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Effect of marine algae supplementation on the fatty acid profile of milk of dairy goats kept indoor and on pasture

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ABSTRACT. The aim of the study was to investigate the effect of the *Schizochytrium limacinum* marine algae on the fatty acid profile of goat milk, with particular reference to n-3 fatty acids, especially docosahexaenoic acid (DHA) and rumenic acid. Forty dairy goats were randomly allocated to four groups: C – fed with 1500 g alfalfa hay and 600 g concentrate; CMA – received the same forages and concentrate supplemented with 15 g/head/day microalgae; P – kept on pasture with 600 g concentrate; PMA – kept on pasture with 600 g concentrate with microalgae inclusion (15 g/head/day). The C and CMA groups were housed indoors, while the goats from P and PMA groups were kept on a natural pasture. The experiment lasted 31 days, including the last 10 days of sampling period. Marine algae feeding had no negative effect on milk yield and milk composition. The microalgae inclusion considerably increased DHA concentration in milk in both marine algae groups (0.40% in CMA and 0.39% in PMA), and additionally the n-6/n-3 ratio was also more favourable in the microalgae supplemented groups (1.25 and 1.37 in CMA and PMA groups, respectively) as compared to the C and P groups respectively in which this ratio was 2.30 and 1.44 ($P < 0.01$). Also, marine algae supplementation increased the concentration of rumenic acid (0.89% and 1.07% in CMA and PMA groups, respectively) in milk in comparison to C (0.46%) and P (0.77%) groups. So, it can be concluded that diet supplemented with microalgae significantly increased the concentration of beneficial fatty acids in goat milk.

Introduction

Nowadays, there has been increased interest in the modification of the fatty acid composition of milk and milk products by different feeding strategies. Composition of n-3 polyunsaturated fatty acids (PUFA) and rumenic acid (*c9t11* C18:2) in milk, which could be improved by different way of feeding methods is the main concern. Increasing the supply of n-3 PUFA (such as grazing, oils, seeds or algae supplements) in the diet is one of the most essential ways of improving the bioactive composition of goat milk. Earlier reports showed that grazing considerably increased the rumenic acid and n-3 fatty acids contents in goat milk (Pajor et al., 2009; Delgadillo Puga et al., 2015). However, the most popular way to improve composition of foodstuffs by n-3 PUFA is supplementing animal diets with different plant oils, seeds, fish oil, freshwater and marine algae (Moate et al., 2013; Tsiplakou et al., 2017; Białek et al., 2018a). Moreover, fish oil and marine algae supplements in diets of ruminants are

a good source of long chain PUFA (LC-PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Toral et al., 2017) that have beneficial effect for human health, including reduced risk of coronary heart disease (Li et al., 2003). Presently recommended EPA+DHA daily intake for adults is 250 mg (EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA), 2010). However, previous studies reported that ruminant diet enriched with marine algae (e.g., *Schizochytrium limacinum*) resulted in decreased dry matter intake, milk yield and milk fat depression in dairy animals (Or-Rashid et al., 2008; Bichi et al., 2013). *Schizochytrium limacinum* is a well-known heterotrophic marine alga with widespread supplementing use, because of its high concentration of DHA (Ren et al., 2010). Nevertheless, data on feeding marine algae supplements are limited, particularly focused on dairy cows and ewes (Toral et al., 2010; Moran et al., 2017a,b). We hypothesized that goat milk fatty acid profile could be significantly improved when the animals are fed 15 g/head/day dried *Schizochytrium limacinum* marine algae, with no effect of feeding marine algae on milk fat content.

So, the aim of this study was to investigate the effect of the feeding of 15 g/head/day marine algae on milk fatty acid composition of goats kept indoor and pasture.

