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Posted Date: 9 August 2023

doi: 10.20944/preprints202308.0714.v1

Keywords: goat, milk, cheese, sensory, supplementation



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## Article

# Influence of Diets Supplemented on Milk and Fresh Cheese of Murciano-Granadina Goats

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**Abstract:** Three dietary treatments were assayed in 350 milking Murciano-Granadina multiparous goats in full-lactation: a control diet and two experimental diets, one including flaked linseed (FL) and the other salmon oil (SO). Neither dietary treatment affected the daily milk yield, cheese yield or the physicochemical parameters of the milk and cheese. With regard to the fatty acid profile (FA), the milk and cheese from animals whose diet was supplemented with SO had a higher percentage of fatty acids than those obtained with the FL supplemented diet, except for C18:0, C18:1, C18:2 n-6, trans-9, trans-12 C18:2, cis-9, trans-11 C18:2, C18:3 and C19:0, which reached their highest levels in milk obtained with the diet supplemented with FL. The decrease in the percentage of C16:0 was greater in the milk derived from the FL diet than from the SO diet. The FL supplemented diet improved the nutritional value of milk due to a reduction in saturated fatty acids (SFA) and an increase in polyunsaturated acids (PUFA) and conjugated linoleic acid (CLA). The decrease in n-6/n-3 in observed milk was more pronounced with the FL diet. No differences in the sensory profile were found for the milk and cheese derived from the different dietary treatments.

**Keywords:** goat; flaked linseed; salmon oil; fatty acid; sensory profile

## Introduction

Goat milk is the third most produced variety in the world, with an increase more than twofold in the last decades, and with an increase market probability of 53% by 2030 (Pulina et al., 2018). There are several differences in its composition compared to cow's milk that determine their low allergenic potential, digestibility and nutritional value, and contribute that goat milk can be considered a natural functional food that its consumption should be promoted.

Murciano-Granadina goat milk is characterized by an excellent technological aptitude to produce different cheese varieties, where cheese quality is determined by fat and protein composition (Garcia et al., 2014). Numerous studies have shown that modification of the basal diet, especially, with dietary fat sources in ruminant diets could be a good strategy to reduce levels of the saturated fatty acids (FAs). Grasses and vegetable seeds or oils such as linseed, hemp seed, chia seed and rubber seed are sources of  $\alpha$ -linoleic acid (ALA, 18:3n-3) while fishmeal or oil, algal oil or microalgae biomass are sources of eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22: n6-3).

This strategy are in accordance with public health policies which recommend a reduction in consumption of saturated fatty acids (SFA) and an increase in the consumption of n-3 FAs, especially  $\alpha$ -linoleic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22: n6-3) which have health benefits (Gebreyowhans et al., 2019).

However, most of these studies were performed in dairy cow and, it is known that the responses regarding goat milk production and mammary lipid metabolism are different from those observed in cow. One problem observed with lipid supplementation in dairy cows is the decrease in protein

content that modify the coagulation properties and may alter the texture of the final product (Ferlay and Chillard, 2020).

Although most studies agree that the final concentration of PUFAs and CLA in processed dairy products is mainly related to their content in milk, the fatty acid profile of milk and dairy products, especially the CLA content, may also be associated with the effects of technological processes applied to obtain products like cheese (Collomb et al., 2006).

Nowadays it is technically feasible to modify FAs by feeding strategies, there is a gap of knowledge about the effects of such supplementation on the properties of the resulting milk and cheese need to be further investigated and constitute the proposal of this study.

Several articles have studied the influence of diet supplementation on goat milk quality, but the number of animals included is normally small or do not reflect real commercial conditions. Moreover, little technological and sensory input is provided. Thus, our study included 350 goats divided into three groups (control and two supplemented with ingredients rich in n-3). Dietary n-3 treatments modified the fatty acid profile without making any sensory difference on milk and fresh cheese, accompanied by marginal modifications to the physicochemical profile. Therefore, milk obtained from animals receiving dietary supplementation can be provided to the dairy industry.

The main aim of this work was to improve the nutritional value of the FAs of goat milk and fresh cheese by supplementing the diet of Murciano-Granadina goats with flaxed linseed (FL) and salmon oil (SO) as lipid sources. This study as a preliminary stage to validate the use of this supplementation at an industrial level in the production of matured cheeses.

