Agricultural Sciences, 2018, 9, 200-220
http://www.scirp.org/journal/as
ISSN Online: 2156-8561
ISSN Print: 2156-8553

Effect of Diet Supplementation with Combinations of Soybean and Linseed Oils on Milk Production and Fatty Acid Profile in Lactating Dairy Ewes

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How to cite this paper: Antonacci, L.E., Bussetti, M., Rodriguez, M.A., Cano, A.V. and Gagliostro, G.A. (2018) Effect of Diet Supplementation with Combinations of Soybean and Linseed Oils on Milk Production and Fatty Acid Profile in Lactating Dairy Ewes. *Agricultural Sciences*, **9**, 200-220.
https://doi.org/10.4236/as.2018.92015

Received: December 27, 2017
Accepted: February 11, 2018
Published: February 14, 2018

Abstract

Thirty-six Pampinta ewes were used in a completely randomized design to examine the effectiveness of soybean (SO) and linseed (LO) oils to reduce the concentration of the atherogenic fatty acids (FA) of milk (C12:0 to C16:0) and increase the content of conjugated linoleic (*cis*-9, *trans*-11 C18:2) also called rumenic acid (RA) and vaccenic acids (*trans*-11C18:1, VA). Six ewes per treatment received a Control diet alone (71% alfalfa hay and 29% concentrate) or supplemented (0.24 kg/ewe·day) with pure oils (SO100 or LO100) or their blend at (%) SO75-LO25, SO50-LO50 and SO25-LO75. Milk yield, milk fat content and milk fat secretion were not affected. Milk protein content resulted higher in SO75-LO25, SO50-LO50 and SO25-LO75 without changes in milk protein yield. Total solid content of milk tended ($p < 0.10$) to increase after oil intake. Concentration of total atherogenic FA decreased and stearic, oleic and linolenic acids increased after oil intake. Milk content of VA and RA resulted higher in treatments with oils without differences between oil blends. The atherogenicity index (AI) in Control milk (2.23) was reduced ($p < 0.001$) by oil intake (1.15 to 1.37). The n-6/n-3 ratio averaged 7.27 in Control milk and was reduced ($p < 0.001$) by oils reaching a minimum value of 1.89 in LO100. Feeding polyunsaturated oils at 7% of total dry matter (DM) intake did not affect the productive response of dairy ewes resulting in an effective tool to improve the healthy value of milk fat. The SO50-LO50 blend showed the highest number of healthy changes in milk FA composition.

Keywords

Ewe Milk, Soybean Oil, Linseed Oil, Conjugated Linoleic Acid

1. Introduction

Milk from ewes (9.584 million tons per year on average for 2007-2011) represents about 1.4% of the whole world production (FAOSTAT, 2013, http://faostat.fao.org/site/569) and is characterized by a low allergenic activity, a high concentration of total solids and the presence of nutraceutical compounds what gives the ewe's cheese a high market value and a growing interest in countries like USA, Brazil and China [1]. Since a large part of the ewe's milk is processed into yoghurt and cheese, its industrial quality is evaluated mainly in terms of its technological and coagulation properties which in turn depend on the fat and protein contents as well as the number of somatic cells [1].

Consumers and dairy industry are highly interested in the healthy value of products which in part depends on levels of those milk FA having a potential negative effect on human health like the saturated FA lauric (C12:0), myristic (C14:0), palmitic (C16:0) and some *trans* FA (*trans*-9 and *trans*-10 C18:1) and concentration of antiatherogenic [2] [3] or anticarcinogenic FA like butyric acid (C4:0), oleic (*cis*-9 C18:1) and RA [3] [4] [5] [6] [7].

Ovine milk is a highly valued product for its nutritional quality and aptitude for industrial technology based on its high solids content. In Argentina, the main use of ewe's milk is the production of cheese with other industrial destinations being minority [8] [9]. The Pampinta breed is a double purpose ewe (dairy and meat) developed at INTA during the 80's in the Province of La Pampa (Argentine) from the crossing of Corriedale sheep with East Frisian rams [10]. These sheep provide milk with a solid content higher than 19 g/100g averaging 6.7 g/100g for protein and 7.4 g/100g for fat which confers an excellent cheese making quality [11]. As reported for dairy cows [12], goats [13], and buffaloes [14], supplementation of dairy ewes with polyunsaturated FA sources (PUFA) reduce milk content of C12:0 to C16:0 and consequently the AI of milk [15].

Studies *in vitro* showed that the partial substitution of linoleic acid (*cis*-9, *cis*-12 C18:2) for linolenic acid (*cis*-9, *cis*-12, *cis*-15 C18:3) would increase the conversion rates of linoleic to RA and VA to RA with a higher isomerization rate of linoleic acid when it is combined with linolenic acid [16]. In dairy ewes, the addition of SO at 6% to a low forage/concentrate (20:80) diet increased milk concentration of RA and AV which decreased after the first week post-supplementation when the *trans*-10 C18:1 increased up to 6 g/100g [17].

By the other hand, supplementation with LO showed to reduce the n-6/n-3 ratio in milk from goats [13] and sheep [15] with an increase in the levels of CLA in milk and ruminal fluid [17]. This strategy leads to the formation of VA with a

lower risk of undesirable shifts towards the *trans*-10 C18:1 that is unfavorable for human health. Previous studies in goats showed that SO and LO supplied at 5% - 6% of total DM intake induced the desired effects to obtain healthy functional milk [13].

The effect of supplementary PUFA on the FA profile of ewe's milk is scarce when compared to studies conducted in cows and goats [18] [19]. In our knowledge, experimental results that examine the potential advantage of combining supplementary SO and LO in the diet of dairy ewes to improve the healthy value of milk fat are still lacking. The aim of this work was to evaluate the effect of different combinations of SO and LO in order to increase milk RA content reducing the presence of those FA which have a potential negative effect on human health without affecting milk yield and composition in dairy ewes.

