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Dietary supplementation with a phytocomplex affects blood parameters and milk yield and quality in grazing goats

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ABSTRACT

A phytocomplex composed by Schizochytrium limacinum, a species of marine alga, Galega officinalis, an herbaceous plant of the Faboideae subfamily, and linseeds, was administered to lactating grazing goats in order to evaluate its effects on milk yield and quality, and to investigate possible effects on goat health status. The hypothesis was that, by using a phytocomplex including plants known to exert an improvement of milk yield and milk quality, a synergism should be possible in order to obtain such effects using low doses, thus avoiding adverse effects. Milk fat was significantly ($p<0.05$) higher in treated group (4.02 vs. 3.61 %), in particular, levels of MUFA (24.55 *vs* 22.56 %), total n3 (1.34 vs 1.19 %) and total CLAs (0.52 vs. 0.40 %), were increased while n6/n3 (1.71 vs. 2.21 %) ratio was decreased. Concerning biochemical parameters, a significant ($p<0.05$) decrease of creatinine (0.73 vs. 0.84 mg/dl) was observed in treated animals, but the levels fell within the normal range for this species. Thus, the addition of the proposed phytocomplex to the diet of grazing goats may be successfully used to improve milk fatty acids profile without negative effects on animal health.

1. Introduction

Animal feeding strategies influences milk yield and nutritional characteristics (Falconnier et al., 2018), including the ratio of n6 and n3 fatty acids as well as the conjugated linoleic acid (CLAs) isomers, that are considered beneficial for human health (Simopoulos, 2002; Dilzer and Park, 2012). Grazing or feeding high percentage of forage containing multiple herbs were shown to increase n3 fatty acids leading to a n6:n3 ratio closer to that recommended (between 2 and 4) for human health (Ellis et al., 2005; Cavaliere et al., 2018; Trinchese et al., 2019). Similarly, the CLAs level in the milk of sheep (Meľuchová et al., 2008), cows (White et al., 2001) and goats (Tsiplakou et al., 2014; Tudisco et al., 2014, 2019b) were significantly increased when animals were fed with fresh forage.

Other ways to improve milk nutritional value are supplementing diet with plant oil and meal, fish oil, and marine algae (Moate et al., 2013; Tsiplakou et al., 2017; Białek et al., 2010).

Schizochytrium limacinum is a marine alga with high concentration of docosahexaenoic acid (DHA) that provides beneficial effects for human health, including the reduced risk of coronary heart disease (Li et al., 2003). According to Pajor et al. (2019), supplementing the diet with 15 g/head of Schizochytrium limacinum improved the fatty acid content of goat milk with no detrimental effect on milk yield and fat content either in indoor or grazing animals. Galega officinalis, an herbaceous plant of the Faboideae subfamily, has been reported to increase milk yield in sheep (Gonzalez-Andres et al., 2004). however, toxic effects have been shown for one of its components, thus, the dose of supplementation is critical (Mooney et al., 2018). Linseed (*Linum usitatissimu* L.), containing a high level of alfa linolenic acid (ALA), additioned to the diet of grazing goat increased milk CLA (Tudisco et al.,

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2019a).

The mechanisms by which nutraceuticals act on animals' metabolism often need further elucidation. Also, the combination of several plants (phytocomplex) to obtain multiple and/or synergic effects on milk yield and quality has been poorly studied, as well as their medium and long term effects on animal health.

The study hypothesis was that, by using a phytocomplex including plants known to exert an improvement of milk yield and milk quality, a synergism should be possible in order to obtain such effects using low doses, thus avoiding adverse effects (Zhou et al., 2016). The aim of the present study was to examine determine the effects of a phytocomplex consisted of Schizochytrium limacinum, Galega officinalis and linseed on milk yield and quality, but also on the health status (Hanuš et al., 2018) of grazing goats, especially during two critical periods: the first decline of lactation curve and when the vegetative stasis of the pasture occurs.

To this purpose, the feeding supplement was administered in lactating grazing goats and milk yield and quality, and several blood parameters were evaluated.

2. Material studied, area descriptions, methods, techniques

2.1. Animals and phytocomplex

The trial was performed, according to the Animal Welfare and Good Clinical Practice (Directive 2010/63/EU) and approved by the local Bioethics Committee (PG/20200016570), from April to August 2018. The experiment was carried out in a farm located at Casaletto Spartano (Salerno province, Southern Italy, at 832 m s.l., 40°09' N; 15°37'E with 70 to 35 mm average rainfall and 9.6–21 °C mean temperature). Thirty Cilentana pregnant grazing goats (3rd parity. 50 ± 2.3 kg body weight) were randomly allocated into two groups (CTR, control; T, treated, n = 15 each) homogeneous for milk yield at the previous lactation (1200 ± 114 vs. 1188 ± 112 g/h/day for group CTR and T, respectively). As farm practice, the goats were fed ad libitum with oat hay and 100–200 or 300 g/day of corn meal 45−30 or 15 days before the parturition, respectively. After delivery, the two groups were housed separately and they had free access (9.00 a.m. to 4.00 pm) to pasture (10 ha) constituted by 60 % Leguminosae (Trifolium alexandrinum, Vicia spp.) and 40 % Graminae (*Bromus catharticus, Festuca arundinacea, Lolium perenne*). To prevent overgrazing, animals only weekly return in the same grazing area. After grazing the animals returned to the stall and received 400 g/head/day of corn meal. Corn refusals were measured each day to assess the group intake. All parturition occurred during the first week of February. The diet of group T was supplemented two times (15th of March, after the lactation peak, and 5th July, when vegetative stasis of pasture occurred) for 30 consecutive days, with 4.4 g/head/d (3.1 g/kg DM intake) of a phytocomplex (PHC) prepared by Divergentvet (Terni, Italy) that consisted of: 11.4 % *Schizochytrium limacinum* with 15.0 % total n3 (14.0 % DHA, 0.12 % Eicosapentaenoic acid EPA, 1.30 % ALA). 13.6 % *Galega officinalis* L. 15.9 %. *Linum usitatissimum* L. (linseed) and 59.1 % dehydrated alfalfa hay. The phytocomplex was premixed with 500 g of corn meal with water and then mixed with the remaining meal for all T group animals. All the goats were weighted, and health status was assessed by clinical examination each 30 days from the onset of the experiment.