## Material and methods

### *Animals and treatments*

The experiment was conducted in a farm located in the southeast of Spain. A total of 350 milking Murciano-Granadina multiparous goats in full-lactation, distributed in three groups of 150, 100 and 100 animals, according to parity and their daily milk yield (recorded one week before the trial). The study followed a 3 × 3 crossover design, with 3 periods of 21 d each (14 d for adaptation and 7 d for sampling and data recording). The three dietary treatments were: a control diet, supplemented with calcium soaps of palm oil FA (MAGNAPAC®, Norel Animal Nutrition, Madrid, Spain); and two experimental diets supplemented with ingredients rich in n-3 - one that included FL (seeds broken and hydrothermally processed from Agrocava SL company, Caravaca de la Cruz, Murcia, Spain), an ingredient rich in ALA; and another that contained a concentrate of SO (Optomega-50, Optivite International Ltd., Nottinghamshire, United Kingdom), a supplement rich in DHA and EPA. Each diet was in the form of a total mixed ration (TMR) consisting of alfalfa hay, citrus pulp, a concentrate mixture and a lipid-rich supplement partially substituting the concentrate (1.55, 3.88 or 2.64 % of diet on a dry basis) with calcium soaps of palm oil FAs, FL or SO concentrate, respectively. All the met the energy and protein requirements of dairy goats. The ingredients and chemical composition of the diets are shown in Table 1. The animals were fed the TMR *ad libitum* twice daily (at 08:00 and 16:00 h) with sufficient feed for approximately 5% to remain uneaten. The amount of feed offered and refused was recorded daily throughout the experimental period. Goats had unlimited access to water and were milked twice a day. Milk yield per group and day was recorded daily during the sampling period, and samples of the offered and refused diet of each period and group were collected to determine the dry matter (DM) content and for chemical analysis.

**Table 1.** Ingredients and chemical composition of experimental diets

	Control	Flaked linseed	Fish oil
Ingredients (% of DM)			
Alfalfa hay	16.92	16.92	16.92
Citrus pulp	15.86	15.77	15.77
Soaps of palm oil fatty acids <sup>1</sup>	1.55		
Flaked linseed <sup>2</sup>		3.88	
Fish oil concentrate <sup>3</sup>			2.64
Concentrate mixture <sup>4</sup>	65.67	63.43	64.67
Nutritive value calculated <sup>5</sup>			
UFL <sup>6</sup> /kg DM	1.01	1.01	1.01
CP, % of DM	16.58	17.01	16.52
PDIE <sup>7</sup> , % of DM	10.8	10.3	10.3
PDIN <sup>8</sup> , % of DM	10.3	11.0	10.7
PDIA <sup>9</sup> , % of DM	4.6	4.7	4.6
Chemical composition analyzed			
DM, %	57.84	57.97	57.79
CP, % of DM	15.50	16.38	16.17
Ether extract, % of DM	4.01	5.21	4.18
NDF, % of DM	37.17	37.70	37.67
ADF, % of DM	21.52	22.46	22.56
ADL, % of DM	3.39	3.41	3.74
Starch. % of DM	20.01	20.00	20.00
Fatty acids (g/100 g of total FA)			
C14:0	1.91	1.38	3.31
C16:0	20.20	16.49	18.35
C16:1	1.69	1.20	3.47
C18:0	9.11	8.16	9.60
C18:1	31.41	17.2	20.80
C18:2 n-6	27.00	21.56	26.57
C18:3n-3	6.97	32.71	14.43
C20:4n-6	0.00	0.00	0.13
C20:5n-3	0.00	0.00	0.09
C22:6 n-3	0.00	0.00	0.10
Saturated	31.23	26.04	31.27
Monounsaturated	33.10	18.42	24.27
Polyunsaturated	33.97	54.27	41.32

<sup>1</sup>Calcium soaps of palm oil fatty acids (84% EE) (MAGNAPAC® NOREL SA, Madrid, Spain).

<sup>2</sup>Linseed flakes (34% EE).

<sup>3</sup>Fish oil concentrate (salmon source) (OPTOMEGA-50, Optivite, International Ltd., Spain) (50% EE).

<sup>4</sup>Concentrate mixture containing barley grain (233.1 g/kg), sunflower meal (165.70 g/kg), soybean hulls (225.50 g/kg), wheat grain (40.11 g/kg), dehydrated sugar beet pulp (25.49 g/kg), corn grain (103.25 g/kg), soybean meal (37.38 g/kg), corn gluten feed (121.21 g/kg), urea (4.87 g/kg), animal fat (lard) (7.83 g/kg), dicalcium phosphate (6.20 g/kg), calcium carbonate (8.88 g/kg), sodium bicarbonate (4.93 g/kg), magnesium oxide (2.87 g/kg) and 12.68 g/kg of trace minerals and vitamins supplement, containing copper (900 mg/kg), manganese (6,000 mg/kg), zinc (5,000 mg/kg), vitamin A (800,000 IU/kg), vitamin D (175,000 IU/kg), vitamin E (6,000 IU/kg).

<sup>5</sup>According to FEDNA (2010).

<sup>6</sup>UFL: forage unit for milk production (1 UFL = 7.1128 MJ of net energy for lactation).

<sup>7</sup>PDIE: protein digestible in the intestine, with energy as limiting factor for rumen microbial growth.

<sup>8</sup>PDIN: protein digestible in the intestine, with nitrogen as limiting factor for rumen microbial growth.

<sup>9</sup>PDIA: protein digestible in the intestine supplied by rumen-undegraded dietary protein.

