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Immune response, productivity and quality of milk from grazing goats as affected by dietary polyunsaturated fatty acid supplementation

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ABSTRACT

This study was undertaken to assess how diet supplemented with fish oil and linseed improve the immune profile, the production performance, and milk quality of grazing goats by a diet supplementation of fish oil or linseed. Twenty-four Garganica grazing goats were divided into three groups named control (CON), fish oil (FO) and linseed (LIN) according to the fat supplement received in their diet. *In vivo* immune responses were evaluated by monitoring cell-mediated and humoral immune responses in order to verify the effects of polyunsaturated fatty acids supplementation on goats' health status. Goat milk samples were analysed weekly to determine milk chemical composition, fatty acid profile, and somatic cell count. Diet based on linseed supplementation (LIN) significantly increased milk yield by 30%, milk fat yield by 67%, protein yield by 34%, and casein yield by 41% as compared with CON. Fat content increased by 30% in LIN milk as compared with CON milk, and by 12% as compared with FO milk. Linseed modified milk fatty acid profile; LIN milk showed lower SFA and higher PUFA than FO milk. The modified fatty acid composition of LIN milk resulted in lower AI and TI indexes than FO and CON milk. Linseed and fish oil administration can reduce humoral immunity of goats, but has no effect in their cellular immunity. Dietary linseed supplementation in grazing dairy goat supports feeding programs to improve milk composition and quality, and a modulation of their immune responses.

1. Introduction

Goat rearing systems are traditionally linked to grazing, thus enriching goat milk and cheeses in health promoting factors, vitamins, and volatile compounds such as flavors and terpenes (Morand-Fehr et al., 2007). However, grazing can have detrimental effects on goats' health status caused by uncontrolled parasitic diseases. The control of parasites has been achieved over the past fifty years with the use of anthelmintic drugs; to meet public concern and to stimulate a sustainable agriculture, a number of *in vivo* experiments with bioactive plants able to reduce parasite population have been carried out, even if very few studies addressed the immunological mechanisms implicated in the host responses, and with inconsistent results (Hoste et al., 2006).

Goat milk is known for its nutritional properties and healthy features, referring to both fatty acid (FA) composition and protein, and amino acid composition (Silanikove et al., 2010). In a comparative analysis carried out by Ruiz et al. (2009) it appeared that grazing dairy goats have different feeding management among western region countries of Mediterranean area. The absence of standardized feeding programs and the variability linked to pasture composition lead to high variability in milk yield and milk composition. This is a critical point because goat milk from grazing animals is characterized by higher levels of beneficial fatty acids than milk from goats with no pasture access. Fat supplementation helps to reduce differences between grazing animals and animals fed hay and concentrate (Bustos et al., 2013). A moderate forage diet supplementation with fish oil, providing 11.1 g fish oil/day/animal, to dairy goats did not result in a reduction of milk yield (Tsiplakou and Zervas, 2013). Adequate supplementations also modulate the content of nutritional components in milk and cheese. Recently, a number of natural fat sources in the ruminant diet have been proposed to enrich milk with polyunsaturated fatty acids. Several studies have been focused on the effects of dietary n − 3 polyunsaturated fatty acids (PUFA) contained fish oil and linseed on milk composition and immune response (Caroprese et al., 2009, 2012; Savoini et al., 2010). Extruded linseed was used as supplement in dairy goats and an increase of the levels of milk FA n − 3 was found (Nudda et al., 2013; Bernard et al., 2015).

Dietary fatty acids may be able to modulate the immune system causing a reduction of lymphocyte proliferation, and of cytokine synthesis, an increase in phagocytic activity and a modification of natural killer activity by several mechanisms (De Pablo and Álvarez De Cienfuegos,

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2000). Dietary fatty acid manipulation of the diet is able to interfere with the modulation of immune responses with potential beneficial effects on animals (Caroprese et al., 2015). The infusion of fish oil or linseed to fasted dairy cows contributed to reduce the alteration of lymphocytes to mitogens (Lacetera et al., 2007). Supplementation of whole linseed in dairy cows and sheep enhanced humoral and cellular immune responses, improving health status during heat stress (Caroprese et al., 2009, 2012).

This study was undertaken to evaluate the effects of different sources of PUFA such as fish oil and linseed supplementation on goat milk yield and composition and on goats' cell-mediated and humoral immune responses.

2. Materials and methods

2.1. Experimental design

The experiment lasted 5 weeks, and was conducted during the spring season (May–June) in a commercial dairy farm located in the Gargano area (Apulia, Southern Italy), with an elevation of about 450 m above sea level (longitude: 16°11′; latitude: 41°53′). The Gargano promontory is characterized by a Mediterranean climate, with about 600 mm annual rainfall, a mean maximum temperature near 20 °C (often over 30 °C in July and August) and a mean minimum temperature of about 10 °C. Twenty-four Garganica goats (60.74 ± 0.71 days of lactation, mean ± SE) were divided into three groups of eight each, balanced for parity (3.8 ± 0.44), milk yield (861.17 ± 29.07 g/day), and body weight (43.98 ± 1.38 kg). Goats had free access to pasture (09.00–17.00) constituted by *Crataegus monogyna*, *Trifolium pratense*, *Prunus spinosa*, *Clematis vitalba*, *Cirsium acaule*, *Carthamus lanatus*, *Cnicus benedictus*, and *Carduus defloratus*.

