ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume 65 162 Number 5, 2017
https://doi.org/10.11118/actaun201765051585

EFFECT OF *CHLORELLA VULGARIS* AND *JAPONOCHYTRIUM* SP. MICROALGAE SUPPLEMENTATION ON COMPOSITION AND FATTY ACID PROFILE OF GOAT MILK

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Abstract

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The aim of this study was to investigate the effect of two species of the microalgae on the milk yield, the basic composition and the fatty acid profile of goat milk, with focus on n-3 fatty acids. Forty-five White short-haired goats were randomly allocated to three groups; the control group (C) with no supplementation microalgae to the diet. The first experimental group (Ch) was supplemented with *Chlorella vulgaris* and second experimental group (J) has been supplemented with *Japonochytrium* sp. The *Japonochytrium* supplementation negatively affected milk yield, but the amount of milk fat (+0.1%; +0.45%) and solids-not-fat (+0.27%; +0.86%) were higher than in group C and Ch. The amount of polyunsaturated (5.527% ± 0.378) and saturated (71.560% ± 0.861) fatty acids was also highest in group J. An increase of C20:4, C20:5 was detected in J and Ch, and the concentration of C22:6 was highest in group J (+0.019%; $P < 0.001$).

Keywords: goat's milk, milk composition, fatty acid, microalgae, *Chlorella vulgaris*, *Japonochytrium* sp.

INTRODUCTION

Producers of goat milk and dairy products are constantly trying to increase yield and change the ratio of protein and milk fat (Kennelly *et al.*, 2005). In recent years, there has been increased effort to affect the fatty acid (FA) profile of the goat milk and dairy products. Modifications in ruminant diet can multiply concentrations of bioactive compounds (conjugated linoleic acid (CLA) or n-3 and n-6 fatty acids) in milk and dairy products (Chilliard *et al.*, 2003). Such enriched milk could be used for the development of new functional foods and nutritional supplements, and it could improve the health of consumers (Hardman, 2002; Póti *et al.*, 2016). The high intake of n-3 fatty acids can reduce human blood triglycerides and the risk factor of coronary heart disease, minimise the possibility of thrombosis leading to a heart attack (Hardman, 2002; Li *et al.*, 2003). Linoleic acid (C18:2, n-6) and alpha-linolenic acid (C18:3, n-3) are considered essential to humans because they cannot be synthesised in the human body (Chilliard *et al.*, 2003). Alpha-linolenic acid is the precursor of n-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (C20:5, n-3, EPA) and docosahexaenoic acid (C22:6, n-3, DHA), which are required for many metabolic processes in human body and effectively prevent coronary heart diseases (Hardman, 2002; Póti *et al.*, 2015). CLA has anticarcinogenic and antiatherogenic effects of the anti-obesity properties enhancing, antidiabetic and immune system (Tsiplakou *et al.*, 2008). The most efficient strategies involved supplementing animal feed with different oils. Results of such studies reported that n-3 PUFA enriched supplemental fat decreased in feed consumption, milk yield and

milk fat depression in cows (Or-Rashid *et al.*, 2008), however, in goat reported no effect (Zhang *et al.*, 2006). Nevertheless, data on feeding microalgae additions are limited, mainly in dairy cows and ewes, such as Papadopoulos *et al.* (2002); Boeckaert *et al.* (2008); Toral *et al.* (2010). The microalgae cultivating have been developed over the last decades because it is a straightforward and inexpensive method for their cultivation. Microalgae can produce valuable metabolites, such as n-3 fatty acids for nutraceutical and pharmaceutical purposes (Guerin *et al.*, 2003; Hu, 2004). Green freshwater microalgae *Chlorella vulgaris* belonging to the class *Trebouxiophyceae*, family *Chlorellaceae* contains high concentrations of beneficial fatty acids, and it is a primary source of linoleic acid and alfa-linolenic acid (Petkov et Garcia, 2007). The second tested microalgae *Japonochytrium* sp. is in Kingdom *Thraustochytrid*, saprophytic species occurring in marine and brackish waters on the surface of algae, organic detritus and vascular plants. *Thraustochytrid* produce and accumulate high concentration of lipids in their biomass, especially DHA (Humhal *et al.*, 2016; Jasuja *et al.*, 2010). Microalgae can be included in foods or feeds, because of their easy digestibility (Luy et Rusing, 2007). We supposed that the milk amount and the composition with emphasis on the fatty acid profile could be improved when goats are fed with the microalgae supplemented the diet. Therefore the aim of this study was to compare the effect of the supplemented two species of microalgae on the milk yield and the basic composition as well as the fatty acid profile of the goat milk, concerning the health of consumers.

