EFFECT OF FEEDING WITH ALGAE ON FATTY ACID PROFILE OF GOAT'S MILK*

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The study was conducted to determine whether the inclusion of algae *Chlorella vulgaris* in dairy goats' diets would change the fatty acid profile and increase the proportion of unsaturated fatty acids in goat's milk. White short-haired dairy goats on 2nd and 3rd lactations were fed 5 and 10 g of dried algae supplementation for six weeks. The fatty acids profile of milk was analyzed using gass chromatography (flame ionization detector (FID)). The addition of dried algae caused changes of the profile of fatty acids in the milk. The more algae were added to the diet, the greater the changes in the fatty acids profile of milk were found. A statistically significant effect (P = 0.0390) was found between the control group and the group supplemented with 10 g of *Chlorella vulgaris* per goat per day. The greatest effect of dietary treatment was seen in the relative reduction of palmitic acid content and increased oleic, linoleic, and linolenic acids content. Results suggested that the addition of algae also increased the nutritional quality of goat's milk. There was a positive change in the ratio of SFA:MUFA:PUFA in terms of reducing the proportion of saturated fatty acids, as well as a change in the ratio of n-6 and n-3 PUFAs.

white shorthaired goat; nutritional value; saturated fatty acids; unsaturated fatty acids; *Chlorella vulgaris*

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INTRODUCTION

The use of goat's milk could help to resolve many human health problems (Reynolds, 2009). For example, it was found that the consumption of goat's milk reduces the amount of total and LDL cholesterol because of triacylglycerols with medium chain fatty acids (FA). Their content in goat's milk is 36% compared to 21% in cow's milk (Haenlein, 2004). Goat's milk is also easier to digest than cow's milk due to its smaller fat globules size and this is one of the reasons why it is better tolerated (Jandal, 1996).

Goat's milk fatty acids (FA) profile differs from the cow's milk FA profile, but there are still a low level of polyunsaturated FAs (PUFAs) and a relatively high amount of saturated FAs (SFAs) in it. Goat's milk fat contains 53–72% of SFAs, 26–42% of monounsaturated (MUFA), and 2–6% PUFAs. More than 75% of the total FAs of goat's milk are capric, myristic, palmitic, stearic, and oleic acids. Therefore, we are constantly striving to increase the content of polyunsaturated FAs and looking for new ways of achieving it (Antunac et al., 2001; Park et al., 2007; Samková et al., 2009).

The typical flavour of goat's milk is created by short chain FAs (caproic, caprylic, and capric) together with medium chain FAs. They are used in the treatment of malabsorption syndromes of various origin (Chow, 2000; Alferez et al., 2001; Cattaneo et al., 2006; Samková et al., 2009). SFAs with longer chains (12–16 carbon atoms per molecule) mostly act in a negative way, because they increase the synthesis of LDL cholesterol after their absorption in the body (Berner, 1993). The most represented SFA in goat's milk is palmitic acid. Goat's milk contains 23.2–34.8% of this FA but its content can be effectively reduced by increasing the proportion of protein in the feeding portion (Park et al., 2007; Czauderna et al., 2010). The stearic acid content in goat's milk is also quite high but this fatty acid is probably very quickly converted by desaturase into oleic acid (Samková et al., 2009). Oleic acid is the most prevailing MUFA in goat's milk with its content being 15.4–27.7%. Some studies indicate that this acid can help reduce weight, because its absence in the diet leads to increased fat deposits in the body (Park et al., 2007; Pauwels, Kostkiewicz, 2010). PUFAs are not too much

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represented in goat's milk fat, but they are important in human nutrition and essential for normal growth and development in mammals (Williams, 2000; Papadopoulos et al., 2002; Bourre, 2005). The recommended ratio of SFA, MUFA, and PUFA is < 1:1.4 > 0.6. The ratio of n-6: n-3 PUFAs should be maximally 5:1. Although there is relatively large amount of PUFAs in ruminants' feeding portions, their content in milk is relatively low due to their biohydrogenation in the rumen.

