SCIENTIFIC NOTE

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# Effect of micro-alga supplementation on goat and cow milk fatty acid composition

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The microalgae cultivation has been developed over the last decades because it is capable of producing valuable metabolites, such as *n*-3 fatty acids for nutraceutical purposes. Aim of this study was to investigate the effect of the micro-alga as fat supplement on fatty acid profile of goat and cow milk, with particular reference to *n*-3 fatty acids and rumenic acid. Twenty dairy goats and 16 dairy cows were randomly allocated to two isonitrogenous treatment groups to investigate the effect of micro-alga supplementation on the composition and fatty acid profile of milk. The 1st goat group was fed with alfalfa hay and concentrate; the 2nd goat group received the same forages but the concentrate was supplemented with 10 g $kg^{-1}$ DM intake micro-alga. The control group cows were fed with alfalfa hay, corn silage and concentrate, while the experimental animals were fed with the same forages but the concentrate was supplemented with 7.4 g $kg^{-1}$ DM intake micro-alga. The experimental periods lasted for 17 d. The micro-alga supplements considerably increased rumenic acid concentration in milk (1.20% *vs*. 1.54%, $P < 0.001$ for goats; 0.75% *vs*. 0.85%, $P < 0.05$ for cows). The *n*-3 fatty acids were higher in milk (1.02 *vs*. 1.35; $P < 0.001$ for goats; 0.47 *vs*. 0.56; $P < 0.05$ for cows) and in addition the *n*-6/*n*-3 ratio was also more favorable in the micro-alga supplemented groups (3.53 *vs*. 2.88; $P < 0.01$ for goats; 4.18 *vs*. 3.36; $P < 0.05$ for cows). It is concluded that the diet with micro-alga supplementation significantly increased the concentration of beneficial fatty acids in both goat and cow milk.

**Key words:** Conjugated linoleic acid, n-3 fatty acids, micro-alga, milk.

## INTRODUCTION

The microalgae cultivating have been developed over the last decades because it is a simple and inexpensive method for $CO_2$ management, which is currently an important global issue. Otherwise, microalgae are capable of producing valuable metabolites, such as *n*-3 fatty acids for nutraceutical purposes (Guerin et al., 2003; Hu, 2004). In recent years there has been increased interest in ways to manipulate the fatty acid composition of foods such as milk and milk products, because it contains a lot of health promoting components, such as *n*-3 fatty acids and conjugated linoleic acid (rumenic acid). These components could improve health of consumers. The high intake of *n*-3 PUFAs are able to reduce the risk factor of coronary heart disease, like the formation of blood clots leading to a heart attack (Li et al., 2003). The rumenic acid (*c9t11*CLA isomer) has a range of positive health properties such as anticarcinogenic and antiatherogenic effects (Lock et al., 2009). Modifications in ruminant diet can multiply concentrations of bioactive compounds (e.g., cis-9 trans-11 18:2 or omega-3 fatty acids) in dairy products. The most effective strategies involved supplementing animal feed with different oils. In contrast, effects of processing conditions, storage, and aging on the fatty acid profile of dairy products are negligible (Ryhänen et al., 2005; Luna et al., 2007; 2008). Previous studies reported that different type of dietary fat supplements (*n*-3 or *n*-6) have altered effects on the feed consumption, milk composition and rumen function among ruminant species. Some researcher reported that *n*-3 PUFA enriched supplemental fat resulted in a decrease in feed consumption, milk yield and milk fat depression in cows (Or-Rashid et al., 2008), however, in goats, others reported no effect (Zhang et al., 2006). While *n*-6 enriched diet (such as sunflower) unmodified or increased the milk composition in both species (Dai et al., 2011; Martínez et al., 2012). Several studies investigated the effects of diet, such as green forages (Tsiplakou et al., 2006; Pajor et al., 2009) or oilseeds (Brzóska, 2006) on fatty acids, however, data on feeding micro-alga supplements are limited (mainly in dairy cows and ewes, such as Boeckaert et al., 2008; Papadopoulos et al., 2002 and Toral et al., 2010, respectively). *Spirulina platensis* and *Chlorella kessleri* are freshwater micro-algae species. Both algae contain high concentrations of beneficial fatty acids: *Spirulina*