2. Material and Methods

2.1. Treatments, Animals and Experimental Design

The experiment was carried out at the National Institute of Agricultural Technology (INTA) at the "Guillermo Covas" Experimental Station located in Anguil, province of La Pampa, Argentina. Thirty-six Pampinta ewes (3 lactations, 50 ± 2.5 days in milk), producing 1.058 (±0.28) kg milk per day and averaging 72.3 (± 2.3) kg live weight (LW) were used in a 36 days trial. The first 7 days were used as a covariate period without supplementary oils, followed by 7 days of adaptation at 50% of the target oil dose and 22 days at full oil dose. Milk production and LW were recorded prior to the start of the experiment in order to homogeneously allocate the animals to the treatments. The ewes were milked once a day in the early morning and kept separate by treatment in pens of 10 m^2 at open sky with natural shade and clean water *ad libitum*. The presence of mastitis and the somatic cell count was monitored throughout the trial. The sheep were fed once a day with alfalfa hay (2.3 kg DM/sheep) and 1.2 kg of a commercial concentrate (18% crude protein) at milking time. The concentrate was composed (% as fed) by corn grain (38.7%), sunflower meal (25.3%), soybean meal (5.0%), wheat bran (29%), salt (0.8%) and a commercial mineral mixture (AF Mix, Milk ACA, 1.2%). Six sheep per treatment received one of six combinations (% by weight) of SO and LO in a completely randomized design at 0-0 (Control, without oils), 100% SO, 75 - 25, 50 - 50, 25 - 75 and 100% LO. The pure oils or their blends were individually fed at 6% of estimated total DM intake (4 kg) manually mixed to the concentrate at milking time.

2.2. Sampling Measurements and Laboratory Procedures

Two samples of alfalfa hay and concentrate were dried in an oven with forced air circulation (60˚C during 48 hours) to determine DM, crude protein (CP) [20] with a LECO FP-528 analyzer. Neutral (NDF) [21] and acid detergent fiber (ADF) [22] were analyzed by the filter bag technique using an autoanalyzer

(ANKOM Corp., Fairtport, New York, USA, 1970). Ether extract (EE) was obtained by extraction with solvents at high temperature [23] using an autoanalyzer (ANKOM Corp., Fairtport, New York, USA). Digestibility of DM (DMD) was measured at 48 hours of *in vitro* incubation (Daisy II equipment, ANKOM).

Milk production was individually recorded (5 consecutive days per week) throughout the trial. Chemical composition of milk was measured from samples (100 ml) collected during two non-consecutive days in each week. They were analyzed for fat, protein, lactose and total solid content by mid-infrared spectrophotometry (Milko Scan-Minor, Foss Electric, Hillerod, Denmark). Intake of alfalfa hay was grouply measured within each treatment during 5 consecutive days in each experimental week. Concentrate and oil consumption were daily and individually measured by quantities offered and refused at the end of milking throughout the experimental period. Samples of milk (36) and foods (1) were collected on days 7, 15, 22, 29 and 36 of the trial, stored at −20˚C and analyzed for FA composition by gas-liquid chromatography (GLC) as described in [24].

2.3. Statistical Analyses

The average value of the last three weeks of data collection was used for the analysis of milk production, milk composition and FA profile adjusted for covariate using the PROC GLM program of SAS/STAT® [25] according to the following model:

$$Yi = \mu + Ti + Cov + Ei$$

where Yi = dependent variable; μ = overall mean; Cov = covariate (milk yield and composition over the first 7 days), Ti = treatment effect and Ei = residual error associated with the i^{th} experimental unit.

3. Results and Discussion

The quality of the alfalfa hay was adequate (**Table 1**) considering its digestibility and CP values with moderate contents of NDF, EE and DM resulting comparable to those reported by [26].

The concentrate showed a high CP content and digestibility (**Table 1**). These results compared well with the quality of the foods used in the meta-analysis of 21 experiments by [27] when sheeps were supplemented with seeds and PUFA oils. The FA profile of feeds and oils is shown in **Table 2**.

As expected, SO was characterized by a high content of linoleic acid (50%) that resulted lower than that reported by other authors [28] [29] [30] but comparable to that used in the work of [30]. The saturated FA content of SO was low while the level of oleic acid (*cis*-9 C18:1) resulted important (19.81%). The linolenic acid represented 46.8% of the total FA in LO (**Table 2**), a value that resulted lower than that reported in other experiments [27] [29] [31] [32] [33]. In the alfalfa hay, the observed level of linoleic acid was low (12.82%) and lower than that reported by [34] although near to the value of 13.59% reported by [35].

Table 1. Chemical composition and *in vitro* dry matter digestibility of pasture and commercial concentrate.

Parameter[1]	Alfalfa hay	Concentrate
Dry matter,%	87.09 ± 3.43	87.50 ± 2.53
Crude protein,% DM	19.39 ± 1.65	19.00 ± 1.74
NDF,% DM	43.65 ± 5.53	35.10 ± 4.60
ADF,% DM	32.40 ± 3.16	11.40 ± 3.12
In vitro DM digestibility,%	64.83 ± 1.30	80.00 ± 2.48
Ether extract,% DM	1.59 ± 0.09	5.50 ± 0.05
Metabolic energy, Mcal/kg DM	2.34 ± 0.05	2.89 ± 0.06

[1]Values are expressed as the mean ± standard deviation.

Table 2. Fatty acid composition of alfalfa hay, commercial concentrate soybean (SO) and linseed (LO) oils.

Fatty acid g/100g FA	Alfalfa hay	SO	LO	Concentrate[1]
C16:0	13.25	10.13	6.85	9.48
C18:0	2.55	4.86	5.47	3.79
cis-9 C18:1	28.18	19.81	20.08	22.96
cis-11 C18:1	1.13	1.79	1.47	1.95
cis-9 *cis*-12 C18:2	11.78	49.99	18.66	48.19
cis-9 *cis*-12 *cis*-15 C18:3	12.82	12.15	46.80	2.50

[1]Commercial concentrate showed in **Table 1**.

An average decrease of the order of 20% in the content of linolenic acid in the conserved forages has been reported [36].

Average milk production in oil-supplemented ewes (877 g/sheep/day) was numerically greater (+12.2%) compared to Control treatment (782 g/day) although this difference was not significant ($p < 0.54$, **Table 3**).

Results indicated the absence of negative effects on milk production of feeding free vegetable oils in the ewe's diet when the forage:concentrate ratio (F:C) was close to 80:20. When this ratio was 20:80, a lower milk yield without differences in fat and milk protein contents was observed by [37] feeding 167 g/sheep·day of sunflower oil. In our experiment, no changes in milk fat content or yield were detected (**Table 3**). This suggests that the important drop (27%) in the concentration of *de novo* synthesized FA (**Table 4**) was compensated by an increase in the uptake of the preformed FA from supplementary oil since its concentration in milk increased (39%) after oil intake (**Table 4**). These findings were consistent with that reported by [27].