MATERIALS AND METHODS

Experimental design

This experiment used 45 White short-haired dairy goats reared on an organic farm in the Czech Republic. The animals were balanced for parity and period of kidding. The control and experimental goats were kept indoors and were fed with 4 kg grass, hay (*ad libitum*) and 300 ggrain mix (50% wheat, 25% oats and 25% maize). Goats in the first experimental group (Ch, n = 15) were fed the same ration with the addition of 10 g/head/day dried granulated microalgae *Chlorella vulgaris*, while goats in the second experimental group (J, n = 15) were fed a ration with the addition of 10 g/head/day dried granulated microalgae *Japonochytrium* sp. Goats in

I: *Chemical composition and fatty acid profile of standard ration and microalgae supplementation (% of total fatty acids).*

	Grasslands	Hay	Concentrate feed	*Chlorella vulgaris*	*Japonochytrium* sp.
Chemical composition					
Dry matter, g.kg^{-1}DM	968.41	931.14	876.34	949.38	968.51
Crude protein, g.kg^{-1} DM	159.79	80.94	88.59	96.12	94.41
Crude fat, g.kg^{-1} DM	18.72	10.14	29.38	13.67	5.4
Crude fiber, g.kg^{-1} DM	219.73	345.41	42.28	17.83	14.09
Crude ash, g.kg^{-1} DM	106.13	46.98	17.34	20.05	24.5
Main fatty acids					
C 16:0, %	14.775	22.829	11.587	16.17	22.93
C 18:0, %	1.992	5.835	1.803	0.92	1.474
C 18:1, n-9, %	6.348	21.624	23.38	0.49	14.869
C 18:2, n-6, %	15.424	17.935	58.307	28.34	1.682
C 18:3, n-6, %	0.175	0.283	0.024	0.00	0.116
C 18:3, n-3, %	56.051	21.99	2.183	33.66	0.085
C 20:2, n-6, %	0.056	0.053	0.046	0.00	0.014
C 20:3, n-6, %	0.00	0.098	0.005	0.00	0.174
C 20:4, n-6, %	0.012	0.00	0.003	0.14	0.209
C 20:5, n-3, %	0.343	0.552	0.123	0.00	0.575
C 22:6, n-3, %	0.094	0.187	0.091	0.81	41.954
SFA, %	23.41	23.72	19.83	19.2	28.842
MUFA, %	10.98	11.28	35.12	18.43	15.853
PUFA, %	65.61	65	45.056	62.14	55.307
n-3, %	50.07	41.11	2.89	33.66	43.456
n-6, %	15.36	23.84	42.17	28.48	11.634

Notes: DM – dry matter, SFA – saturated fatty acid, MUFA – monounsaturated fatty acid, PUFA – polyunsaturated fatty acid.

control group (C, n = 15) were fed mentioned ration without supplements. Chemical composition and fatty acid profile of standard ration and microalgae supplementation are shown in Tab. I. Goats were individually fed their diet and their microalgae, the supplements were fed during milking from May to July 2015. Individual milk samples for analysis were taken four times during the experiment during the morning milking every two weeks. The first samples were collected 60 days after kidding before microalgae were fed. The second samples were taken 74, and the third samples were taken 88 days after kidding, during the supplementation with microalgae. The fourth milk samples were taken 102 days after kidding, and it was 14 days after supplementation with microalgae was finished. Milk samples (200 ml per animal) were collected into standard plastic sample tubes, cooled to 5–8 °C, and transported in a thermo-box to the milk laboratory at Czech University of Life Sciences in Prague for analysis.

Determination of basic components in milk

Milk samples were assayed immediately on arrival for solids-not-fat solids (SNF), fat (F), total protein (P) and lactose (L) contents. Daily milk yield (DMY) was determined from both milk sampling of the day. Milk was assayed using the infrared Fourier transform analyser operating in the central part of the infrared spectrum (Milkoscan FT2, FOSS, Hilleroed, Denmark). Milk samples were analysed for milk compositions by gravimetric method (ČSN EN ISO 1211 – Reference method) according to the Czech Standards Institution (2011).

