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Effect of *Schizochytrium limacinum* marine alga supplementation on fatty acid profile of goat cheese

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Abstract

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Twenty multiparous Hungarian native goats (71 days in milk) were randomly allocated to two treatment groups. The animals were balanced for parity, and time of kidding. The control and experimental goats (n=10 in both groups) were kept indoors and were fed with 1.5 kg alfalfa hay, while the control animals received daily 600 g concentrate. The experimental group was fed with the same amount of hay and concentrate, and additionaly 15 g dried *Schizochytrium limacinum* marine alga supplementation. The investigation period lasted 31 days; the first 21 days was the period of adaptation to the marine algae enriched diet and the last 10 days was the samples collecting period. During the last 10 days, pooled milk samples were collected for cheese processing every two days, and 5–5 cheese samples were prepared from each groups. The cheese samples were selected for 4-week-ripening period, and they were frozen and stored at -20°C until further analysis. Fat, dry matter and fatty acid profile of them were determined. The *Schizochytrium limacinum* marine alga supplementation resulted in significantly higher concentrations of rumenic acid and docosahexaenoic acid in the cheese samples (0.35% vs 0.03%; $P < 0.001$; 0.44% vs 0.82%; $P < 0.001$). Consumers could have benefit from cheese produced by marine alga supplemented animals' milk due to increasing of rumenic acid and DHA acid concentration, which improves the human health.

Keywords: goat; milk; *Schizochytrium limacinum;* cheese; DHA

Introduction

Goat milk production is only 2% (app. 15 million tonnes/year) of the global milk production and supply (FAOSTAT, 2016). Despite this fact, it is interesting that most part of the human population who consume goat milk cite a lower incidence of allergies and digestive complaints. Production of goat milk has played an important role in the economic viability, especially in developing countries during the last few decades. In recent years, the goat milk cheeses have gained popularity due to the increased interest of consumers in both the tradition of cheese making and the typical sensory characteristics attributed to goat milk (Apostu et al., 2014). Additionally, due to the relatively low investment needs and its high nutrition and health benefits, production of goat milk and its products is not the privilege of the developing countries. Goat cheese is traditionally the main commercial goat milk product produced and consumed in large quantities around the World. The goat cheeses are generally produced by small-scale enterprises, therefore artisan cheese and other milk based products may be able to help to develop the goat sector and to improve the rural conditions (increases the ratio of labour) in the World.

Increasing the beneficial fatty acids (e.g. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) in the animals' diet (e.g. oils seed or micro algae supplements) is one of the most frequent solutions to improve the bioactive components of animal products. Microalgae is a good dietary source of n-3 fatty acids like marine algae (Papadopoulos et al., 2002;Toral et al., 2010) and freshwater algae (Póti et

al., 2015;Tsiplakou et al., 2017; Novotna et al., 2017) in ruminant feeds. Nevertheless, data on feeding *Schizochytrium limacinum* supplements are limited, mainly focused on dairy animals'milk composition rather cheese fatty acids composition (Toral et al., 2010). The *Schizochytrium limacinum* is a valuable marine alga; it is capable of producing valuable metabolites, such as docosahexaenoic acid (DHA) fatty acid for nutraceutical purposes (Guerin et al., 2003). The DHA is a well-known beneficial fatty acid for human health. This fatty acid was associated with a lower risk of cardiovascular disease (Isaksen et al., 2019). Recommended daily intake of DHA and EPA is 250 mg for adults and 100 mg for infants according to EFSA (2010).

Aim of this study was to investigate the effect of the marine algaesupplementation on the fatty acid profile of goat cheese, with particular reference to DHA and rumenic acid.