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is rich in C18:3 (*n*-6), whereas *Chlorella* contains high proportion C18:3 (*n*-3) fatty acids. We hypothesized that milk fatty acid profile can be improved when the animals are fed with about 1% micro-algae supplemented diet and the same time serious negative effect on DM intake, milk composition and rumen function can be avoided.

Aim of this study was to investigate the effect of the supplemented micro-alga on fatty acid profile of the goat and cow milk, with particular reference to health promoting components (*n*-3 fatty acids, *c9t11*CLA).

## MATERIALS AND METHODS

### Experimental design

The study was carried out in a University's farm in Gödöllő (Pest County, Hungary; 47°35'40.0" N 19°22'09.7" E). Twenty multiparous Hungarian native goats (1st investigation) (62 average days in milk) and 16 multiparous Holstein-Friesian cows (2nd investigation) (174 average days in milk) were randomly allocated to two treatment groups. The animals were balanced for parity, and time of kidding and calving. The experimental period lasted for 17 d, which encompassed the first week as the period of adaptation to the diet and the last 10 d as the experimental period. The control and the fat supplemented diets in both investigations were approximately isonitrogenous. The ingredients and composition of the experimental feedings are shown in Table 1.

Samples of pooled milk were collected twice a day during the 10 d of the experimental period at 06:00 and 18:00 h, milk samples were frozen and stored at -20 °C until further analysis. Before laboratory investigation, milk samples gathered twice a day were combined for analysis of chemical composition.

### First investigation

The diets were adjusted to the NRC (2007) recommendations of energy and protein requirements for dairy goats (60 kg body weight; 2.0 kg milk $d^{-1}$). The control and experimental goats (n = 10-10) were kept indoors and were fed with 2 kg alfalfa hay, while the control animals received 1 kg grain mix: 15% winter wheat, 31% corn, 15% extracted sunflower meal (37% crude protein), 10% extracted soy meal (46% crude protein), 24% wheat bran, 5% premix. The experimental group was fed with 1000 g $d^{-1}$ grain mix: 16.6% winter wheat, 31% corn, 15% extracted sunflower meal (37% crude protein), 5.4% extracted soy meal (46% crude protein), 24% wheat bran, 5% premix with 3% dried *Chlorella kessleri* micro-alga (10 g $kg^{-1}$ DM intake).

### Second investigation

The diets were adjusted to the NRC (2001) recommendations of energy and protein requirements for dairy cows (700 kg body weight; 20.0 kg milk $d^{-1}$). The control and experimental cows (n = 8-8) were kept indoors and were fed with 8 kg alfalfa hay and 25 kg corn silage and received 3000 g grain mix. In the control group the grain mix contained 15% winter wheat, 31% corn, 15% extracted sunflower meal (37% crude protein), 10% extracted soy meal (46% crude protein), 24% wheat bran, and 5% premix; in experimental group: 17.8% winter wheat, 31% corn, 14.5% extracted sunflower meal (37% crude protein), 2.7% extracted soy meal (46% crude protein), 24% wheat bran, 5% premix, and 5% dried *Spirulina platensis* micro-alga (7.4 g $kg^{-1}$ DM intake).

