyunsaturated fatty acids from oxidation. To determine the possible effect on some milk components, nutritional value and lipid stability of including carrots in the diet of lactating cows, ten cows were selected in a dairy farm and divided into 2 groups (control and experimental) by 5 cows in each. In experimental group's diet additionally 7 kg carrots per cow per day were included. Individual cow milk samples were obtained 1 day before feed enrichment, in days 7, 24, 35, 42 (during the feed enrichment), and 1 week after feed enrichment. Analyses of milk fat, protein content, somatic cell count (SCC), retinol, BC, vitamin C, tocopherols, immunoglobulins (Ig), lysozyme, fatty acids (FA) were made, and milk yield was measured. Supplying cow diet with carrots showed a tendency to improve milk quality by promoting the faster decrease of SCC, and significantly ($p<0.05$) increasing Ig and lysozyme content, thus potentially improving milk nutritional value. The vitamin content rose significantly ($p<0.05$) by retinol and tocopherol contents, but β-carotene content increase in milk was not observed. Also milk fatty acid (FA) stability changes during 5 day storage in temperature of 4 - 6 °C were not observed.

**Key words**: dairy products, nutritional value, antioxidants, carotenoids, forages.

## Introduction

The oxidative and lipolytic stability of milk and dairy products are of concern to the dairy industry. The deterioration processes can result in strong off-flavours and in decreased nutritional quality of dairy products, making them unacceptable to consumers. The oxidative stability of milk and dairy products is the result of delicate balance between anti- and pro-oxidative processes in milk influenced by factors such as degree of FA unsaturation, content of antioxidants as tocopherols and carotenoids, dissolved oxygen, storage temperature, metal prooxidants (especially copper) (Bergamo et al., 2003; Havemose et al., 2004; Smet et al., 2008; Żegarska, 2003). Carotenoids besides their well-known function as vitamin A precursors have other valuable properties. These properties include their anti-oxidant functions (radical and singlet oxygen quenching), role in cell differentiation, precursor of nuclear receptor ligand, and induction of cell-communication (Hulshof et al., 2006).

Concerning milk nutritional value, milk fat is one of the main sources of retinol, BC, vitamin E in the human diet; better absorption of BC from milk than from many other foods is also known (Swensson et al., 2007; Żegarska, 2003). Higher levels of α-tocopherol and BC in milk fat have positive implications in human nutrition: besides the specifically protecting polyunsaturated FA, it reduces cholesterol oxidation, and, therefore, its cytotoxicity and atherogenecity (Bergamo et al., 2003).

Carotenoids are abundant in plant material and appear in milk as the result of their ingestion by cow. There are often seasonal and regional variations of retinol and carotenoid concentrations, which reflect the nature of the forage used. However, BC is easily oxidized and concentrations decrease quickly during storage. Thus, to achieve a high-concentration of BC in milk, the importance of a cow diet with high-quality roughage, especially silage or pasture, cannot be underestimated (Swensson et al., 2007). Since animals cannot synthesize carotenoids and animal feed is generally poor in carotenoids, about 30 - 120 ppm of total carotenoids, are added to animal feed to improve animal health, enhance meat colour and quality, and increase vitamin A levels in milk and meat (Ananda and Vadlani, 2010). Animals in transition period or challenged with stress may benefit of a feeding strategy including antioxidants to inhibit free radical attacks and enhance the antioxidant status. High producing dairy cows are prone to oxidative stress, and the situation can be exacerbated under certain environmental, physiological, and dietary conditions. However, specific feeding strategies may contribute to enhance immunity and the antioxidant status (Petit, 2009). It has been demonstrated that carotenoids and retinol are able to reduce mastitis in dairy cows although the effect of BC was not systematic. Carotenoids also have a positive role in fertility independent of the role of retinol (Chew, 1995; Weiss, 2010).

The spontaneous oxidized flavour (SOF) of milk has been known for many years, predominantly as a seasonal problem arising in spring. It tends to occur in well-managed, high-yielding dairy herds. It has been suggested that the susceptibility of milk to SOF depends on the balance between α-tocopherol, BC and the polyunsaturated FA in the neutral lipids and phospholipids (Żegarska, 2003). Several reports have highlighted the beneficial action of α-tocopherol supplementation in cows feed on spontaneous oxidized flavour-related problems in milk,

but the influence of carotenoids is not as clearly defined (Nozière et al., 2006).