Determination of fatty acids in milk

For fatty acids analysis, milk was frozen at –18 °C until analysis. Milk samples were defrosted in a water bath at 20 °C, homogenised and centrifuged at 5000 g per minute for 15 min. The cell pellet was frozen at –70 °C and lyophilized (Lyovac GT2, Hilleroed, Denmark) for 15 hours. Fatty acids in isolated milk fat were re-esterified to their methyl esters. For esterification, approximately 40 mg milk fat, 0.5 ml methanol, and 0.5 ml methanolic-base (0.5 N) were placed in a 25ml centrifuge tube. The solution was agitated and heated for 2 min at 80 °C. Next, 1.5 ml of hexane and 10 ml of saturated sodium chloride solution were added. The tube was agitated again, and the hexane layer was separated and analysed. FAs were determined using a gas-chromatographic method (GC) using an Agilent 7890A apparatus with an SP-2560 Supelco column (100 m × 0.25 mm × 0.2 μ) with helium carrier gas (flow 1.2 ml/ min), with injection and detector temperatures at 280 °C. The column temperature was 140 °C held for 5 min and then increased 4 °C/ min to 240 °C. The identification of FAME was carried out using the analytical standards (Supelco, Christiansburg, Virginia). The proportions of individual FAs were calculated from the ratio of each peak area to the total area of all observed FAs. Furthermore, the total amount of saturated (SFA), mono (MUFA) and polyunsaturated (PUFA) fatty acids including subgroups n-3 and n-6 fatty acids have been determined.

Statistical analysis

Effects of addition microalgae *Chlorella vulgaris* and microalgae *Japonochytrium* sp. in the diets on selected milk production traits were assessed by analysis of variance (ANOVA) using the GLM procedure of the SAS 9.2 software (SAS Institute Inc. 2002–2005). For the calculations, the following model was designed:

$$Y_{ij} = \mu + A_i + \beta_j + e_{ijk}$$

Y_{ij} – measured values of dependent variable (milk yield (kg), milk fat, protein, lactose and solids-not-fat (%), fatty acids (%)), μ – overall mean of dependent variable,

A_i – fixed effect of diet (microalgae addition, 3 levels), $_i$ = *Chlorella vulgaris* (Ch), n = 15; *Japonochytrium sp.* (J), n = 15; control group without microalgae (C), n = 15), β_j – fixed effect of order of sample (an average of group Ch, J, C), e_{ijk} – random residual effect. The differences between the variables estimated were tested at the levels of significance $P < 0.05$ and $P < 0.01$.

RESULTS

Basic statistical parameters of the model applied to different microalgae supplementation, and the order of sample on the composition of goat milk are shown in Tab. II. Model equations and the group were statistically significant for the daily milk yield, the milk fat and the solids-not-fat ($P < 0.001$). The order of sample was statistically significant only for fat ($P < 0.001$). The effect of sample affected the amount of milk yield ($P < 0.001$), the milk fat ($P < 0.001$), and the solids-not-fat ($P < 0.05$). Nevertheless, other parameters did not show the significant effect of factors differences in all groups (model, group and order of sample). The amount of the milk yield and the composition of goat milk is shown in Tab. III. There was a higher amount of milk yield in the control group than in group Ch (+1.24 kg, $P < 0.001$) and in group J (+0.89 kg, $P < 0.001$). The order of sample affected the milk yield, too. The highest amount of milk was measured in the second specimen (2.06% ± 0.101) in contrast with the fourth sample when the lowest amount of milk (1.57% ± 0.101) was measured. This difference was statistically significant ($P < 0.001$). The amount of fat and solids-not-fat was measured the highest in group J and the lowest in group Ch, in both cases. The differences between groups were statistically significant ($P < 0.001$). The order of sample affected the amount of fat and solids-not-fat in milk, too. The lowest amount of milk was measured in the last specimen; however, the amount of fat and protein slightly increased.

II: *Basic statistical parameters of model applied to different microalgae supplementation and the order of sample on composition of goat milk.*

	MODEL		Group		Order of sample	
	r^2	P	F-test	P	F-test	P
milk yield (kg)	0.402	<0.001	61.93	<0.001	4.95	0.002
fat (%)	0.167	<0.001	10.65	<0.001	6.60	<0.001
protein (%)	0.031	0.255	0.28	0.753	2.02	0.112
lactose (%)	0.014	0.715	0.16	0.854	0.86	0.464
SNF (%)	0.136	<0.001	11.39	<0.001	3.2	0.024

Notes: SNF – solids-not-fat.