Green algae are one possible source of bioactive components. Freshwater alga *Chlorella vulgaris* therefore represents an interesting source of nutritionally valuable substances, including unsaturated fatty acids. Its cultivation is easy and relatively cheap. Algae grow very fast, have high yields, and contain very valuable nutrients, especially proteins, antioxidants, and fatty acids (Table 1). *Chlorella vulgaris* contains about 10% fat. Its major part consists of essential unsaturated fatty acids. The advantage of algae production is in their huge growth potential, their small requirements for growing area, and a residue-free harvest (Petkov, Garcia 2007; Görs et al., 2010; Doucha, Lívanský, 2012).

Algae represent one of the most efficient converters of solar energy to biomass (Masojídek, Prášil, 2010). The use of algae has a great potential not only in the pharmaceutical and food industries (Lee, 2001), but also as an additive to livestock feed (Rasoul-Amini et al., 2009). Such supplementation of ruminants is an effective method for reducing SFAs and increasing concentrations of conjugated linoleic acid (CLA) and other PUFAs in the milk of ruminants. Changes in the fatty acid profile probably relate to changes in the population of rumen bacterial flora (Toral et al., 2012).

Although considerable evidence about the health benefit of unsaturated fatty acids has been presented, they are still present in the diets of most European countries at a less than optimal requirement. Milk fat is an easily changeable component which provides the possibility to produce new dairy products from milk with higher content of n-3 and n-6 fatty acids (Kennelly et al., 2005; Park et al., 2007). Omega-3 fatty acids are in fish meat (Cant et al., 1997; Donovan et al., 2000; Shingfield et al., 2006), wheat germ, fresh fruit and vegetables, garlic, olive oil, linseed, linseed oil, walnuts, soybeans, soybean oil, and rapeseed oil (Chichlowski et al., 2005; Jones et al., 2005) and seaweed (Rasoul-Amini et al., 2009). There are several studies focused on increasing the content of PUFAs in cow's milk usually by fish oil supplementation (Cant et al., 1997; Doreau, Chilliard, 1997; Donovan et al., 2000; Keady et al., 2000), by linseed (Nudda et al., 2006) or by marine algae. Milk from cows fed fish oil contained higher concentrations of conjugated linoleic acid (CLA) and total unsaturated fatty acids (Baer et al., 2001). Dietary marine algae (*Schizochytrium* sp.) also increased concentrations of CLA and docosahexaenoic acid in the milk of dairy cows (Franklin et al., 1999). Or-Rashid et al. (2008) monitored changes in fatty acids in rumen fluid of cattle fed a diet containing red algae *Crypthecodinium cohnii*. The percentage of SFA decreased when the diet was supplemented with algae. On the contrary, there was an increase in rumenic CLA. The enrichment of goat's milk with fish oil was reported by Kitessa et al. (2001), Chilliard et al. (2003), and Cattaneo et al. (2006). According to Papadopoulos et al. (2002), dairy ewe's milk composition was significantly affected by the dietary inclusion of algae *Schizochytrium* sp. The milk was significantly enriched with PUFAs and the ratio of n-6:n-3 was 2.5:4.5. However, the response of dairy goats to freshwater algae supplementation is not very well known.

Table 1. Major fatty acids in algae *Chlorella vulgaris*

Fatty acid	% of total FA
C 4:0	0.20
C 6:0	2.77
C 8:0	0.26
C 12:0	0.87
C 14:0	0.69
C 16:0	14.42
C 16:1	4.04
C 18:0	1.57
C 18:1	17.62
C 18:2	11.97
C 18:3 ω-3	15.79
C 20:0	0.14
C 22:6	0.30
C 24:0	0.22

Source: Ötleş, Pire, 2001

The addition of algae to the diet of ruminants resulted in the reduction of SFAs and increase of CLA and other PUFAs content in their milk. A higher daily milk yield and higher fat and protein content in milk were registered, too. The addition of algae to the diet had a positive effect on cellular nutrition and the central nervous system (Vahmani, 2013).

