

Effects of dairy cow diet supplementation with carrots on milk composition, concentration of cow blood serum carotenes, and butter oil fat-soluble antioxidative substances

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Abstract. Fat-soluble constituents of milk – β -carotene and α -tocopherol – are essential for quality and nutritional value of milk and dairy products. Provision of fat-soluble antioxidants and vitamins such as carotenoids and vitamin E necessary for cow organism and milk synthesis depends on their concentration in fodder. The aim of this study was to estimate the effect of cow feed supplementation by carrots on the total carotene concentration in cow blood serum, on fat, protein, lactose concentration in milk, and milk yield, as well as to investigate the effects on β -carotene and α -tocopherol concentration in butter oil and intensity of its yellow colour. A total 20 cows of Latvian brown ($n = 8$) and Danish red ($n = 12$) breed were divided into control (CG) and experimental group (EG). In the EG, cow feed was supplemented with seven kg of carrots per cow per day for six weeks at the end of the indoor period (March–May). Milk samples from indoor period ($n = 100$) and grazing ($n = 20$) were used for butter oil extraction.

The carotene concentration observed in blood of animals before the experiment was insufficient taking into account that the recommended β -carotene concentration in serum is above 3.0 mg l^{-1} level. During indoor period the increase in carotene concentration in blood of cows was significant in both groups ($P < 0.05$) but in EG it was more explicit showing the positive effect of carrot supplementation. Carrot supplementation did not change milk fat, protein, lactose concentration, and yield ($P > 0.05$). At the same time it contributed in more stable β -carotene, as well as 30% higher α -tocopherol concentration and more intense yellow colour of butter oil samples during the indoor period of the experiment ($P < 0.05$).

Key words: dairy cow, milk, butter oil, carrot, fat-soluble antioxidants.

INTRODUCTION

The fat fraction of milk is an effective delivery system for fat-soluble vitamins and antioxidative substances in human food. Their content influences not only nutritional value, but also the complex interplay of pro- and antioxidants in milk and dairy products that is important for quality maintenance (Baldi & Pinotti, 2008). However, it has been noted that sensorial properties and oxidative stability during indoor period, especially winter and spring, are declining (Jensen, 2002; Schreiner & Windisch, 2006). Provision of fat-soluble antioxidants and vitamins such as carotenoids and vitamin E necessary for cow organism and milk synthesis depends on their concentration in fodder. Carotenoid concentrations in feeds are highly variable and decrease during the feed storage (National Research Council, 2001; Nozière et al., 2006a; Calderón et al., 2007). Also higher amounts of certain types of feed, especially maize silage or feed based on grains and concentrates increase the risk of insufficient provision of carotenoids (Nozière et al., 2006a; Georges, 2009). The concentration of antioxidative substances in milk and butter oil is also affected by cow health condition and welfare. Because of the current intensification of milk production, problems caused by the oxidative stress of animals are increasing. Mastitis is a major source of economic loss on dairy farms (Meglia, 2004; Beecher et al., 2013). It is known that fat-soluble vitamins, E, A, and carotene are crucial to increase the resistance of the cow to mastitis (Chew, 1995; Meglia, 2004). Various studies indicate that carotenoids may have their own independent role in fulfilling specific animal health functions in order to maintain udder and reproductive health (Arechiga et al., 1998; de Ondarza et al., 2009; Kaewlamun et al., 2011). Many bioactives belonging to carotenoid class neutralise singlet oxygen, other reactive oxygen species, and inhibit free radical and light-initiated oxidative reactions (Pokorny & Parkanyiova, 2005). β -carotene, independent of its provitamin A function, as an antioxidant hinders superoxide formation within the phagocyte (Sordillo et al., 1997) and can enhance the bactericidal ability of neutrophils as reviewed by Chew, 1995. Yet there are not many studies in the world about usage of natural sources of carotene and even more – in practice emphasis usually is put only on the necessity to avoid from the deficit of vitamins A and E. Administration of the natural vs. synthetic form of carotene and other bioactives has potential of better bioavailability and enhanced transfer to milk (Meglia, 2004; Baldi et al., 2008; Politis, 2012). One of the richest sources of carotene is carrot roots historically used as indoor season feed for dairy cattle to enhance yellow colouring in butter and other dairy products. However, because of their varying quality during the spring months and labour-consuming preparation process, carrots are usually replaced by silage. Nevertheless, the economic benefits of carrot feeding may be indirect. Carrot roots are used to provide energy for livestock; although low in protein they enhance forage intake levels. Also owing to soluble sugars and other components contained in carrots, digestive processes, as well as milk secretion can be improved (Fuller et al., 2004; de Ondarza et al., 2009). The aim of the study was to estimate the effect of cow feed supplementation by carrot roots on the total carotene concentration in cow blood serum, on fat, protein, lactose concentration in milk, and milk yield. As well our aim was to investigate the effects on β -carotene and α -tocopherol concentration in butter oil and intensity of its yellow colour.