Material and methods

Experimental design

The animal care was in accordance with the guidelines on the protection of animals used for scientific purposes (Directive 2010/63/EU of the European Parliament and of the Council). The study was carried out on a Hungarian native goat farm (Pest County, Hungary; geographical coordinates: 47°33’77.41”N 19°37’26.11”E) (average annual temperature 12.3 °C with a total precipitation of 580 mm). The animals were balanced for parity and time of kidding. The kidding started at the beginning of March. The weaning of the kids was carried out at the age of 8 weeks on average. After weaning, all goats were milked twice a day by machine milking. Forty multiparous Hungarian native goats (days in milk (DIM) 71) were randomly allocated to four treatment groups. The first and the second group were housed indoors, while the goats from other groups were kept on a natural pasture. The animals in the control group (C, n = 10) were fed 1500 g alfalfa hay and 600 g concentrate; in the second group (CMA, n = 10) goats received the same forages and concentrate with addition of 15 g/head/day dried *Schizochytrium limacinum* marine algae. The does in the third group were kept all day long on pasture (P, n = 10) and were fed 600 g concentrate without microalgae supplementation. Finally, in the fourth group (PMA, n = 10) goats were kept on pasture and fed additionally the same amount of concentrate with the addition of 15 g/head/day dried microalgae. The dried microalgae supplement was produced by Alltech Inc. (ALL-G-RICH®; Dunboyne, Co Meath, Ireland) and contained 30.19 and 58.72 g/100 g of total fatty acids (FA) of DHA and palmitic acid, respectively. In each group, the concentrate was given individually twice a day in equal amounts during milking. A commercial trace-mineralized salt block and drinking water were provided free of choice to all animals.

The experiment lasted 31 days with the first 21 days of adaptation to the diet and the last 10 days of sampling. The control and the experimental concentrates were approximately isonitrogenous and balanced by energy content. The diets were adjusted to the National Research Council recommendations of energy and protein requirements for dairy goats (NRC, 2007). The composition of the experimental diets is shown in Table 1.

Utilization of native pasture is extensive in order to avoid over-grazing of the grassland. The main species were *Festuca pseudovina*, *Cynodon dactylon*, and the main legume was *Lotus corniculatus*. The annual grass yield (green) was 1.4 t/ha green yield. The stocking density of the pastures grazed by the goat was about 0.5 AU/ha. Before starting the grazing, the grass yield was estimated by the clipping method using a 2 m^2 frame. Five random samples were taken (3 cm above ground) from each plot. After grazing, herbage residuals were cut, weighted and yields were calculated as before. Consumed grass yield was calculated by the difference of the pre-grazing grass yield per m^2 and the post-grazing grass yield per m^2.

Milk samples were taken from each animal twice a day during 10 days of the experimental period at 06:00 and 18:00. Two milk samples were collected into 50 ml plastic tubes from every goat (one tube for analysis of milk composition, other tube for determination of fatty acid composition), and the samples were immediately transported to the laboratory. Before laboratory investigation, morning and evening milk samples for each animal were combined for analysis of chemical composition. The milk samples for determination of milk

Table 1. Chemical composition and fatty acid (FA) profile of forage

Indices	Indoor		Pasture	
	C[1]	CMA[2]	P[3]	PMA[4]
Ingredients, %				
pasture	–	–	74.34	74.33
alfalfa hay	71.29	71.28	–	–
concentrate	28.71	27.98	25.66	25.01
microalgae[5,6]	–	0.74	–	0.66
DMI[7], kg/day	1.89	1.89	2.12	2.12
Chemical composition				
dry matter (DM), g/kg forage	902	902	378	378
crude protein, g/kg DM	188.70	189.00	154.00	154.27
crude fat, g/kg DM	25.03	28.27	38.34	41.23
crude fibre, g/kg DM	193.82	193.64	212.14	211.98
crude ash, g/kg DM	65.62	65.78	68.78	68.92
NE_l[8], MJ/kg DM	6.44	6.44	6.13	6.13
Main FA, % of total FA				
C12:0	0.23	0.23	0.25	0.25
C14:0	0.62	0.83	0.52	0.71
C16:0	13.26	15.38	11.50	13.39
C18:0	2.82	2.81	1.93	1.92
C18:1n-9	30.70	31.96	37.61	38.74
C18:2n-6	29.97	24.66	27.78	23.03
C18:3n-3	16.85	17.08	14.50	14.71
C22:6n-3 (DHA)[9]	–	3.79	–	2.32