2.2. Feed analysis

Pasture samples were monthly collected cutting the grass of 4 different 2.5 m^2 areas, at 3 cm from the ground; four representative of 1 kg (obtained balancing the amount from the 4 different areas) was dried at 65 °C using air-oven and milled through a 1 mm screen and stored. Feeds chemical composition was determined according to AOAC (2012) (ID numbers: DM 934.01. CP 954.01, EE 920.39) and fiber was fractionated according to Van Soest et al. (1991). The nutritive value (UFL = 1700 kcal of net energy for lactation) was calculated according to INRA (1978).

For the fatty acid profiles of feeds and phytocomplex, the total fat was extracted according to Folch et al. (1957). Transmethylation of fatty acids was conducted by a base-catalysed procedure according to Christie (1982), with modifications as described by Chouinard et al. (1999). Fatty acids methyl esters were quantified using a gas chromatograph according to the method described by Tudisco et al. (2014). Fatty acids peaks were identified using pure methyl ester external standards (Larodan Fine Chemicals, AB, Limhamnsgardens Malmo, Sweden). Fatty acids in samples were identified by comparing the retention times of peaks with those of the standard mixture.

2.3. Milk analysis

As farm practice, for the first 60 days the milk was entirely ingested by the kids. From day 60 of lactation, milk yield was recorded daily, and representative milk samples from the two daily milkings were collected and analyzed monthly, from April to August, using the infrared method (Milkoscan 133B, Foss Matic, Hillerod, Denmark) standardized for goat milk. In addition, total fat of milk samples was separated using a mixture of hexane/isopropane (3/2 v/v), as described by Hara and Radin (1978).

Transmethylation and quantification of fatty acids were carried out as described above for the feeds. additional standards for CLA isomers were obtained from Larodan (Larodan Fine Chemicals, AB, Limhamnsgardens Malmo, Sweden).

2.4. Blood analysis

Before kidding and every two months up to the end of the trial, blood samples were collected and analyzed for biochemical parameters. Blood sampling was carried out at 8.00 a.m., before feeding, always by the same practitioner following the rules of good veterinary practice under farm conditions (FVE, 2005). Animals were fasted overnight, at least 8 h before the blood collection. Blood samples were taken from the jugular vein in 8 mL Vacucheck tubes with gel separator and clot activator that promote blood clotting with glass or silica particles, stored at 4 °C and immediately transported to the laboratory. Serum was obtained by centrifugation at 2000 rpm for 15 min, and then serum samples were frozen in small aliquots at – 80 °C. Blood chemistry analyses were performed by an automatic biochemical analyzer AUTOLAB PM4000 (AMS Rome, Italy) using reagents from Spinreact (Santa Coloma, Spain) to determine blood urea nitrogen (BUN), creatinine (CREA), total proteins (TP), albumin (ALB), aspartate amino transferase (AST) and albumin/globulin ratio was also calculated.

2.5. Statistical analyses

Data concerning chemical composition and fatty acids profile of pasture were analyzed by one-way ANOVA according to this model:

$$Yi = \mu + Si + \varepsilon ik$$

where Yi = mean of response variable, μ = population mean, Si = effect of sampling (i = 5. April, May, June, July, August), and ε = experimental error.

ANOVA for repeated measures over time was utilized for the analysis of body weight, milk yield and composition and milk fatty acids profile, according to the following model:

$$Yijk = \mu + Gi + Sj + (G + S)\, ij + \mathcal{E}ijk$$

where Yijk = mean of response variable, μ = population mean, Gi = effect of diet (i = 2. CTR and T), Sk = effect of sampling (j = 5. April, May, June, July, August), (G × S) ij = fixed effect of interaction between diet and time, and ε = experimental error. The means were compared using Tukey's test and the differences were considered statistically significant at P < 0.05. All the analyses were performed by JMP software v.11 (SAS Insitute, NC, USA, 2000).

3. Results

No corn meal refusals were observed and no differences were seen neither for body weight nor for BCS that resulted similar between groups at the beginning and at the end of the trial (April: 2.85 and 2.70 – August 3.00 and 2.90 for T and CTR group, respectively, BCS Scale 1–5 points). The chemical composition and fatty acids profile of feeds and phytocomplex are reported in Table 1. Concerning the chemical composition of the pasture, the level of crude protein in the pasture was around one percentage point lower than those presented by Tudisco et al. (2012) and Zicarelli et al. (2016) for the same area. At the same time, the fiber fractions were slightly lower. The nutritive value was unchanged. Corn showed high levels of total monounsaturated (MUFA), total polyunsaturated (PUFA) and C18:2n6. Conversely, C18:3n3 was 53.0 % and 64.2 % of total PUFA in the pasture and phytocomplex, respectively.

The pasture chemical composition, and fatty acid profile changed during the trial (Table 2). In August crude protein and neutral detergent fiber (NDF) contents were the highest and lowest, respectively. The samples collected in July showed opposite values and lower percentage of PUFA and C18:3n-3 compared to those collected in May, June and August. In April the pasture showed the highest values of MUFA and the lowest of PUFA while in August PUFA, C18:2n-6 and C18:3n-3 were higher than in the other periods.

The milk yield was similar between groups (Table 3), whit exception of the third sampling (1702 vs. 1427 kg for T and CTR group, respectively). Milk fat was significantly higher in group T (4.02 vs. 3.61 % for T and CTR, respectively. $p < 0.05$)

As illustrated in Table 4, C4:0 (0.011 vs. 0.016 %$p < 0.01$) and C6:0 (0.040 vs. 0.0136 % $p < 0.01$) were significantly lower in group T compared to CTR, while MUFA (24.55 vs. 22.56 %. $p < 0.05$), total n3 (1.34 vs. 1.19 % $p < 0.05$) and total CLAs (CLA c9 t11 + CLA t10 c11: 0.52 vs. 0.40 %. $p < 0.05$) were significantly higher in milk from treated group. n6/n3 ratio was more favourable in the treated group (1.71 vs. 2.21 % $p < 0.05$). In contrast, group CTR showed significantly ($p < 0.05$) higher SFA (73.18 vs. 71.28 %), PUFA (4.26 vs. 4.16 %) and n6 (2.63 vs. 2.29 %) levels. In addition, significant sampling effects were observed for several FA.

