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Extra Virgin Olive Oil: Does It Modify Milk Composition of Hair Sheep?

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Abstract: The aim of this study was to determine the effect of olive oil addition on the production, chemical composition, and fatty acid profile of sheep's milk. Twenty-four lactating ewes with a live weight of 34.6 ± 4.61 kg were used. The animals were randomly distributed into four treatments (n = 6) with dietary addition of 0%, 2%, 4%, and 6% (dry matter basis) olive oil for 45 days. Milk samples were taken every 7 days for fatty acid (FA) and chemical analyses. A decrease ($p < 0.05$) in dry matter and crude protein intake was observed with 4% oil inclusion. Milk production and milk components were similar between treatments. The kilograms of meat from weaned lambs linearly increased as the oil inclusion increased. Milk C4:0 to C17:0 decreased with 2% olive oil. The monounsaturated and polyunsaturated FA content in the milk increased with the oil inclusion. There was an increase in the milk's linoleic acid, linolenic acid, and eicosapentaenoic acid content with 2% olive oil. Overall, the addition of 2% extra virgin olive oil is recommended to improve milk's FA profile without negative effects on animal performance.

Keywords: olive; atherogenic; milk; sheep; lipids; small ruminants; supplementation

1. Introduction

In the tropical regions of Mexico, sheep production is of socioeconomic importance and represents an activity of relevance in rural areas, as sheep can adapt to different regions and climates [1]. In these regions, lactating hair sheep feeding is mainly based on grazing, and during drought periods, the supply of nutrients from these forages is low (<7% of CP and NDF >70%) [2]. These factors reduce animal productivity (low milk production and low pre- and post-weaning weight gain) [3]. In this sense, when the feeding of these animals is of poor quality, milk saturated fatty acids (SFAs), mainly lauric acid (C12:0), myristic acid (C14:0), and palmitic acid (C16:0), can increase, and these FAs are associated with high cholesterol blood levels, generating cardiovascular problems and hypertension in humans [4–6]. In addition, due to rumen biohydrogenation, most dietary unsaturated

fatty acids (UFAs) are converted to saturated fats upon entering the rumen, which limits the UFA content in milk and dairy products [7].

In the last few years, strategies have been sought to reduce the amount of SFA and increase UFA in milk; these include adding oilseeds (i.e., linseed, soybean, and safflower oil) rich in linoleic and linolenic acid to ruminant diets [8–11]. This is because during the rumen biohydrogenation process, some by-products are generated, such as α-linolenic acid (ALA), linoleic acid (LA), rumenic acid (conjugated linoleic acid; CLA), and vaccenic acid (VA; t11-18:1), and these can promote anti-inflammatory and anticancer processes and prevent heart problems [5,12–14].

There are studies on the use of oleic acid-rich lipids, such as the silage of olive pastes, olive oil, calcium salts of olive oil, and lampante olive oil [9,15–17], added to the diet of dairy sheep and dual-purpose breeds. However, no studies related to dietary extra virgin olive oil and its effects on the fatty acid profile and composition of milk have been conducted in hair sheep from the Mexican tropics. Therefore, the objective of this study was to determine the effect of the addition of olive oil on the productive traits, milk composition, and milk fatty acids of lactating hair sheep.

2. Materials and Methods

2.1. Localization

The study was conducted at Rancho San Francisco, located at 21°14′48″ N and 89°02′35″ W longitude at five masl in the municipality of Dzidzantun (Yucatán, Mexico). The average temperature was 26 °C with 980 mm of rain during the experimental months, and the extremes of relative humidity were between 66% and 89% [18,19].

2.2. Animal Handling and Experimental Design

The animals were treated according to the guidelines and regulations for animal experimentation of the Academic Division of Agricultural Sciences, Universidad Juárez Autónoma de Tabasco (project ID PFI: UJAT-DACA-2015-IA-02). Twenty-four ewes (Pelibuey × Katahdin) with a live weight of 34.6 ± 4.61 kg were used. In a completely randomized experimental design [20], the animals were divided into four groups (n = 6). Animals were housed with their lambs in individual cages (2 × 3 m) and provided with feeders and drinkers. The study lasted 45 days.