Milk protein content (g/100g) resulted lower in Control (5.69) ($p < 0.05$) compared to SO50-LO50 (6.10) and SO25-LO75 (6.10) treatments without effects ($p > 0.05$) on milk protein yield (**Table 3**). The increase in milk protein

Table 3. Milk production and composition in dairy ewes supplemented or not (Control) with combinations of soybean (SO) and linseed (LO) oils at different percentages (w/w).

Parameter	Treatment[1]							$p <$[2]
	Control	SO100	SO75-LO25	SO50-LO50	SO25 LO75	LO100	SEM	
Milk yield, g/d	782	963	854	805	902	862	21.3	0.54
Fat, g/100g	6.42	5.96	6.56	6.75	7.09	6.59	0.37	0.18
Protein, g/100g	5.69^{c}	5.67^{c}	5.79^{bc}	6.10^{ab}	6.10^{a}	5.18^{abc}	0.11	0.05
Lactose, g/100g	5.68	5.37	5.22	4.98	5.26	5.14	0.10	0.07
Solids, g/100g	16.79	17.07	17.50	17.77	18.53	17.58	0.19	0.10
Fat yield, g/d	50	60	60	60	60	60	0.006	0.87
Protein yield, g/d	50	50	50	50	50	50	0.003	0.71

[1]Values are expressed as least squares means and standard error of least squares means. Ewes were fed a basal diet (Control) without oils or the basal diet supplemented with pure oils or blends at 6% of estimated total DM intake: SO100 = 0.24 kg SO; SO75LO25 = 0.18 kg SO and 0.6 kg LO; SO50LO50 = 0.12 kg SO and 0.12 kg LO; SO25LO75 = 0.6 kg SO and 0.18 kg LO and LO100 = 0.24 kg LO. [2]Treatment (T) effect. a, b, c = Means in the same row with different superscripts differ significantly for treatment effect with P-value as mentioned in column for significance at $p < 0.05$ (Test Tukey-Kramer).

Table 4. Milk fatty acid (FA) composition from dairy ewes supplemented or not (Control) with combinations of soybean (SO) and linseed (LO) oils at different percentages (w/w).

FA (g/100g FA reported)	Treatment[1]							$p <$[2]
	Control	SO100	SO75-LO25	SO50-LO50	SO25-LO75	LO100	SEM	
C4:0	2.62^{bc}	2.84^{a}	2.53^{c}	2.51^{c}	2.75^{ab}	2.72^{abc}	0.074	0.03
C6:0	2.74^{a}	1.78^{d}	2.17^{b}	2.12^{bc}	2.18^{b}	1.90^{cd}	0.08	<0.0001
C8:0	2.94^{a}	1.50^{d}	2.21^{b}	2.14^{b}	2.13^{bc}	1.78^{cd}	0.12	<0.0001
C10:0	9.21^{a}	4.18^{d}	6.43^{bc}	6.45^{b}	6.11^{bc}	5.30^{cd}	0.39	<0.0001
C10:1	0.39^{a}	0.11^{c}	0.19^{b}	0.20^{b}	0.18^{b}	0.14^{bc}	0.02	<0.0001
C12:0	5.38^{a}	2.91^{b}	3.58^{b}	3.73^{b}	3.59^{b}	3.36^{b}	0.19	<0.0001
C12:1	0.10^{a}	0.06^{c}	0.07^{b}	0.08^{b}	0.08^{b}	0.07^{bc}	0.005	<0.0001
C14:0	10.68^{a}	8.39^{c}	7.27^{b}	9.07^{bc}	9.28^{b}	8.85^{bc}	0.28	0.001
C14:1	0.29^{a}	0.14^{c}	0.17^{bc}	0.17^{bc}	0.18^{b}	0.16^{bc}	0.01	<0.0001
IsoC15:0	0.13^{a}	0.09^{b}	0.08^{b}	0.09^{b}	0.10^{b}	0.09^{b}	0.01	0.02
C15:0	1.03^{a}	0.65^{c}	0.75^{b}	0.75^{b}	0.78^{b}	0.75^{b}	0.03	<0.0001
C15:1	0.22^{a}	0.12^{c}	0.13^{bc}	0.13^{bc}	0.14^{b}	0.13^{bc}	0.005	<0.0001
C16:0	25.34^{a}	20.01^{b}	20.07^{b}	20.11^{b}	20.40^{b}	20.38^{b}	0.63	<0.0001
C16:1	1.04^{a}	0.47^{b}	0.59^{b}	0.59^{b}	0.57^{b}	0.52^{b}	0.04	<0.0001
C17:0	0,55^{a}	0,44^{b}	0.42^{b}	0.41^{b}	0.44^{b}	0.43^{b}	0.02	<0.0001
C17:1	0.24^{a}	0.11^{b}	0.11^{b}	0.11^{b}	0.12^{b}	0.12^{b}	0.009	<0.0001
C18:0	6.11^{c}	7.89^{a}	6.95^{abc}	6.01^{c}	7.27^{ab}	6.79^{bc}	0.36	0.01