III: *Effect of microalgae supplementation and order of sample on milk yield and composition of goat milk (LSM ± SD).*

effect		Milk yield (kg)	Fat (%)	Protein (%)	Lactose (%)	SNF (%)
		LSM ± SD	LSM ± SD	LSM ± SD	LSM ± SD	LSM ± SD
Group	C	$2.59 \pm 0.070^{A,C}$	2.95 ± 0.053^{A}	2.89 ± 0.022	4.37 ± 0.029	10.81 ± 0.093^{A}
	Ch	$1.35 \pm 0.097^{B.a}$	$2.60 \pm 0.073^{B.C}$	2.86 ± 0.031	4.34 ± 0.040	$10.22 \pm 0.128^{B.C}$
	J	$1.70 \pm 0.105^{D.b}$	3.05 ± 0.079^{D}	2.88 ± 0.033	4.36 ± 0.043	11.08 ± 0.079^{D}
Order of sample	1	1.88 ± 0.101	2.73 ± 0.076^{A}	2.81 ± 0.032	4.36 ± 0.042	10.49 ± 0.134^{a}
	2	2.06 ± 0.101^{A}	$3.13 \pm 0.076^{B.C}$	2.90 ± 0.032	4.37 ± 0.042	11.02 ± 0.134^{b}
	3	2.00 ± 0.101^{a}	2.70 ± 0.076^{D}	2.88 ± 0.032	4.39 ± 0.042	10.56 ± 0.134
	4	$1.57 \pm 0.101^{B.b}$	2.90 ± 0.077	2.91 ± 0.032	4.30 ± 0.042	10.74 ± 0.135

Notes: SNF – solids-not-fat. Different letters in the columns are statistical significance of the A-B, C-D…P < 0.001; a-b, c-d...P < 0.05.

Neither the microalgae supplementation nor the order of sample had any effect on the amount of protein and lactose in goat milk.

From the basic statistic model equation (Tab. IV) the microalgae supplementation positively affected the concentrations of lauric (C12:0), myristic (C14:0), palmitoleic (C16:1), oleic (C18:1), linoleic (C18:2), eicosatrienoic (C20:3) and n-6 PUFA, however the order of sample affected C6-16:0, C18:1, C18:2, C20:3, SFA, MUFA on the level of significance P < 0.01. The results of fatty acid analysis of milk samples are presented in Tab. V. The highest amount of SFA was measured in group J (71.560% ± 0.861), it was caused by the increase of caproic (C6:0), caprylic (C8:0), capric (10:0), lauric (12:0), myristic (C14:0) and decreased concentrations of palmitic acid (C16:0) and stearic acid (C18:0). The order of sample affected the concentration of SFA significantly as well (P < 0.001). The amount of MUFA was lower in group J than in Ch and C (P>0.05). However, the effect of the order of sample was significant (P < 0.001). The decrease of MUFA between the first and the second sample was –4.509%, it was due to a decrease of C16:1- *trans*, C16:1, C18:1, n-9 in the second sample. Higher concentration of MUFA was measured in the last sample (+3.921%, P < 0.001). PUFA increased in the experimental group J unlike C (+0.189%) and Ch (+0.525%), but the amount of both subgroups n-3 and n-6 was the lowest in group J. The amount of PUFAs, n-3, n-6 fatty acids was not statistically significant for the effect of different microalgae supplementation neither for the order of sample. The differences in concentration of C18:2 were significant between groups J (+0.311%; P < 0.05) and C and in groups J (+0.444%; P < 0.001) and Ch. The effect of the order of sample was significant for concentrations of eicosatrienoic acid (C20:3, n-6 and C20:3, n-3), their concentrations increased in the last sample. The concentration of DHA was highest in group J, it was statistically significant (+0.019%; P < 0.001). DHA decreased during the experiment, the highest concentration of DHA was in the first sample and the last sample was lowest (–0.017%, P < 0.05).