MATERIAL AND METHODS

Animals and experimental design

The trial was performed in a low-input conventional dairy farm in Latvia specializing on the red cattle breeds – Latvian Brown and Danish Red – that represent the typical herd of Latvia. A total of twenty dairy cows were used in the study. Two cow groups – one experimental and one control group – with 10 cows in each of them were formed taking into account following factors: 1) cow breed, 2) parity (1–6 times), 3) stage of lactation (3–6 months), 4) milk fat and protein concentration, as well as 5) – productivity. Cow distribution by breeds – Latvian Brown ($n = 4$) and Danish Red ($n = 6$) – in each group was similar. During indoor period cows were housed in a tie-stall barn and fed individually. The feeding and milking of cows was held twice a day. The basic feed of both groups was equal (see Table 1); also cow's mineral salt licks were freely available. EG feed was supplemented with carrots – 7 kg per cow per day.

Table 1. The composition of cows feed during experiment (per cow per day)

Cow groups	Indoor period			Outdoor period		
	basic feed composition	supplemental feed	total carotene, mg α -tocopherol, mg	basic feed composition	total carotene, mg α -tocopherol, mg	
CG	haylage*, hay- <i>ad libitum</i> ; mixed feed concentrate** –	–	217	pasture grass – <i>ad libitum</i> ,	590	1053
EG	2 kg; beet molasses – 0.5 l	carrots 7 kg	299	hay – 10 kg		

CG = control group, EG = experimental group; * mainly consisting of perennial ryegrass; ** feed additive for dairy cattle 'LUX' ('Tukuma Straume', Latvia), contained following: wheat, barley, sunflower meal, wheat bran, rapeseed cake, fodder yeast, calcium carbonate, sugar beet molasses, sodium chloride, etc. vitamin and mineral supplements.

Feed supplementation was conducted at the end of the indoor period from the end of March and during April and May. The total period of the feed supplementation was 42 days. During outdoor period cows were grazed between both milkings, as well as received hay in the barn. Both groups received equal feed; the total concentration of carotene and α -tocopherol in forage of the respective sampling day is shown in Table 1.

Sample collection and preparation for analyses

A total of 120 milk samples were obtained from the morning milking both on indoor period and when the animals were on the pasture. Milk samples were obtained from healthy cows with somatic cell count less than 400,000 in ml. During the indoor period, milk samples were taken one day before the feed supplementation ($n = 20$) and several times repeatedly – 1, 3, 5, and 6 weeks from the beginning of the feed supplementation ($n = 80$). Milk samples from the pasture period were taken 7 weeks after the end of the feed supplementation experiment and the beginning of the grazing period or 13 weeks from the launch of the whole experiment ($n = 20$).

Milk fat, protein, lactose concentration and yield

Concentration of milk fat, protein, and lactose was determined in line by infrared spectroscopy with the ISO 9622:1999 standard method (International Organization for Standardization, 1999) at the Laboratory of Milk Quality Control of the joint-stock company 'Stock Breeding and Artificial Insemination Station of Sigulda'.