[1] C – control diet (hay and concentrate), [2] CMA – control diet supplemented with 15 g/head/day microalgae, [3] P – pasture-based diet, [4] PMA – pasture-based diet supplemented with 15 g/head/day microalgae; [5] microalgae chemical composition: DM – 929 g/1000 g, crude protein – 148 g/kg DM, crude fat – 482 g/kg DM, crude fibre – 23 g/kg DM, ash – 38 g/kg DM; [6] microalgae contained, g/100 g FA: C12:0 0.14, C14:0 4.37, C16:0 58.72, C18:0 1.35, C18:1n-9 1.33, C18:2n-6 0.47, C18:3n-3 0.17, C22:6n-3 30.19, [7] DMI – dry matter intake; [8] NE_l – net energy; [9] DHA daily intake: 2027.8 mg

composition were stored at 4 °C for the latter analysis, the other milk samples were frozen and stored at −20 °C for the analysis of fatty acids.

Chemical analysis

The forage samples were analysed for dry matter, crude protein, crude fat, crude fibre and crude ash according to the procedure of the Hungarian Feed Codex (2004).

Fat, protein, lactose and total solids contents of milk were determined using a LactoScope™ analyser (Perten Instruments, Hägersten, Sweden). Milk fat was extracted with the method of Folch et al. (1957). Fatty acids were re-esterified to methyl esters using sodium methanol and boron trifluoride (BF_3) (Park and Goins, 1994). Methyl esters of FA were determined by gas chromatography (gas chromatographer GC 2010, Shimadzu, Kyoto, Japan) with a flame ionization detector (FID) and a column (CP-SIL-88, 100 m × 0.25 mm × 0.2 μm). The split injection ratio was 50:1. Helium was used as the carrier gas, applying a flow rate of 28 cm/s. The injector and detector temperatures were 270 and 300 °C, respectively. The oven temperature programmed run started at 80 °C, then was increased 2.5 °C/min up to 205 °C and held for 20 min and then increased again to 225 °C at 10 °C/min, and held for 5 min. Peaks were identified on the basis of the retention times of standard methyl esters of individual FA (Mixture Me 100, Larodan Fine Chemicals AB, Limhamn, Sweden). The individual FA were calculated by the ratio of their peak area to the total area of all observed acids. The FA were quantitatively and qualitatively determined (mg/100 ml and % of total FA); in this study the results were presented as % of total FA. The selected FA combinations were calculated by using FA data: saturated fatty acids (SFA); monounsaturated fatty acids (MUFA); PUFA; total n-6 and n-3 PUFA and n-6:n-3 ratio.

DHA transfer from feed to milk efficiency was calculated according to Moate et al. (2013): DHA in milk yield (g/day) / DHA intake (g/day).

Statistical analysis

Statistical analysis, processed by the SPSS 23.0 software package (IBM Corporation, Armonk, NY, USA), was carried out in order to determine the effect of diets on milk composition and FA profile by the two different keeping treatments (indoor and pasture). The significance of differences was assessed by Student's t-test in case of normal distribution (Shapiro-Wilk's test). Since data were not normally distributed, variables were subjected to Mann-Whitney U test. Data were expressed as mean ± SD. Differences are shown when $P < 0.05$.

Results

The daily milk yield was similar among treatments at the start of the trial. During the study, dairy goat milk production was unaffected by marine algae supplementation. Average value of daily milk was 1.5 l/day. The marine algae feeding caused higher ($P < 0.05$) fat (3.93 and 3.87 g/100 g) content as compared to milk from goats kept indoors or on pasture wthout marine algae supplementation (3.46 and 3.50 g/100 g, respectively) (Table 2). In contrast, only in grazing groups marine algae addition significantly influenced the milk protein and total solids non-fat content (3.24 and 8.66 g/100 g in P group *vs* 2.94 and 8.28 g/100 g in PMA group, respectively).