Material and Methods

Experimental design

The study was carried out in a goat farm (Pest County, Hungary). Twenty multiparous Hungarian native goats (71 days in milk) were randomly allocated to two treatment groups. The parity and kidding time of animals were similar in both groups. The control and experimental goats (n = 10–10) were kept indoors and were fed with 1.5 kg alfalfa hay with an additional ratio of 600 g/d concentrate. The 600 g/day fodder of the experimental group was mixed with 15 g dried *Schizochytrium limacinum* marine alga. The investigation period lasted for 31 days; the first three weeks were the period of adaptation to the marine algae enriched diet, then the last 10 day was the experimental period. The control and the fat supplemented diets were approximately isonitrogenous. Pooled goat milk samples from both groups were collected and processed into cheese at days 1, 3, 5, 7 and 9 of the experimental period, so in total 5–5 cheese samples were prepared per group. The cheese samples were collected after the 4-week-ripening period. The milk and cheese samples were frozen and stored at -20°C until further analysis. Description of cheese processing is shown at Figure 1.

Chemical analysis

Determinations for dry matter, fat and protein content of cheese samples are described in the Hungarian Standards (1978, 1980, 2002).

Fat, protein, lactose, and total solids contents of milk were determined using a LactoScope™ device (Delta Instruments Ltd., Netherlands).

Milk fat was extracted form cheese samples by the method of Folch et al. (1957). Fatty acids were re-esterified to methyl esters using sodium methanol and boron trifluoride (BF3) (Park & Goins, 1994). Methyl esters of fatty acids were determined by gas chromatography (gas chromatographer GC 2010, Shimadzu Kyoto, Japan) with a flame

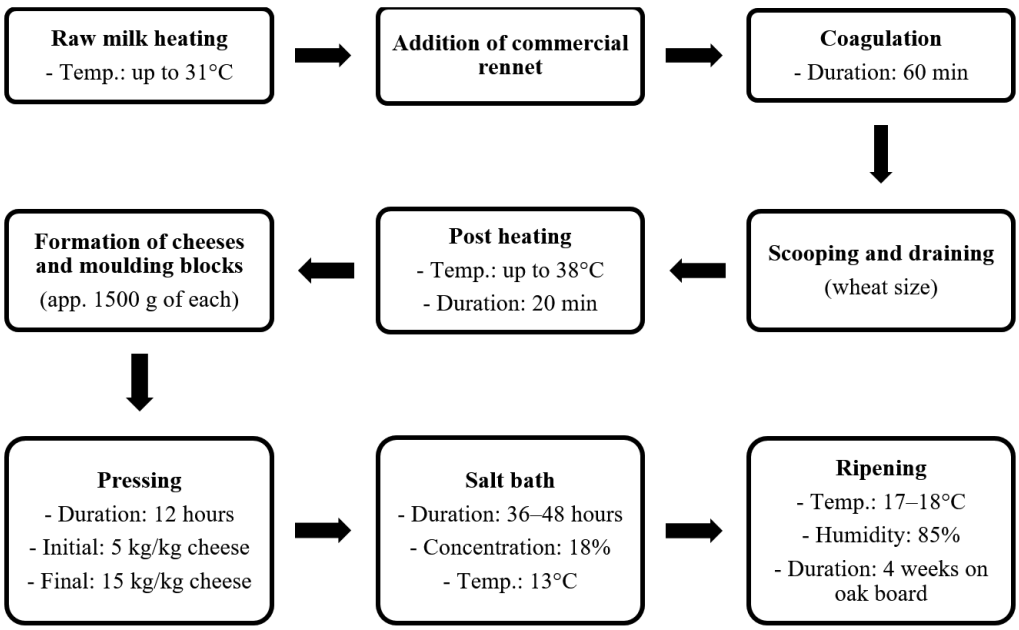

Fig. 1. Description of experimental cheese processing

ionization detector (FID) and column (CP-SIL-88, 100 m x 0.25 mm × 0.2 μm). Helium was used as the carrier gas, applying a flow rate of 28 cm/s. The split injection ratio was 50:1. The injector and detector temperatures were 270 and 300°C, respectively. The oven temperature was heated at a rate of 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 fatty acids (Mixture Me 100, Larodan Fine Chemicals AB, Limhamn, Sweden). The individual fatty acids were calculated by the ratio of their peak area to the total area of all observed acids and the results were presented as g/100 g of total fatty acids. The selected fatty acid combinations were calculated by using fatty acids data, where SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; total n-6 and n-3 fatty acids and n-6/n-3 ratio.