**Table 1. Chemical composition and fatty acid profile of fed forage.**

| | Goat | | Cow | |
|---|---|---|---|---|
| | Control | Experimental | Control | Experimental |
| Ingredients | | | | |
| Concentrate, % DM[1,2] | 33.1 | 33.1 | 14.6 | 14.6 |
| Winter wheat, % DM | 5.1 | 5.6 | 2.2 | 2.7 |
| Corn, % DM | 10.5 | 10.5 | 4.6 | 4.6 |
| Extracted soybean, % DM | 3.3 | 1.6 | 1.5 | 0.4 |
| Extracted sunflower, % DM | 4.9 | 4.9 | 2.1 | 2.1 |
| Wheat bran, % DM | 7.9 | 7.9 | 3.5 | 3.5 |
| Premix, % DM | 1.6 | 1.6 | 0.7 | 0.7 |
| Alfalfa hay, % DM[3] | 66.9 | 66.9 | 38.1 | 38.1 |
| Corn silage, % DM[4] | - | - | 47.3 | 47.3 |
| Microalgae, % DM[5] | - | 1.0 | - | - |
| Microalgae, % DM[6] | - | - | - | 0.7 |
| DIM, kg $d^{-1}$ | 2.7 | 2.7 | 18.3 | 18.3 |
| Chemical composition | | | | |
| Dry matter, g DM $kg^{-1}$ forage | 898 | 896 | 508 | 508 |
| Crude protein, g $kg^{-1}$ DM | 210.4 | 209.2 | 165.8 | 165.5 |
| Crude fat, g $kg^{-1}$ DM | 20.9 | 20.8 | 22.0 | 21.9 |
| Crude fiber, g $kg^{-1}$ DM | 254.8 | 253.8 | 259.2 | 258.4 |
| Crude ash, g $kg^{-1}$ DM | 76.9 | 75.8 | 75.3 | 74.5 |
| NE, MJ $kg^{-1}$ DM | 5.91 | 5.91 | 5.87 | 5.88 |
| Main fatty acids (FA) | | | | |
| C16:0, % | 26.68 | 26.78 | 22.44 | 22.54 |
| C18:0, % | 4.27 | 4.26 | 2.47 | 2.50 |
| C18:1*n*-9, % | 10.67 | 10.54 | 7.93 | 7.82 |
| C18:2*n*-6, % | 32.88 | 32.45 | 31.64 | 31.46 |
| C18:3*n*-3, % | 15.64 | 16.46 | 23.13 | 23.11 |
| C18:3*n*-6,% | 0.03 | 0.04 | 0.01 | 0.24 |

DM: Dry matter, DMI: dry matter intake, NE: net energy.
[1]Concentrate (control diet) for goats contained (g 100 $g^{-1}$ FA) C16:0 (12.1), C18:0 (2.5), C18:1*n*-9 (23.6), C18:2*n*-6 (56.2), C18:3*n*-3 (4.3); for cows contained (g 100 $g^{-1}$ FA) C16:0 (22.8), C18:0 (2.0), C18:1*n*-9 (19.4), C18:2*n*-6 (49.8), C18:3*n*-3 (2.6)
[2]Concentrate (experimental diet) for goats contained (g 100 $g^{-1}$ FA) C16:0 (11.6), C18:0 (2.86), C18:1*n*-9 (21.5), C18:2*n*-6 (51.1), C18:3*n*-3 (8.7); for cows contained (g 100 $g^{-1}$ FA) C16:0 (18.7), C18:0 (2.4), C18:1*n*-9 (20.7), C18:2*n*-6 (50.4), C18:3*n*-3 (2.3)
[3]Alfalfa hay contained (g 100 $g^{-1}$ FA) C16:0 (28.7), C18:0 (2.8), C18:1n-9 (2.7), C18:2*n*-6 (19.5), C18:3*n*-3 (28.3)
[4]Silage contained (g 100 $g^{-1}$ FA) C16:0 (17.3), C18:0 (2.4), C18:1*n*-9 (8.6), C18:2*n*-6 (35.8), C18:3*n*-3 (25.3)
[5]Microalga (*Chlorella kessleri*) contained (g 100 $g^{-1}$ FA) C16:0 (21.3), C18:0 (1.0), C18:1*n*-9 (11.2), C18:2*n*-6 (14.3), C18:3*n*-3 (37.4), C18:3*n*-6 (0.6)
[6]Microalga (*Spirulina platensis*) contained (g 100 $g^{-1}$ FA) C16:0 (36.2), C18:0 (5.1), C18:1*n*-9 (4.6), C18:2*n*-6 (26.2), C18:3*n*-3 (0.7), C18:3*n*-6 (22.7)

### Chemical analysis

The forage samples (n = 3-3) were analyzed for DM, crude protein, crude fat, crude fiber and crude ash according to the procedure of the Hungarian Feed Codex (2004).