Carrot roots are rich source of carotenoids. Carotenes are the main representatives of carotenoids in carrot roots and constitute about 5.33 mg 100 $g^{-1}$ (Kotecha et al., 1998) or BC - 5.650 – 16.300 mg 100 $g^{-1}$ (Danish Food Composition Databank, 2009). The proportions of individual carotenoids reported are BC (45 - 80 g 100 $g^{-1}$), α-carotene (15 - 40 mg 100 $g^{-1}$), γ-carotene (2 - 10 mg 100 $g^{-1}$), and others (3 - 6 mg 100 $g^{-1}$) from the sum of all carotenoids. Thiamine, riboflavin, niacin, folic acid, and vitamin C are also present in appreciable amounts in carrot roots. Carrots are also good sources of carbohydrates and minerals like calcium, phosphorus, iron, and magnesium (Kotecha et al., 1998). In absorption of carotenoids as fat soluble constituents the presence of lipids is important. Fats and oils have been included in concentrate mixtures for dairy cows principally to increase the energy density of the diet, enabling high yielding cows in early lactation to attain their full milk yield potential. More recently lipids were included in an attempt to alter the fatty acid (FA) composition of milk fat, thereby improving its nutritional and physical properties. However, lipids, especially those containing polyunsaturated FA, have an adverse effect on rumen microflora and fibre digestibility, and at high levels in the diet usually result in reduced yields of milk and milk constituents. In practical diets the levels of fat added to concentrates generally do not exceed 5 g 100 $g^{-1}$ (Murphy et al., 1990).

The aim of this study was to assess the influence of cow feed enrichment with carotenoids on milk quality, nutritional value and lipid stability.

## Materials and Methods

***Experimental design***. Ten cows were selected in a conventional dairy farm "Strautiņi" and divided into control group (CG) and experimental group (EG) by 5 cows in each. Cow breed (Holstein, Latvian Brown), lactation number (3 - 5), stage of lactation and productivity were as similar as possible in both groups. The basic feed was equal in both groups, i.e. silage was fed to ad libitum and rapeseed animal feed – 2 kg per cow per day. In the EG diet additionally 7 kg carrots per cow per day were included (Table 1). Both groups also received rapeseed oil 100 g per cow per day, mixed in dry forage for better carotenoid absorption. The carotenoids content in cow feed is given in Table 1.

Table 1

**The characterization of cow feed**

| Cow groups | Basic feed | Additional feed | Content of carotenoids, mg per cow per day | | |
|---|---|---|---|---|---|
| | | | β-carotene | α-carotene | Total carotenes |
| Control | Silage – ad libitum; rapeseed animal meal – 2 kg per cow per day | Rapeseed oil – 100 g per cow per day | 207 | - | 225 |
| Experimental | | Carrots – 7 kg, rapeseed oil – 100 g per cow per day | 1090 | 221 | 1325 |

***Milk sample collection and storage***. Individual cow milk samples were obtained in days 0 (1 day before additional feed enriched with carotenoids was administered to the herd), 7, 24, 35, 42 (during the feed enrichment), and 1 week after special feeding (day 56) from afternoon milking. Equal amounts of each cow's milk were pooled together getting 1 sample of each group that were immediately cooled to 4 - 8 °C temperature and transported to the laboratory next morning. Milk was stored at 4 - 6 °C temperature until analyses of milk fat, protein, SCC, and FA, or frozen at – 18 °C temperature until analyses of retinol, α- and γ-tocopherols, BC, Ig, and lysozyme.

***Feed analyses***. Total carotene content in feed was determined by the extraction of carotene from the sample (3 g) with petroleum ether (50 ml) and measuring the concentration on the photometer FEK-56 M by the wave length 450 nm, in accordance with ГОСТ 13496.17-95 method.

The extraction of total lipids from feed for analyses of α- and β- carotene concentration was performed by the method of Hara and Radin, 1978. Concentration of α- and β- carotene in feed was determined by HPLC using the technique consisting of a Waters Alliance 2695 HPLC with photodiode array detector, monitoring between 280 and 600 nm, using a 150 x 4.6 mm, RP C18 column and Empower Pro software. The flow rate was 2 ml $min^{-1}$ and the mobile phase consisted of acetonitrile, methanol-acetate ammonium 50 m*M*, dichloromethane and water (70:15:10:5). Concentration of carotenoids was calculated by using external standards.