Continued

C18:1								
Trans-8	0.54^{c}	0.87^{a}	0.79^{ab}	0.74^{b}	0.76^{ab}	0.78^{ab}	0.04	0.0002
Trans-9	0.45^{c}	0.56^{b}	0.58^{b}	0.67^{a}	0.58^{b}	0.54^{b}	0.03	0.0007
Trans-10	2.07^{c}	6.20^{a}	4.84^{b}	3.94^{bc}	3.51^{bc}	3.11^{bc}	0.69	0.004
Trans-11 (VA)	2.26^{b}	4.98^{a}	5.50^{a}	5.63^{a}	5.76^{a}	5.20^{a}	0.53	0.006
Total *trans*	5.32^{c}	12.61^{a}	11.71^{ab}	10.98^{b}	10.61^{b}	9.63^{b}	0.52	<0.0001
cis-9 C18:1	16.57^{b}	18.40^{a}	16.94^{b}	16.89^{b}	17.32^{ab}	18.48^{a}	0.46	0.03
cis-11 C18:1	0.71^{b}	1.09^{b}	1.06^{a}	1.03^{a}	1.06^{a}	1.14^{a}	0.04	<0.0001
C18:2 (n-6)	6.51^{c}	11.25^{a}	9.34^{b}	9.31^{b}	8.08^{b}	9.18^{b}	0.45	<0.0001
C18:3 (n-3)	0.63^{d}	1.97^{c}	2.40^{c}	3.24^{b}	3.49^{b}	5.18^{a}	0.22	<0.0001
cis-9 trans-11 C18:2 (RA)	1.50^{b}	2.42^{a}	2.79^{a}	3.04^{a}	2.72^{a}	2.50^{a}	0.30	0.02
C20:4 (AA)	0.26^{a}	0.18^{bc}	0.19^{b}	0.18^{bc}	0.15^{c}	0.15^{bc}	0.012	<0.0001
C20:5 (EPA)	0.07^{b}	0.06^{c}	0.07^{b}	0.09^{a}	0.07^{b}	0.10^{a}	0.005	<0.0001
C22:6 (DHA)	0.06^{a}	0.04^{b}	0.06^{a}	0.06^{a}	0.06^{a}	0.05^{ab}	0.004	0.03
Short chain FA[3]	17.88^{a}	10.42^{d}	13.57^{b}	13.43^{b}	13.36^{bc}	11.79^{cd}	0.54	<0.0001
Medium chain FA[4]	44.76^{a}	33.34^{b}	35.51^{b}	35.37^{b}	35.74^{b}	34.46^{b}	0.94	<0.0001
Long chain FA[5]	37.38^{a}	56.13^{c}	49.76^{b}	51.71^{b}	50.55^{b}	52.58^{b}	1.01	<0.0001
Saturated FA (SFA)	66.5^{a}	50.68^{c}	54.19^{b}	53.82^{b}	54.94^{b}	52.29^{bc}	0.97	<0.0001
Unsaturated FA (UFA)	33.48^{c}	49.30^{a}	45.83^{b}	46.15^{b}	45.05^{b}	47.70^{ab}	0.96	<0.0001
SFA/UFA	2.03^{a}	1.03^{c}	1.19^{bc}	1.18^{bc}	1.23^{b}	1.12^{bc}	0.05	<0.0001
AI[6]	2.23^{a}	1.15^{c}	1.30^{bc}	1.32^{bc}	1.37^{b}	1.26^{bc}	0.07	<0.0001
Δ9-D products	25.42^{c}	35.48^{a}	33.28^{ab}	32.67^{b}	32.70^{b}	32.96^{b}	0.80	<0.0001
Substrates	48.74^{ab}	58.18^{a}	48.35^{ab}	47.58^{b}	48.60^{ab}	47.47^{b}	0.82	<0.0001
Índex[7]	0.34^{b}	0.41^{a}	0.41^{a}	0.41^{a}	0.40^{a}	0.41^{a}	0.006	<0.0001
De novo FA (C4:0-C15:1)	35.41^{a}	22.59^{c}	26.87^{b}	27.30^{b}	27.33^{b}	25.19^{bc}	0.90	<0.0001
Preformed FA (>17:0)	38.11^{c}	56.67^{a}	51.85^{b}	51.52^{b}	51.55^{b}	54.06^{a}	0.70	<0.0001
n-6/n-3 FA	7.27^{a}	5.66^{b}	3.79^{c}	2.87^{d}	2.32^{de}	1.89^{e}	0.20	<0.0001
AR/AV	0.69^{a}	0.46^{c}	0.47^{bc}	0.54^{b}	0.48^{bc}	0.47^{bc}	0.03	<0.0001
∑(C12:0-C16:0)	41.26^{a}	31.32^{b}	33.04^{b}	33.15^{b}	33.29^{b}	32.31^{b}	0.89	<0.0001

[1]Values are expressed as least squares means and standard error of least squares means. Ewes were fed a basal diet (Control) without oils or the basal diet supplemented with pure oils or blends at 6% of estimated total DM intake: SO100 = 0.24 kg SO; SO75-LO25 = 0.18 kg SO and 0.6 kg LO; SO50-LO50 = 0.12 kg SO and 0.12 kg LO; SO25-LO75 = 0.6 kg SO and 0.18 kg LO and LO100 = 0.24 kg LO. [2]Treatment effect. [3]Short chain FA (C6:0 to C10:0). [4]Medium chain FA: (C12:0 to C17:1). [5]Long chain FA: (C18:0 to C22:6). [6]Atherogenicity index: (C12 + 4 * C14 + C16)/(ΣUFA). UFA: cis-9 C14:1, C16:1, cis-9 C18:1, cis-11 C18:1, trans-11 C18:1, C18:3, C18:2, C18:2 cis-9 trans11 CLA. The detrimental FA trans-6-8, 9, 10 C18:1 were excluded. [7]Index: ([ΣΔ9Dproducts]/[ΣΔ9D products + Susbstrates]). [8]Substrates:C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + Trans 11 C18:1. [ad]Means in the same row with different superscripts differ significantly for treatment effect with P-value as mentioned in column for significance at $p < 0.05$ (Test Tukey-Kramer).

concentration with linseed oil intake was comparable to that reported in [27]. Total milk solid content tended to increase after oil intake (**Table 3**) an important result for cheese making as reported by [37] after the inclusion of increasing levels (60, 117 and 167 g/sheep·day) of sunflower oil in the ration. Feeding sunflower oil at 2.5% of the diet did not change milk production nor fat, protein, lactose and total solids yields [38].

Ovine milk has a high industrial aptitude for its high yield (20% or 5:1) for the production of cheese compared to 14% (7:1) of goat's milk and 10% (10:1) of cow's milk [9]. In the present work, the milk cheese extract (fat and protein) resulted higher in ewes supplemented with oil mixtures (12.80 g/100g) compared to Control (12.11 g/100g) with the lowest values observed in treatments with pure soybean (11.63 g/100g) and linseed (11.77 g/100g) oils (**Table 3**). Therefore, the inclusion of a mixture of PUFA-rich oils in the diet of dairy sheep would not affect the commercial value of the milk in a payment system referenced to the cheese extract as proposed by [39]. In addition, the fat:protein ratio resulted optimal (1 ± 0.1) according to that reported in [37] guaranteeing an adequate level of fat for industrial processing and cheese maturation [40].