Fat, protein, lactose, and solids non-fat contents of milk were determined using a MilkoScan device (Combi Foss 5000 apparatus, Foss Electric, Denmark).

The milk fat was dissolved in a sodium hydroxide-methanol solution and re-esterified to methyl-esters

according to the AOAC (1990) method using boron trifluoride ($BF_3$) (Park and Goins, 1994). Methyl esters of fatty acids were determined by gas chromatography (gas chromatographer GC 2010, Shimadzu Kyoto, Japan) with a flame ionization detector (FID) and 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 28 cm $s^{-1}$. The split injection ratio was 50:1. The injector and detector temperatures were 270 and 300 °C, respectively. The oven temperature was held at 80 °C for 0 min, then programmed at a rate of 2.5 °C $min^{-1}$ up to 205 °C and held for 20 min and then increased again to 225 C at 10 °C $min^{-1}$, and held for 5 min (total time of oven program: 77 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 proportions of the individual acids were calculated by the ratio of their peak area to the total area of all observed acids. The selected fatty acid combinations were calculated by using fatty acids data: SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; CLA: *c9t11*CLA conjugated linoleic acid (rumenic acid) (no evaluated other CLA isomers) total *n*-6 and *n*-3 fatty acids and *n*-6/*n*-3 ratio. Atherogenic index (AI) was calculated using the equation elaborated by Ulbricht and Southgate (1991).

### Statistical analysis

Statistical analysis was processed by the SPSS 21.0 software package. Statistical analysis was carried out in order to determine the effect of diets (fixed effect) on milk content and fatty acid composition as dependent variables. Data were expressed as mean ± SD. 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. Differences are shown when $P < 0.05$.

## RESULTS AND DISCUSSION

Based on our results, the milk composition was slightly affected by diet (Table 2). The control diet caused lower ($P < 0.05$) fat content (3.04 *vs*. 3.67 g 100 $g^{-1}$ milk) compared with the experimental goats, but the cow milk from the micro-alga supplementation group had significantly lower ($P < 0.05$) fat (3.49 *vs*. 3.79 g 100 $g^{-1}$ milk) compared with the control group, nevertheless other parameters not showed differences.

The results of the fatty acid analysis of milk samples are presented in Table 3. In the present study, the experimental period was relatively short, but some reports conducted that fatty acid concentrations have changed markedly within a first week and remained relatively constant on different lipid diets (Roy et al., 2006; Toral et al., 2010). In the 1st investigation the experimental diet significantly increased concentrations of butyric (C4:0), vaccenic (C18:1t), linoleic (C18:2), α-linolenic (C18:3), eicosatrienoic (C20:3), eicosapentaenoic (C20:5), docosahexaenoic (C22:6), monounsaturated (MUFA), polyunsaturated (PUFA), and *n*-3 fatty acids and significantly decreased the concentrations of eicosatetraenoic (C20:4) and saturated fatty acids (SFA) in goat milk. In contrast, the concentrations of medium chain fatty acids (e.g. lauric and myristic fatty acids) and other long chain fatty acids such as palmitic acid (C16:0), oleic acid (C18:1), did not show any significant differences during the experimental period.