Statistical analysis

Statistical analysis was processed by the SPSS 25.0 software package (IBM Corporation, Armonk, NY, USA). Statistical analysis was carried out in order to determine the effect of treatments (fixed effect) on milk and cheese composition and cheese fatty acid profile as dependent variates. The equality of variances was assessed by F test. The significance of differences was calculated by 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 ± SEM. Significance was taken at an alpha level of 0.05.

Results and Discussion

Milk composition was not affected significantly by marine algae supplementation (Table 1). The fat and protein content in milk samples of control group were 3.65 g/100 g milk and 2.89 g/100 g milk, and in experimental animals' milk samples were 3.74 g/100 g milk and 2.95 g/100 g milk, respectively. The milk fat and protein content are important for milk producers, because these have great effect on cheese composition. Values of fat, protein and lactose content are within the normal ranges for dairy goats reported by several authors (Park et al., 2006; Kuchtik et al., 2015).

Table 1. Effect of marine algae supplementation on chemical composition of goat milk (mean ± SEM)

Items, g/100g	Control diet	Experimental diet	SEM	P
Fat	3.65	3.74	0.063	N.S.
Protein	2.89	2.95	0.021	N.S.
Lactose	4.67	4.61	0.018	N.S.
Dry matter	11.91	12.01	0.064	N.S.

Fat and dry matter composition of goats' cheeses fed bymarine algae supplementation were 20.50 g and 51.15 g/100 g cheese, respectively, while control goats had 20.25 g and 50.85 g/100g cheese (Table 2). Fat and dry matter contents of cheeses did not show any significant differences during the experimental period. Cheese composition was consistent with previous study (Pajor et al., 2012).

Table 2. Effect of marine algae supplementation on chemical composition of goat cheese (mean ± SEM)

Items, g/100g	Control diet	Experimental diet	SEM	P
Moisture	49.15	48.85	0.844	N.S.
Dry matter	50.85	51.15	0.746	N.S.
Fat	20.25	20.50	0.448	N.S.
Fat in DM	39.82	40.08	0.738	N.S.
Protein	22.50	22.20	0.466	N.S.

The results of the fatty acid analysis of cheese samples are presented in Table 3. Marine algae enriched diet increased the concentrations of caprylic (C8:0), capric (C10:0), palmitic (C16:0), vaccenic (18:1),linolenic (C18:3), docosahexaenoic (C22:6), total n-3 fatty acids and SFA in samples. While, marine algae supplementation significantly decreased the concentrations of lauric (C12:0), myristic (C14:0), myrsistoleic (C14:1), palmitoliec (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), MUFA, PUFA and n-6/n-3 ratio in goat cheese samples, respectively.

As it is shown in Table 3, the experimental diet significantly increased the concentrations of the most interesting fatty acids as short chain fatty acids (C8:0 and C10:0), vaccenic (C18:1*t11*), rumenic acid (C18:1*c9t11*), n-3 fatty acids and docosahexaenoic (C22:6) in goat cheeses.

The marine algae supplementation had a great effect on the concentrations of short and medium-chain fatty acids (from C4 to C14) in milk, that were lower in control group. The short chain fatty acids are hydrolysed shortly and they are directly absorbed from intestinal wall to the liver portal vein; thereby these fatty acids can decreased the malabsorption syndrome in human patients (Papamandjaris et al., 1998). Recently it was reported by Gómez-Cortes et al. (2018) that the short and medium-chain fatty acids can help to maintain the balance of the gut microflora and the body weight control of human consumers. Moreover, Zan et al. (2006) reported that the short-chain fatty acids (mainly caproic, caprylic and capric acids) have a significant effect on the flavour of dairy products.