The biochemical parameters are illustrated in Table 5. Creatinine was significantly ($p < 0.05$) lower in group T (0.78 and 0.72 g/dl for CTR and T groups, respectively).

4. Discussion

The goats' body weight did not change during the trial in both groups for which the energy requirements were satisfied. Indeed, the pasture intake of the local genotype goats in that area during spring and summer period account to 20 g DM/kg BW and their energy requirements to 0.0365 UFL/kg metabolic weight (MW═BW 0.75) for maintenance and 0.41 UFL/kg fat-corrected milk (4% fat) (Rubino, 1996). In this experiment, the goats weighed 50 kg BW, thus, pasture intake was 1 kg DM, equal to 0.76 UFL. Goats average milk yield was 1.3 kg with around 4.0 % of fat, thus, their average energy requirement was 1.22 UFL (0.69 UFL maintenance, plus 0.53 UFL milk synthesis). Corn meal (1.1 UFL/kg DM) covered the energy deficiencies. Actually, in April and May the energy requirements were underestimated but the difference between UFL requirements vs ingested was very little (Table 6).

Table 1
Chemical composition and energy values of feeds.

Chemical composition (g/kg DM)	Corn	Pasture	Phytocomplex
CP	99.6	155.8	178.4
EE	41.2	20.6	121.5
NDF	123.3	458.5	251.6
ADF	31.2	308.2	131.2
ADL	6.0	41.0	12.0
UFL/kg DM	1.1	0.76	0.80
Fatty acid profile (% total FA)			
∑SFA	15.31	18.02	18.13
∑MUFA	27.99	4.92	24.47
∑PUFA	56.69	77.06	57.40
C18:3n3	1.45	40.85	37.09
C18:2n6	54.88	27.02	15.51

DM: dry matter, CP: crude protein, EE: ether extract. NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, UFL: unit feed for lactation, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

Table 2
Chemical composition, nutritive value (UFL/kg DM) and fatty acid profile of pasture during the trial.

	April	May	June	July	August	P
Chemical composition (g/kg DM)						
CP	156.0 ± 12.32b	157.0 ± 18.90b	151.0 ± 17.09b	149.0 ± 14.61b	165.8 ± 18.11a	*
EE	20.10 ± 1.86	20.18 ± 1.65	20.12 ± 1.11	19.0 ± 1.63	18.6 ± 1.22	NS
NDF	477.5 ± 26.78ab	470.5 ± 30.71ab	491.5 ± 32.90ab	515.5 ± 37.12a	453.5 ± 33.01b	*
ADF	303.2 ± 21.12c	321.5 ± 26.78b	347.2 ± 25.12a	368.2 ± 30.09a	331.2 ± 19.08b	*
ADL	40.10 ± 2.04a	42.03 ± 3.41a	44.0 ± 1.90a	46.0 ± 2.05a	33.0 ± 0.98b	*
UFL/kg DM	0.76	0.76	0.76	0.75	0.76	NS
% total FA						
∑SFA	19.7 ± 1.13a	19.2 ± 2.07a	16.9 ± 0.88b	19.8 ± 1.31a	14.5 ± 1.62b	*
∑MUFA	7.8 ± 0.67a	4.5 ± 0.36b	4.0 ± 0.66b	4.0 ± 0.23b	4.3 ± 0.41b	*
∑PUFA	70.7 ± 5.67	77.0 ± 4.88	80.2 ± 6.71	75.1 ± 6.12	81.2 ± 4.90	NS
C18:2n-6	10.6 ± 1.23c	21.6 ± 1.89b	34.2 ± 3.03b	20.7 ± 2.41b	47.2 ± 4.78a	*
C18:3n-3	30.5 ± 3.08c	41.2 ± 2.59b	42.8 ± 2.12b	39.6 ± 4.56b	53.5 ± 5.87a	*

CP: crude protein, EE: ether extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, UFL: unit feed for lactation, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.
Means ± SD.
* $p < 0.05$. NS, Not Significant.

Average milk yield was not significantly different between the groups. Among the ingredients of PHC, Galega officinalis is well known to increase milk production in goats and other mammals (Castroviejo, 1999) possibly through its phytoestrogens that increase the prolactin receptor in the mammary gland. On the other hand, despite the lactogenic value of Galega officinalis, several studies showed that one of its components, the Galegina, possesses a toxic effect, thus, the doses used to improve milk yield should be reduced to the minimum (Mooney et al., 2008). In all cases, doses of Galega officinalis over 5 g kg^{-1} were considered toxic (Khodadadi, 2016). Thus, our phytocomplex provided a dose of 0.59 g/head/day that was not toxic but was not able to significantly increase milk yield.

Concerning milk composition, the higher fat values in treated group could be attributed to the presence of Schizochytrium limacinum in the phytocomplex. Such results is in agreement with those reported by Papadopoulos et al. (2002) who showed that fat was significantly increased after marine algae dietary supplementation in ewe milk. Similarly, other authors obtained a higher fat content by supplementing goats with freshwater algae (10 g/kg DM intake) (Póti et al., 2015) or ewes with marine algal oil (30 g/kg DM intake) (Reynolds et al., 2006). On the other hand, contradictory results are reported in the literature

Table 3
Body weight, milk yield and chemical composition in treated group (T) and control group (CTR) along the trial.