2.3. Experimental Diets

The basal diet (offered at 0800 h) consisted of ground sorghum, ground corn, soybean meal, cane molasses, soybean husk, minerals, vitamins, and chopped fresh Taiwan grass (*P. purpureum*; offered at 1800 h), using only the stems to reduce the nutritional variation throughout the experimental phase, at a ratio of 80:20 (concentrate:forage) (Table 1).

The concentrate was formulated according to the AFRC recommendations [21] for sheep with an average weight of 45 kg and a milk yield of 1.74 kg/d; the protein and total fat contents were 4.5% and 7.0%, respectively. The concentrate had a metabolic energy of 10.0 MJ and 13% crude protein per kg DM (Table 2). The dietary treatments had increasing levels of 0, 2, 4, and 6% of the DM of extra virgin olive oil (Sesajal®, Guadalajara, Jalisco, Mexico).

2.4. Handling and Feeding

At the beginning of the study, the sheep were weighed and dewormed with 5% Closantel® (Wyeth LLC, Madison, NJ, USA) and given an ADE vitamin supplement (Vigantol ADE® intramuscularly; 1 mL/10 kg BW). The offered diets were adjusted to allow a minimum rejection of 10% to estimate voluntary intake. The animals were fed twice daily, at 0800 and 1500 h.

Table 1. Formulation and chemical composition (g/kg DM) of the concentration and forage offered to lactating hair sheep.

Ingredient	Concentrate (g/kg)	*P. purpureum* (g/kg)	Olive Oil (g/kg)	Total
P. purpureum	0	200	0	
Ground sorghum	181	0	0	
Soybean meal	198	0	0	
Molasses	100	0	0	
Wheat bran	300	0	0	
Mineral blend	20	0	0	
ADE vitamins	1	0	0	
Total	800	200		1000
Chemical composition (g/kg)				
DM	847	283	0	
OM	919	953	0	
CP	161	31	0	
EE	43	19.2	999	
NDF	447	693	0	
ADF	245	470	0	
ME (MJ/kg DM)	10.61	7.6	37.7	
Fatty acid composition (g/kg)				
C16:0	0.00	2.44	6.69	
C18:0	0.26	0.27	4.40	
C18:1	9.39	0.33	695	
C18:3	1.62	10.71	2.20	
C20:0	6.03	0.36	1.60	
∑SFA	6.29	3.07	12.69	
∑MUFA	9.39	0.33	695	
∑PUFA	1.62	10.71	2.20	

SFAs (saturated fatty acids) = C16:0 + C18:0 + C20. MUFAs (monounsaturated fatty acids) = C18:1. PUFAs (polyunsaturated fatty acids) = C18:3. DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; ADF = acid detergent fiber; NDF = neutral detergent fiber; ME= Metabolizable energy.

Table 2. Chemical composition (g/kg DM) of dietary treatments with increasing levels of olive oil.

Component	% Olive Oil			
	0	**2**	**4**	**6**
DM	734.2	722.6	711.4	700.6
OM	925.0	911.2	897.1	883.4
CP	135.0	132.9	130.8	128.8
EE	38.2	53.4	68.0	82.2
NDF	496.2	488.4	480.8	473.5
ADF	290.0	285.4	281.0	276.7
ME (MJ)	10.0	10.4	10.9	11.3
Fatty acid profile (g/kg DM)				
C16:0	0.01	0.11	0.22	0.32
C18:0	0.01	0.08	0.15	0.21
C18:1	0.32	11.3	21.84	32.11
C18:3	0.10	0.13	0.16	0.19
C20:0	0.21	0.23	0.25	0.27
∑SFA	0.23	0.42	0.61	0.80
∑MUFA	0.32	11.25	21.84	32.11
∑PUFA	0.10	0.129	0.16	0.19

SFAs (saturated fatty acids) = C16:0 + C18:0 + C20; MUFAs (monounsaturated fatty acids) = C18:1; PUFAs (polyunsaturated fatty acids) = C18:3 (Tsiplakou et al., 2017); DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; ADF = acid detergent fiber; NDF = neutral detergent fiber; ME= Metabolizable energy.