Table 3. Effect of marine algae supplementation on selected fatty acid profile of goat cheese (mean ± SEM) (% of total fatty acids)

Fatty acids	Control diet	Experimental diet	SEM	P
Butyric acid	1.72	1.91	0.131	N.S.
Caproic acid	2.31	2.72	0.134	N.S.
Caprylic acid	2.31	2.82	0.099	**
Capric acid	9.22	10.41	0.266	*
Lauric acid	4.56	4.24	0.074	*
Myristic acid	11.67	10.82	0.165	**
Myristoleic acid	0.22	0.12	0.016	***
Palmitic acid	33.24	34.15	0.190	**
Palmitoleic acid	0.80	0.71	0.018	**
Stearic acid	8.67	6.95	0.310	***
Oleic acid	19.99	16.78	0.595	**
Pamitic acid / Oleic acid	0.49	0.60	0.020	***
Vaccenic acid	0.85	1.83	0.163	***
Linoleic acid (n-6)	2.62	1.93	0.117	***
Linolenic acid (n-3)	0.41	0.50	0.099	***
Eicosapentaenoic acid	0.07	0.06	0.002	*
Docosahexaenoic acid	0.03	0.35	0.054	***
Short chain fatty acids	15.56	17.87	0.545	*
C14:1/(C14:1+C14:0)	0.018	0.011	0.001	***
Odd fatty acids	2.31	2.01	0.052	***
SFA	73.50	76.54	0.602	**
MUFA	21.90	19.48	0.488	**
PUFA	4.60	3.98	0.115	***
PUFA ω-6	2.80	2.12	0.115	***
PUFA ω-3	0.68	1.04	0.064	***
n-6/n-3	4.13	2.04	0.028	N.S.
rumenic acid	0.44	0.82	0.064	***

SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids

Concentrations of lauric and myristic acids were lower in experimental cheese samples compared to the control ones. These fatty acids are known to be hypercholesterolemic fatty acids and they are most strongly associated with higher blood cholesterol (German & Dillard, 2004) and increased level of LDL cholesterol concentrations in blood serum (Ulbricht & Southgate, 1991).

The fodder enriched with *Schizochytrium limacinum* affected positively the concentration of vaccenic and rumenic acid in cheese. The rumenic and vaccenic acids concentrations in the cheese samples were 0.44 and 0.82% vs. 0.85 and 1.83% for hay based and experimental diet, respectively. The polyunsaturated fatty acids, such as linoleic and linolenic acid, are partly saturating in the rumen by biohydrogenation. During this process, the vaccenic (*t11*C18:1)(TVA) and various isomers of C18:1 are formed as intermediates. Beyond that, the long chain n-3 PUFAs (LC-PUFA) in the diet inhibits vaccenic acid saturation in the rumen (Or-Rashid et al., 2008). LC-PUFA inhibition is causing high level of other specific biohydrogenation intermediates, which have been associated with decreased milk fat content (Toral et al., 2017) and reduced viability of the rumen bacteria community (Białek et al., 2018). One of the indicator of ruminal function is odd chain fatty acids concentrations in milk. These fatty acids are produced by ruminal bacterial populations. The marine algae treatment has a significant effect on viability of microbiota in rumen. It was found that the odd chain fatty acids concentrations are lower in experimantal group than in control one.