The aim of the present study was therefore to determine whether the addition of algae *Chlorella vulgaris* in the goat's diet results in fatty acids changes and improves nutritional and health properties of goat's milk. Attention was paid to reducing the proportion of SFAs and increasing the proportion of nutritionally beneficial unsaturated FAs, mainly n-3 PUFAs. The production of milk with beneficial fatty acids may have a desirable impact on the health of consumers. Autotrophic cultivation of algae for feeding can also significantly contribute to reducing carbon dioxide emissions, because the algae use it as a source of carbon.

Table 2. Silage composition (analysed by AGRO CS a.s. EKOAKVA laboratory Česká Skalice, Czech Republic)

Parameter	Units	In the mass	In dry matter
Original mass	g . kg-1	509.50	100.00
N substances	g . kg-1	46.85	91.95
SNLs	g . kg-1	22.24	43.66
fat	g . kg-1	16.30	32.00
Fiber	g . kg-1	170.58	334.82
Ash matter	g . kg-1	29.39	57.69
BNVL	g . kg-1	248.58	487.92
Starch value		21.17	41.55
MEs/BE	MJ . kg-1	4.61/9.47	
NEL/NEV	MJ . kg-1	2.67/2.52	
Lactic acid	g . kg-1	20.40	
Acetic acid	g . kg-1	3.90	
Butyric acid	g . kg-1	0.00	
pH		4.40	

BNVL - nitrogen-free substances, SNLs - digestible nitrogen substances, MEs/BE - metabolisable energy of feed/ brutto energy of feed, NEL/ NEV - netto energy for lactation/ netto energy for fattening

MATERIAL AND METHODS

Chlorella vulgaris Beij., 1996/H 14 (denoted as strain H 14) was selected in the laboratory of the Institute of Botany, Academy of Sciences of the Czech Republic, Třeboň. The alga is deposited at the Culture Collection of Autotrophic Organisms. Basic growth parameters of the strain are: maximum specific growth rate $\mu_{max} = 0.18\ h^{-1}$ at culture temperature 35–37°C and pH 6–7.5 (Doucha, Lívanský, 2012).

Algae cultivation took place in the photobioreactor, where the algae with nutrient solution were exposed to sunlight and a CO_2 bubbler. Algae were harvested when their culture reached about 30 g of dry matter per 1 l of nutrient solution. After pre-concentration and washing with water the algae were dried using a spray dryer to 60°C for a few seconds.

Three-year old white shorthaired dairy goats on 2nd and 3rd lactation and at the beginning of the lactation period were selected for the experiment. Goats were fed *ad libitum* hay, silage, and then mashed concentrated feed in organic quality. The concentrate consisted of 50% wheat, 25% oats, and 25% maize and its portion was about 0.3 kg per animal per milking (goats were milked twice a day). Silage composition is shown in Table 2. Selected individuals were divided into three groups per 12 animals marked with blue, green, and red. The blue group was a control group with no addition of algae to the diet. The second group marked in green were the animals supplemented with 5 g of dried algae *Chlorella vulgaris* daily. Animals of the third group, marked red, were fed 10 g algae per head per day during the test. Added algae were ground and given to goats individually during milking in the form of dried powder. Samples for fatty acid profile analysis were taken at the beginning of the experiment (March 15th) and six weeks later by its end (April 26th). Milk was collected into standard plastic sample tubes, cooled down, and kept frozen at –18°C until analysis.