In the 2nd investigation the micro-alga supplemented diet significantly decreased the concentrations of butyric (C4:0), caproic (C6:0), caprylic (C8:0) and *n*-6/*n*-3 ratio and significantly increased the stearic acid (C18:0), vaccenic (C18:1t), α-linolenic (C18:3), rumenic acid (*c9t11*C18:2), eicosapentaenoic (C20:5), docosahexaenoic

**Table 2. Composition of goat and cow milk from different feeding technologies (mean ± SD).**

| | Goat milk | | | Cow milk | | |
|---|---|---|---|---|---|---|
| | Control diet | Experimental diet | P | Control diet | Experimental diet | P |
| Fat, % | 3.04 ± 0.62 | 3.67 ± 0.68 | * | 3.79 ± 0.18 | 3.49 ± 0.11 | * |
| Protein, % | 2.85 ± 0.22 | 3.02 ± 0.19 | NS | 3.34 ± 0.07 | 3.38 ± 0.12 | NS |
| Lactose, % | 4.42 ± 0.12 | 4.51 ± 0.13 | NS | 4.97 ± 0.14 | 5.03 ± 0.12 | NS |
| Total solids without fat, % | 8.06 ± 0.35 | 8.32 ± 0.32 | NS | 9.11 ± 0.19 | 9.21 ± 0.20 | NS |

NS: Nonsignificant difference; *P < 0.05.

**Table 3. Effect of micro-alga supplementation on fatty acid profile of goat and cow milk (mean ± SD) (% of total fatty acids).**

| | Goat milk | | | Cow milk | | |
|---|---|---|---|---|---|---|
| Fatty acids | Control diet | Experimental diet | P | Control diet | Experimental diet | P |
| C4:0 | 1.03 ± 0.12 | 1.23 ± 0.10 | ** | 2.04 ± 0.07 | 1.47 ± 0.11 | *** |
| C6:0 | 2.12 ± 0.11 | 2.03 ± 0.11 | NS | 1.70 ± 0.13 | 1.37 ± 0.12 | ** |
| C8:0 | 3.03 ± 0.17 | 2.93 ± 0.27 | NS | 1.26 ± 0.05 | 1.13 ± 0.06 | ** |
| C10:0 | 12.43 ± 0.64 | 11.98 ± 0.76 | NS | 3.45 ± 0.14 | 3.37 ± 0.29 | NS |
| C12:0 | 5.26 ± 0.70 | 5.15 ± 0.67 | NS | 4.23 ± 0.11 | 4.37 ± 0.17 | NS |
| C14:0 | 11.28 ± 0.90 | 11.42 ± 0.97 | NS | 14.59 ± 0.43 | 14.99 ± 0.44 | NS |
| C14:1 | 0.17 ± 0.03 | 0.18 ± 0.03 | NS | 1.07 ± 0.15 | 1.21 ± 0.09 | NS |
| C16:0 | 33.56 ± 2.64 | 31.09 ± 2.83 | NS | 39.25 ± 1.76 | 37.84 ± 2.04 | NS |
| C16:1 | 0.49 ± 0.14 | 0.49 ± 0.12 | NS | 1.68 ± 0.28 | 1.37 ± 0.26 | NS |
| C18:0 | 6.18 ± 0.55 | 5.85 ± 0.70 | NS | 7.24 ± 0.39 | 7.90 ± 0.49 | * |
| C18:1*n*-9c | 12.66 ± 1.88 | 13.79 ± 1.96 | NS | 14.68 ± 0.50 | 15.60 ± 0.42 | NS |
| C18:1*n*-11t | 0.84 ± 0.50 | 2.11 ± 0.86 | *** | 0.62 ± 0.20 | 1.61 ± 0.22 | *** |
| C18:2*n*-6 | 3.29 ± 0.30 | 3.59 ± 0.33 | * | 1.71 ± 0.09 | 1.62 ± 0.10 | NS |
| C18:3*n*-3 | 0.88 ± 0.09 | 1.17 ± 0.14 | *** | 0.26 ± 0.03 | 0.26 ± 0.06 | NS |
| C18:3*n*-6 | ND | ND | | 0.02 ± 0.01 | 0.05 ± 0.02 | ** |
| C20:3*n*-3 | 0.03 ± 0.02 | 0.12 ± 0.03 | *** | 0.07 ± 0.01 | 0.11 ± 0.02 | ** |
| C20:4*n*-6 | 0.21 ± 0.03 | 0.13 ± 0.03 | *** | 0.14 ± 0.01 | 0.15 ± 0.03 | NS |
| C20:5*n*-3 | 0.03 ± 0.01 | 0.07 ± 0.01 | *** | 0.03 ± 0.02 | 0.03 ± 0.01 | NS |
| C22:6*n*-3 | 0.02 ± 0.01 | 0.04 ± 0.01 | ** | 0.01 ± 0.01 | 0.02 ± 0.01 | ** |
| SFA | 77.47 ± 2.44 | 74.32 ± 3.03 | * | 75.62 ± 1.61 | 74.34 ± 1.64 | NS |
| MUFA | 16.09 ± 1.99 | 18.18 ± 2.03 | * | 17.83 ± 0.56 | 18.61 ± 0.51 | * |
| PUFA | 4.60 ± 0.36 | 5.20 ± 0.40 | ** | 3.13 ± 0.10 | 3.25 ± 0.13 | NS |
| *n*-6 | 3.58 ± 0.31 | 3.85 ± 0.35 | NS | 1.91 ± 0.10 | 1.85 ± 0.09 | NS |
| *n*-3 | 1.02 ± 0.10 | 1.35 ± 0.14 | *** | 0.47 ± 0.07 | 0.56 ± 0.06 | * |
| *n*-6/*n*-3 | 3.53 ± 0.35 | 2.88 ± 0.37 | *** | 4.18 ± 0.73 | 3.36 ± 0.43 | * |
| *c*9*t*11CLA | 1.20 ± 0.19 | 1.54 ± 0.15 | *** | 0.75 ± 0.06 | 0.85 ± 0.08 | * |
| AI | 4.10 ± 0.49 | 3.53 ± 0.51 | * | 4.86 ± 0.21 | 4.67± 0.08 | * |