Table 2. Chemical composition of goat milk from different keeping treatments (mean ± SD), %

Indices	Indoor		Pasture	
	C[1]	CMA[2]	P[3]	PMA[4]
Fat	3.50 ± 0.19[a]	3.93 ± 0.45[b]	3.46 ± 0.26[a]	3.87 ± 0.28[b]
Protein	2.92 ± 0.10	2.93 ± 0.08	3.24 ± 0.07[b]	2.94 ± 0.07[a]
Lactose	4.70 ± 0.08[b]	4.66 ± 0.02[a]	4.72 ± 0.04[b]	4.64 ± 0.03[a]
Total solids non-fat	8.32 ± 0.18	8.29 ± 0.10	8.66 ± 0.08[b]	8.28 ± 0.10[a]
Total solids	11.82 ± 0.30	12.22 ± 0.50	12.12 ± 0.28	12.15 ± 0.36

[1] C – control diet (hay and concentrate), [2] CMA – control diet supplemented with 15 g/head/day microalgae, [3] P – pasture-based diet, [4] PMA – pasture-based diet supplemented with 15 g/head/day microalgae; [ab] – means with different superscripts within each row separately for indoor and pasture are significantly different at $P < 0.05$

The enrichment of experimental diets with marine algae significantly influenced the FA profile of milk fat (Table 3). The marine algae supplementation increased the concentrations of caprylic acid (C8:0) (but only in goats kept on pasture), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), vaccenic acid (*t11* C18:1), docosahexaenoic acid (C22:6), total n-3 PUFA and rumenic acid (*c9t11* C18:2), while significantly decreased the concentrations of stearic acid (18:0), oleic acid (*c11* C18:1) and linoleic (C18:2) (but only in indoor kept goats) in goat milk.

The n-6/n-3 ratio was more favourable (lower) in CMA and PMA groups (1.25 and 1.37, respectively) in comparison to C (2.30) and P (1.44) groups, respectively.

Based on fat and DHA content of milk in CMA and PMA groups (3.93 and 0.40% *vs* 3.87 and 0.39%, respectively), daily 15 g/head marine algae supplementation resulted in 15.7 and 15.1 mg DHA content in 100 mg of milk in CMA and PMA treatments, respectively (Table 4). Moreover, the DHA conversion efficiency ratio from marine algae fodder to milk was 11.6 and 11.2% in CMA and PMA group, respectively.

Table 3. Effect of marine algae supplementation on fatty acid (FA) profile of goat milk (mean ± SD), % of total FA (except n-6/n-3 ratio)

Fatty acids	Indoor		Pasture	
	C[1]	CMA[2]	P[3]	PMA[4]
C6:0	0.11 ± 0.05	0.15 ± 0.11	0.11 ± 0.05	0.15 ± 0.11
C8:0	1.08 ± 0.18	1.27 ± 0.26	1.06 ± 0.25[a]	1.54 ± 0.29[b]
C10:0	6.09 ± 0.58[a]	8.27 ± 0.64[b]	6.90 ± 1.71[a]	10.00 ± 0.81[b]
C12:0	3.04 ± 0.34[a]	3.89 ± 0.27[b]	4.13 ± 0.94[a]	4.66 ± 0.49[b]
C14:0	9.72 ± 0.66[a]	12.51 ± 0.40[b]	10.41 ± 1.18[a]	11.97 ± 0.57[b]
C14:1	0.09 ± 0.01[a]	0.13 ± 0.02[b]	0.14 ± 0.04[a]	0.15 ± 0.03[b]
C16:0	32.24 ± 1.54[a]	39.59 ± 1.74[b]	34.49 ± 1.73[a]	37.16 ± 1.21[b]
C16:1	0.57 ± 0.02[a]	0.69 ± 0.04[b]	0.76 ± 0.05	0.75 ± 0.05
C18:0	13.59 ± 1.16[b]	7.64 ± 1.03[a]	10.78 ± 1.62[b]	7.78 ± 1.11[a]
c11 C18:1n-9	24.77 ± 1.95[b]	17.29 ± 1.33[a]	22.53 ± 3.04[b]	16.30 ± 1.40[a]
t11 C18:1n-7	1.38 ± 0.23[a]	1.72 ± 0.23[b]	1.69 ± 0.34[a]	2.24 ± 0.71[b]
C18:2n-6	2.49 ± 0.15[b]	1.78 ± 0.17[a]	1.98 ± 0.27	2.00 ± 0.14
C18:3n-3	0.71 ± 0.08	0.59 ± 0.13	0.71 ± 0.13	0.67 ± 0.12
C20:3n-6	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01[b]	0.02 ± 0.01[a]
C20:4n-6	0.17 ± 0.02[a]	0.18 ± 0.01[b]	0.19 ± 0.02[b]	0.16 ± 0.02[a]
C20:5n-3 (EPA)	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.04	0.06 ± 0.01
C22:5n-3	0.13 ± 0.02[b]	0.08 ± 0.02[a]	0.17 ± 0.02[b]	0.10 ± 0.01[a]
C22:6n-3 (DHA)	0.00 ± 0.00[a]	0.40 ± 0.06[b]	0.07 ± 0.01[a]	0.39 ± 0.07[b]
odd FA	2.54 ± 0.10[a]	2.37 ± 0.17[b]	2.26 ± 0.12	2.22 ± 0.11
SFA	69.12 ± 1.91[a]	76.14 ± 1.74[b]	70.85 ± 3.59[a]	76.06 ± 1.66[b]
MUFA	26.85 ± 1.80[b]	19.86 ± 1.52[a]	25.16 ± 3.28[b]	19.46 ± 1.60[a]
PUFA	4.04 ± 0.19	4.00 ± 0.36	4.00 ± 0.50[a]	4.48 ± 0.42[b]
n-6	2.69 ± 0.16[b]	1.98 ± 0.18[a]	2.20 ± 0.29	2.18 ± 0.15
n-3	0.89 ± 0.09[a]	1.13 ± 0.14[b]	1.02 ± 0.12[a]	1.22 ± 1.12[b]
n-6/n-3 ratio	2.30 ± 0.28[b]	1.25 ± 0.13[a]	1.44 ± 0.19[b]	1.37 ± 0.15[a]
c9t11 C18:2 (rumenic acid)	0.46 ± 0.07[a]	0.89 ± 0.08[b]	0.77 ± 0.12[a]	1.07 ± 0.21[b]