Milk samples were defrosted in a water bath at 20°C. Fat was extracted by the modified Gerber method (ISO 2446:2008 (IDF 226:2008). The method was modified using butyrometers for cheese, allowing fat removal from the top of the butyrometer after its centrifugation. Separated milk fat was esterified by 0.25M methanolic KOH according to ISO 15884:2002 (IDF 182:2002). After that, methyl esters of fatty acids were extracted into 10 ml of n-heptane and dried using anhydrous sodium sulphate. The fatty acid profile analysis was performed on Agilent 7890 GC-FID system (Agilent Technologies Inc., Santa Clara, USA) using biscyanopropyl polysiloxane Rt®-2560 capillary column (100 m × 0.25 mm × 0.2 µm) (Restek Corp., Bellefonte, USA). The instrumental conditions were: inlet and detector temperatures 250°C, oven temperature program 100°C (4 min), 3°C per min to 240°C (10 min). The results were expressed in relative percentage of each fatty acid, and were calculated using the internal normalization of the chromatographic peak area. The Food Industry FAME Mix (AOAC 996.06 Standard, 2000) was used for peak identification.

The obtained data were processed using MS Excel 2007 and STATISTICA Version 9 software. To compare different groups of samples (blue, green, and red), the relative content of each fatty acid was finally calculated (100% = FA content at the beginning of the experiment). The individual groups were then compared using a *t*-test for two independent variables. Differences were considered significant at $P < 0.05$.

Table 3. Fatty acids profile of goats' milk samples (in %) for each experimental group at the beginning of the experiment

Sampling date 15. 3.	Blue group		Green group		Red group	
	x	SD	x	SD	x	SD
C4:0	1.7	0.6	1.5	0.2	1.2	0.2
C6:0	1.9	0.3	2.2	0.3	1.6	0.3
C8:0	2.5	0.4	3.0	0.4	2.3	0.3
C10:0	10.9	3.2	11.5	1.4	9.4	1.6
C12:0	4.7	1.0	5.6	1.0	4.9	1.3
C14:0	12.3	1.6	13.3	1.4	13.7	1.6
C14:1	1.1	0.1	1.2	0.1	1.4	0.2
C16:0	29.3	2.6	32.6	2.6	35.4	2.4
C16:1	1.0	0.1	0.9	0.1	1.1	0.2
C18:0	11.6	1.2	12.8	0.7	12.8	1.7
C18:1	15.5	2.6	12.3	2.3	13.2	2.8
C18:2	2.0	0.5	1.4	0.3	1.6	0.3
C20:0	1.1	0.3	0.6	0.2	0.8	0.4
C18:3	1.5	0.3	2.3	0.3	2.0	0.4

Blue group = control, Green group = 5 g *Chlorella vulgaris*/goat/day, Red group = 10 g *Chlorella vulgaris*/goat/day.

RESULTS

Basic statistical characteristics (arithmetic mean (x) and standard deviation (SD)) of fatty acids content in goat's milk at the beginning and the end of the experiment are given in Tables 3 and 4. The content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in all samples was below the detection limit of the method used (0.1%). The differences of total SFA, MUFA, and PUFA at the beginning and at the end of the experiment are presented in Fig. 1.

As *Chlorella vulgaris* contains mainly longer chain fatty acids with 16 carbons and more (Table 1), the effect of feed supplementation was focussed on these fatty acids. Fat extraction and fatty acids analysis conditions could probably cause a partial evaporation of highly volatile short chain fatty acids and therefore their changes are not discussed in this paper.

Table 4. Fatty acids profile of goats' milk samples (in %) for each experimental group at the end of the experiment

Sampling date 26. 4.	Blue group		Green group		Red group	
	x	SD	x	SD	x	SD
C4:0	0.9	0.7	1.4	0.2	0.9	0.5
C6:0	0.9	0.8	1.8	0.3	1.5	0.8
C8:0	1.4	0.6	2.2	0.3	1.4	0.5
C10:0	7.4	0.8	8.4	1.0	6.5	1.1
C12:0	4.1	0.5	4.0	0.6	3.6	0.6
C14:0	13.0	1.1	11.9	0.5	11.0	1.4
C14:1	1.4	0.2	1.3	0.2	3.8	0.1
C16:0	34.6	2.5	31.9	0.6	28.4	2.0
C16:1	1.1	0.1	1.0	0.1	0.8	0.3
C18:0	14.3	2.4	12.1	0.7	10.8	0.3
C18:1	17.5	3.2	19.4	2.8	21.2	1.2
C18:2	1.9	0.1	2.4	0.3	2.6	0.3
C20:0	0.7	0.2	1.3	0.2	3.8	0.2
C18:3	1.1	0.3	0.8	0.3	3.6	0.4

Blue group = control, Green group = 5 g *Chlorella vulgaris*/goat/day, Red group = 10 g *Chlorella vulgaris*/goat/day.