DISCUSSION

The nutrition is one of the most important factors affecting goat milk composition (Heanlein, 2004). The aim of this study was to investigate the influence of supplementations of freshwater microalgae *Chlorella vulgaris* and marine microalgae *Japonochytrium* sp. into the goat diet on the milk yield, the basic composition and the fatty acid profile of goat's milk. The chemical composition of microalgae depends especially on their environmental and cultivation conditions. About 30% of world production of algae and microalgae is used as animal feed; it is a non-traditional source of the protein, fatty acids, vitamins, minerals and others bioactive components (Lee, 2001; Petkov *et* Garcia, 2007; Christaki *et al.*, 2010). *Chlorella vulgaris* is a source of linoleic and alpha-linolenic acid (Petkov et Garcia, 2007) and *Japonochytrium* sp. is an excellent source of DHA (Schmitt *et al.*, 2012; Humhal *et al.*, 2016). It corresponds with our results, *Chlorella vulgaris* contained 33.66% alpha-linolenic

IV: *Basic statistical parameters of model applied to effect of microalgae supplementation and order of sample on fatty acid profile of goat milk (% of total fatty acids).*

Fatty acids	MODEL		Group		Order of sample	
	r^2	P	F-test	P	F-test	P
C4:0	0.43	0.056	2.28	0.131	2.93	0.062
C6:0	0.61	0.003	1.61	0.227	8.43	0.001
C8:0	0.63	0.002	3.42	0.055	7.94	0.001
C10:0	0.65	0.001	3.53	0.051	8.63	0.001
C12:0	0.83	<0.001	10.88	0.001	21.72	<0.001
C14:0	0.64	0.001	5.19	0.017	7.13	0.002
C16:0	0.69	0.000	0.15	0.863	13.18	<0.001
C16:1; t	0.54	0.009	10.30	0.001	0.31	0.820
C16:1	0.29	0.238	0.25	0.783	2.34	0.107
C18:0	0.70	0.000	2.26	0.133	12.41	0.000
C18:1; t	0.10	0.856	0.52	0.603	0.28	0.836
C18:1, n-9; c	0.89	<0.001	9.72	0.001	41.82	<0.001
C18:1; c	0.22	0.431	2.43	0.116	0.09	0.963
C18:2; t	0.10	0.840	0.20	0.824	0.54	0.660
C18:2, n-6; c	0.64	0.001	8.75	0.002	4.75	0.013
C18:3, n-6	0.26	0.331	0.62	0.548	1.66	0.212
C18:3, n-3	0.23	0.023	0.75	0.485	1.33	0.296
CLA	0.27	0.296	0.24	0.791	2.06	0.141
C20:3, n-6	0.53	0.013	7.19	0.005	1.85	0.175
C20:3, n-3	0.52	0.013	0.62	0.550	6.19	0.004
C20:4, n-6	0.37	0.107	1.38	0.277	2.65	0.080
C22:2	0.21	0.483	0.87	0.438	0.98	0.424
C20:5, n-3	0.14	0.721	0.64	0.538	0.52	0.672
C22:6, n-3	0.59	0.004	7,65	0.004	3.67	0.032
SFA	0.68	0.001	1.00	0.387	11.86	0.000
MUFA	0.80	<0.001	2.80	0.088	21.85	<0.001
PUFA	0.21	0.476	0.78	0.474	1.06	0.392
n-6	0.52	0.015	7.84	0.004	1.25	0.320
n-3	0.22	0.432	0.58	0.570	1.32	0.298

Notes: CLA – conjugated linoleic acid, SFA – saturated fatty acid, MUFA – monounsaturated fatty acid, PUFA – polyunsaturated fatty acid, t – trans, c – cis.