[1] C – control diet (hay and concentrate), [2] CMA – control diet supplemented with 15 g/head/day microalgae, [3] P – pasture-based diet, [4] PMA – pasture-based diet supplemented with 15 g/head/day microalgae; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids, odd FA – odd-chain fatty acids; [ab] – means with different superscripts within each row separately for indoor and pasture are significantly different at $P < 0.05$

Table 4. Calculated docosahexaenoic acid (DHA) conversation efficiency from diet to milk

Treatment	Daily intake of DHA g/day	Average milk DHA content, mg/100 g milk	Milk production, l	DHA in milk yield, g/day	DHA efficiency ratio, %
CMA[1]	2.028	15.7	1.5	0.236	11.6
PMA[2]	2.028	15.1	1.5	0.226	11.2

[1] CMA – control diet supplemented with 15 g/head/day microalgae, [2] PMA – pasture-based diet supplemented with 15 g/head/day microalgae

Discussion

Daily milk yield did not differ among feeding treatments. Lack of significance in this trait may be due to the low amount of supplemented marine algae (15 g/head/day). Milk fat and protein contents were consistent with previous study (Kuchtík et al., 2015). Supplementation of marine algae in indoor group had no effect on the milk protein content as in previous reports (Franklin et al., 1999). However, decreased protein content was measured in the pastured animals. The goats in the algal groups had lower lactose content. Also total solid non-fat was different between groups but only in pasture kept animals. Total solids did not show any differences. Pajor et al. (2009) reported that the grazing slightly increased the milk protein and solids non-fat content of goat milk. The milk protein content is crucial not only for consumers but also for farmers and milk processors, because it has a great influence on cheese composition. Papadopoulos et al. (2002) reported that ewe milk fat and protein contents were significantly increased by treatment containing marine algae at doses: 23.5, 47 and 94 g/day/animal. The same positive effect was also found by Póti et al. (2015) with freshwater algae supplementation (10 g/kg DM intake) and by Reynolds et al. (2006), where ewe milk fat content was higher for soybean and marine algal oil supplementation (2:1 ratio w/w; 30 g/kg DM). In contrary, Boeckaert et al. (2008) reported that algae supplementation level of about 10 g/kg DM intake significantly reduced the cow milk fat content. In addition, Bichi et al. (2013) found milk fat depression in dairy ewes fed algae containing fodder (8 g/kg of DM).