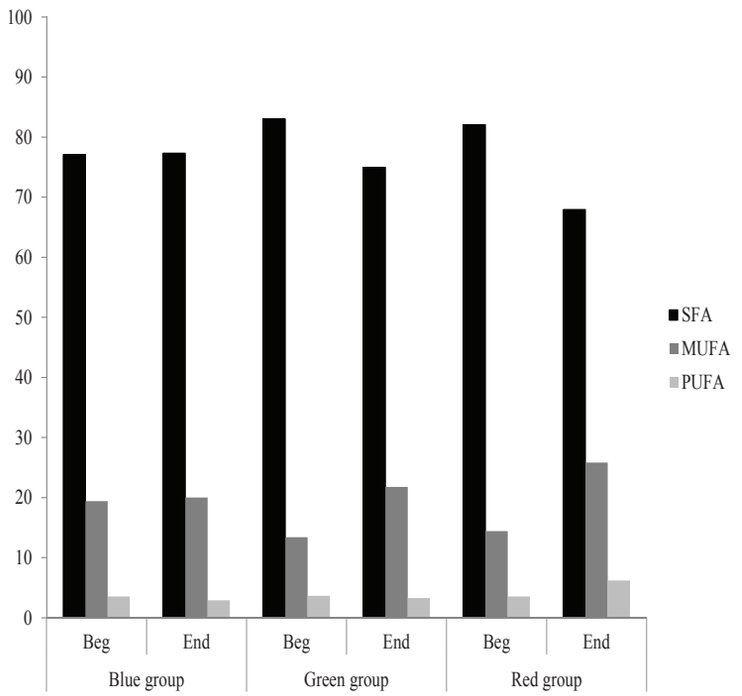

Fig. 1. Comparison of SFA, MUFA and PUFA content (in %) in experimental goat groups at the beginning (Beg) and at the end (End) of experiment. Blue group = control, Green group = 5 g *Chlorella vulgaris*/goat/day, Red group = 10 g *Chlorella vulgaris*/goat/day

DISCUSSION

Park et al. (2007) reported that the content of capric, myristic, palmitic, stearic, and oleic acids should exceed 75% in goat's milk, which corresponds to the results obtained (over 80% on the average). The content of palmitic acid in goat's milk should be 23.2–34.8% (Park et al., 2007). There was a 5.32% difference in this fatty acid between the control group samples taken at the beginning and at the end of the experiment which is within the range given above. The green group showed a negligible decrease of 0.71%. The largest decrease in palmitic acid content (from 35.45 to 28.44%) was observed in the red group. This downward trend was also described by Cattaneo et al. (2006) in their study focused on the addition of fish oil diet to goats. They reduced the content of palmitic acid by 3.04%. According to Czadeurna et al. (2010), palmitic acid content can be also reduced by increasing the protein content in the feed, which happened by adding algae to the diet, too. *Chlorella vulgaris* is a good source of valuable proteins which make up to 60% of its dry matter content (Doucha, 1998).

The content of oleic acid showed almost identical values in the case of the blue (control) group at the beginning and at the end of the measurement. There was an 8.15% increase in both oleic acid isomers in the green group. The largest increase (by 9.33%) was recorded in the red group, where the value was 11.89% at the beginning and 21.22% at the end of the experiment. A similar tendency was also described by Chilliard et al. (2003). The addition of encapsulated canola seeds to goats feeding portion increased the oleic acid content by 7.9%. In the control group, the linoleic acid content decreased by 0.16% during the experiment. The green group showed an increase of 1.14%. The red group recorded a 1.06% increase of all C 18:2 isomers. The content of C 18:3 isomers in the control group and the green group with the addition of *Chlorella vulgaris* (5 g per animal per day) decreased by 0.45 and 1.55%, respectively. On the other hand, the red group (10 g per animal per day) showed the 1.61% increase of both these isomers. Chilliard et al. (2003) also described the increase (by 3.1%) of linolenic acid in the case of canola seed supplementation.