### *Fresh goat cheese-making*

Bulk milk samples were collected from each group during the sampling period. On two days of each period 15 L samples of milk were used for chemical and sensory analysis of the milk, and for cheese-making. The milk was pasteurized (78 °C for 15 s) by a plate heat exchanger (100 L Alfa Laval, Lund, Sweden) in the pilot plant of the Food Technology Department the same day of milking, and immediately stored at 4 °C. Cheese-making<sup>9</sup> was carried out the following day according to Garcia et al. (2012).  $T_{max}$  is an optical parameter derived from a CoAguLite™ optical sensor coupled to the vat and is useful for predicting milk clotting time.

Cheese yield was defined as the amount of milk needed to obtain a given number of kilograms of cheese (L kg<sup>-1</sup>).

### *Physicochemical analysis of milk and cheese*

All the analyses were made in triplicate. The milk pH measurements were made with a Crison® pH meter (micro pH 2001, Barcelona) connected to a previously calibrated Crison® glass combined electrode (1952-2002). The pH of the cheeses was measured by suspending 5 g of grated cheese in 30 ml of distilled water and stirring for 10 min. DM was determined according to IDF (2004). The fat content of the milk and cheese was measured by the Van Gulik method (ISO, 2008). The milk and cheese protein content were determined by the Kjeldahl method (IDF, 2008). To obtain FAs content of each type of milk and cheese, lipids were extracted according to Röse-Gottlieb method before derivatization and quantified by gas chromatography (IDF, 2002), using a Finnigan Trace GC ULTRA gas chromatograph equipped with an AS3000 auto-sampler (both from Thermo Finnigan, Spain), a capillary column with cross-linked 70 % Cyanopropyl Polysilphenylene-siloxane, 60 m long, 0.25 mm internal diameter and 0.25 µm film thickness (BPX70, SGE, Australia) and an ionization flame detector. The methyl esters of FA were identified by comparison with the retention times of reference standards (Sigma-Aldrich, San Luis, Missouri, EEUU). The FA integration was processed by software from Chrom Card Fisons Instruments (Italy), and the fatty acid composition was expressed as a weight percentage of total fatty acids. The injections were performed in triplicate. The atherogenic (AI) and thrombogenic indices (TI) were calculated according to Ulbricht and Southgate (1991).  $AI = (12:0 + 4 \times 14:0 + 16:0) / (MUFA + [n-3 + n-6])$ ;  $TI = (14:0 + 16:0 + 18:0) / (0.5 \times MUFA + 0.5 \times n-3 + 3 \times n-6 + n-3 / n-6)$ .

### *Texture and sensory analysis*

Cheese texture profile analysis was determined according to García et al. (2012). Sensory profile of milk and cheese were analysed by ten trained panellists according to García et al. (2012).

### *Statistical analysis of the results*

Statistical treatment of the physicochemical data was performed using IBM SPSS Statistics 19 (IBM Spain, S.A., Madrid, Spain). The data were analyzed with a repeated measures linear mixed model, with the diet as fixed effect and group and phase as random factors. Pairwise comparisons among means were performed using least significant difference (LSD). The level of significance was taken as  $P < 0.05$  and a trend towards significance at  $P < 0.1$ . Statistical treatment of the sensory data was performed with Minitabv15.0 (Addlink Software Scientific, S.L. Barcelona, Spain). One-way ANOVA was used to determine significant differences.

## **Results**

### *Goat milk and cheese composition*

DM intake with the SO diet tended ( $P < 0.1$ ) to decrease compared with the control treatment, but dietary intake with the FL diet did not significantly differ from the other treatments (Table 2). The dietary treatments had no significant effect ( $P > 0.05$ ) on daily milk yield. As shown in Table 2, in general, the supplementation of goat diets had no effect on the physicochemical parameters of the

resulting milk, since no significant differences ( $P > 0.05$ ) were observed in pH, protein or fat. However, significant differences were observed in the dry matter content of the milk ( $P < 0.05$ ), which was lower in the milk from animals receiving the SO diet than in the corresponding milk from the FL diet.

**Table 2.** Influence of diet on dry matter intake, yield milk and the physicochemical parameters of goat milk

	Control	Flaked linseed	Fish oil	SEM <sup>1</sup>	P-value <sup>2</sup>
DM intake (kg/animal and day)	2.07a	2.02ab	1.99b	0.010	†
Milk yield (kg/animal and day)	2.42	2.46	2.47	0.110	ns
pH	6.69	6.68	6.68	0.037	ns
Dry matter (g/100g)	13.54ab	13.75a	13.34b	0.373	*
Protein (g/100g)	3.14	3.14	3.10	0.039	ns
Fat (g/100g)	5.28	5.35	5.03	0.241	ns

<sup>1</sup>SEM= standard error of the mean (n=3)

<sup>2</sup>Probability of significance: ns,  $P > 0.05$ ; †,  $P < 0.1$ ; \*,  $P < 0.05$

<sup>ab</sup> Means with different letters indicate differences ( $P < 0.05$ ) between columns

The type of supplementation did not influence the pH, DM, protein or fat content of the fresh cheese ( $P > 0.05$ ) (Table 3). Although a slight decrease in fat were observed in cheeses derived from animals with SO supplemented diet. Neither no significant differences ( $P > 0.05$ ) were observed in the clotting time ( $T_{max}$ ) or cheese yield. A higher  $T_{max}$  and lower yield were determined in cheeses derived from animals fed with supplemented FL diet.