Extraction of butter oil

Each group's pooled milk samples for butter oil extraction were obtained by pouring together equal quantities (1 l) of fresh milk from individual cows. For butter oil extraction, milk was warmed up to $42 \pm 3^\circ\text{C}$. Subsequently milk was creamed with a separator obtaining cream having approximately 30% fat content. Then cream was ripened at $4\text{--}6^\circ\text{C}$, for 20 ± 1 hour, and churned till the formation of butter. Buttermilk was removed, and butter was rinsed with cold distilled water. Afterwards butter was warmed up to $45 \pm 5^\circ\text{C}$ and centrifuged $14,360 \times g$, for 10 minutes at 40°C to separate pure butter oil that was used for β -carotene, α -tocopherol, and colour analyses. Till further analyses butter oil samples were frozen at -20°C temperature.

β -carotene and α -tocopherol concentration in butter oil

β -carotene and α -tocopherol concentration in butter oil was found out based on the standard methods ISO 9936:2006 (International Organization for Standardization, 2006), EN 12823-2:2000 (European Committee for Standardization, 2000), and method of Granelli and Helmersson (1996) with slight modifications. In a glass baker 0.4 grams of oil were weighted adding 5 ml of ethanol with butylated hydroxytoluene (0.2%) (Supelco) and 10 ml of 2 M ethanolic KOH solution, then mixed. Saponification was performed in $54 \pm 1^\circ\text{C}$ temperature for 1 h. Then 20 ml of deionized water and 4 ml of hexane (Sigma Aldrich, Chromasolv) were added and tubes were centrifugated for 5 min $9,400 \times g$, at $4 \pm 1^\circ\text{C}$ temperature. The upper lipid layer was transferred in a test tube, and the extraction was repeated two more times. The extract was evaporated in vacuum at $40 \pm 1^\circ\text{C}$ temperature. In the test tube containing the residue, 0.2 ml of methanol (Sigma Aldrich, Chromasolv) was added. The solution was centrifugated for 5 min $34,000 \times g$ at $4 \pm 1^\circ\text{C}$ temperature. The samples prepared were analysed by high-performance liquid chromatograph (Waters Alliance 2695) with a photodiode detector (Waters) using YMC Carotenoid column ($5\mu\text{m}$, 4.6×250 mm) at a temperature of $40 \pm 1^\circ\text{C}$. Sample injection volume was 50 μl . The mobile liquid phase was prepared from methanol, methyl *tert*-butyl ether (Sigma Aldrich, CHROMASOLV), and deionized water in various proportions with the flow rate 1 ml min^{-1} . Analyses of β -carotene and α -tocopherol concentration in butter oil were made in the Agency of the Latvia University of Agriculture 'Research Institute of Biotechnology and Veterinary Medicine 'Sibra'.

Blood sample collection and storage

Cows blood samples were taken from *the v. jugularis externa* after morning milking and collected into 5 ml vacutainers without stabilizer. Then samples were stored at room temperature until the blood coagulates and serum separates (1h). Thereafter the tubes were centrifugated (3,000 rpm, 4°C , 10 min) and serum was stored at -18°C until analyses.

Total carotene concentration in cow blood serum

Experiment covered a comparison of the total carotene concentration in blood serum of animals one day before and at the end of the feed supplementation experiment – after 6-week feed supplementation with carrots – that was determined by the spectrophotometric method. Carotene determination with spectrophotometric method was performed in the Institute of Food Safety, Animal Health and Environment ‘BIOR’.

Yellow colour intensity of butter oil

Samples were heated to $40 \pm 1^\circ\text{C}$ before carrying out analyses. Then oil was poured into a transparent plastic Petri plates so as to avoid the formation of air bubbles and held in $25 \pm 1^\circ\text{C}$ for the temperature stabilization. The colour of butter oil samples was measured using a ColorTec – PCM colour meter, USA (CIE 1976 $L^*a^*b^*$ colour model), which has been calibrated according to a standard. The color was determined in 7 different surface points. Colour measurements were made at the Faculty of Food Technology of the Latvia University of Agriculture in the Research Laboratory of Packaging Materials’ Attributes. The data were processed with ColorSOF QCW data program. Colors range was read in three coordinate system: L^* , which characterizes the degree of brightness, where $L = 100$ - white, but $L = 0$ - black, a^* is the measure of the – a (green) to + a (red) and b^* factor – from - b (blue) to + b (yellow). The factor b^* values were used to compare yellow colour intensity of samples.