***Milk vitamin A, tocopherol, and BC analyses***. Milk (10 ml) was mixed with 10 ml of isopropanol and 5 ml of hexane: toluene (10: 8 $vv^{-1}$). The tubes were centrifuged at temperature of 4 °C at 2800 rpm for 5 min. The top layer was transferred to a clean container, residue mixed with 3 ml of hexane: toluene mix, centrifuged, extraction repeating 3 times. Supernatants were collected, evaporated under a gentle stream of nitrogen on a warm plate at 40 °C until dryness and dissolved in 5 ml of ethanolic butylhidroxitoluene (2 g $l^{-1}$) and 5 ml 2 *M* saturated potassium hydroxide solution. Samples were placed in a water bath at 40 °C for 30 min, then cooled in an ice water

bath. The samples were added to 10 ml deionized water, centrifuged. The extraction was repeated 3 times, and supernatants were collected. The extracts were evaporated to dryness under $N_2$. The residues were dissolved in 2 ml of methanol, filtered, and 80 µl was injected for HPLC analysis using the technique consisting of a Waters Alliance 2695 HPLC with diode array detector, vitamin A monitoring at 325 nm, tocopherols at 292 nm, and BC at 475 nm.

***Vitamin C or L-ascorbic acid in milk*** was determined in accordance with method of Tillmans (Matiseks et al., 1998). All reagents and standards for vitamin analyses were at least analytical grade from Sigma Aldrich.

***Analyses of milk FA***. Milk fat was obtained by centrifugation of milk 15 min at 4 °C and 3500 rpm. The upper cream layer was centrifuged 30 min at 40 °C and 13000 rpm. 7-15 mg of the upper butter oil layer was mixed with 1 ml of hexane and 10 µl of Na methilate (12.5 g 100 $ml^{-1}$ $wv^{-1}$), shaken 1 min, left for 10 min in room temperature, and centrifuged 5 min at 4 °C and 13000 rpm. The upper layer was used for further analysis by gas-liquid chromatography using an ACME model 6100, GLC (Young Lin Instrument Co.) gas chromatograph fitted with flame ionization detector, automatic sample injector, and a 30 m long, 0.25 mm i.d. Alltech AT-FAME analytical column. The carrier gas (He) flow rate was 2 ml $min^{-1}$. The injector and detector temperatures were 225 °C and 250 °C, resp. The oven temperature was programmed from 50 °C (initial delay 4 min) till 170 °C at a rate of 8 °C $min^{-1}$ (held 15 min), till 240 °C at a rate of 6 °C $min^{-1}$. Peaks were identified according to similar peak retention times using standard mixture Supelco FAME Mix C4-C24, Sigma Aldrich. Results were evaluated with a conventional integrator program (Autochro-2000, Young Lin Instrument Co.)

FA content was analyzed in days 1 and 5 to compare the lipid stability in milk stored in 4-6 °C temperature (poured into 25 ml glass bottles, in dark). The sum of polyunsaturated FA (linoleic C18:2 *cis* n-6, C18:2 *trans* and linolenic acids C18:3 *cis* n-6, and n-3) in storage days 1 and 5 were compared.

The above mentioned feed and milk analyses were carried out in the Scientific Laboratory of Biochemistry and Microbiology of the Research Institute of Biotechnology and Veterinary Medicine 'Sigra' of the LLU.

***Immunoglobulin (IgA, IgG, IgM) and lysozyme*** concentrations were determined by turbodimetric method (Грант Х., 1973), using pH-meter Jenway 3520 and spectrophotometer Jenway 6705 UV/VIS (UK) in the Petera Delles Food Processing laboratory of the Faculty of Food Technology of the LLU.

***Milk fat and protein content*** was determined by automated infrared analysis using Milcoscan equipment (Standard method ISO 9622-1999), and SCC was determined by "Somacount 300" in the laboratory of Milk Quality Control of the Sigulda CMAS. ***Milk yield*** was registered once a month from cow supervision data.

Samples were analyzed at least in triplicate. The results were calculated, analyzed, and graphs were made using MS Office program Excel or Microsoft Windows for SPSS software packages.