2.5. Weight Gain and Feed Conversion

The animals were weighed every eight days with a Torrey® scale with an accuracy of 0.100 kg. The weight gain of the sheep was determined by the difference between the final weight (FW) and the initial weight (IW) divided by the days between weightings (g/day). The feed conversion was calculated by the difference between the intake (kg) and weekly weight change of the sheep (kg).

2.6. Production and Chemical Composition of Milk

The daily milk production was determined by manual milking from the first week of the study until day 45; a total of 6 samplings were obtained. The lambs were separated from the mothers one day before the mother's milk sampling starting at 1900 h, for 12 h, starting the milking 0700 h. Before milking, the sheep received 3 IU of oxytocic intramuscularly (Pisa, Mexico). The teats of the animals were disinfected with an iodized solution, and after 30 s, they were dried with paper towels. The daily milk yield (DMY, kg) of the sheep was calculated using the milk obtained over a period of 12 h multiplied by 2.

Samples of 100 mL of milk were taken from each sheep milked weekly, of which 5 mL was taken for protein and fat analysis, analyzed in duplicate by infrared methodology with the aid of an automatic milk analyzer (Lactoscan LS—60, Milkotronic Ltd., Nova Zagora, Bulgaria), and the rest of the milk sample was kept frozen. In addition, the milk yield with 6% fat correction (FCM) and energy (ECMY) was calculated according to the formula [22]:

$$FCMY = (0.28 + 0.12F) \times MY$$

where F = fat percentage. The energy-corrected milk yield (ECMY, kg) was calculated according to the formula:

$$ECMY = (0.071 \times F + 0.043 \times P + 0.2224) \times MY$$

where P is the protein percentage.

The protein correction was calculated according to the formula proposed by Pulina et al. [23]:

$$FPCM\ 6.5, 5.8\%, kg/d = [milk\ (kg/d)\ (0.25 + 0.085\ fat\% + 0.035\ protein\%)].$$

2.7. Fatty Acid Profile

The composition of fatty acids in the milk was carried out in the environmental engineering laboratory of the Faculty of Engineering of the Autonomous University of Yucatan (UADY). Milk samples were taken using 1 mL of the sample, which was stirred for one minute in a vortex to homogenize the sample. The total fat was extracted with a mixture of methanol chloroform (2:1) and quantified by gravimetry using the method of Folch et al. [24]. Total fat concentrations are expressed in g/100 g of milk. The fatty acids were derived using boron trifluoride and extracted with hexane [24]. The derivatized extracts were injected into a Hewlett Packard gas chromatograph 5890 Series II, which used a Supelco SP 2560 column, 100 m long × 0.25 mm internal diameter [24]. Peaks were identified by means of Supelco reference standards (FAME).

2.8. Chemical Analysis

The DM determination of the forage samples was performed in a forced air oven at 55 °C for 48 h (constant weight) (#7.007) AOAC [25]. The N content was realized (CP, N × 6.25) by combustion using a LECCO CN-2000 series 3740 (LECCO, Corporation, Saint Joseph, MI, USA) (#2.057) AOAC [25]. MO was determined by incineration in a muffle at 600 °C for 6 h, and the contents of FND and FDA were determined as suggested by Van Soest et al. [26].

2.9. Statistical Analysis

The data were subjected to analysis of variance in a randomized design [20] using the PROC GLM procedure of the SAS [27]. The means of the treatments were compared with Tukey's test with an alpha of 0.05:

$$Y_{ij} = \mu + \tau_i + E_{ij}$$

$$i = 1, 2, 3, \ldots, t$$

$$j = 1, 2, 3, \ldots, n$$

where Y_{ij} = variable response in the j-th repeat of the i-th treatment, μ = general mean, τ_i = effect of the i-th treatment, and E_{ij} = random error.

Milk production and weight gain data were subjected to a PROC MIXED time repeated measures analysis of the SAS [27]. Tukey's test was performed when a significant treatment effect was detected ($p < 0.05$). Additionally, a response surface analysis was performed to evaluate the linear, quadratic, and cubic effects of the treatments (0, 2, 4, and 6% olive oil in the ration) [20].