**Table 3.** Influence of diet on the physicochemical parameters of fresh goat cheese

	Control	Flaked linseed	Fish oil	SEM <sup>1</sup>	P-value <sup>2</sup>
pH	6.88	6.90	6.89	0.019	ns
Dry matter (g/100g)	41.27	41.22	41.18	1.304	ns
Protein (g/100g)	13.77	13.94	14.11	0.320	ns
Fat (g/100g)	24.71	25.11	22.87	1.143	ns
Coagulation time ( $T_{max}$ )	7.42	7.70	7.50	0.217	ns
Yield (L/Kg)	5.40	5.33	5.47	0.098	ns

<sup>1</sup>SEM= standard error of the mean (n=3)

<sup>2</sup>Probability of significance: ns,  $P > 0.05$

#### Fatty acid profile

Regarding to milk FA content (Table 4), significant differences ( $P < 0.01$ ) among treatments were found for all FAs, except C4:0. The milk from goats fed the SO supplemented diet showed higher percentages of most FAs than the milk from goats fed the control diet, although C16:0, C18:0 and C18:1 had lower values than the control. It should be noted that the SO diet was rich in DHA and EPA, so an increase in fatty acids in the milk was to be expected. If we compare the FA values of the milk obtained from the SO and FL diets, higher percentages were found with SO diet, except for C18:0, C18:1, C18:2, *trans*-9, *trans*-12 C18:2, *cis*-9, *trans*-11 C18:2; C18:3 and C19:0, which reached their highest levels in milk derived from goat fed with FL diet. The 249% increase in concentration of ALA in the diet supplemented with FL it has been expected due to the high concentrations of this FA of this type of seed. By contrast, a lower percentage of palmitic acid (C16:0) was obtained in the milk derived animals fed with FL than with the SO supplemented diet.

**Table 4.** Influence of diet on fatty acid profile of goat milk (g/100g of total fatty acids)

	Control	Flaked linseed	Fish oil	SEM <sup>1</sup>	P-value <sup>2</sup>
C4:0	1.27	1.29	1.25	0.018	ns
C6:0	2.14b	2.27a	2.30a	0.056	***
C7:0	0.02c	0.03b	0.04a	0.001	***
C8:0	2.99c	3.24b	3.42a	0.070	***
C9:0	0.07c	0.08b	0.09a	0.001	***
C10:0	10.82c	11.50b	12.53a	0.120	***
C12:0	4.78c	4.96b	5.90a	0.090	***
C13:0	0.12b	0.13b	0.14a	0.004	***
C14:0	8.70c	9.00b	10.13a	0.138	***
C14:1	0.15b	0.14c	0.19a	0.005	***
C15:0	0.80c	0.85b	0.97a	0.016	***
C16:0	35.43a	31.40c	34.45b	0.371	***
C16:1	0.89b	0.74c	1.19a	0.020	***
C17:0	0.53c	0.55b	0.59a	0.011	***
C18:0	7.91b	9.27a	5.23c	0.309	***
C18:1	17.63a	17.38a	14.20b	0.225	***
<i>cis</i> -11 C18:1	0.48b	0.47b	0.75a	0.027	***
C18:2 n-6	3.19c	3.80a	3.66b	0.157	***
<i>trans</i> -9, <i>trans</i> -12 C18:2	0.24c	0.57a	0.31b	0.016	***
<i>cis</i> -9, <i>trans</i> -11 C18:2	0.66c	0.97a	0.90b	0.036	***
<i>trans</i> -10, <i>cis</i> -12 C18:2	0.01c	0.02b	0.03a	0.003	***
C18:3 n-3	0.28c	0.99a	0.47b	0.014	***
C19:0	0.03b	0.06a	0.04b	0.003	***
C20:0	0.19b	0.19b	0.20a	0.003	**
C20:1	0.11b	0.10c	0.31a	0.011	***
C20:2 n-6	0.06c	0.07b	0.10a	0.003	***
C20:4 n-6	0.36b	0.33c	0.43a	0.027	***
C20:5 n-3	0.03c	0.05b	0.09a	0.014	***
C22:0	0.05b	0.05b	0.07a	0.003	***
C22:5 n-3	0.09c	0.12b	0.22a	0.017	***
C22:6 n-3	0.00b	0.00b	0.16a	0.059	**
Saturated	75.84b	74.42c	77.16a	0.189	***
Monounsaturated	19.25a	18.82b	16.63c	0.187	***
Polyunsaturated	4.24c	5.77a	5.35b	0.189	***
CLA	0.66c	0.99a	0.93b	0.038	***
n-3	0.39c	1.17a	0.90b	0.065	***
n-6	3.61b	4.04a	4.14a	0.200	**
n-6/ n-3	9.34a	3.50c	4.99b	0.553	***
Atherogenicity index	3.19b	2.95c	3.70a	0.078	***
Saturated: unsaturated (ratio)	3.23b	3.03c	3.52a	0.043	***

<sup>1</sup>SEM= standard error of the mean (n=3)

<sup>2</sup>Probability of significance: ns, P>0.05; \*\*, P<0.01; \*\*\*, P<0.001

<sup>abc</sup>Means with different letters indicate differences (P<0.05) between columns.