## Results and Discussion

The average milk yield, fat, protein and SCC content before (D0) and during (D7, 24, 35, and 42) the cow feed enrichment (D7, 24, 35, 42) are given in Table 2.

Table 2

**Characterization of milk before and during feed enrichment with carotenoids**

| Parameters | Control group | | Experimental group | |
|---|---|---|---|---|
| | Before enrichment | During enrichment | Before enrichment | During enrichment |
| Milk yield, kg $day^{-1}$ | $27.65 \pm 1.131^{a}$ * | $26.12 \pm 1.895^{ac}$ ** | $22.08 \pm 1.626^{b}$ * | $23.44 \pm 0.962^{c\ b}$** |
| Fat content, g 100 $g^{-1}$ | $4.78 \pm 0.239^{a}$ | $3.66 \pm 0.218^{b}$ | $4.49 \pm 0.225^{ac}$ | $4.28 \pm 0.180^{c}$ |
| Protein content, g 100 $g^{-1}$ | $3.05 \pm 0.153^{a}$ | $2.92 \pm 0.046^{a}$ | $3.53 \pm 0.177^{b}$ | $2.98 \pm 0.155^{a}$ |
| Somatic cell count, $10^3$ $ml^{-1}$ | $246 \pm 12.300^{a}$ | $152.20 \pm 35.172^{c}$ | $397 \pm 19.850^{b}$ | $128.00 \pm 72.365^{c}$ |

Means within rows marked with the same letter did not differ significantly at $p<0.05$.
* – The average milk yield from January and February months.
** – The average milk yield from March and April months.

Before feed enrichment (D0) the average milk yield was significantly ($p<0.05$) lower in EG, but protein content and SCC were significantly ($p<0.05$) higher in EG in milk as in CG. Comparing results before and during experiment, milk yield remained similar in both groups; fat content significantly ($p<0.05$) decreased in CG, but in EG remained similar; protein content significantly ($p<0.05$) decreased in EG, but in CG remained similar; SCC significantly ($p<0.05$) decreased in both groups. During feed enrichment there were no significant ($p<0.05$) differences on average milk yield, protein content and SCC between both groups. However, the fat content was significantly ($p<0.05$) higher in EG milk during the feed enrichment. The SCC in all milk samples did not exceed 400 000 $ml^{-1}$, and, during the experiment, decreased in both groups (Figure 1) that can be related with the lactation period and seasonal changes (Piena Lopkopība, 2001). Fluctuations of results can be also related to cow health, mobility, other factors, as well as analytical accuracy. As seen from trend line slope coefficients, the tendency of milk SCC decrease was greater

in EG: -51.46 versus -21.26 of CG. This tendency of SCC decrease is in accordance with previous findings (Chew, 1995), showing that feed enrichment with carotenoids have positive role on cow health, especially mastitis prevention.

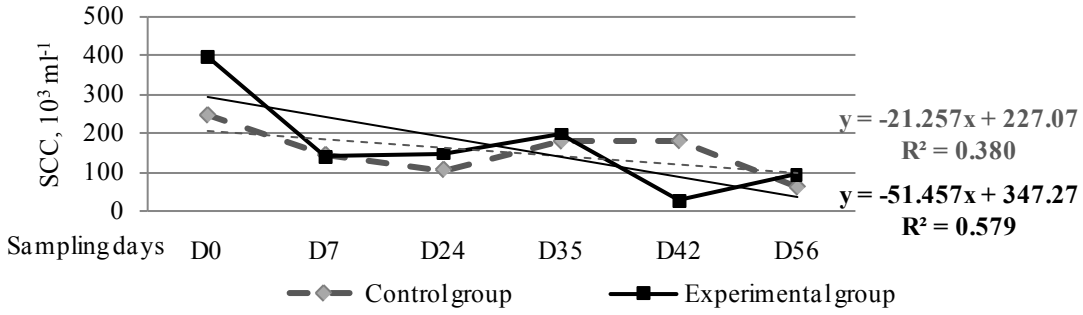


Figure 1. Somatic cell count of milk during the experiment.