Group effect	Body weight kg		Milk yield kg		Fat %		Protein %		Lactose %	
T	50.31		1346.75		4.02a		3.16		4.07	
CTR	50.64		1261.83		3.61b		3.30		4.13	
P	NS		NS		*		NS		NS	
RMSE	2.419		175.25		0.796		0.294		0.224	
	T	**CTR**	**T**	**CTR**	**T**	**CTR**	**T**	**CTR**	**T**	**CTR**
Time effect										
April	51.10	50.54	1761.33a	1693.33a	3.62b	3.09b	2.90	3.06	4.52a	4.52a
May	50.39	50.01	1702.00a	1427.14b	3.72ab	3.34ab	3.01	3.12	4.20a	4.20a
June	50.29	50.27	1389.33b	1311.42b	4.136a	3.75a	3.23	3.25	3.98b	4.13ab
July	50.16	50.39	1125.33b	1211.42b	4.184a	3.80a	3.37	3.29	3.83b	4.04b
August	50.05	51.60	755.76c	665.83c	4.457a	4.045a	3.30	3.78	3.85b	3.75b
P	NS		**		*		NS		**	
GxT										
P	NS		NS		NS		NS		NS	

** $p < 0.01$.

* $p < 0.05$. NS, Not Significant. RMSE, root mean square error.

about this effect of Schizochytrium limacinum: Boeckaert et al. (2008) found that algae supplementation (in ratio of 43.0 g/kg DM) significantly decreased the fat content in cow milk and Bichi et al. (2013) described a similar result in dairy ewes fed algae (in ratio of 8 g/kg DM) containing fodder. These authors hypothesised that such fat decrease was due to the high content of DHA in the Schizochytrium limacinum, thus reducing DM intake and, consequently, milk yield and milk fat. In such a contest, the low amount of Schizochytrium limacinum in the proposed phytocomplex may be critical to obtain the fat increase. The improvement of fatty acid profile obtained by the phytocomplex supplementation is particularly important in terms of healthy promoting properties. On the other hand, a decrease of short-chain fatty acids (C4:0 and C6:0) was detected. These SFA possess health-promoting effects for humans, inhibiting bacterial and viral growth and dissolving cholesterol deposits (Sun et al., 2003).

In human nutrition, the increase in MUFA is an important target for the prevention of cardiovascular diseases (Shingfield et al., 2010). These results could be also attributable to the presence of the linseed in the phytocomplex. According to other authors, in milk of goats (Nudda et al., 2006) and sheep (Caroprese et al., 2016) fed linseed significantly lower SFA and significantly higher MUFA and PUFA were observed. MUFA, total n3 and total CLAs were significantly higher in the treated group, suggesting benefits of the phytocomplex administration. In particular, the significant increase of C18:1trans 11 is important in determining the health properties of milk by lowering triglycerides and LDL cholesterol and by increasing the HDL one (Ohlsson, 2010). Concerning the significant sampling effect observed for several FA, it must be underlined that milk FA profile is mainly affected by feeding but it is difficult to evaluate separately the influence of feeding and lactation stage (Solaiman, 2010; Currò et al., 2019).

Several studies assessed that marine algae supplements in the diet of ruminants represents a good source of long chain polyunsaturated fatty acids (LC-PUFA) (Pajor et al., 2019). Such fatty acids, mainly DHA, affects the biohydrogenation of C18:2n-6 and C18:3n-3 fatty acids in the rumen (Boeckaert et al., 2007). Their supplementation reduced C18:0 production, resulting in the accumulation of various hydrogenation intermediates, predominantly C18:1 trans 11 (t11) and C18:1 t10 (Boeckaert et al., 2007). DHA is required for many metabolic processes and has been shown to effectively prevent coronary heart disease in humans. moreover, it is considered one of the most valuable health promoting components (Sokoła-Wysoczańska et al., 2018). Previous studies showed that Schizochytrium limacinum supplementation is able to increase DHA levels in milk (Toral et al., 2010; Moran et al., 2017). The doses reported in the literature to obtain such effect ranged from 15 g/head/day in goat (Pajor et al., 2019) up to 910 g/head day in dairy cows (Franklin et al., 1999) whereas only 0.501 g/head day were contained in our phytocomplex. This suggests that the combined actions of the different components contained in the phytocomplex may exert their positive effects even at very low doses. In the present study, the n6/n3 ratio was significantly lower in the group supplemented by the phytocomplex. The n6/n3 ratio is nowadays accepted as a tool to assess the nutritional value of fats. Importantly, the lower n6/n3 ratio in the milk of the animals from both groups was in line with the recommendations for human nutrition (EFSA Panel on Dietetic Products et al., 2010). As indicated in Fig. 1, a significant improvement of n6/n3 ratio after the PHC administration in April was observed.

The phytocomplex supplementation also significantly affected total CLAs in milk, mainly represented by the cis-9 trans-11, which is formed through biohydrogenation from linoleic and alfa-linolenic acids in the rumen by anaerobic bacteria that form vaccenic acid (t11 C18:1) as intermediate. Grazing with algae supplementation may improve the rumen environment for bacteria that, in a favourable pH had a positive effect on vaccenic acid and conjugated linoleic acid (CLA) isomer production (Tsiplakou et al., 2006). As illustrated in Fig. 2, the level of CLA was significantly higher in T group after the first PHC administration up to July. In the same group, the decrease of milk CLA was lower after the second PHC administration.

In April, when PUFA in the pasture reach the lowest levels, CLA content in milk was significantly higher in the treated group. This suggests that the phytocomplex may compensate pasture quality still guaranteeing the highest milk nutritional value.

Interestingly, in both groups, CLA level was the highest in July when both linoleic and alfa-linolenic acids were lower than in May and June in the pasture. This could be due to the down-regulation of SCD activity exerted by PUFA during the previous months (Kuhnt et al., 2006). This phenomenon did not occurs at the first sampling probably due to the lactation stage effect, according to Lock and Garnsworthy (2003). Within blood parameters, creatinine was found significantly decreased in the treated group, and a sampling effect was found for BUN, CREA and AST. Even though these parameters may change according to feeding and lactation stage, the goal of this study was to assess possible adverse effects due to the phytocomplex supplementation. Thus, since all levels fell in the normal range for goats (Piccione et al., 2010), the slight change of creatinine has low diagnostic value.

5. Conclusions

Overall, the proposed phytocomplex supplementation seemed to be able to improve milk quality in terms of nutritional value and possible beneficial effects for human health by acting on MUFA, n3, n6/n3 ratio

Table 4
Milk fatty acids profile (% of total FA) along the trial between treated group (T) and control group (CTR).