acid and *Japonochytrium* sp. contained 41.954% DHA. The majority of the previous papers are focused on the influence of algae and microalgae on the qualitative indicators of milk of cows and ewes. According Papadopoulos *et al.* (2002) adding seaweed to the diet affected the cellular nutrition and the central nervous system, and that affects all metabolic processes in the body. The use of algae increases the activity of the thyroid gland, which affects the formation and use of acetic acid and butyric acid, which has a major influence on the amount of fat produced. Ewe milk fat and protein contents were significantly increased by supplementation with algae. According to Bichi *et al.* (2013), dietary marine algae have been associated with the milk fat depression in the milk of dairy ewes, on the other hand, marine algae positively affected the fatty acid profile, par example the effect of addition of algae *Schizochytrium* sp. on fatty acid composition in sheep milk that content of n-3 and n-6 FAs was increased (Papadopoulos *et al.*, 2002). It corresponds with the study of Shingfield *et al.* (2013) that some species of algae have been linked to depression of milk fat in dairy ewes and the Boeckaert *et al.* (2008), they reported that algae supplementation (10 $g.kg^{-1}$ DM intake) significantly reduced the cow milk fat content. On the other hand, goats seem to be less sensitive to the milk fat depression than sheep (Franklin *et al.*, 2013). The content of the milk fat was the highest in a group with supplementation with *Japonochytrium* sp. However, the group with supplementation with *Chlorella vulgaris* the milk fat depression was proven. According to Póti *et al.* (2015), in dairy cows, the microalgae supplementation hurt rumen fermentation; it reduced fermentation to

V: *Effect of microalgae supplementation and order of sample on selected fatty acid profile of goat milk (LSM ± SELSM) (% of total fatty acids).*