In next figures (2 and 3) we can see that Ig concentration was significantly higher ($p<0.05$) in EG milk, as in CG milk in D24, 42 and 56, but the lysozyme concentration – in D24, 35, 56 and, particularly, in D42 when the duration of feed enrichment was the most lengthy. The decrease of Ig in D35 can be related with sample storage or analytical error (similar tendency is seen in both groups). The higher amounts of antimicrobial proteins in EG milk can be derived from feed enrichment with carotenoids thus strengthening cow health. In D7 a higher lysozyme and Ig concentration in EG milk was not observed yet, showing that the favourable effect can not be obtained immediately, but after a longer adaptation period to feed enrichment. As seen from Fig. 4, the growth of PB in this sampling day was significantly higher in EG milk, as in CG milk.

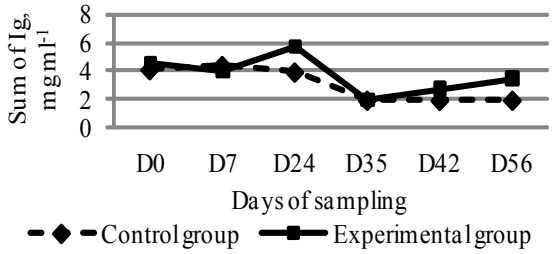


Figure 2. The sum of immunoglobulins in milk.

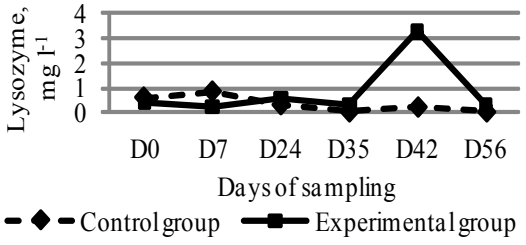


Figure 3. Lysozyme content in milk.

In spite of a wide diversity of xanthophylls and carotenes in forages, BC (especially the all-*trans* isoform) is the main circulating carotenoid in bovines (Nozière et al., 2006). The 9-*cis* and 13-*cis* isoforms have been also observed, as well as α-carotene and lutein (Nozière et al., 2006). In our experiment the BC was measured as the main representative of milk carotenoids. As seen from Figure 4, the content of BC in milk of both groups was significantly ($p<0.05$) lower during and after feed enrichment, as before.

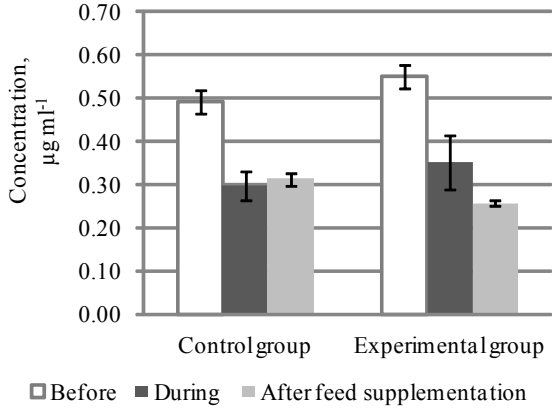


Figure 4. β-Carotene content in milk.

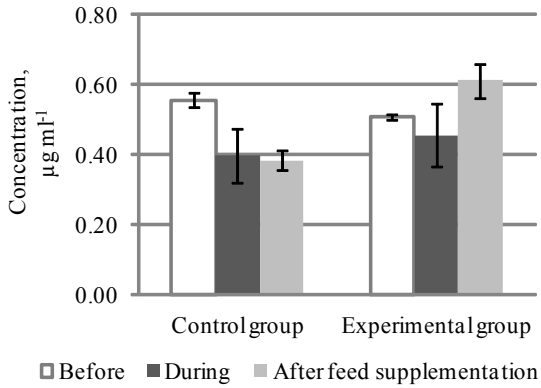


Figure 5. Retinol content in milk.

The BC decrease by 36% in EG, and by 39% in CG looks surprisingly, because the concentration of BC in milk is highly dependent on the concentration of BC in the diet. However, different studies confirm that carotenoid transfer from diet to milk is relatively low (Nozière et al., 2006). The low content of BC in EG milk could be connected with BC conversion and accumulation processes in cow body, and may depend on both - the initial retinol and the BC status of the animals. Carotenoid availability for secretion in milk is governed by their transport into lymph and plasma, their metabolism within tissues (especially conversion into vitamin A and by utilization as pigments or antioxidants), as well as their storage in adipose tissues or secretion into bile by the liver. The extent of carotenoid degradation by microorganisms in the rumen also remains uncertain because of the wide range of results. Also a specific effect of stage of lactation has not been clearly established, and this could be an important factor affecting concentrations of BC, and other micronutrients, in milk (Nozière et al., 2006). Other possible factors, as analytical error also have to be mentioned.