All analytical reagents used in the analysis were of analytical or higher purity.

Statistical data analyses

Samples were analysed at least in duplicate. Statistical data processing of the acquired results was carried out using MS Excel and SPSS 17 application. Data are presented as the mean \pm standard error of means (s.e.m.). The hypotheses suggested were tested by Student’s t test; factors were considered significant if $P < 0.05$.

RESULTS AND DISCUSSION

Total carotene concentration in cow blood serum

In order to better assess the impact of feed supplementation on quality and composition of the milk, it was necessary to find out whether the additionally fed carrots increase the carotene concentration in cow organism. Results of the total carotene concentration in blood serum of animals before and after the 6-week feed supplementation with carrots are shown in Fig. 1. The concentration of β -carotene in cow blood can serve as an indicator showing whether the amount of carotenoids in feed is sufficient. A serum β -carotene level of 3.0 mg l^{-1} has been suggested as the level below which the supplementation is beneficial for udder health (Frye et al., 1991; Jukola et al., 1996). Before the experiment, the average carotene concentration in blood serum of animals of both groups was similar ($P > 0.05$), 2.5 and 2.6 mg l^{-1} in the CG and EG, respectively, and it was below the recommended level.

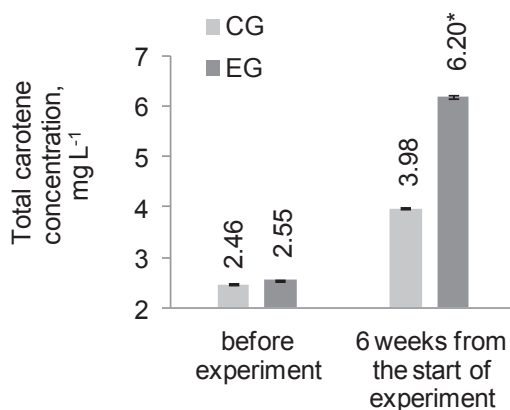


Figure 1. Cow's blood serum total concentration of carotene before the supplementation experiment and six weeks later. CG = control group, EG = experimental group. *Significant difference between dietary treatments (Student's *t* test, $P < 0.05$). Means \pm s.e.m. for 10 cows per group are presented.

After the 6-week feed supplementation period, the carotene concentration in CG and EG cow blood serum was 4.0 mg l⁻¹ and 6.2 mg l⁻¹, respectively. A significant increase ($P < 0.05$) may be associated with a composition differences in separate lots of the basic feed. Therefore the concentration increase in the EG accounted for 2.6 times, while in the CG – 1.7 times. This demonstrates that diet supplementation with 7 kg of carrots may improve dairy cow carotene status. Insufficient carotene concentration (below 3.0 mg l⁻¹ level) has been observed also in other studies giving a cause to a warning. A total of 35% of cows in a study on herds from various regions of France had less than 1.5 mg l⁻¹ β -carotene in the blood plasma showing a strong β -carotene deficiency, while 71% had less than 3.5 mg l⁻¹ β -carotene (Georges, 2009). The status was improved by the contribution of grass silage or alfalfa and the best amelioration was attained by cow grazing. Similar observations were made in Canada, where mean serum β -carotene concentration from 20 Holstein cow herds was lower than preferable – 1.12 mg l⁻¹ (LeBlanc et al., 2004). Previous studies showed that also in organic farm system, β -carotene and vitamin E concentration in feed may be insufficient to provide the preferable β -carotene and α -tocopherol level in cow blood serum during the calving period, as well as in blood of calves (Johansson et al., 2012). Therefore farms working under this system need to supplement animal food with β -carotene, vitamin A, and E that could reduce the incidence of mastitis. The observation was made in several studies about the effect of cow feed enrichment with β -carotene additives. Supplementation during the dry period showed positive effect on β -carotene level in cow blood and colostrum that is important also for the health of calves (Kaewlamun et al., 2011). However, there is a lack of relevant information in the scientific literature about effects of carrot supplementation on cow blood carotene level.