Fatty acids (%)	Group			Order of sample			
	C	Ch	J	1	2	3	4
	LSM ± SELSM	LSM ± SELSM	LSM ± SELSM	LSM ± SELSM	LSM ± SELSM	LSM ± SELSM	LSM ± SELSM
C4:0	2.602 ± 0.063	2.683 ± 0.077	2.399 ± 0.109	2.353 ± 0.091^{a}	2.619 ± 0.091	2.712 ± 0.091^{b}	2.561 ± 0.091
C6:0	2.520 ± 0.049	2.604 ± 0.061	2.689 ± 0.086	2.485 ± 0.072^{a}	2.821 ± 0.072$^{A.b}$	2.725 ± 0.072^{c}	2.385 ± 0.072$^{B.d}$
C8:0	2.495 ± 0.067^{a}	2.599 ± 0.082	2.846 ± 0.117^{b}	2.534 ± 0.098^{a}	2.939 ± 0.098$^{A.b}$	2.782 ± 0.098^{c}	2.331 ± 0.098$^{B.d}$
C10:0	8.205 ± 0.246^{a}	8.492 ± 0.302	9.514 ± 0.426^{b}	8.165 ± 0.358^{A}	9.975 ± 0.358$^{B.C}$	9.131 ± 0.358^{a}	7.677 ± 0.358$^{D.b}$
C12:0	3.208 ± 0.051^{A}	3.178 ± 0.062^{C}	3.644 ± 0.088$^{B.D}$	3.093 ± 0.074^{A}	3.743 ± 0.074$^{B.C}$	3.496 ± 0.074$^{B.E}$	3.044 ± 0.074$^{D.F}$
C14:0	9.721 ± 0.075	9.560 ± 0.091^{a}	10.070 ± 0.129^{b}	9.473 ± 0.108$^{A.a}$	10.044 ± 0.108$^{B.c}$	9.100 ± 0.108^{b}	9.618 ± 0.108^{d}
C16:0	27.586 ± 0.252	27.721 ± 0.308	27.436 ± 0.436	25.827 ± 0.366$^{A.a}$	28.776 ± 0.366^{B}	28.281 ± 0.366^{B}	27.441 ± 0.366^{b}
C16:1; t	0.407 ± 0.020^{A}	0.463 ± 0.024^{C}	0.273 ± 0.034$^{B.D}$	0.380 ± 0.029	0.361 ± 0.029	0.383 ± 0.029	0.399 ± 0.029
C16:1	0.541 ± 0.023	0.515 ± 0.028	0.532 ± 0.040	0.555 ± 0.034	0.504 ± 0.034	0.472 ± 0.034	0.584 ± 0.034
C18:0	10.536 ± 0.176	10.369 ± 0.216	9.787 ± 0.305	11.429 ± 0.256$^{A.a}$	9.345 ± 0.256^{B}	9.925 ± 0.256^{B}	10.223 ± 0.256^{b}
C18:1; t	2.171 ± 0.127	2.277 ± 0.155	2.004 ± 0.220	2.135 ± 0.184	2.154 ± 0.184	2.041 ± 0.184	2.274 ± 0.184
C18:1, n-9; c	19.494 ± 0.229^{A}	19.578 ± 0.280^{C}	17.616 ± 0.396$^{B.D}$	21.122 ± 0.333^{A}	16.647 ± 0.333$^{B.C}$	17.657 ± 0.333$^{B.E}$	20.158 ± 0.333$^{D.F}$
C18:2; t	0.912 ± 0.048	0.881 ± 0.059	0.943 ± 0.083	0.856 ± 0.070	0.946 ± 0.070	0.883 ± 0.070	0.962 ± 0.070
C18:2, n-6; c	2.085 ± 0.050^{a}	1.952 ± 0.061^{A}	2.396 ± 0.087$^{b.B}$	2.267 ± 0.073^{a}	1.918 ± 0.073$^{b.c}$	2.188 ± 0.073	2.204 ± 0.073^{d}
C18:3, n-6	0.046 ± 0.002	0.048 ± 0.003	0.044 ± 0.004	0.052 ± 0.003	0.045 ± 0.003	0.044 ± 0.003	0.043 ± 0.003
C18:3, n-3	1.18 ± 0.063	1,11 ± 0,078	1,04 ± 0.110	0,97 ± 0,092	1,11 ± 0,092	1.14 ± 0.092	1.22 ± 0.092
CLA	0.716 ± 0.071	0.641 ± 0.087	0.706 ± 0.123	0.620 ± 0.103	0.634 ± 0.103	0.594 ± 0.103	0.903 ± 0.103
C20:3, n-6	0.104 ± 0.004	0.104 ± 0.005	0.086 ± 0.007	0.091 ± 0.006	0.079 ± 0.006$^{a.A}$	0.109 ± 0.006^{b}	0.113 ± 0.006^{B}
C20:3, n-3	0.051 ± 0.005	0.047 ± 0.006	0.041 ± 0.008	0.036 ± 0.007^{a}	0.033 ± 0.007^{A}	0.051 ± 0.007	0.068 ± 0.007$^{B.b}$
C20:4, n-6	0.095 ± 0.009	0.114 ± 0.011	0.117 ± 0.015	0.135 ± 0.013	0.114 ± 0.013	0.096 ± 0.013	0.091 ± 0.013
C22:2	0.013 ± 0.005	0.002 ± 0.007	0.003 ± 0.009	0.000 ± 0.008	0.000 ± 0.008	0.009 ± 0.008	0.015 ± 0.008
C20:5, n-3	0.091 ± 0.007	0.102 ± 0.008	0.102 ± 0.012	0.103 ± 0.010	0.105 ± 0.010	0.092 ± 0.010	0.093 ± 0.010
C22:6, n-3	0.055 ± 0.003^{A}	0.055 ± 0.003^{B}	0.074 ± 0.004^{A,B}	0.071 ± 0.004^{a}	0.061 ± 0.004	0.060 ± 0.004	0.054 ± 0.004^{a}
SFA	70.154 ± 0.497	70.524 ± 0.608	71.560 ± 0.861	68.692 ± 0.722^{A}	73.336 ± 0.722$^{B.C}$	72.279 ± 0.722$^{B.E}$	68.676 ± 0.722$^{D.F}$
MUFA	24.451 ± 0.338	24.390 ± 0.414	22.913 ± 0.585	26.113 ± 0.491^{A}	21.604 ± 0.491$^{B.C}$	22.430 ± 0.491$^{B.E}$	25.525 ± 0.491$^{D.F}$
PUFA	5.338 ± 0.218	5.002 ± 0.267	5.527 ± 0.378	5.157 ± 0.317	5.019 ± 0.317	5.236 ± 0.317	5.746 ± 0.317
n-6	3.298 ± 0.121^{a}	3.657 ± 0.148^{A}	2.641 ± 0.209$^{b.B}$	3.322 ± 0.176	2.924 ± 0.176	3.211 ± 0.176	3.338 ± 0.176
n-3	1.399 ± 0.071	1.317 ± 0.087	1.260 ± 0.123	1.180 ± 0.103	1.310 ± 0.103	1.353 ± 0.103	1.459 ± 0.103

Notes: CLA - conjugated linoleic acid, SFA – saturated fatty acid, MUFA – monounsaturated fatty acid, PUFA – polyunsaturated fatty acid, t – trans, c – cis.