Rumenic acid (RA, *cis*-9, *trans*-11 C18:2) levels were highest in the diet supplemented with FL. Non-conjugated isomers of C18:2 were detected, and the percentages of *trans*-9, *trans*-12 C18:2 were higher in the milk of animals receiving FL/SO diets. One of the highest PUFA concentrations in our

study was found for arachidonic acid (C20:4 n-6). In the milk obtained from goats given FL, the concentrations of SFAs (-1.88%) were lower, while those of PUFAs (36.03%), CLAs (48.81%), n-3 (197.59%) and n-6 (11.87%) were higher than in the control milk. The n-6/n-3 ratio was the lowest (-62.58%). Goat milk obtained with the diet supplemented with SO showed higher concentrations of SFAs (1.73%), PUFAs (25.96%), CLAs (40.07%), n-3 (129.83%) and n-6 (14.56%) than the control milk, while the SO diet significantly reduced the amount of monounsaturated fatty acids (-13.49%) and the n-6/n-3(-46.62%) ratio. In addition, the atherogenicity index determined in the diet enriched with FL was lower than control diet due to a decrease in the saturated/unsaturated ratio.

Table 5 details the FA profile of the fresh goat cheeses. As can be seen, there were significant differences ( $P < 0.05$ ) in all the FAs determined, except C7:0 and C17:0, between the cheeses. The FA profile was similar to that observed in milk. An increase in the PUFA and CLA contents and a decrease in the n-6/n-3 ratio and atherogenicity index compared with the control diet were observed, particularly in the cheeses made with milk from the animals receiving FL.

**Table 5.** Influence of diet on fatty acid profile of fresh goat cheese (g/100g of total fatty acids)

	Control	Flaked linseed	Fish oil	SEM <sup>1</sup>	P-value <sup>2</sup>
C4:0	1.24a	1.24a	1.15b	0.047	***
C6:0	2.11b	2.19a	2.12b	0.078	*
C7:0	0.03	0.03	0.03	0.001	ns
C8:0	2.92b	3.21a	3.24a	0.080	***
C9:0	0.07c	0.08b	0.09a	0.002	***
C10:0	10.66c	11.44b	12.00a	0.085	***
C12:0	4.74c	5.03b	5.70a	0.080	***
C13:0	0.12c	0.13b	0.14a	0.005	***
C14:0	8.68c	8.99b	10.04a	0.102	***
C14:1	0.14b	0.14b	0.19a	0.011	***
C15:0	0.82c	0.85b	0.96a	0.007	***
C16:0	35.90a	31.61c	34.79b	0.481	***
C16:1	0.91b	0.77c	1.21a	0.048	***
C17:0	0.53	0.54	0.57	0.019	ns
C18:0	7.90b	9.00a	5.68c	0.547	***
C18:1	17.48a	17.14b	14.51c	0.300	***
<i>cis</i> -11 C18:1	0.50b	0.47b	0.75a	0.068	***
C18:2 n-6	3.14c	3.74a	3.58b	0.035	***
<i>trans</i> -9, <i>trans</i> -12 C18:2	0.25c	0.55a	0.30b	0.018	***
<i>cis</i> -9, <i>trans</i> -11 C18:2	0.72c	0.97a	0.95b	0.097	***
<i>trans</i> -10, <i>cis</i> -12 C18:2	0.03b	0.04a	0.03b	0.010	***
C18:3 n-3	0.28c	0.97a	0.48b	0.036	***
C19:0	0.04c	0.06a	0.05b	0.003	***
C20:0	0.19b	0.18c	0.20a	0.007	***
C20:1	0.11b	0.10b	0.32a	0.023	***
C20:2 n-6	0.07c	0.07b	0.10a	0.005	***
C20:4 n-6	0.36b	0.32c	0.40a	0.022	***
C20:5 n-3	0.03c	0.05b	0.09a	0.015	***
C22:0	0.04b	0.05b	0.08a	0.002	***
C22:5 n-3	0.08c	0.12b	0.21a	0.020	***
C22:6 n-3	0.00b	0.01b	0.21a	0.047	***
Saturated	75.95b	74.59c	76.77a	0.283	***
Monounsaturated	19.15a	18.62b	16.98c	0.286	***
Polyunsaturated	4.18c	5.82a	5.28b	0.103	***
CLA	0.72b	0.98a	0.97a	0.095	***
n-3	0.38c	1.14a	0.94b	0.087	***
n-6	3.56c	4.12a	4.03b	0.088	***
n-6/ n-3	9.43a	3.63c	4.51b	0.467	***
Atherogenicity index	3.23b	2.97c	3.63a	0.044	***
Saturated: unsaturated (ratio)	3.26b	3.06c	3.45a	0.045	***

<sup>1</sup>SEM= standard error of the mean (n=3)

<sup>2</sup>Probability of significance: ns, P>0.05; \*, P<0.05; \*\*\*, P<0.001

<sup>abc</sup> Means with different letters indicate differences (P<0.05) between columns.