Milk fat, protein, lactose concentration and yield

Milk quality parameters of samples obtained before and during the feed supplementation experiment, as well as milk yield between the groups were compared. The summary of indicators characterising concentration of milk fat, protein, lactose, and yield is given in the Table 2. The average bulk milk quality parameters and yield before

the feed supplementation experiment as well as during it did not indicate significant differences between groups except lactose concentration that was significantly lower in EG ($P < 0.05$) milk. The significant difference in lactose concentration, as well as insignificant differences in milk yield and other parameters between groups before the experiment may be related to the individual characteristics of the animals, as feed composition was the same before the experiment.

Table 2. Comparison of average milk quality parameters and yield before and during feed supplementation experiment

Indicators	Before experiment				During supplementation			
	CG	EG	pooled s.e.	<i>P</i> -value	CG	EG	pooled s.e.	<i>P</i> -value
Fat, g kg ⁻¹	41.6	44.8	2.4	ns	43.5	43.7	2.2	ns
Protein, g kg ⁻¹	32.9	33.7	1.4	ns	32.6	34.0	1.0	ns
Lactose, g kg ⁻¹	47.8	46.5	0.5	*	47.7	46.5	0.4	*
Milk yield, kg day ⁻¹	19.65	16.51	1.73	ns	19.63	17.99	1.65	ns

CG = control group, EG = experimental group, Pooled s.e. = pooled standard error, * = significant difference, ns = non-significant difference between dietary treatments (Student's *t* test, $P > 0.05$).

Comparison of the average bulk milk quality parameters and yield before the feed supplementation experiment and during it did not indicate significant changes in any of the groups ($P > 0.05$). The possible explanation of the results could be that the effect of supplemental feeding may occur only after a longer period of time and is affected by individual health condition of each animal. The similar to our results were obtained in the study of Schreiner and Windisch (2006). Feeding additionally 10 kg carrots with and without 1 kg rapeseed oil per cow per day in iso-caloric diets showed no effects on the total milk yield, fat and protein concentration. At the same time the contradictory results were acquired by other authors. Increase in milk yield by 11% was observed in a study of Arechiga et al. (1998) in which heat-stressed cows feed was supplemented with 400 mg of β -carotene per cow per day. Oldham et al. (1991) observed that after supplementing the feed with 300 mg of β -carotene per cow per day, milk production increased by 6.4% and fat concentration reduced by 4.6%. In the study of de Ondarza et al. (2009), supplementation of 425 mg β -carotene per day did not affect the milk production, but significantly ($P < 0.05$) higher fat concentration (+0.1%) was observed, especially for early lactation cows and cows in their third or greater lactation. Dissimilar basic feed uptake of β -carotene, duration of supplementation or stage of lactation can be some of the explanations of such diverse effects of feed supplementation with carotenoid additives in different studies.

β -carotene and α -tocopherol concentration in butter oil

Changes of the β -carotene and α -tocopherol concentration in butter oil samples during the experiment are presented in Fig. 2. Average β -carotene concentration before the experiment was higher in the CG butter oil – 5.2 vs. 4.3 mg kg⁻¹ fat in the EG oil samples ($P < 0.05$). The average concentration of α -tocopherol did not differ significantly ($P > 0.05$) and was 9.6 and 10.0 mg kg⁻¹ fat in the CG and EG, respectively. During the supplementation with carrots, average concentration of β -carotene in butter oil samples acquired from the EG did not change, compared to β -carotene concentration before the experiment ($P > 0.05$), but in samples