3. Results

3.1. Voluntary Intake

The dry matter intake (DMI) and crude protein (CP) intake were affected ($p < 0.05$) by the addition of olive oil; these levels were reduced with the addition of 4% olive oil in the ration, whereas for 0%, 2%, and 6% olive oil, they were similar with a cubic effect. A linear effect ($p < 0.0001$) was found for ether extract (EE) intake as the levels increased (0, 2, 4, and 6% of the DM). The same pattern was observed when DMI, OM, and CP were expressed in $g/kg^{0.75}$ (Table 3).

3.2. Productive Behavior of Sheep and Lambs

No differences were found in the productive behavior of lactating sheep when adding extra virgin olive oil to the diet (Table 4). On the other hand, differences ($p < 0.03$) were observed in the intake of concentrated feed as oil levels (0%, 2%, 4%, 6% of DM) were increased (Table 4). No differences ($p > 0.05$) were found in the growth of lambs, but a linear trend ($p < 0.02$) was observed in the kg of meat weaned per sheep as the oil was increased in the ration (Table 5).

3.3. Production and Chemical Composition of Milk

Milk production and milk components were similar between treatments, but quadratic effects were detected in milk production and corrected milk production for protein, fat, and energy, and except for the percentage of milk fat, the non-fat solids as well as daily fat and protein yields in our study showed no changes (Table 6).

3.4. Fatty Acid Profile in Milk

The addition of extra virgin olive oil at levels of 0, 2, 4, and 6% caused changes ($p < 0.05$) in the fatty acid profile of the milk of hair sheep (Table 7). The oil concentrations used showed linear and quadratic effects ($p < 0.05$); mostly, short- and medium-chain fatty acids (C4:0 to C17:0) showed a reduction when 2% of the DM of olive oil was added. Long-chain fatty acids with greater than 17 carbons increased ($p < 0.05$) with 2% oil inclusion, and this level seemed to improve the quality of the milk fat (Table 7). According to our data (Table 7), the control treatment (0% olive oil) resulted in the highest levels of saturated fatty acids; this content showed a linear effect ($p < 0.0001$), in which the concentration decreased as the amount of olive oil added increased. The saturated fatty acid with the highest presence was C16:0. Monounsaturated fatty acids showed linear ($p < 0.01$) and

quadratic ($p < 0.001$) effects. In this sense, the percentage of polyunsaturated fatty acids increased as the addition of olive oil in the diet increased; this was related to the high concentration of oleic acid contained in olive oil, which caused the decrease in the activity of the enzyme Δ^9-desaturase, which participates in de novo synthesis and the synthesis of saturated fatty acids and possibly increases the microbial activity in the rumen (Table 7).

In this study, an increase in various fatty acids, such as oleic, linoleic, and linolenic acids, was found with 2% olive oil ($p < 0.05$), while an increase in eicosapentaenoic acid was observed as more olive oil was integrated into the diet ($p < 0.05$), as shown in Table 7. These fatty acids are relevant because they have various health benefits, and the human body cannot synthesize them.

The milk of hair sheep had quadratic ($p < 0.004$) and cubic ($p < 0.001$) effects in the atherogenic index (AI) analyses, while for the thrombogenic index (TI), a lower concentration was found in the 2 and 6% olive oil groups compared with the control group.

Table 3. Feed intake from hair sheep fed with different levels of olive oil inclusion.