Fatty acids profile	T	CTR	Group effect	Sampling effect	Interaction GxS	RMSE
C4:0	0.011	0.016	**	**	**	0.014
C6:0	0.040	0.136	**	**	**	0.130
C8:0	0.569	0.754	NS	**	**	0.474
C10:0	7.028	7.219	NS	**	**	2.212
C11:0	0.059	0.058	NS	NS	*	0.036
C12:0	4.017	4.262	NS	**	NS	0.872
C13:0	0.065	0.068	NS	NS	NS	0.0277
C14:0	11.722	11.908	NS	NS	NS	1.313
C14:1 cis 9	0.114	0.100	NS	**	NS	0.039
C15:0	0.929	0.922	NS	**	*	0.114
C15:1	0.206	0.227	NS	**	NS	0.055
C16:0	32.06	32.44	NS	**	**	2.357
C16:1 cis9	0.678	0.661	NS	**	**	0.116
C17:0	0.718	0.713	NS	**	*	0.066
C17:1	0.167	0.156	NS	NS	**	0.049
C18:0	13.655	14.406	NS	NS	NS	1.990
C18:1 cis9	1.387	1.325	NS	**	**	0.250
C18:1trans 11	21.058	19.128	**	**	**	2.222
C18:2 trans9 trans 12 n6	0.304	0.318	NS	**	NS	0.078
C18:2 cis9 cis 12 n6	1.773	2.166	**	**	**	0.376
C20:0	0.083	0.108	NS	*	*	0.106
C20:2	0.008	0.007	NS	NS	NS	0.011
C20:3n3	0.104	0.118	NS	NS	NS	0.036
C20:3n6	0.136	0.120	NS	NS	NS	0.037
C20:4n6	0.002	0.008	*	NS	NS	0.014
C20:5n3	0.040	0.059	*	NS	NS	0.024
C18:3 n3	1.105	0.982	**	**	NS	0.223
C18:3 n6	0.027	0.039	NS	NS	NS	0.057
C21:0	0.059	0.066	NS	NS	NS	0.213
C21:1	0.288	0.304	NS	**	NS	0.160
C22:0	0.011	0.018	**	**	NS	0.008
C22:1n9	0.008	0.014	NS	NS	NS	0.016
C22:2n6	0.034	0.040	NS	NS	NS	0.018
C22:6n3	0.062	0.064	NS	NS	NS	0.020
C22:6 n6	0.064	0.062	NS	NS	*	0.021
C23:0	0.007	0.007	NS	NS	NS	0.020
C24:0	0.0036	0.0001	**	**	**	0.006
C24:1n9	0.159	0.146	NS	NS	NS	0.048
cis-9 trans-11 CLA	0.490	0.399	**	**	**	0.121
trans-10 cis-12 CLA	0.034	0.023	**	**	NS	0.014
SFA	71.28	73.18	**	*	**	0.650
MUFA	24.55	22.56	**	NS	**	0.620
PUFA	4.16	4.26	*	NS	**	0.109
DHA	0.010	0.012	NS	NS	NS	0.002
∑ CLA	0.52	0.40	**	*	*	0.035
PUFA n3	1.34	1.19	**	**	NS	0.051
PUFA n6	2.29	2.63	**	**	**	0.090
n6/n3	1.71	2.21	**	NS	NS	0.851

SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, DHA: docosahexaenoic acid, CLAs: conjugated linoleic acids.
** $p < 0.01$.

* $p < 0.05$. NS, Not Significant. RMSE, root mean square error.

Table 5
Blood profile of goats of treated group (T) and control group (CTR) along the trial.

	BUN mg/dl		CREA mg/dl		TP g/l		ALB g/l		BIL mg/dl		AST U/l		A/G	
	CTR	T	CTR	T	CTR	T	CTR	T	CTR	T	CTR	T	CTR	T
	27.85	23.30	0.78a	0.72b	8.42	7.81	2.89	2.83	0.38	0.37	99.50	107.21	0.54	0.57
Group effect	NS		*		NS		NS		NS		NS		NS	
February	27.00	21.21	0.84	0.73	8.02	7.77	2.89	2.89	0.36	0.33	91.29	90.93	0.57	0.60
May	34.86	30.33	0.73	0.67	8.58	7.66	2.90	2.74	0.43	0.37	107.5	127.53	0.52	0.56
August	20.67	17.77	0.78	0.77	8.69	8.02	2.89	2.88	0.35	0.43	99.67	102.54	0.51	0.56
Sampling effect	*		*		NS		NS		NS		*		NS	
Interaction GxS	NS		NS		NS		NS		NS		NS		NS	
RMSE	1.304		0.012		0.116		0.051		0.011		7.363		0.016	

** $p < 0.01$, * $p < 0.05$. NS, Not Significant. RMSE, root mean square error.

Table 6
Goats 'energy requirements [(1)] and energy intake [(2)].

T group	Body weight, kg	Milk (4% fat)	UFL requirements	UFL intake at pasture	Total ingested UFL
April	51.10	1.661	1.38	0.78	1.22
May	50.39	1.630	1.36	0.77	1.21
June	50.29	1.418	1.27	0.76	1.20
July	50.16	1.155	1.16	0.75	1.19
August	50.05	0.824	1.02	0.76	1.20
CTR group					
April	50.54	1.461	1.29	0.77	1.21
May	50.01	1.285	1.21	0.76	1.20
June	50.27	1.261	1.21	0.76	1.20
July	50.39	1.175	1.17	0.77	1.21
August	51.60	0.670	0.98	0.78	1.22

Energy requirements for lactation: 0.41 UFL/kg fat-corrected milk (4% fat).
[(1)] Energy requirements for maintenance: 0.0365 UFL/kg metabolic weight ($MW = BW^{0.75}$).

[(2)] Pasture DM intake: 20 g DM/kg BW; corn UFL intake: 0.44 UFL unchanged during the trial.