of the CG it decreased ($P < 0.05$). It comprised 3.7 and 4.2 mg kg⁻¹ fat in the CG and EG. The difference between the groups was not significant ($P > 0.05$); however, a tendency of marginally higher β -carotene concentration in samples of the EG was observed. The negative tendency regarding changes in β -carotene concentration in the CG butter oil may be related to carotene deficit in diet as described above considering that fat yield – the average quantity of the milk fat daily produced by a cow – remained unchanged (0.86 kg day⁻¹ during supplementation vs. 0.85 kg day⁻¹ before the experiment). At the same time, β -carotene concentration in butter oil samples of the EG was more stable with respect to the similar fat yield before and during supplementation (0.80 and 0.79 kg day⁻¹). Within the supplementation experiment (indoor period), average concentration of α -tocopherol in butter oil samples acquired from the EG, increased ($P > 0.05$) reaching average 12.4 mg kg⁻¹ fat. The tendency of changes in average α -tocopherol concentration in the CG was opposite – it decreased ($P > 0.05$) to average 8.6 mg kg⁻¹ fat and thus was lower by 31% if compared to the EG ($P < 0.05$).

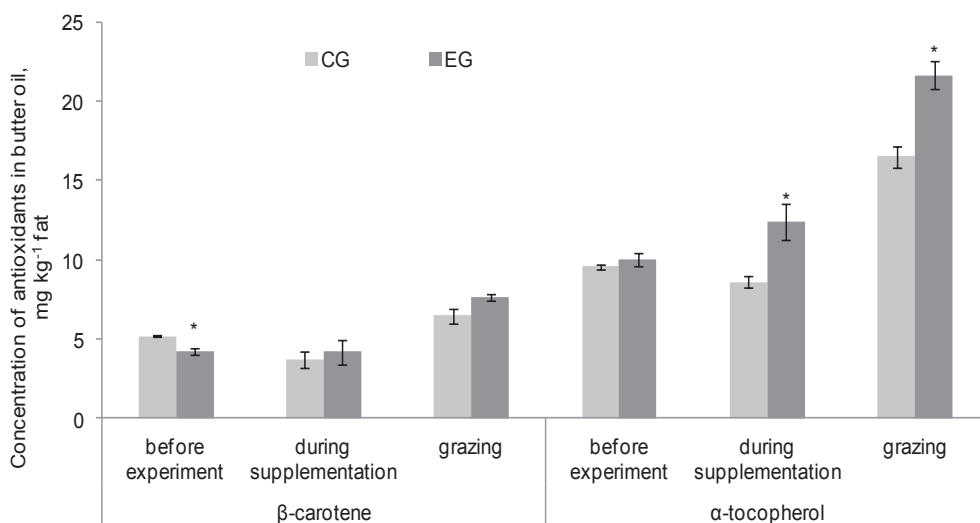


Figure 2. β -carotene and α -tocopherol concentration (means \pm s.e.m.) in butter oil. CG = control group, EG = experimental group. *Significant difference between dietary treatments (Student's *t* test, $P < 0.05$).

The positive effect towards more stable β -carotene concentration in butter oil samples of the EG, as well as its higher α -tocopherol concentration during the experiment ($P < 0.05$) may be related to higher total amount of fat-soluble antioxidative substances ingested by feed. The antioxidant system is an integrated system and deficiencies of one component can affect antioxidative efficiency of others (Baldi et al., 2008). It is likely that greater quantities of α -tocopherol remain and can be utilised in milk lipid synthesis because body's necessity for antioxidative substances may be met much better. Similar effect towards increase in tocopherol concentration in milk was observed in another earlier experiment that covered supplementation of Holstein and Latvian brown cow feed with carrots and red palm oil feed additives (Antone et al., 2011). Also the study of Nałecz-Tarwacka et al. (2003) conducted with Black & White

cows showed higher levels of vitamins A, D, E, and carotene in cow milk achieved by carrot supplementation, yet the statistically significant differences ($P < 0.05$) were confirmed only for vitamin A. Experiment of Schreiner and Windisch (2006) also showed that carrot supplementation increases carotene content in butter. However, it is known that there is a limitation of daily secretion of α -tocopherol and β -carotene being independent of milk yield and fat content. Thus, diet supplementation can change the vitamin content of the milk only within certain limits (Baldi & Pinotti, 2008).