The inhibited biohydrogenation leads to lower level of the stearic acid concentration in experimental cheese samples. Reduced availability of stearic acid is associated with a lower oleic acid concentration in cheese samples. Oleic acid mainly synthetized in mammary gland by stearoyl-CoA desaturase (SCD) enzyme. By Shingfield et al. (2010) 60% of amount of oleic acid (C18:1c9) in milk is synthesised by SCD enzyme, remained part is originated from diet. SCD enzyme activity is mostly estimated by Δ9 desaturase index operation. The Δ9 desaturase index is commonly expressing as C14:1/(C14:1+C14:0) ratio. Bernard et al. (2008) reported that this index is the closest relation with the Δ 9-desaturase activity. The utmost proportion of miristoleic acid (C14:1) (~90%) is synthesised by SCD enzyme (Shingfield et al., 2010) in milk. The C14:1/(C14:1+C14:0) ratio in control goat group milk samples was 0.018, while, in marine algae treatment was 0.011. In this study, marine algae supplementation has significant effect on stearoyl-CoA desaturase enzyme, resulted in reduced amount of unsaturated fatty acids. Well known fact that the oleic acid is the main determining factor of MUFA concentrations, therefore these fatty acid concentrations are significantly lower in experimental samples compared to control cheeses. In addition, Dai et al. (2011) reported that the higher ratio of oleic acid to palmitic acid resulted in a softer fat. Earlier, softer butter produced was reported from milk that contains high concentration of unsaturated fatty acids and reduced the starter culture activity in cheese (Ryhanen et al., 2005). These results suggested that the ratio of oleic acid to palmitic acid is a substantial factor for milk producers. In our study, the ratio of oleic acid to palmitic acid was more favourable in the marine algae group; this value was 0.49 compared to control cheese samples (0.60; $P < 0.001$), respectively.

The vaccenic acid is converted to rumenic acid by Δ9-desaturase in mammary gland (Kuhnt et al., 2006). Therefore, marine algae feeding increased markedly the vaccenic

and rumenic acids content. Nevertheless, the rumenic acid is interesting in human health; it suppresses carcinogenesis and reduces atherogenesis (Lock et al., 2009).

Increasing the content of n-3 PUFAs in the milk or cheese is one of the most investigated areas of ruminant husbandry. In our study, the n-6/n-3 ratio dramatically decreased in marine algae enriched group. This is in correspondence with earlier literatures. Toral et al. (2010) found considerably decreased n-6/n-3 ratio in milk when feeding marine algae supplementations. Nevertheless, the value of n-6/n-3 ratio is interesting in evaluation of fats according to their nutritional value. The recommended value of this ratio is less than 4 by nutritionists (Simopoulos, 2004).

In the present study, the concentrations of n-3 fatty acids and LC-PUFA fatty acid (DHA) increased significantly by marine algae supplements. DHAis required for many metabolic processes in human and effectively prevent coronary heart disease (CHD).

The long-chain PUFA, including eicosapentaenoic acid (EPA and DHA) synthesis is possible but not very efficient in humans (Tvrzicka et al., 2011). Therefore, EPA and DHA are mostly of exogenous source in human diet (Brenna et al., 2009). Recently, the recommended intake of EPA+DHA is 250 mg for adults and 100 mg for infants (European Food Safety Authority, 2010). Average amount of EPA and DHA was 12.3 and 71.8 mg/100 g cheese in the samples of the experimental group (Table 4). The aggregated value of EPA+DHA was 84 mg/100 g cheese. Zhang et al. (2018) reported that the average EPA+DHA intake for adult estimated at 88.1 mg/day. The difference between the recommended and average intake of EPA+DHA was 161.9 mg/d. This is equivalent to 193 g of cheese obtained from the marine algae enriched goat milk.

Table 4. Cheese fat content and calculated amount of EPA and DHA level of goat cheeses by feeding methods (mg/100 g cheese)

Fatty acids	Control diet	Experimental diet
Cheese fat content, %	20.25	20.50
EPA, mg	14.18	12.30
DHA, mg	6.08	71.75
Total EPA+DHA, mg	20.25	84.05

Conclusion

There were significant differences in cheese fatty acid profile between the control and experimental groups. The *Schizochytrium limacinum* marine algae supplementation resulted significantly higher concentrations of caprylic and capric fatty acids, total n-3 PUFA, DHA and rumenic acid and lower value of n-6/n-3 ratio.

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