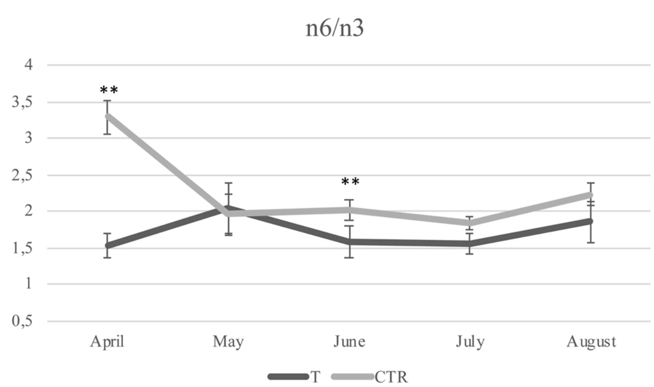

Fig. 1. n6/n3 ratio (means ± SD) along the trial for T (Treated) and CTR (Control) groups **, $p < 0.01$.

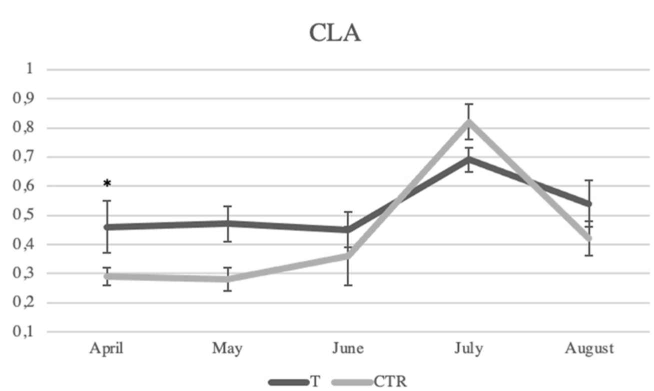

Fig. 2. CLA trend during the trial (means ± SD) for T (Treated) and CTR (Control) groups. *, $p < 0.05$.

and CLA content. In particular, when PUFA and mainly n3 amount in the pasture decreases. The amount of Galega officinalis, linseed and Schizochytrium limacinum contained in the complex were much lower than those reported in the literature to exert similar effects by themself, thus suggesting a possible synergism between the different compounds, but such hypothesis needs to be further explored. Indeed, at the dose used in this trial, the phytocomplex contains very low amount of plants, it is therefore possible that a stronger effect could be obtained by changing the administered dose. Importantly, the blood profiles suggest the phytocomplex had no negative effects on goat's health.

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Declaration of Competing Interest

The authors report no declarations of interest.

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References

AOAC, 2012. Official Methods of Analysis, 19th edition. Association of Official Analytical Chemists, Arlington, VA.
Białek, A., Białek, M., Lepionka, T., Kaszperuk, K., Banaszkiewicz, T., Tokarz, A., 2010. The effect of pomegranate seed oil and grapeseed oil on cis-9, trans-11 CLA (rumenic acid), n-3 and n-6 fatty acids deposition in selected tissues of chickens. J. Anim. Physiol. Anim. Nutr. 102, 962–976. https://doi.org/10.1111/jpn.12902.
Bichi, E., Hervás, G., Toral, P.G., Loor, J.J., Frutos, P., 2013. Milk fat depression induced by dietary marine algae in dairy ewes: persistency of milk fatty acid composition and animal performance responses. J. Dairy Sci. 96, 524–532. https://doi.org/10.3168/jds.2012-5875.
Boeckaert, C., Vlaeminck, B., Mestdagh, J., Fievez, V., 2007. In vitro examination of DHA-edible micro algae 1. Effect on rumen lipolysis and biohydrogenation of linoleic and linolenic acids. Anim. Feed Sci. Technol. 136, 63–79.
Boeckaert, C., Vlaeminck, B., Dijkstra, J., Issa-Zacharia, A., Van Nespen, T., Van Straalen, W., Fievez, V., 2008. Effect of dietary starch or micro algae supplementation on rumen fermentation and milk fatty acid composition of dairy cows. J. Dairy Sci. 91, 4714–4727. https://doi.org/10.3168/jds.2008-1178.
Caroprese, M., Ciliberti, M.G., Santillo, A., Marino, R., Sevi, A., Albenzio, M., 2016. Immune response, productivity and quality of milk from grazing goats as affected by dietary polyunsaturated fatty acid supplementation. Res. Vet. Sci. 105, 229–235. https://doi.org/10.1016/j.rvsc.2016.02.018.
Castroviejo, S., 1999. Flora Iberica. Vol. VII Leguminosae. Madrid. Real Jardín Botánico, CSIC, p. 578.
Cavaliere, G., Trinchese, G., Musco, N., Infascelli, F., De Filippo, C., Mastellone, V., Morittu, V.M., Lombardi, P., Tudisco, R., Grossi, M., Monda, V., Cutrignelli, M.I., Messina, A., Calabrò, S., Moni, H.B., Stradella, L., Messina, G., Monda, M., Crispino, M., Mollica, M.P., 2018. Milk from cows fed a diet with a high forage: concentrate ratio improves inflammatory state, oxidative stress, and mitochondrial function in rats. J. Dairy Sci. 101, 1843–1851. https://doi.org/10.3168/jds.2017-13550.
Chouinard, P.Y., Corneau, L., Barbano, D.M., Metzger, L.E., Bauman, D.E., 1999. Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows. J. Nutr. 129, 1579–1584. https://doi.org/10.1093/jn/129.8.1579.
Christie, W.W., 1982. A simple procedure of rapid transmethylation ofglycerolipids and cholesteryl esters. J. Lipid Res. 23, 1072–1075.
Currò, C., Manuelian, L., De Marchi, M., Claps, S., Rufrano, D., Neglia, G., 2019. Effects of breed and stage of lactation on milk fatty acid composition of italian goat breeds. Animals (Basel) 9, 764. https://doi.org/10.3390/ani9100764.
Dilzer, A., Park, Y., 2012. Implication of conjugated linoleic acid (CLA) in human health. Crit. Rev. Food Sci. Nutr. 52, 488–513. https://doi.org/10.1080/10408398.2010.501409.