Values of fat-soluble antioxidant concentration in milk fat reported by other scientists during the indoor period are similar to or higher than our data. β -carotene concentration in milk fat found by Butler et al. (2008) was higher (5.5–6.3 mg kg⁻¹ fat) but that of Calderón et al. (2007) – similar to our results: by feeding grass silage, β -carotene comprised 4.2 mg kg⁻¹ fat. Regarding the milk fat α -tocopherol concentration during the indoor period, butter oil in the CG in our experiment was noticeably lower than in the studies mentioned above. In the study of Butler et al. (2008), α -tocopherol concentration was 23.1–23.9 mg kg⁻¹ fat, and in the study of Calderón et al. (2007) – 11.31 mg kg⁻¹ fat. The differences of results between different studies may be explained by variations in feed quality, composition (the usage of hay and haylage in our experiment), cow breeds, or other factors.

In comparison with the indoor period, concentration of fat-soluble antioxidants in butter oil samples obtained in grazing season grew considerably in both groups ($P < 0.05$). Average concentration of β -carotene increased to 6.5 and 7.7 mg kg⁻¹ fat, whereas concentration of α -tocopherol – to 16.5 and 21.7 mg kg⁻¹ fat, in the CG and EG, respectively. Observations of animal feeding and housing impact have been reported very widely. Results of the study on changes in milk fat-soluble antioxidant concentration during grazing period conducted by other scientists are similar to our results. It has been ascertained that milk fat-soluble antioxidant concentration largely depends upon the animal nutrition, and most notably it increases in summer during the grazing season (Coulon et al., 2003). For example, Butler's et al. (2008) findings show that β -carotene concentration in milk fat increased from 5.5–6.3 mg kg⁻¹ fat during the indoor season to 6.0–9.3 mg kg⁻¹ fat during the grazing season, but α -tocopherol concentration in milk fat during the grazing season reached 21.4–32.0 mg kg⁻¹ fat compared to 23.1–23.9 mg kg⁻¹ fat during the indoor season. Although pasture grass is the richest source of natural antioxidants used in cattle diets since the ancient times, nowadays smaller use of grazing and higher milk production intensity, as well as other reasons can adversely affect quantity of antioxidants in butter oil.

Yellow colour intensity of butter oil

As carotenoids also are yellow-, orange- and red-coloured pigments, changes in yellow colour intensity of butter oil may indicate carotene richness of the feed. Feeding animals with carrots may contribute to the colour of milk and products thereof as one of the most important sensory properties. Regression analysis indicated a positive relationship between the total carotene amount ingested by feed and yellow colour intensity of butter oil ($P < 0.05$, $r^2 = 0.55$) (Fig. 3.). The medium close relationship shows that other factors could affect the colour as well.

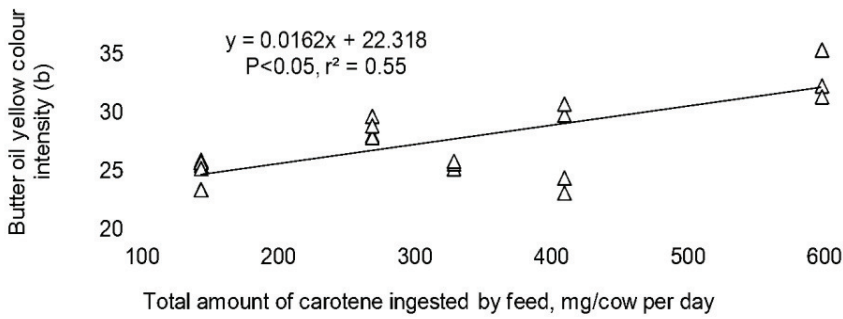


Figure 3. Correlation between yellow colour intensity (b) of butter oil and total amount of carotene (mg per cow per day) ingested by feed.