BC concentrations measured in milk of both groups during and after feed enrichment were similar or higher to earlier findings – BC in Netherlands raw milk in March was 0.186 ± 0.021 μg $g^{-1}$ (Hulshof et al., 2006), but in France BC in milk of cows whose diet was based on hay and grass-silage in March and April was (0.056-0.142) ± 0.008 μg $g^{-1}$ (Nozière et al., 2006). BC concentration in milk of cows fed grass-clover silage was 0.440 ± 0.023 μg $ml^{-1}$, but in milk of cows fed hay was 0.264-0.445μg $ml^{-1}$ (Havemose et al., 2006). In our experiment the concentration of BC measured in day 0 was remarkably higher (0.490 ± 0.027 for CG and 0.550 ± 0.028 μg $ml^{-1}$ for EG), as literature data. BC is one of the most fluctuating milk constituents and its content depends on cow breed, feeding regimen, lactation period, health status, milk fat content, as mentioned before (Nozière et al., 2006).

Comparing the retinol content in milk during the experiment, we can see that it significantly ($p<0.05$) decreased in CG milk, while in EG milk remained similar (Fig. 5), and after the end of feed enrichment slightly but significantly increased, and was significantly ($p<0.05$) higher than that in CG milk. That can be explained by the fact that for retinol synthesis for EG cows BC is more available. The results of other authors show, that retinol content in Swedish raw milk was 0.307 μg $ml^{-1}$ in March (Swensson et al., 2007), retinol in Netherlands' raw milk in March was 0.396 ± 2.8 μg $100g^{-1}$ (Hulshof et al., 2006). According to Haug et al. (2007), the milk retinol content is about 0.280 μg $ml^{-1}$. During our experiment the retinol content in both groups' milk was similar or above previously mentioned quantities.

The sum of α- and γ-tocopherols was determined (see Figure 6). Vitamin E is not a single compound; it includes tocopherols and tocotrienols. In whole milk, α-tocopherol is the major form of vitamin E (> 85 g 100 $g^{-1}$); γ-tocopherol and α-tocotrienol are present to a lesser extent, about 4 g 100 $g^{-1}$ each of the sum of tocopherols and tocotrienols (Haug et al., 2007). According to Haug et al. (2007), the vitamin E concentration in milk is about 0.6 μg $ml^{-1}$, but may increase 3-4 folds by proper feeding regimes. Before the experiment the sum of α- and γ-tocopherols was significantly higher ($p<0.05$) in CG milk than in EG milk. During the experiment it changed significantly ($p<0.05$) in milk of both groups, decreasing in CG milk, but increasing in EG milk. An adverse shift occurred after the end of feed enrichment. Results are not easily explicable because carrots are not regarded as important source of tocopherols, still they contain a small amount of α-tocopherol, i.e. 0.320 - 0.950 mg 100 $g^{-1}$ (Danish Food Composition Databank, 2009). Oil supplementation also could enhance the absorption of this vitamin from feed. Compared to previous literature data, the sum of tocopherols in both groups before, during and after feed enrichment was rather below 0.6 μg $ml^{-1}$.

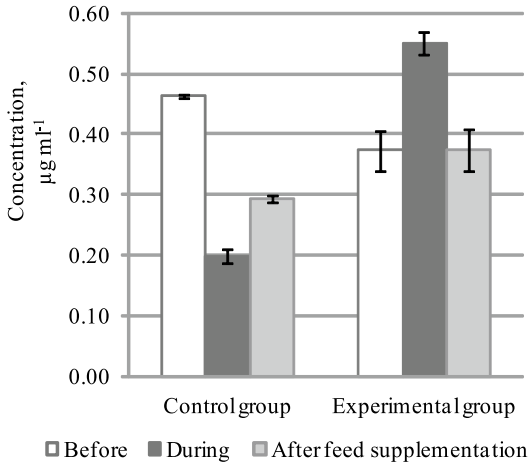


Figure 6. The sum of α- and γ-tocopherols in milk.