Goats in CON group received 1.5 kg of concentrate; goats in FO group received 1.5 kg of concentrate and were supplemented with 50 g/day of microencapsulated fish oil (Orovital Cod, Ascor Chimici srl, Capocolle di Bertinoro, Italy); goats in LIN group were supplemented with 150 g/day of whole linseed (Lin Tech, Tecnozoo srl, Torreselle di Piombino Dese, Italy) in substitution of an equal amount of concentrate. Water was available *ad libitum* for all groups from drinking troughs at any time of day.

2.2. Chemical composition of diet ingredients

Chemical analyses of concentrate, fish oil and whole linseed were carried out using standard procedures. Crude protein (CP) content was measured according to the Kjeldahl method (proc. 988.05; AOAC, 2000), fat (ether extract) by the Soxhlet method (proc. 920.39; AOAC, 2005) and neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) were performed following the method of Van Soest et al. (1991), using an Cap, FC 221, FOSS. The chemical composition of the diet ingredients is reported in Table 1.

Lipid extraction for fatty acid analysis of the diet ingredients was carried out according to a modified Folch method (Arvidsson et al., 2009). Separation and quantification of the methyl esters were carried out using a gas chromatograph (Agilent 6890N) equipped with a flame ionization detector (FID), autosampler, split injection port and a fused silica capillary column (100 m, internal diameter: 0.25 mm, film thickness: 0.25 μm) (HP-88 capillary column, Agilent Technologies Spa, Italy). Helium was used as the gas carrier (0.42 ml/min). Injector temperature was maintained at 220 °C whereas the detector temperature was of 250 °C. Fatty acid profile was determined using the following temperature gradient program: initial temperature of 150 °C that was increased to 220 °C at 1 °C/min. Each peak was identified and quantified using pure methyl ester standards: 37 component methyl ester fatty acid (FAME) (Matreya Inc., Pleasant Gap, PA). Results were expressed as g/100 g of total FAME analysed (Table 2).

Table 1
Formulation and chemical composition of dietary ingredients of ingested experimental diets in dairy goats.

Item	Ingredient, kg		
	Concentrate	Fish oil[a]	Linseed[b]
Diet			
CON[c]	1.50	0	0
FO	1.50	0.05	0
LIN	1.35	0	0.15
Chemical composition			
Dry matter (DM, g/kg)	953	905	951
Lipid extract (g/kg DM)	25	16	37
CP (g/kg DM)	123	90	183
ADF (g/kg DM)	90	14	131
NDF (g/kg DM)	524	843	234
ADL (g/kg DM)	23	7	50

DM = dry matter; CP = crude protein; ADF = acid detergent fiber; NDF = neutral detergent fiber; ADL = acid detergent lignin.
[a] Orovital Cod (Ascor Chimici srl, Capocolle di Bertinoro, Italy).
[b] Lin Tech (Tecnozoo srl, Torreselle di Piombino Dese, Italy).
[c] CON = control group with no fat supplementation, FO = fat supplementation based on fish oil, LIN = fat supplementation based on linseed.

2.3. Sampling and chemical analyses of milk

Goats were milked twice daily (07.00 and 14.00) using a milking machine. Milk samples from each goat were collected at morning and afternoon milking once a week, in the same day, throughout the experiment. Fresh samples were used for the following chemical analysis: pH (GLP 21 Crison, Spain), total protein, casein, fat, and lactose content using an infrared spectrophotometer (MilkoScan™ FT120, Foss Electric, DK-3400 Hillerød, Denmark) according to the International Dairy Federation standard (IDF, 1990), and SCC using a Fossomatic™ Minor (Foss Electric, DK-3400 Hillerød, Denmark) (IDF, 1995).

2.4. Milk fatty acid analysis

At 1, 3, and 5 week of the experiment each milk sample was analysed for milk fatty acid (FA) composition. Milk fat was extracted according to the procedure of Luna et al. (2005) and trans-esterification of fatty acids according to ISO-IDF (2002) procedures, as reported in Caroprese et al. (2011). Briefly, fatty acid methyl esters were separated and measured using a gas-chromatograph (Agilent 6890N) equipped with CP-Sil 88 fused-silica capillary column [100 m × 0.25 mm (i.d.) with 0.25 μm film thickness]. Operating conditions were: a helium flow rate of 1 ml/min; a flame ionization detector (FID) at 260 °C; a split-splitless injector at 260 °C and an injection volume of 1 μl with a split ratio of 1:50.

Table 2
Fatty acid profile of dietary ingredients of ingested experimental diets in dairy goats.

Fatty acids (g/100 g of total FAME[a])	Ingredient		
	Concentrate	Fish oil[b]	Linseed[c]
16:0	15.5	20.2	6.1
18:0	1.8	5.0	3.4
18:1$^{cis\text{-}9}$	29.3	20.4	21.0
18:2n−6$^{cis\text{-}9,\ cis\text{-}12}$	48.6	7.6	16.0
18:3n−3	2.3	1.5	51.7
20:5n3 (DHA[d])	0.0	6.15	0.01
22:6n3 (EPA[e])	0.0	6.72	0.01

[a] FAME = fatty acid methyl esters.
[b] Orovital Cod (Ascor Chimici srl, Capocolle di Bertinoro, Italy).
[c] Lin Tech (Tecnozoo srl, Torreselle di Piombino Dese, Italy).
[d] DHA = docosahexaenoic acid.
[e] EPA = eicosapentaenoic acid.