The somatic cell count (SCC) is a technique used to diagnose subclinical mastitis and in the case of sheep's milk a healthy reference value of 10 to 200 × 10^3 cells/ml was established in the USA [9]. In the present work, the average values of SCC in the oil supplemented sheep (99 × 103 cells/ml, **Figure 1**) were within the reference values and lower than those observed in the Control treatment (128 × 10^3 cel/ml) and also to the value of 191 × 10^3 reported by [9].

Concentrate intake (kg DM/ewe·day) resulted higher ($p < 0.05$) in supplemented ewes receiving pure oils (SO-100 = 0.950, and LO-100 = 0.965) compared to Control (0.933) and also in the 50:50 treatment (964) being numerically lower ($p > 0.05$) in SO-75 and SO-25 treatments (0.925 and 0.932 kg MS respectively). Total DM intake averaged 3.24 kg/ewe·day comprising 2.30 kg of alfalfa

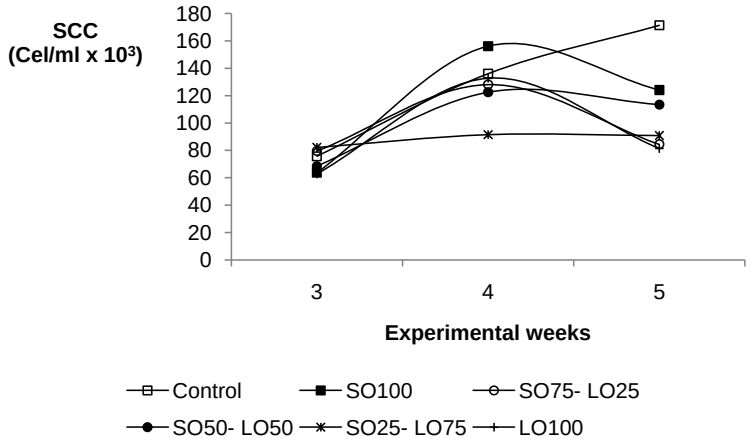

Figure 1. Somatic cell count (SCC) in dairy ewes supplemented or not (Control) with combinations of soybean (SO) and linseed (LO) oils at different percentages (w/w) during the last three weeks of the experiment.

hay and 0.94 kg concentrate. Voluntary DM intake was not affected in sheeps consuming 83.6 (±33.6) g of lipid [27] or after the inclusion of 6% soybean oil in the ration [19] results that were consistent with those observed in the present work. A reduction in DM intake after vegetable oil feeding is a frequently observed result [41] that may be linked to detrimental effects on ruminal fermentation [42].

The inclusion of lipids in ruminants diets usually reduces fiber digestion when the level is higher than 4% of DM [43]. In the present trail, the lack of differences in DM at 7% of oil supply suggests the absence of any negative effect on ruminal digestion as was observed in dairy cows supplemented with polyunsaturated oils [24]. The results available on forage type or processing are scarce since most of the work in sheeps has been done using hay and the number of plant species involved is relatively low [44]. Intake (g/sheep·day) of linoleic and linolenic acids from the concentrate, hay and oils averaged 106 and 18 g/day in SO100, 96 and 27 in SO75-LO25, 79 and 46 in SO50-LO50, 71 and 51 in SO25-LO75 and 58 and 63 in LO100 respectively.

Milk content of butyric acid (C4:0) did not decrease or even increase (SO100 and SO25) after oil intake (**Table 4**) according to [3]. The result can be considered as relevant considering the favorable effects of C4:0 on human health [3]. Butyric acid is partly synthesized by a malonyl-CoA independent way and therefore not associated with the activity of the enzyme acetyl CoA carboxylase which is inhibited by exogenous FA [3] [45]. Compared to Control, total concentration of FA from C6:0 to C12:0 was significantly reduced by intake of pure oils or their mixtures (**Table 4**). This was a relevant result considering the characteristic flavors and aromas that these FA's confer to dairy products from ewes being in turn partially responsible for the economic value of them [9]. Caprylic (C8:0) and capric (C10:0) FA represent between 3% to 18% of total FA in ewe's milk while in cow's milk this contribution is only 3% to 5% [9]. The content of these two FA's in milk from Control ewes comprised 12.15% (**Table 4**) and the decrease after oil intake averaged 7.65% ($p < 0.01$) a result frequently observed when free oils are fed [3].

Concentration of saturated medium chain FA (44.76 g/100g) decreased (−22%, $p < 0.05$) to an average value of 34.88 g/100g after oil intake without differences between blends ($p > 0.05$). The observed decrease of *de novo* synthesized FA (C4:0 to C15:1) after oil intake was important in all treatments with the lowest values observed in SO100 and LO100. This effect can be explained by the inhibition in the activity of lipogenic mammary enzymes such as acetyl-CoA carboxylase [46] [47]. The reduction was apparently compensated by a concomitant increase in mammary uptake of preformed FA's since milk fat concentration or yield was not decreased (**Table 3**) despite of the important increase in milk content of *trans*-10 C18:1 in SO100 and SO75 tretments (**Table 4**). A negative correlation ($R^2 = 0.46$, $p < 0.05$) between this *trans*-10 isomer and milk fat concentration was observed (**Figure 2**) according to [48] [49].

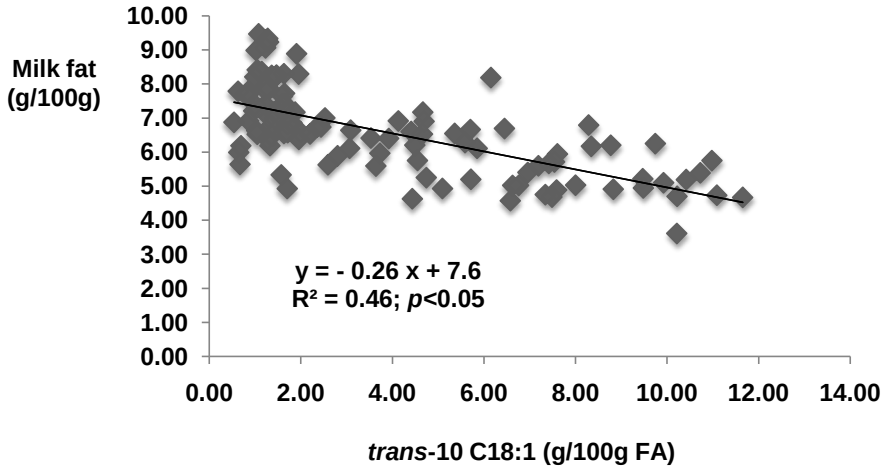

Figure 2. Relationship between milk fat content and concentration of *trans*-10 C18:1 in milk from dairy ewes supplemented with polyunsaturated vegetable oils.