Though the rumen microbiota could change the structure of fatty acids from the diet, the differences measured between the beginning and the end of the experiment showed that fatty acid profile of milk was influenced by algae supplementation. The more algae added to the diet, the greater changes in the fatty acids profile of goat's milk were found. The statistical comparison of total fatty acids in each group showed that the difference between blue and green groups (no *Chlorella vulgaris* and 5 g of *Chlorella vulgaris*) was statistically insignificant ($P = 0.3262$), the same as the difference between green and red groups (5 and 10 g of *Chlorella vulgaris*) ($P = 0.1764$). In contrast, a statistically significant difference between the blue group and the control group was found ($P = 0.0390$). The addition of 10 g of algae into the feed thus demonstrated statistically significantly different fatty acid composition of dairy goats' milk.

The ratio of SFA:MUFA:PUFA in the samples analyzed was compared with nutritionally recommended values (< 1:1.4:> 0.6). In the case of the

control group, this ratio was almost identical at the beginning (5.56:1.4:0.25) and at the end of the experiment (5.41:1.4:0.20). The green group with the addition of 5 g of algae per animal per day showed a positive change in this ratio from 8.66:1.4:0.38 to 4.82:1.4:0.21. The red group, with the highest addition of algae (10 g per day), showed the greatest positive change in the ratio of SFA:MUFA:PUFA, from 7.97:1.4:0.34 to 3.68:1.4:0.34. There was a significant (by more than 50%) reduction in the SFA content. However, it should be noted that short chain fatty acids were not taken into account because of their volatility and possible loss during the applied esterification method.

The red group also exhibited a positive shift of n-6:n-3 PUFA ratio, which should be maximally 5:1 in the human diet. This experimental group exhibited the ratio of 3.72:1 at the beginning of the experiment and 2.36:1 at the end of the experiment, while the blue control group started at 3.04:1 and finalized at 4.70:1. The results may, however, be regarded only as orientation values because the total PUFA content was relatively low. If this tendency is proved, the increasing proportion of n-3 PUFA in milk from goats supplemented with algae could bring about a positive effect in the prevention of hypertension, tumour growth and development, coronary disease, and diabetes (Simopoulos, 1999; Hardman, 2002).

Fatty acids play also an important role in cholesterol regulation in blood. Fatty acids with 12 to 16 carbon atoms significantly increase the level of LDL and HDL cholesterol and thus the total cholesterol in the blood plasma (Foster et al., 2009). Novel milk with reduced SFA could therefore decrease LDL cholesterol fraction which significantly lowers total plasma cholesterol (Noakes et al., 1996). Increasing the content of PUFAs could be beneficial not only to humans, but also to neonatal goat kids, especially in organic farming systems where the diet of the new-born animals consists of maternal milk for at least 45 days.

CONCLUSION

Our results showed that dietary composition affected the fatty acid profile of dairy goat's milk. The addition of dried algae *Chlorella vulgaris* to the diet of goats caused changes in both saturated and unsaturated fatty acids in milk. The differences measured between the beginning and the end of the experiment showed that the more algae was added to the diet, the greater changes in the fatty acids profile of goat's milk were found. A statistically significant effect was found between the control group and the group supplemented with 10 g of *Chlorella vulgaris* per goat per day. The greatest effect was seen in the relative reduction of palmitic acid content and increased oleic, linoleic, and linolenic acids content. The addition of algae also increased the nutritional quality of goat's milk, because there was a positive change in the ratio of SFA:MUFA:PUFA in terms of reducing the proportion of SFAs, as well as a the tendency in the change of n-6 and n-3 PUFAs ratio.

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