Changes in yellow colour intensity of butter oil during the experiment are presented in the Fig. 4. During the feed supplementation, yellow colour of butter oil samples acquired from the EG was stronger ($P < 0.05$) than the one of butter oil yielded from the CG. After 1-, 3- and 5-week supplementation periods, the intensity of yellow colour in the EG butter oil gradually increased ($P < 0.05$), while in samples of the CG it remained constant also showing a tendency to decrease insignificantly ($P > 0.05$) when compared to the colour intensity before the experiment. Measurements showed the decrement in colour intensity of butter oil at the end of the indoor period. It may be related to the deficit of yellow pigments – β -carotene and retinol – due to reduced body reserves or to increased milk yield resulting in dilution effect (Nozière et al., 2006a and 2006b; Calderón et al., 2007).

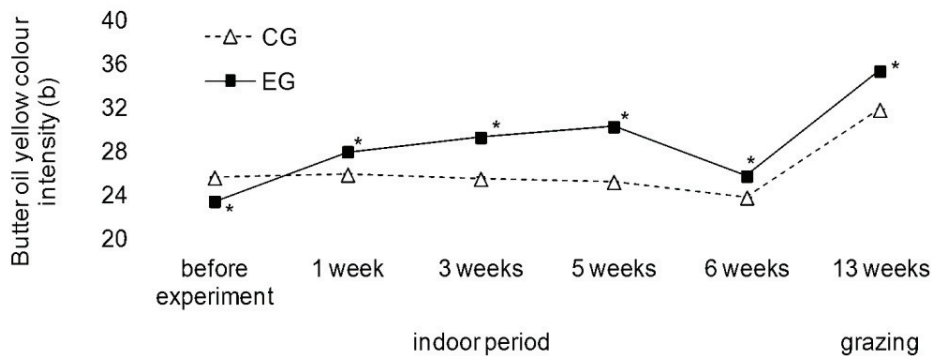


Figure 4. Changes in yellow colour intensity (mean \pm s.e.m.; b) of butter oil. CG = control group, EG = experimental group. *Significant difference between dietary treatments (Student's t test, $P < 0.05$).

During the pasture period, yellow colour intensity of butter oil produced from milk of both groups increased ($P < 0.05$). Such pronounced colour changes coincide with the increase of β -carotene concentration in butter oil samples obtained from the pasture period. Intensity of yellow colour sometimes may also serve as an indicator of the basic feed type consumed by herd. It is believed that colour measurements should be taken 4–5 weeks after changing composition of basic feed because effect left by carotenoid intake

may be dilatory (Nozière et al., 2006b). Our study shows that butter oil colour differences, compared to the control samples, were apparent earlier – after 1–3 weeks from the cow feed supplementation with carrots. This can be explained by the fact that studies were conducted with the red varieties of cows that are either more efficient at absorbing β -carotene or less efficient at converting it to retinol (National Research Council, 2001; Nozière et al., 2006a). Subsequently their carotene transition in milk should be more noticeable.

CONCLUSIONS

Carrots as a natural source of carotene are relatively inexpensive and can be grown in many parts of Europe and other regions. Milk producers can be suggested to add this vegetable to the feed of dairy cows in order to improve cow carotene status and ensure increased quality and nutritional value of milk and high-fat dairy products. Cow feed supplementation with carrot roots can help to maintain optimal β -carotene level in cow blood, as well as to provide a more stable or higher level of fat-soluble antioxidants (β -carotene and α -tocopherol) in butter oil and its yellow colour intensity during the indoor period. Increased concentration of biologically active compounds such as β -carotene and α -tocopherol can give higher nutritional value to butter and other dairy products. Yellowish colour may improve consumption of dairy products, especially among consumers preferring goods produced within the environmentally friendly organic farming system since such products are associated with natural or pasture feeding. Nevertheless, further research should be necessary regarding effects thereof on milk composition and dairy product shelf-life.

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