The fish oil and marine algae supplements in diets of ruminants are definitely good source of LC-PUFA, such as DHA. It was reported that these fatty acid affect the biohydrogenation of C18:2n-6 and C18:3n-3 fatty acids in the rumen. LC-PUFA inhibit the step of hydrogenation from *trans* C18:1 to C18:0 causing higher level of *trans* C18:1 fatty acids, and other specific biohydrogenation intermediates have been associated with milk fat depression and negatively influenced protozoa and cellulolytic bacteria inhabiting in rumen (Toral et al., 2017; Białek et al., 2018b). In the present study, the effect of 15 g/head/day marine algae supplementation on rumen viability and fermentation was not so unequivocal; marine algae feeding elevated fat content of milk and concentrations of small and medium chained fatty acids (e.g., C8:0 and C10:0) of milk. On the contrary, the odd chain fatty acids (OCFAs) concentrations were slightly decreased in the milk of the experimental goats supplemented with marine algae in comparison to milk from control animals. The OCFAs are also an indicator of ruminal fermentation, produced by ruminal bacterial populations. Nevertheless, the effect of marine algae supplementation on animal health parameters have been poorly studied.

The short and medium chain fatty acids are hydrolysed rapidly and absorbed directly by the liver *via* portal vein, thereby these fatty acids have been used for patients with malabsorption syndrome (Papamandjaris et al., 1998). The marine algae supplementation resulted in significantly higher C8:0 and C10:0 fatty acids. The short and medium-chain fatty acids become more and more interesting for nutritionists. Recently, Gómez-Cortés et al. (2018) summarized the milk fatty acids and their potential health benefits in a review report, and stated that short and medium-chain fatty acids help to maintain the gut microbiota and body weight control.

Concentration of the DHA, which is required for many metabolic processes and effectively prevent coronary heart disease (CHD) in humans was increased by the experimental diet. Additionally, the DHA is one of the most valuable health promoting components. In the present study, the DHA values were markedly higher due to marine algae supplementation regardless animal husbandry system. Also Toral et al. (2010) found that feeding marine algae supplementations considerably increased the DHA content in milk. In the present study, the average values of DHA transfer efficiency in PMA and CMA treatments were 11.2 and 11.6%, respectively. Previously, Moran et al. (2017a) found that DHA concentration and conservation efficiency from algae to milk dramatically raised up to 20% during 21 days, then reached the plateau (between 21–22%) 7 days later. In contrast, Moran et al. (2017b) found higher average efficiencies ratio (7 and 18%) in dairy cows fed *Aurantiochytrium limacinum* enriched

fodder (105 or 146 g/day/cow) during the 12-week treatment. Other study reported that transfer efficiency was 8.9 in cows fed 125–375 g/day marine algae (Moate et al., 2013). However, the transfer efficiency ratio of DHA from fish oil contains diet to milk was less than 5% in cows (Palmquist, 2009). These results suggest that the biohydrogenation level of DHA in *Schizochytrium limacinum* algal meal in the rumen is less than other type of n-3 fatty acid supplements. This may be related to the structure of *Schizochytrium limacinum* cell membrane (Moran et al., 2017a). The authors speculated that algal cell membrane protects the DHA contents during drying processing. The DHA transfer efficiency from diet to milk is influenced by fat protection against biohydrogenation. Franklin et al. (1999) found that the DHA transfer efficiency ratio of dairy cows fed *Schizochytrium limacinum* marine algae coated by xylose was higher (16.7%) than in animals fed by uncoated (unprotected) marine algae (8.4%). However, in this study, the experimental period was relatively short, but some reports stated that DHA fatty acid concentration and the transfer coefficient for DHA have changed markedly within 21–28 days and remained relatively constant during observation period on different marine algae diets (Franklin et al., 1999; Moate et al., 2013; Moran et al., 2017a,b). Nevertheless, further research need to determine the long-term effect of marine algae supplementation on DHA concentration in milk and DHA transfer efficiency from marine algae diet to milk.