Items	% Olive Oil						Contrast		
	0	2	4	6	SE	*p*-Value	L	Q	C
LW (kg)	38.42	37.88	38.08	36.01	0.93	0.26	0.09	0.41	0.47
MDW (kg)	15.40	15.25	15.26	14.68	0.28	0.27	0.08	0.43	0.55
TDMI (kg/d)	2.08	1.96	1.96	2.06	0.07	0.45	0.79	0.11	0.93
DMI (% PV)	5.44 ^{a}	5.04 ^{ab}	4.89 ^{b}	5.32 ^{ab}	0.12	0.01	0.37	0.001	0.58
OM (kg/d)	1.93	1.77	1.73	1.78	0.06	0.13	0.08	0.10	0.93
CP (kg/d)	0.29 ^{a}	0.27 ^{ab}	0.26 ^{b}	0.27 ^{ab}	0.008	0.04	0.03	0.06	0.89
EE (kg/d)	0.08 ^{ab}	0.12 ^{b}	0.166 ^{ab}	0.214 ^{a}	0.004	<0.0001	<0.0001	0.38	0.86
NDF (kg/d)	1.02	0.93	0.90	0.94	0.034	0.26	0.166	0.14	0.96
ADF (kg/d)	0.59	0.54	0.53	0.54	0.02	0.37	0.22	0.19	0.97
DMI (g/kg$^{0.75}$)	135.24 ^{a}	125.04 ^{ab}	121.11 ^{b}	130.30 ^{ab}	3.33	0.02	0.21	0.004	0.65
OM (g/kg$^{0.75}$)	124.98 ^{a}	115.60 ^{ab}	111.97 ^{b}	120.50 ^{ab}	3.12	0.02	0.22	0.005	0.64
CP (g/kg$^{0.75}$)	19.10 ^{a}	17.52 ^{b}	16.95 ^{b}	18.16 ^{ab}	0.42	0.004	0.07	0.001	0.68
EE (g/kg$^{0.75}$)	5.32 ^{b}	7.95 ^{b}	10.83 ^{b}	14.46 ^{a}	0.20	<0.0001	<0.0001	0.01	0.59
NDF (g/kg$^{0.75}$)	65.55	60.86	58.99	63.61	2.004	0.10	0.39	0.02	0.68
ADF (g/kg$^{0.75}$)	37.80	35.19	34.10	36.83	1.31	0.20	0.49	0.04	0.70

$^{a, b}$ Separate literals in the same column indicate statistical differences ($p < 0.05$); SE, standard error of the mean; LW, live weight; MDW, metabolic body weight; TDMC, total dry matter intake; DMI, dry matter intake; OM, organic matter; CP, crude protein; EE, ether extract; ADF, acid detergent fiber; NDF, neutral detergent fiber. L, linear contrast; Q, linear quadratic; C, cubic contrast.

Table 4. Productive parameters from hair sheep fed with different levels of olive oil inclusion.

Items	% Olive Oil						Contrast		
	0	2	4	6	SE	*p*-Value	L	Q	C
IW (kg)	33.05	34.74	35.98	34.78	2.17	0.82	0.52	0.52	0.84
FW (kg)	41.20	39.75	40.86	40.84	3.47	0.99	0.99	0.84	0.81
TWP (kg)	8.15	5.01	4.88	6.06	1.68	0.51	0.41	0.22	0.82
DMI (kg/d)	2.08	1.96	1.96	2.06	0.07	0.45	0.80	0.11	0.93
CC (kg/d)	1.76 ^{a}	1.60 ^{ab}	1.56 ^{b}	1.60 ^{ab}	0.05	0.03	0.02	0.06	0.87
FC(g/d)	492.81	469.88	461.94	490.13	20.98	0.67	0.86	0.23	0.82
OOC (g/d)	0.00 ^{d}	46. 62 ^{c}	93. 98 ^{b}	138. 21 ^{a}	2.59	<0.0001	<0.0001	0.65	0.74

$^{a–d}$ Separate literals in the same column indicate statistical differences ($p < 0.05$); SE, standard error of the mean; IW, initial weight; FW, final weight; TWG, total weight gain; DMI, dry matter intake; CC, concentrate consumption; FC, forage consumption; OOC, olive oil consumption; L, linear contrast; Q, linear quadratic; C, cubic contrast.

Table 5. Productive traits from hair sheep lambs fed with different levels of olive oil inclusion.