EFSA Panel on Dietetic Products, Nutrition, Allergies (NDA), 2010. Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA J. 8, 1461.

Ellis, W.C., Mahlooji, M., Lascano, C.E., Matis, J.H., 2005. Effects of size of ingestively masticated fragments of plant tissues on kinetics of digestion of NDF. J. Anim. Sci. 83, 1602–1615. https://doi.org/10.2527/2005.8371602x.

Falconnier, G.N., Descheemaeker, K., Traore, B., Bayoko, A., Giller, K.E., 2018. Agricultural intensification and policy interventions: exploring plausible futures for smallholder farmers in Southern Mali. Land Use Policy 70, 623–634. https://doi.org/10.1016/j.landusepol.2017.10.044.

Folch, J., Lees, M., Sloane, G.H., 1957. A simple method for isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Franklin, S., Martin, K.R., Baer, R.J., Schingoethe, D.J., Hippen, A.R., 1999. Dietary marine algae (Schizochytrium sp.) increases concentrations of conjugated linoleic, docosahexaenoic and transvaccenic acids in milk of dairy cows. J. Nutr. 129 (11), 2048–2054.

FVE, 2005. Codice delle buone pratiche veterinarie. Supp. 5.

Gonzalez-Andres, F., Redondo, P.A., Pescador, R., Urbano, B., 2004. Management of Galega officinalis L. and preliminary results on its potential for milk production improvement in sheep. New Zealand J. of Agric. Res. 47, 233–245. https://doi.org/10.1080/00288233.2004.9513591.

Hanuš, O., Samková, E., Křížová, L., Hasoňová, L., Kala, R., 2018. Role of fatty acids in milk fat and the influence of selected factors on their variability—a review. Molecules 23, 1636. https://doi.org/10.3390/molecules23071636.

Hara, A., Radin, N.S., 1978. Lipid extraction of tissues with a low-toxicity solvent. Analyt. Biochem 90, 420–426.

INRA, 1978. Alimentation Des Ruminants. INRA, Paris, France.

Khodadadi, S., 2016. Administration of Galega officinalis in experimental and clinical investigations. A narrative review. Ann. Res. Antioxidants 1 (1), e03.

Kuhnt, K., Kraft, J., Moeckel, P., Jahreis, G., 2006. Trans-11–18: 1 is effectively δ9-desaturated compared with Trans-12–18: 1 in humans. Br. J. Nutr. 95, 752–761. https://doi.org/10.1079/bjn20051680.

Li, D., Bode, O., Drummond, H., Sinclair, A.J., 2003. Omega-3 (n-3) fatty acids. In: Gunstone, F.D. (Ed.), Lipids for Functional Foods and Nutraceuticals. Oily Press, Bridgwater (UK), pp. 225–262.

Lock, A.L., Garnsworthy, P.C., 2003. Seasonal variation in milk conjugated linoleic acid and D9-desaturase activity in dairy cows. Livestock Prod. Sci. 79, 47–59. https://doi.org/10.1016/S0301-6226(02)00118-5.

Meľuchová, B., Blaško, J., Kubinec, R., Górová, R., Dubravská, J., Margetín, M., Soják, L., 2008. Seasonal variations in fatty acid composition of pasture forage plants and CLA content in ewe milk fat. Small Rumin. Res. 78, 56–65. https://doi.org/10.1016/j.smallrumres.2008.05.001.

Moate, P.J., Williams, S.R.O., Hannah, M.C., Eckard, R.J., Auldist, M.J., Ribaux, B.E., Jacobs, J.L., Rales, W.J., 2013. Effects of feeding algal meal high in docosahexaenoic acid on feed intake, milk production, and methane emissions in dairy cows. J. Dairy Sci. 96, 3177–3188. https://doi.org/10.3168/jds.2012-6168.

Mooney, M.H., Fogarty, S., Stevenson, C., Gallagher, A.M., Palit, P., Hawley, S.A., Hardie, D.G., Coxon, G.D., Waigh, R.D., Tate, R.J., Harvey, A.L., Furman, B.L., 2008. Mechanisms underlying the metabolic actions of galegine that contribute to weight loss in mice. Br. J. Pharmacol. 153 (8), 1669–1677. https://doi.org/10.1038/bjp.2008.37.

Moran, C.A., Morlacchini, M., Fusconi, G., 2017. Enhancing the DHA content in milk from dairy cows by feeding ALL-G-RICHTM. J. Appl. Anim. Nutr. 5, e11. https://doi.org/10.1017/jan.2017.9.

Nudda, A., Battacone, M.G., Usai, G., Fancellu, S., Pulina, G., 2006. Supplementation with extruded linseed cake affects concentrations of conjugated linoleic acid and vaccenic acid in goat milk. J. Dairy Sci. 89, 277–282. https://doi.org/10.3168/jds.S0022-0302(06)72092-6.

Ohlsson, L., 2010. Dairy products and plasma cholesterol levels. Food Nutr. Res. 54 https://doi.org/10.3402/fnr.v54i0.5124.

Pajor, F., Egerszegi, I., Steiber, O., Bodnár, Á., Póti, P., 2019. Effect of marine algae supplementation on the fatty acid profile of milk of dairy goats kept indoor and on pasture. J. Anim. Feed Sci. Technol. 28, 169–176. https://doi.org/10.22358/jafs/109955/2019.

Papadopoulos, G., Goulas, C., Apostolaki, E., Abril, R., 2002. Effects of dietary supplements of algae, containing polyunsaturated fatty acids, on milk yield and the composition of milk products in dairy ewes. J. Dairy Sci. 69, 357–365. https://doi.org/10.1017/s0022029902005599.

Piccione, G., Casella, S., Lutri, L., Vazzana, I., Ferrantelli, V., Caola, G., 2010. Reference values for some haematological, haematochemical, and electrophoretic parameters in the Girgentana goat. Turk. J. Anim. Sci. 34, 197–204. https://doi.org/10.3906/vet-0902-1.