*Texture and sensory profile*

The results obtained for the texture profile (Table 6) indicate that no significant differences were observed in any of the parameters studied ( $P > 0.05$ ), except adhesiveness ( $P < 0.05$ ), the cheeses made with SO or FL milks being less adhesive than the control cheeses. Although no significant differences were found regarding hardness, the cheeses derived from the supplemented diets were less firm probably because of the PUFA content.

**Table 6.** Influence of diet on texture profile analysis (TPA)

	Control	Flaked linseed	Fish oil	SEM <sup>1</sup>	P-value <sup>2</sup>
<b>Hardness1</b>	8.51	7.76	7.96	0.185	ns
<b>Hardness2</b>	4.67	4.23	4.77	0.325	ns
<b>Cohesiveness</b>	0.36	0.37	0.39	0.029	ns
<b>Gumminess</b>	3.02	2.85	3.18	0.239	ns
<b>Chewiness</b>	35.26	33.48	37.26	2.871	ns
<b>Adhesiveness</b>	0.97a	0.74b	0.66b	0.086	*
<b>Springiness</b>	11.65	11.72	11.72	0.153	ns

<sup>1</sup>SEM= standard error of the mean (n=3)

<sup>2</sup>Probability of significance: ns,  $P > 0.05$ ; \*,  $P < 0.05$

<sup>ab</sup> Means with different letters indicate differences ( $P < 0.05$ ) between columns.

In a milk sensory analysis conducted by a trained panel (Table 7), no significant differences were observed between the different milks for any of the determined sensory attributes ( $P > 0.05$ ). However, while no significant differences were found, the milk with the highest overall acceptance was that obtained from the control group and the least acceptable was the milk from goats fed the SO diet.

**Table 7.** Influence of diet on the sensorial parameters of milk

	Control	Flaked linseed	Fish oil	SEM <sup>1</sup>	P-value <sup>2</sup>
<b>Whiteness</b>	9.87	9.77	9.77	0.079	ns
<b>Goaty odour</b>	8.99	9.05	9.07	0.318	ns
<b>Foreign odours</b>	0.02	0.08	0.06	0.030	ns
<b>Goaty taste</b>	8.94	8.77	8.87	0.377	ns
<b>Foreign flavours</b>	1.09	1.05	1.20	0.278	ns
<b>Fatty sensation</b>	3.12	3.07	3.20	0.285	ns
<b>Overall score</b>	7.82	7.60	7.38	0.483	ns

<sup>1</sup>SEM= standard error of the mean (n=3)

<sup>2</sup>Probability of significance: ns,  $P > 0.05$

No significant differences were also found in the sensory profile ( $P > 0.05$ ) between the different types of cheeses (Table 8). However, the cheeses with the highest overall score were the control ones and the lowest score corresponded to those made with milk from animals given a diet supplemented with SO, reflecting the results obtained in milk (Table 7).

**Table 8.** Influence of diet on the sensorial parameters of cheese

	Control	Flaked linseed	Fish oil	SEM <sup>1</sup>	P-value <sup>2</sup>
<b>Whiteness</b>	9.61	9.61	9.47	0.202	ns
<b>Goaty odour</b>	0.90	1.03	0.98	0.283	ns
<b>Foreign odours</b>	0.03	0.08	0.06	0.038	ns
<b>Goaty taste</b>	1.37	1.77	1.65	0.342	ns
<b>Foreign flavours</b>	0.08	0.15	0.18	0.055	ns
<b>Hardness</b>	2.59	2.52	2.57	0.328	ns
<b>Fatty sensation</b>	1.76	1.64	1.63	0.263	ns
<b>Overall score</b>	7.30	7.28	7.15	0.374	ns

<sup>1</sup>SEM= standard error of the mean (n=3)

<sup>2</sup>Probability of significance: ns, P>0.05

## Discussion

### *Diet intake and physicochemical milk/cheese composition*

The slight decrease in the intake of the SO diet was not statistically significant. However, studies carried out (Bernand et al., 2015) showed that feed consumption decreased by 6% in goats in response to a mixture of extruded linseeds and fish oil (representing 14.7 and 1.7% of the DM in the respective diets), while adding 21% extruded linseed alone did not affect the intake, although both diets had the same ether extract content (6.9% DM). Kitessa et al. (2003) determined that in goat the inclusion of 3% unprotected fish oil reduced feed intake, but supplementation with protected fish oil had no effect on the same parameter. Thus, the effect of adding fatty supplements on intake depends on many factors, including ruminant species, the amount included in the diet, and the type and composition of any supplement.