In dairy cattle, the decrease in milk fat content in the presence of PUFA is frequently associated with an increase in *trans*-10 C18:1 levels [48] [49]. The presence of this FA and/or its related compounds (*trans*-10, *cis*-12 C18:2) have been associated with dysfunctions in the activity of enzymes such as lipoprotein lipase (LPL) and stearyl CoA desaturase (SCD) involved in the capture (LPL) and synthesis of FA which explains the drop in milk fat content.

The basal AI in Control milk (2.23) was reduced by oil intake as the consequence of the significant decrease observed in the concentration of C12:0 to 16:0 FA and the increase in unsaturated FA without important differences between oil blends. Compared to the Control treatment, total concentration of the pro-atherogenic FA (C12:0 to C16:0) decreased (−21%) from 41.26 g/100g to an average of 32.62 g/100g FA. This result could be explained by the inhibitory effect of certain FA (*trans*-10 C18:1, *trans*-10, *cis*-12 C18:2) on *de novo* mammary lipogenesis as already stated. This result contributes to avoid an excessive intake of unhealthy saturated FA improving the nutritional value of milk and reducing the atherogenic potential of ovine milk fat. Compared to Control, the average reduction (19.7%) in milk content of myristic acid (**Table 4**) can be considered important taking into account that its pro-atherogenic role is considered to be very potent [50].

The reduction in milk saturated FA concentration (**Table 4**) improves the nutritional value of milk due to its association with the incidence of cardiovascular diseases [51]. A similar but more accentuated trend to decrease the level of saturated medium chain FA was also observed by [19] after the inclusion of unsaturated FA at 6% of the ration and also by [37] with the inclusion of increasing levels of them to a basal diet with a high Concentrate:Forage ratio (80:20).

The basal levels of *trans*-9 C18:1 (0.45 g/100g FA) were increased ($p < 0.01$) by supplementary oil in all treatments while those of *trans*-10 C18:1 (2.07 g/100g FA) resulted higher only when SO was the predominant oil (SO100 = 6.20 and

SO75 = 4.84 g/100g FA, Table 4). It is advisable to avoid any excessive consumption of *trans*-10 C18:1 due to the increase in the lipid deposition in the aorta artery, the higher VLDL, total and LDL cholesterol and the reduced concentration of HDL cholesterol observed in rabbits after the consumption of a butter rich in *trans*-10 C18:1. In contrast, animals that consumed butter rich in VA and RA presented neutral effects or a tendency to reduce lipid deposition in the artery [52].

In our trial, since the lowest numerical concentration of *trans*-10 C18:1 (3.94 g/100g of FA) and the highest numerical values of RA (3.04 g/100g FA) were found in the 50:50 oil-blend while maintaining a high RA/VA ratio, this oils blend behaved as the most promising. The shift towards the synthesis of the unwanted isomer *trans*-10 C18:1) is linked to starch-rich rations through mechanisms capable of altering the ruminal microbial activity associated with the biohydrogenation of the PUFA and the presence of a source of linoleic acid [45] [53]. In a low forage diet rich in concentrate (F/C ratio = 20:80), intake of increasing amounts of sunflower oil (60, 117 and 167 g/sheep·day) induced significant increases in milk content of *trans*-10 C18:1 which remained constant and below 1% in the control ration [37]. It was reported that increasing levels of concentrate generate significant increases in *trans*-10 C18:1 in sheep's milk [54].

Milk content of oleic acid increased ($p < 0.05$) only in treatments with pure oils (SO100 and LO100) as observed in dairy cattle [28]. This increase did not seem to be explained by a greater desaturation activity of stearic acid [3] [55] since its concentration did not decrease or even increase in SO100 and SO25 (Table 4). It could be explained by a higher intake and mammary uptake of the oleic acid contained in the oils (Table 2). The increases in milk concentration of C18:0 in SO100 and SO25 were consistent with results from [37] after feeding 167 g/sheep·day of sunflower oil in a high concentrate ration and also with the inclusion of 2.5% sunflower oil in a 60:40 F/C diet [38].

Linoleic acid content in Control milk (6.51 g/100g FA, Table 4) resulted higher than the normal range of 2 - 3 g/100g FA observed in bovine milk [3]. In all treatments with supplementary oil, the basal level of this FA was strongly increased (44.9% on average, $p < 0.01$) reaching a maximum record of 11.25 g/100g FA in SO100. These values are higher than the maximums reported [45] for dairy cows supplemented with soybean and linseed oils (4 g/100g FA) or the range (2.74 - 3.92 g/100g FA) observed in grazing dairy cows [29] [49]. These results did not keep with that reported by [37] who showed a lower impact on the levels of linoleic acid in milk (2.63, 2.87 and 2.95 g/100g FA) with increasing intakes (60, 117 and 167 g/sheep·day) of sunflower oil in the diet. In cows or goats supplemented with sources of linoleic acid, the presence of this FA in milk does not generally exceed more than 1.5 percentage units over basal [45] with increases in sheep's or goat's milk between +0.5 and +1.8 g/100g FA at an increase-rate of 0.07% (±0.02) per gram of linoleic acid/kg of DM ingested [44]. Therefore, those results were not consistent with what was observed in the

present experiment. Other studies conducted with sheep [19] [56] were consistent with [44].