*;**, ***Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
ND: not detected, NS: nonsignificant difference, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, CLA: conjugated linoleic acid, AI: atherogenic index.

(C22:6), monounsaturated (MUFA), and *n*-3 fatty acids as well. The proportion of the medium chain fatty acids was similar in the two feeding systems.

In our study, due to the higher concentration of butyric acid and fat content of goat milk, 10 g $kg^{-1}$ DM intake (DMI) of alga supplementation had slightly positive effect on the rumen fermentation. Papadopoulos et al. (2002) reported that ewe milk fat and protein contents were significantly increased for treatment containing algae. In dairy cows, 7.4 g $kg^{-1}$ DMI of micro-algae supplementation has slightly negative effect on rumen fermentation; it results reduced fermentation in order to decrease fat content and concentrations of C4:0, C6:0 and C8:0 fatty acids of cow milk. Boeckaert et al. (2008) reported that algae supplementation level of about 10 g $kg^{-1}$ DM intake (DMI) significantly reduced the milk fat content. The milk composition determines the composition of cheese, so these results suggested that the milk produced by the supplemented group would have a higher cheese yield. However, since organoleptic examination of milk samples was not evaluated in this study, this should be tested in further researches. This information may be important for consumers.

The butyric acid (BA) is a health promoting component. In our study, the BA values for both treatments were similar to those from earlier studies of goats (1.2%-1.4%) (Delgado-Pertíñez et al., 2013) and cows (1.4%-1.8%) (Puppel et al., 2012). But other author reported higher BA content in goat milk (1.97%-2.44%) (Park et al., 2007). Nevertheless, the reports showing difference in BA content between small ruminant species. It has been shown that there is higher butyric acid content in sheep milk (3%-4%) than in goat milk (Toral et al., 2010).