The dietary treatments had no significant effect on daily milk yield despite the substantial reduction in feed intake when a mixture of extruded linseeds and fish oil was included in the diet (Bernard et al., 2015). As shown in Table 2, supplementation of goat diets with fats with a higher polyunsaturated fatty acid had no effect on the physicochemical parameters of the resulting milk. In goat milk, unlike in cow milk, there is no decrease in the milk protein and fat content when the goat diet is supplemented with PUFA-rich vegetable oils, which can be partially explained by the fact that the inhibition of acetyl-CoA carboxylase and *de novo* lipogenesis are less strong in the goat mammary gland (Ferlay and Chillard, 2020). However significant differences were observed in the dry matter content of the milk, which was lower in the milk from animals receiving the SO diet than in the corresponding milk from FL diet in agreement with studies carried out in goats (Sanz-Sampelayo et al., 2007) and in ewes fed diets supplemented with fish oil (Toral et al., 2010), which may be explained due to the hypophagic effect of long-chain PUFA from fish oil (Ferlay and Chillard, 2020).

As regard the fresh goat cheese, no influence of either diet supplementation was observed on the physicochemical parameters, in agreement with Santurino et al. (2018) and Bennato et al. (2020) when linseed supplementation was used. As regards the technological suitability for cheese-making no significant differences were observed in the clotting time or cheese yield between diets. Milk clotting time is related with the milk physicochemical composition, mainly its protein concentration and so, as expected, no differences between milks were observed in this parameter. The protein content, especially, would explain why no differences were found in the cheese yield.

Our results agree to those obtained by Cosentino et al. (2020) in Padraccio cheese derived from a dietary supplementation with extruded linseed where no significant differences were observed in any rheological characteristics. However, cheese texture derived from cows supplemented with extruded linseed, vitamin E and plant extract produced a less firm and softer, more uniform, meltable and fatty texture in than control cheeses (Sympoura et al., 2009) explaining these results by reference to the lower fat melting point of the cheeses due to the higher PUFA content. In our study, although

no significant differences were found regarding hardness, the cheeses derived from supplemented diets were less firm probably due to the PUFA content, in agreement to Inglingstad et al. (2017) which correlated a higher unsaturation level with a softer texture. However, an increase of hardness was observed by Bennato et al. (2020) with linseed supplements associated to the lower moisture of supplemented cheeses. Changes in the texture of different types of fortified cheese could be explained by the interactions between milk components, enzymes, and sources of fat (Bermúdez-Aguirre and Barbosa-Cánovas, 2011) and by the different technological treatment applied during cheesemaking.

#### *Fatty acid profile*

Diet rich in n-3 FAs affect the FA composition not only by direct assimilation into milk but also modulating the expression of lipogenic enzymes (Bodkowski et al., 2016). Following the same pattern as was described by several authors in dairy cow, the supplementation with sunflowers oil decreased the SFA and increased the total n-3 FA. However, the FL supplementation enhanced the n-3FAs, especially ALA in the diet supplemented with FL would contribute to a decrease in cardiovascular disease risk factors due to reduced levels of serum-low-density lipoprotein cholesterol. In addition, FL diet decreased the level of palmitic acid (C16:0), hypercholesterolemic saturated acid in accordance with the results described by Sanz-Sampelayo et al. (2007). The RA level was highest in the diet supplemented with FL according with the findings obtained in Manchega ewes when the diet was supplemented with extruded linseed (Gómez-Cortés et al., 2009). The greater increase determined may have been due to the levels and form of the linseed because the extrusion process increases the accessibility of ALA to rumen microbiota. The resulting alteration of the rumen metabolism would make biohydrogenation less efficient and may also decrease the saturation ratio, increasing the concentration of C18:3 and *trans*-fatty acids in milk from diets rich in linseed oil (Sanz-Sampelayo et al., 2007). The isomer, *trans*-10 *cis*-12 (another CLA) always remains at trace levels in goat milk because this CLA is converted into *trans*-10 C18:1 in the rumen and an increase in this FA was only observed when it was infused postruminally (Andrade et al., 2006). However, Gómez-Cortés et al. (2009) determined that diets with extruded linseed had a minimal effect on this isomer, which agrees with our results. On the other hand, Shingfield et al. (2003) observed in milk from a diet supplemented with fish oil increased the concentrations of CLA and long chain PUFA, and decreased C18:0 as also shown in our study.

Goat seems to be less sensitive than cow to the shift from *trans*-11 to *trans*-10 C18:1, which would explain the stability in the *cis*-9, *trans*-11 CLA determined in our study. However, an increase in the percentage of *trans*-9, *trans*-12 C18:2 was observed in the milk of animals receiving FL/SO diets, as supported by Gómez-Cortés et al. (2009) in previous studies using diets supplemented with extruded linseed.