The non-conjugated isomers of linoleic acid that escape ruminal biohydrogenation are included in the phospholipids and cholesterol esters that are poorly used (3%) by the mammary gland [57]. The level of VA (hypocholesterolemic, antiatherogenic and precursor of RA) in the milk from Control ewes was 2.26 g/100g AG showing a strong increase (140%, $p < 0.05$) after oil intake in all treatments without differences between oil blends. Numerical values of VA increased with the inclusion of SO in the mixture up to a maximum of 75% and then decreased in LO100 treatment (**Table 4**). In Control milk, VA represented 42.5% of the total *trans*-C18:1 a value that was maintained in the range of 39% - 54% in the treatments with supplementary oil. The observed RA/VA ratios may be considered low if compared to the values observed in milk from grazing dairy cows (77% - 82%) supplemented with the same oil mixtures [49]. The difference could be explained in part by the greater presence of *trans*-9 and especially *trans*-10 C18:1 in milk from the oil-supplemented ewes.

The changes observed in levels of *trans*-10 C18:1 and VA are consistent with that reported in [64] after supplementation with sunflower and fish oils at 2% of the diet (*trans*-10 C18:1 = 6.48 and VA = 8.05 g/100g FA) in a ration with 80% concentrate and similar to that observed by [37] after supplementation with sunflower oil (167 g) to dairy ewes (*trans*-10 = 3.74 and VA = 8.50 g/100g FA). Soybean oil fed at 6% of a concentrate rich ration (F:C = 20:80) induced a transient increase in VA levels during the first week with a significant subsequent increase in levels of C18:1 *trans*-10 (10 g/100g FA) [17]. On the other hand, an increase of 79% in milk VA content (2.36 g/100g FA) was reported over basal value (1.32 g/100g FA) when supplementing with sunflower oil at 2.5% of total DM intake [38].

The highest total *trans*-C18:1 concentrations in ewe's milk would be obtained in pasture based diets (5.7 ± 1.1 g/100g FA) if compared to confined production systems (3.4 ± 2.5 g/100 FA) being the *trans*-11 C18:1 the major isomer (2% to 3.5%) as reported for cows and goats [44]. These average values resulted lower than those obtained in the present work (**Table 4**) without the inclusion of fresh forage in the diet.

In humans, VA can exert direct anticarcinogenic effects [58] or mediated via endogenous conversion to RA at tissue level with an estimated conversion rate of 20% [59] by the Δ-9 desaturase activity [60]. This route has been shown to be an effective prevention of the chemically induced cancer in rats [61] and increases the bioavailability of RA in peripheral tissues [62].

In the present work, the average conversion rate of VA into RA appeared to be 43% (**Figure 3**) and so, higher than the 33% reported by [63] for dairy cows. Taking the RA/VA ratio as an estimator, the average conversion rate in oil treatments was in the order of 48 (±3.2)% (**Table 4**) similar to the 50% value reported by [38] and greater than those informed (35% and 30%) by other authors

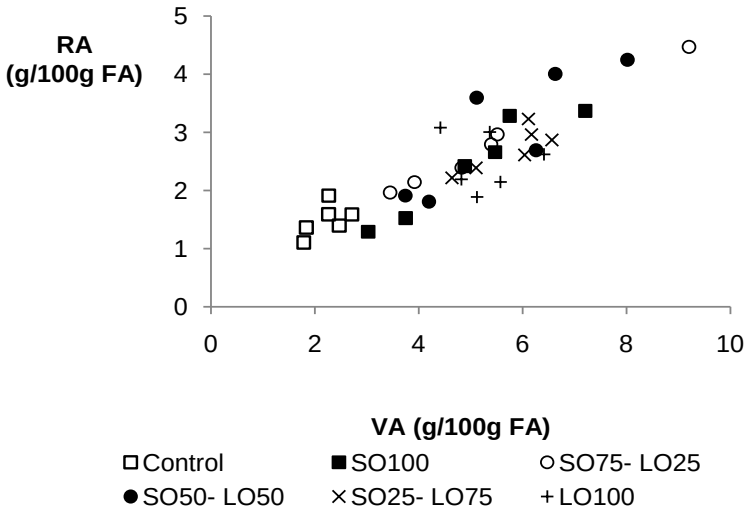

Figure 3. Relationships between vaccenic (VA, trans-11 C18:1) and rumenic (RA, cis-9, trans-11 C18:2) acid contents in ewe's milk.

[37] [64]. In dairy cows, an average conversion rate of 41% has been proposed [44] a value that resulted close to that obtained in the present work (**Figure 3**).

The basal level of RA (1.50 g/100g FA, **Table 4**) was higher than the values reported for dairy ewes fed rations without fresh forage (0.6 g RA/100g) and close to that observed in grazing ewes (1.6 ± 0.53 g/100g) or values of 1.3 (±0.6) g/100g observed with pasture and concentrate [44]. This basal level increased 1.79 times ($p < 0.05$) after oil intake (**Table 4**) without differences ($p > 0.05$) between oil mixtures. The highest numerical value of RA was observed in the SO50-LO50 treatment (3.04 g/100g FA) and the lowest when the oils were supplied in pure form (**Table 4**). Baseline values for RA of 0.69 g/100g AG were reported in diets with 60 forage: 40 concentrate reaching values of 1.18 g/100g after the inclusion of 2.5 sunflower oil at 2.5% of total DM intake [38].

The increase in milk concentration of VA and RA (**Table 4**) resulted relevant for their beneficial effects on cardiovascular health [65] [66] and the anti-carcinogenic properties [7] [67]. The inclusion of sunflower oil at 5.1% of the dairy ewes diet allowed to obtain a milk containing 2.19 g RA/100g FA [37] a result comparable to the 2.31 g/100g obtained in a similar ration with a lower inclusion (2%) of oil by [64]. In a high concentrate (80%) ration, SO supply at 6% induced a transient increase in RA which declined after the first week of oil intake [17]. In the present work, the highest values of RA in milk were observed in week 2 of the trial (**Figure 4**) without a well-defined or different pattern of response between the oil-blends tested.