decrease the fat content and concentrations of butyric (C4:0), caproic (C6:0) and caprylic (C8:0) fatty acids of cow milk. Goat milk fat is rich in fatty acids with short and medium carbon chain which are C6:0, C8:0 and C10:0 (capric acid) (Haenlein, 2004). The supplementation with *Japonochytrium* sp. decreased the amount of C4:0, however, the amount of C6:0, C8:0, C10:0 was the highest. On the other hand, the supplementation with *Chlorella vulgaris* slightly increased the amount of C4:0, C6:0, C8:0, C10:0 compared to control group. The supplementation with *Chlorella kessleri* (10 g/kg^{-1} DM intake) significantly increased concentrations of C4:0 but C6:0–C12:0 were decreased in goat milk (Póti *et al*., 2015). The higher SFA amount does not necessarily mean the worse quality of milk, these SFA with short and medium carbon chain are nutritionally highly beneficial for consumers (Sanz Sampelayo *et al*., 2007). On the other hand, the part of SFA represented by hypercholesterolaemic FAs (lauric C12:0, myristic C14:0, palmitic C16:0) significantly increase the level of LDL (low-density lipoprotein) and HDL (high-density lipoprotein) cholesterol (Heanlein, 2007), increase deposition of fat in the vascular walls, and are related to atherosclerotic diseases (Jensen, 2002; Ducháček *et al*., 2014). The content of C16:0, C18:0 was lower in the group supplemented with *Japonochytrium* sp. than in the group with *Chlorella vulgaris*. According the study of Póti *et al.* (2015) the concentrations of C12:0, C14:0, C16:0 did not show any significant differences during the experimental period. However, the decrease of these acids should have a positive effect on the consumer's health (Kouřimská *et al*., 2014). In Borková *et al.* (2016), the feed supplementation with *Japonochytrium* sp. (5g/head/day) increased the amount of vaccenic acid (t11-C18:1), CLA, C18:3, cis-8,11,14-C20:3, and DHA, on the other hand, C18:0 content was decreased. This is in concordance with Póti *et al.* (2015), the feeding of n-3 fatty acids increased markedly the vaccenic acid content, while decreased the amount of C18:0 in milk fat. In our study, the increase of C20:4, C20:5 in both experimental groups were detected. However, the content of CLA and alpha-linolenic was decreased. The concentration of DHA was highest in group with *Japonochytrium sp.* microalgae. Goat milk fat generally contains about 53–72% of SFA, 26–42% of MUFA and 2–6% PUFA (Toral *et al*., 2012). However the addition of microalgae *Chlorella vulgaris* in the goats diet positively affected the ratio of SFA:MUFA:PUFA, from 7.97:1.4:0.34 to 3.68:1.4:0.34 (Kouřimská *et al*., 2014).The feed enrichment with *Japonochytrium* microalgae positively affected the nutritional quality of goat milk due to increasing the content of PUFA (Borková *et al*., 2016). The n-6:n-3 ratio is generally used to assess the nutritional value of fats (Póti *et al*., 2015).The group J exhibited a positive ratio of n-6:n-3 PUFA, 2.096:1 and for the group Ch it was 2.777:1. This is in concordance with the literature reports, the recommended value is an n-6:n-3 ratio of less than 4 (Simopoulos, 2004). However, our results may be regarded as orientation values because the total PUFAs amount was relatively low. Increasing the amount of PUFAs, especially n-3 PUFA, could be beneficial for consumers health (Hardman, 2002), producers of dairy products (Borková *et al*., 2016), but also to goat kids in organic farming systems (Kouřimská *et al*., 2014).

CONCLUSION

In conclusion, there were significant differences in the composition of milk and fatty acid profile between two experimental groups of goats supplemented with two different species of microalgae. The *Japonochytrium* sp. microalgae supplementation resulted in the significantly higher amount of fat and solids-not-fat than *Chlorella vulgaris* supplementation. The concentration of SFA with short and medium carbon chain was significantly greater in both groups with microalgae supplementations. *Japonochytrium* sp. increased the amount of total PUFAs. The use of microalgae in the nutrition of goats caused changes in the ratio of milk fat and protein and therefore affect the yield in the production of cheese and other dairy products. While the consumers have dietary benefits from consumption of this fortified milk by microalgae fed goats, due to increased concentrations of health promoting fatty acids, can also improve the human health.

Acknowledgements

This work was supported by the grant No. QJ1310107 funded by the NAZV Agency of the Ministry of Agriculture of the Czech Republic and the "S" grant of MŠMT.

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