In our study we found that the highest DHA values are associated with an increase in SFA concentration and a higher saturated:unsaturated fatty acid ratio, contrary to that observed in the milk derived from ewe supplemented with tuna oil (Kittesa et al., 2003). In studies regarding the influence of the type of diet in goat and ewe milk (Sanz-Sampelayo et al., 2007) suggested that the biohydrogenation of PUFAs in soybeans or linseed would occur slowly, producing SFA, and less C18:1 or CLA however, this effect was not observed in our study, possibly due to the way the seeds had been treated, since the process used to obtain flaked linseed breaks the seed and increases the accessibility of ALA. Therefore, the diet supplemented with FL significantly improved the nutritional value of the subsequent milk due to the reduction in SFA and increased levels of PUFA and CLA isomers (Gebreyowhans et al., 2019). Martínez-Marín et al. (2019) determined that a diet rich in linseed oil decreased the n-6/n-3 ratio and significantly increased the CLA levels, which agrees with our results. A nutritional improvement was also observed in milk from goats given the diet supplemented with SO due to the significant increase PUFA and CLA. Although several studies stated that the n-6/n-3 PUFA dietary ratio is of no relevance for modifying the risk of cardiovascular disease, there are studies which determined that the conversion of long-chain omega-3 PUFAs (n-LCPUFAs), such as EPA and DHA, was reduced by a high ratio of linoleic/linolenic acids (Brenna et al., 2009). So, it would be recommended an increase in the dietary intake of preformed n-LCPUFA or

reducing the n-6 PUFA intake, or a combination of both; however, direct DHA intake is more efficient. Indeed, this was the case with our results for the fatty acid profile and n-6/n-3 ratio of the milks from the diets enriched with flaked linseed or fish oil, and for the increase in DHA with the SO diet. In addition, the decrease in the atherogenicity index observed in milk/cheese resulting from the diet enriched with FL, due to a decrease in the saturated/unsaturated ratio, confirms the results obtained in goat using a diet supplemented with unsaturated plant oils and those obtained in grazing goats with diet supplemented with extruded linseed (Caroprese et al., 2016). A decrease was also observed in milk and cheese derived from Manchega ewes fed extruded linseed (Gómez-Cortés et al., 2009).

Unlike milk, few researches have investigated the effect of diet supplementation on FAs profile in cheese. The similar FA patterns observed in milk and cheese agree with the results determined by Gebreyowhans et al. (2019). FA profile of cheeses mainly those FA associated with potential benefits to human health, depend primarily on the FA composition of milk used than the cheese-making technology (Nguyen et al., 2019).

It should be highlighted that the ALA values obtained in our study neither in milk nor cheese derived from a FL supplemented diet are higher than the overall mean value found among the European countries (Zongo et al., 2021).

Although the CLA determined in cheese was seen to be primarily dependent on the CLA level of the unprocessed milk. In our study an increase compared with the control in the PUFA and CLA contents and a decrease in the n-6/n-3 ratio and atherogenicity index were observed, particularly in the cheeses made with milk from the animals receiving FL.

#### *Milk/cheese sensory profile*

No significant differences were observed by a trained panel between the milk and fresh goat cheese resulting from the different diets in any of the sensory attributes determined. These results confirm that it is possible to obtain milk/cheese with better nutritional characteristics without altering the sensory profile according to the results obtained by Dauber et al. (2021) in goat cheese derived from milk supplemented with sunflower oil. Our results are in agreement to those observed by Nguyen et al. (2019) in ewe cheese although they observed that levels of MUFA showed a strongly negative effect on cheese eating quality, which can partly explained the lowest overall acceptance of cheeses derived from SO diet. However, in commercial CLA-fortified dairy products some defects or losses in flavour were determined (Rodríguez-Alcalá and Fontecha, 2007). Differences were determined in Pecorino cheese odour, flavour and toughness as a result of a diet supplemented with extruded linseed, lower odour and higher toughness and flavour values being found for the CLA-enriched cheese (Branciarri et al., 2012). Thus, it should be noted that, in our study while no significant differences were found, the highest overall acceptance was that obtained for the milk and cheese corresponding to the control group according to the results observed by Santurino et al. (2017) and the least acceptable was the milk/cheese from goats given the SO diet.

In conclusion, the study shows that a diet supplemented with FL or SO modifies FA of milk and cheese, with marginal effects on the physicochemical composition. Therefore, for both milk and cheese, dietary supplementation, especially with FL diet, results in a product with a higher nutritional quality than that obtained using the diet routinely fed on farms, especially as far as the fatty acid profile is concerned. Based on a sensory analysis conducted in milk and fresh cheese, no significant differences existed between the control and supplemented groups, which is important from the consumer's point of view because any increase in price because of a product being healthier must be justified by sensory properties, which should equal to or be better than those provided by the traditional product.

**Authors Contributions:** F. Moya: funding, methodology, validation; J. Madrid: conceptualización, methodology, supervision, writing-review and reediting; F. Hernández: conceptualización, methodology, supervision, writing-review and reediting; I. Peñaranda: methodology, validation, formal analysis, investigation, review irene.penaranda@um.es; M.D. Garrido: formal analysis, methodology; M. B. López: conceptualización, methodology, supervision, writing-review and reediting, visualization, project administration

**Financial Support Statement:** This work was carried out thanks to the financial support provided by the Centre for the Development of Industrial Technology (CDTI) and Kpra S. Coop.

**Ethics Approval:** Not applicable. The staff at a Kpra farm facilities looked over the animal continuously.

**Data Availability Statement:** None of the data was deposited in an official repository. Available upon request

**Conflicts of Interest:** The authors declare that funding was received from Kpra s.Coop. . F. Moya work for Kpra S. Coop. All authors contributed to analysing and interpreting the data and therefore declare no conflict of interest.

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