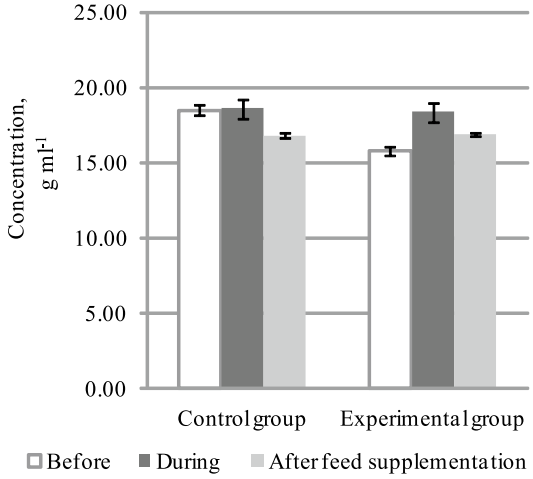


Figure 7. Vitamin C content in milk.

Concerning vitamin C, which also has antioxidant activity (Coultate, 2002; Swensson et al., 2007), its content in milk before additional feeding was slightly higher ($p<0.05$) in CG milk (Fig. 7). During and after the feed enrichment the average vitamin C content resembled in both groups' milk without significant differences. The average vitamin C concentration in milk was 17.49 ± 1.153 μg $ml^{-1}$, that conforms to literature data, i.e. 10-25 μg $g^{-1}$ (Walstra, 2006).

Milk lipid stability was investigated by the storage stability of polyunsaturated FA. Comparing the polyunsaturated FA content in milk stored in 4 - 6 °C temperature in days 1 and 5, there no significant changes of it in milk of both groups (Figure 8) were observed. This can be explained with the induction period when the oxidation process is still slow due to slow oxygen uptake and milk lipids are still protected against oxidative damage due to sufficient antioxidant concentrations (Coultate, 2002). Compared with many edible fats, milk fat is relatively resistant to oxidation because of its low polyunsaturated FA content, and high proportion of saturated FA, and the presence of natural antioxidants, principally α-tocopherol and BC (Żegarska, 2003). The average content of polyunsaturated FA during the feed enrichment was similar in milk of both groups (2.35 ± 0.198 g 100 $g^{-1}$ of total fatty acids in EG and 2.19 ± 0.183 g 100 $g^{-1}$ in CG).

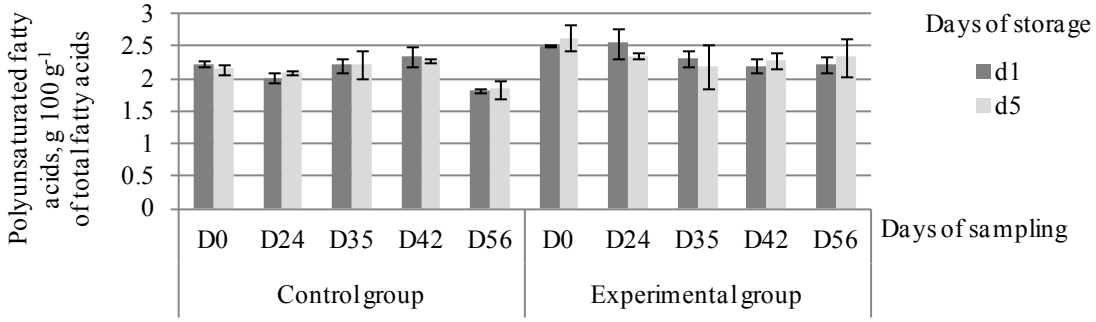


Figure 8. Polyunsaturated fatty acid content in milk stored in 4-6 °C temperature in days 1 and 5.

However, under more unfavorable storage conditions, such as light or high temperature influence, or in a longer period, as in such dairy products, as butter or ice cream storage, milk lipid stability potentially can be ameliorated by increased antioxidant vitamin concentrations.

## Conclusions

Enriching cow diet with carrots showed a tendency to promote the decrease of somatic cell count in milk, significantly ($p<0.05$) increase immunoglobulin and lysozyme content, thus potentially improving milk nutritional value. Milk retinol and tocopherol content in EG milk rose significantly ($p<0.05$), but β-carotene content decreased by 36 and 39% in EG and CG milk, accordingly. Polyunsaturated fatty acid stability changes during 5 day storage in temperature of 4 - 6 °C were not observed.

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