Other valuable fatty acid is eicosapentaenoic acid (EPA; C20:5n-3). Daily supplementation of goats with 15 g marine algae per animal under this trial resulted in 2.36 and 2.32 mg EPA per 100 ml of milk, and 15.7 and 15.1 mg DHA per 100 ml of milk in CMA and PMA groups, respectively. Therefore, the sum of EPA+DHA value was 18.1 mg/100 ml in marine algae enriched diet (CMA) and 17.4 mg/100ml in grazing with marine algae treatment (PMA). The recommended intake of EPA+DHA is 250 mg for adults and 100 mg for infants (EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA), 2010). Currently, the average EPA+DHA intake for humans is estimated at 88.1 mg/day (together with foods and dietary supplements) (Zhang et al., 2018). The difference between the recommended and average intake of EPA+DHA (161.9 mg/day) is equivalent to 895 and 930 ml of milk obtained from the marine algae supplemented goats from CMA and PMA groups, respectively.

In the present study, the n-6/n-3 ratios dramatically decreased in *Schizochytrium limacinum* marine algae enriched groups. This is in concordance with other reports. Toral et al. (2010) found that feeding lipid supplementations considerably decreased the n-6/n-3 ratio in milk. The n-6/n-3 ratio is generally used to assess the nutritional value of fats. The low n-6/n-3 ratio in the milk of animals that received microalgae is in line with the new recommendations for human nutrition (EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA), 2010).

The content of C18:0 fatty acid was significantly lower in the PMA and CMA treatments. According to partial biohydrogenation, the polyunsaturated fatty acids, such as linoleic acid, are biohydrogenated to C18:0 and various isomers of C18:1 in the rumen. In contrast, LC-PUFA n-3 in the diet inhibit vaccenic acid saturation in the rumen (Or-Rashid et al., 2008). As a consequence, the feeding of n-3 fatty acids increased markedly the vaccenic acid content and parallel decreased the proportion of C18:0 in milk fat. The reducing availability of C18:0 exerts influence on the oleic acid (*c11* C18:1) concentration in milk. Oleic acid is mainly performed by stearoyl-CoA desaturase (SCD) enzyme in mammary gland. Shingfield et al. (2010) reported that SCD enzyme was responsible for 60% of amount of oleic acid synthesis in milk; while other part was absorbed by intestinal wall in digestive tract.

The microalgae supplementation have significant effect on the concentration of rumenic acid in milk. The rumenic acid concentration was the most favourable in pasture and marine algae combined diet. Throughout biohydrogenation, rumenic acid is formed from linoleic acid in the rumen by anaerobic bacteria (such as *Butyrivibrio fibrisolvens*), with vaccenic acid (*t11* C18:1) as intermediates. The water content of the grass affects microbiological fermentation and pH in the rumen. It has been shown that grazing with algae combined diet markedly improved the rumen environment for bacteria, such as *B. fibrisolvens*. This is in concordance with previous report where favourable pH (6.0 or above) had a positive effect on vaccenic acid and conjugated linoleic acid (CLA) isomer production (Tsiplakou et al., 2006). The vaccenic acid is converted to rumenic acid by Δ^9-desaturase in the mammary gland and also in some human tissues (Kuhnt et al., 2006). The rumenic acid suppresses carcinogenesis, modulates the immune system, and reduces atherogenesis (Lock et al., 2009).

The milk composition determines the composition of cheese. Results of this report suggested that the cheese produced from bioactive compounds enriched milk could have higher nutritional value.

It is confirmed by Luna et al. (2007), who reported that the cheese processing did not change the fatty acid concentrations, which mainly depended on content of biologically active components of milk.

Conclusions

Daily 15 g/head *Schizochytrium limacinum* marine algae supplementation improved the fatty acid content of goat milk without any negative effect on milk yield and fat content regardless animal husbandry system. In goats fed concentrate with marine algae supplementation significantly higher levels of rumenic acid and n-3 polyunsaturated fatty acids (such as docosahexaenoic acid) in milk were observed. Supplementation with marine algae is suitable for improving the content of bioactive compounds in goat milk, which is important for consumers (from the point of view of dietary benefits).

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