The experimental forage is a dietary source of *n*-3 fatty acids like linseed (Brzóska, 2006), rapeseed (Kudrna and Marounek, 2006) oils and marine lipids (Toral et al., 2010) in ruminant feeds. Increasing the supply of *n*-3 PUFA in the diet is one of the most essential ways of improving the *n*-3 content of goat and cow milk. In the present study, the *n*-6/*n*-3 ratios changed from 3.53 to 2.88 in goat milk and from 4.18 to 3.36 in cow milk samples from animals receiving micro-alga enriched fodder. This is in concordance with the literature 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. Simopoulos (2004) recommended value is an *n*-6/*n*-3 ratio of less than 4. The low *n*-6/*n*-3 ratio in the milk of animals that received micro-alga is in line with the new recommendations for human nutrition. α-Linolenic acid is the precursor of *n*-3 PUFAs such as eicosapentaenoic acid (EPA) ($C_{20:5}$ *n*-3) and docosahexaenoic acid (DHA) ($C_{22:6}$ *n*-3), which are required for many metabolic processes in human and effectively prevent coronary heart disease (CHD).

In the present study, the atherogenic index (AI) was proved to be significantly lower in both experimental treatments compared with control ones. The experimental diet considerably decreased the AI in goat milk (4.10 *vs*. 3.53; $P < 0.05$) and in cow milk (4.86 *vs*. 4.67; $P < 0.05$), respectively. The low AI value in the milk of animals that received micro-algae is in keeping with the new recommendations for human nutrition.

The micro-alga supplementation positively affected the concentrations of rumenic acid in milk. The rumenic acid concentration was 1.20% and 1.54% in goat milk and 0.75% and 0.85% in cow milk samples for hay based and experimental diet, respectively. Throughout biohydrogenation rumenic acid is formed from linoleic acid in the rumen by anaerobic bacteria (such as *Butyrivibrio fibrisolvens*), with vaccenic acid (t11C18:1) as intermediates. The vaccenic acid is converted to rumenic acid by $\Delta^9$-desaturase in mammary gland and also in some human tissues (Kuhnt et al., 2006). Some authors reported that diet can exert influence on rumenic acid concentration in milk, such as high protein level (Czauderna et al., 2010) and supplemental sunflower fat (Martínez et al., 2012). The rumenic acid suppresses carcinogenesis, modulates the immune system, and reduces atherogenesis (Lock et al., 2009). Increased the rumenic acid in milk by diet may provide functional food for human consumption. Functional foods have been defined as foods having the presence of physiologically active components (rumenic acid, *n*-3 fatty acids) that provide a health benefit beyond basic nutrition (Hasler, 2000).

The C18:0 fatty acid was significantly higher in the milk of cows fed with *Spirulina* enriched diet compared with the control diet, in contrast, the milk of the experimental goats supplemented with 1% *Chlorella* slightly (not significant) by decreased in the stearic acid concentration. Because of partial biohydrogenation, the polyunsaturated fatty acids, such as linoleic acid, are biohydrogenated to C18:0 and C18:1 in the rumen. In contrast, long chain *n*-3 PUFAs 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 parallelly decreased the proportion of C18:0 in milk fat. The fact that *Spirulina* was rich in C18:3 (*n*-6) and *Chlorella* was rich in C18:3 (*n*-3) may clarify this observation.

Ryhänen et al. (2005) and Luna et al. (2007; 2008) reported that processing of milk into cheese did not change the fatty acid concentrations. This underlines that alterations in the rumenic acid concentration in cheese are depended on mainly raw materials' rumenic acid content (Luna et al., 2007). This is suggesting that cheese made from micro-alga supplemented milk contains high level of biologically active promoters.

## CONCLUSIONS

In conclusion, there were significant differences in milk fatty acid profile between the two treatments in both investigations. The micro-alga supplementation resulted

in significantly higher concentrations of rumenic acid and *n*-3 fatty acids such as α-linolenic, eicosatrienoic acid and docosahexaenoic acid. Consumers have dietary benefits from consumption of nutraceutical milk by micro-alga fed goats and cows due to increased concentrations of health promoting fatty acids, which improve the human health.

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