Póti, P., Pajor, F., Bodnár, A., Penksza, K., Köles, P., 2015. Effect of micro-alga supplementation on goat and cow milk fatty acid composition. Chil. J. Agric. Res. 75, 259–263. https://doi.org/10.4067/S0718-58392015000200017.

Reynolds, C.K., Cannon, V.L., Loerch, S.C., 2006. Effects of forage source and supplementation with soybean and marine algal oil on milk fatty acid composition of ewes. Anim. Feed Sci. Technol. 131, 333–357. https://doi.org/10.1016/j.anifeedsci.2006.06.015.

Rubino, R., 1996. L'allevamento Caprino. ASSONAPA, Roma, Italy.

Shingfield, K.J., Bernard, L., Leroux, C., Chilliard, Y., 2010. Role of trans fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. Animal 4, 1140–1166.

Simopoulos, A.P., 2002. The importance of the ratio of omega-6/ omega-3 essential fatty acids. Biomed. Pharmacother. 56, 365–379. https://doi.org/10.1017/S1751731110000510.

Sokoła-Wysoczańska, E., Wysoczański, T., Wagner, J., Czyż, K., Bodkowski, R., Lochyński, S., Patkowska-Sokoła, B., 2018. Polyunsaturated fatty acids and their potential therapeutic role in cardiovascular system disorders-A. Nutrients 10 (10), 1561. https://doi.org/10.3390/nu10101561.

Solaiman, S.G., 2010. Goat Science and Production, 1st ed. Wiley-Blackwell, Ames, IA, USA, pp. 193–216.

Sun, C.Q., O'Connor, C.J., Roberton, A.M., 2003. Antibacterial actions of fatty acids and monoglycerides against Helicobacter pylori. FEMS Immunol. Med. Microb. 36, 9–17. https://doi.org/10.1016/S0928-8244(03)00008-7.

Toral, P.G., Hervás, G., Gómez-Cortés, P., Frutos, P., Juárez, M., de La Fuente, M.A., 2010. Milk fatty acid profile and dairy sheep performance in response to diet supplementation with sunflower oil plus incremental levels of marine algae. J. Dairy Sci. 93, 1655–1667. https://doi.org/10.3168/jds.2009-2769.

Trinchese, G., Cavaliere, G., Penna, E., De Filippo, C., Cimmino, F., Catapano, A., Musco, N., Tudisco, R., Lombardi, P., Infascelli, F., Messina, G., Muredda, L., Banni, S., Monda, M., Crispino, M., Mollica, M.P., 2019. Milk from cow fed with high forage/concentrate ratio diet: beneficial effect on rat skeletal muscle inflammatory state and oxidative stress through modulation of mitochondrial functions and AMPK activity. Front. Phys. 9, 1969. https://doi.org/10.3389/fphys.2018.01969.

Tsiplakou, E., Mountzouris, K.C., Zervas, G., 2006. Concentration of conjugated linoleic acid in grazing sheep and goat milk fat. Livestock Sci. 103, 74–84. https://doi.org/10.1016/j.livsci.2006.01.010.

Tsiplakou, E., Abdullah, M.A.M., Alexandros, M., Chatzikonstantinou, M., Skliros, D., Sotirakoglou, K., Flemetakis, E., Labrou, N.E., Zervas, G., 2017. The effect of dietary Chlorella pyrenoidosa inclusion on goats milk chemical composition, fatty acids profile and enzymes activities related to oxidation. Livestock Sci. 197, 106–111. https://doi.org/10.1016/j.livsci.2017.01.014.

Tudisco, R., Calabrò, S., Cutrignelli, M.I., Moniello, G., Grossi, M., Gonzalez, O.J., Piccolo, V., Infascelli, F., 2012. Influence of organic systems on stearoyl-CoA desaturase in goat milk. Small Rumin. Res. 106, 37–42. https://doi.org/10.1016/j.smallrumres.2012.04.031.

Tudisco, R., Grossi, M., Calabrò, S., Cutrignelli, M.I., Musco, N., Addi, A., Infascelli, F., 2014. Influence of pasture on goat milk fatty acids and Stearoyl-CoA desaturase expression in milk somatic cells. Small Rumin. Res. 122, 38–43. https://doi.org/10.1016/j.smallrumres.2014.07.016.

Tudisco, R., Chiofalo, B., Lo Presti, V., Morittu, V.M., Moniello, G., Grossi, M., Musco, N., Grazioli, R., Mastellone, V., Lombardi, P., Infascelli, F., 2019a. Influence of feeding linseed on SCD activity in grazing goat mammary glands. Animals 9, 786. https://doi.org/10.3390/ani9100786.

Tudisco, R., Morittu, V.M., Addi, L., Moniello, G., Grossi, M., Musco, N., Grazioli, R., Mastellone, V., Pero, M.E., Lombardi, P., Infascelli, F., 2019b. Influence of pasture on stearoyl-coa desaturase and mirna 103 expression in goat milk: preliminary results. Animals 9, 606. https://doi.org/10.3390/ani9090606.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3598.

White, S.L., Bertrand, J.A., Wade, M.R., Washburn, S.P., Green, J.T., Jenkins, T.C., 2001. Comparison of fatty acid content of milk from jersey and holstein cows consuming pasture or a total mixed ration. J. Dairy Sci. 84, 2295–2301. https://doi.org/10.3168/jds.S0022-0302(01)74676-0.

Zhou, X., Seto, S.W., Chang, D., Kiat, H., Razmovski-Naumovski, V., Chan, K., Bensoussan, A., 2016. Synergistic effects of chinese herbal medicine: a comprehensive review of methodology and current research. Front. Pharm. 9, 201. https://doi.org/10.3389/fphar.2016.00201.

Zicarelli, F., Addi, L., Tudisco, R., Calabrò, S., Lombardi, P., Cutrignelli, M.I., Moniello, G., Grossi, M., Tozzi, Bm, Musco, N., et al., 2016. The influence of diet supplementation with Saccharomyces cerevisiae or Saccharomyces cerevisiae plus Aspergillus oryzae on milk yield of Cilentana grazing dairy goats. Small Rumin. Res. 135, 90–94. https://doi.org/10.1016/j.smallrumres.2015.12.018.