A high concentration of RA in milk (2.59 g/100g) was obtained feeding sunflower oil at a rate of 117 and 167 g/ewe·day day with concomitant increases of the *trans-10* C18:1 isomer [37]. In our trial, supplementation with the 50% SO-LO blend showed the greatest persistence in milk RA content (**Figure 4**) suggesting to be a usefull dietary strategy. The basal level of VA and RA as well

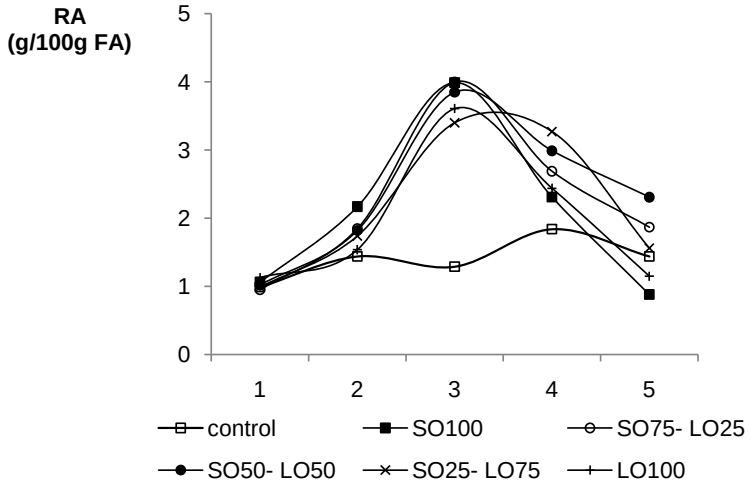

Figure 4. Concentration of rumenic acid (RA, cis-9, trans-11 C18:2) in milk from dairy ewes supplemented or not (Control) with combinations of soybean (SO) and linseed (LO) oils at different percentages (w/w) over five experimental weeks.

as the increase registered after oil intake obtained in ewes (**Table 4**) were lower than those observed in grazing dairy cows consuming similar blends of SO and LO [49]. Enrichment of ewe's milk and cheese with these bioactive compounds (VA, RA) and also with linolenic acid has gained relevance due to the promising results on human health. In clinically healthy subjects, the consumption of 200 g per week of a cheese with a high content of VA (3.26 g/100g FA) and RA (1.56 g/100g FA) during a 10 weeks period produced favorable biochemical changes in the atherosclerotic markers compared to intake of a standard cheese with 0.4 g/100g of VA and 0.19 g/100g of RA [68]. In hypercholesterolemic individuals, the consumption of a cheese rich in rumenic acid (2.5 g/100g FA) decreased (7%) plasma LDL cholesterol compared to a control cheese containing only 1.5 g of RA [69].

In the present work, the reduction in concentration of total saturated FA after oil intake averaged 0.8 times while the increase in concentration of unsaturated FA was 1.4 times ($p < 0.01$) without significant differences between oil mixtures (**Table 4**). Milk fat from Pampinta ewes is characterized by its lower content of long chain FA and a higher level of short chain FA compared to cow's milk. The capric, lauric, myristic, palmitic and oleic acids comprise about 65% of the total FA [9] as observed in milk from Control ewes (67%) (**Table 4**). The majority of the milk FA showed important modifications after supplementary oil intake or their blends showing increased milk content of C18 FA at the expense of saturated FA concentration (**Table 4**). This pattern of response has been reported in cows and goats [13] [45] as well as in sheep fed high levels of SO [17] [54].

Milk content of linolenic acid (C18:3n-3) increased with intake of LO averaging 400% over Control. No significant differences were detected ($p > 0.05$) between the 100SO and SO75-LO25 treatments or between SO50LO50 and

SO25-LO75. Values recorded in LO100 showed to be the highest (**Table 4**). Feeding LO at 4% of DM intake increased (+170%) milk concentration of linolenic acid compared to control without effects of SO alone or the 50:50 mixture of oils [70].

The n-6/n-3 ratio in Control milk resulted relatively high (7.27) and was reduced ($p < 0.05$) after the inclusion of LO in the mixtures. The lowest values (2.32 and 1.89) were observed in LO75 and LO100 (**Table 4**). In dairy sheep supplemented with sunflower oil (2%), this ratio averaged 8.14 [64] resulting therefore much higher than that recorded in SO100 (5.66) with 7% SO in the ration. In dairy cows the lowest n-6/n-3 ratio (2.13) was observed when LO was supplied at 4% of DM intake with an intermediate result (3.44) using the SO50-LO50 mixture [71]. Compared to the Control value of 4.25 no differences were detected in this ratio when pure SO was supplied (4.35, **Table 4**). When the n-6/n-3 ratio is lower than 4 a decrease in mortality due to cardiovascular diseases and breast cancer risk was postulated with healthy effects on chronic diseases such as colon cancer and rheumatoid arthritis [72]. Recent studies also showed positive effects on depression [73]. In the present work, the n-6/n-3 values were below 4 after the SO75 treatment (**Table 4**). Concentration of total unsaturated FA in milk significantly increased ($p < 0.05$) after oil intake averaging 40% over Control without significant differences ($p > 0.05$) between pure oils.

4. Conclusion

The results obtained confirmed the existence of a broad plasticity in the FA composition of ovine milk when PUFA oils are included in the ration an aspect that can be advantageously used to improve the nutritional value of milk and dairy products. Feeding oils at 7% in a forage-concentrate ration (71:29) did not affect the productive response or the yield and content of milk fat, lactose and total solids showing positive increases on milk protein content. The milk cheese extract and the somatic cell count were also not affected by supplementary oil which constitutes a suitable feeding strategy to produce ewe's milk for cheese industrialization. Concerning the nutritional value of the milk, the reduction in the hypercholesterolemic fatty acids (C12:0 to C16:0) and the concomitant increase in bioactive fatty acids like VA, RA and linolenic with absence of important shifts towards the *trans*-9 and trans-10 C18:1 FA represent a potential benefit for the consumer's health and for the addition of value for dairy products at the farm level using a natural way like controlled changes in the diet of ewes. Taking toghether, results suggest that the soybean-linseed oil blend at 50% generated the highest number of favorable nutritional changes in ewe's milk taking into account the decrease in the hypercholesterolemic fraction of milk, the simultaneous increase in vaccenic, rumenic and linolenic acids, the n-6/n-3 ratio lower than 4 and an low atherogenic index. The laws of response to incremental doses of oils and the persistence of the favorable changes induced in the milk merits to be experimentally explored.

Acknowledgements

This work was supported by the National Institute of Agricultural Technology (INTA). This Institute is a decentralized state agency with operational and financial autarchy, under the Ministry of Agroindustry of the Argentine Republic. This publication is part of the requirements to access to the academic degree of Doctor in Agricultural Sciences by the Mar del Plata National University, Argentina.

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