Items	% Olive Oil						Contrast		
	0	2	4	6	SE	*p*-Value	L	Q	C
BW (kg)	3.74	4.58	5.48	5.76	0.52	0.06	0.01	0.60	0.78
KW (kg)	13.04	16.90	16.62	19.63	1.68	0.10	0.02	0.81	0.34
WGL (kg)	9.30	12.32	11.13	13.87	1.41	0.19	0.07	0.92	0.22
DWG (kg/d)	0.21	0.27	0.24	0.31	0.03	0.20	0.07	0.87	0.23

SE: standard error; PIC: birth weight; KW: kilograms weaned; WGL: weight gain in lactation; DWG: daily weight gain; L: linear contrast; Q: linear quadratic; C: cubic contrast.

Table 6. Effect of dietary inclusion of olive oil in hair sheep on the estimated milk production and milk chemical composition.

Items	% Olive Oil						Contrast		
	0	2	4	6	SE	*p*-Value	L	Q	C
DMP (g/d)	1158.78	1314.40	1032.80	1292.00	85.53	0.07	0.75	0.54	0.01
CPC (g/d)	1108.15	1256.61	1058.01	1284.96	91.53	0.21	0.41	0.66	0.05
FC (g/d)	1129.68	1284.61	1079.26	1310.44	70.77	0.20	0.41	0.67	0.05
EC (kcal/d)	1015.46	1004.61	1071.96	1019.18	22.33	0.14	0.44	0.35	0.05
Chemical composition of milk (g/100 g)									
Fat	6.41	6.30	6.87	6.42	0.21	0.22	0.51	0.41	0.06
Protein	5.10	4.98	5.20	5.08	0.08	0.27	0.63	0.92	0.05
Non-fat solids	12.17	11.93	12.02	12.04	0.08	0.24	0.42	0.12	0.27
Daily performance g/d									
Fat	72.26	82.29	71.86	85.81	6.40	0.30	0.29	0.76	0.12
Protein	60.49	67.87	60.74	72.29	5.63	0.36	0.26	0.71	0.18

SE, standard error of the mean; DMP, daily milk production; CPC, protein correction; FC, fat correction; EC, energy correction; L, linear contrast; Q, quadratic linear; C, cubic contrast.

Table 7. Effect of dietary inclusion of olive oil in hair sheep on milk fatty acid profile (g/100 g FA).

Fatty Acid (g/100 g Fatty Acids)	% Olive Oil						Contrast		
	0	2	4	6	SE	*p*-Value	L	Q	C
C4:0	1.02	0.78	1.04	0.66	0.14	0.21	0.24	0.65	0.07
C6:0	0.68	0.63	0.70	0.52	0.05	0.06	0.06	0.21	0.08
C8:0	9.31 ^{a}	7.92 ^{a}	8.30 ^{a}	5.84 ^{b}	0.50	<0.0001	<0.0001	0.28	0.04
C10:0	9.31 ^{a}	7.92 ^{a}	8.30 ^{a}	5.84 ^{b}	0.50	<0.0001	<0.0001	0.28	0.04
C12:0	0.46 ^{ab}	0.45 ^{ab}	0.65 ^{a}	0.31 ^{b}	0.06	0.006	0.38	0.01	0.01
C11:0	0.19 ^{ab}	0.19 ^{ab}	0.24 ^{a}	0.13 ^{b}	0.02	0.03	0.30	0.04	0.04
C13:0	3.66 ^{a}	3.31 ^{ab}	2.89 ^{ab}	2.63 ^{b}	0.22	0.008	0.0007	0.85	0.82
C14:0	0.44 ^{a}	0.47 ^{ab}	0.66 ^{b}	0.37 ^{b}	0.06	0.01	0.94	0.01	0.02
C14:1	0.09 ^{a}	0.09 ^{a}	0.06 ^{b}	0.09 ^{a}	0.007	0.004	0.15	0.04	0.004
C15:0	1.45 ^{ab}	1.19 ^{BC}	1.56 ^{to}	1.06 ^{c}	0.09	0.001	0.06	0.22	0.001
C15:1	0.35 ^{a}	0.29 ^{ab}	0.25 ^{b}	0.27 ^{b}	0.02	0.005	0.001	0.07	0.70
C16:0	14.98 ^{a}	14.63 ^{a}	13.74 ^{a}	11.53 ^{b}	0.50	<0.0001	<0.0001	0.06	0.72
C16:1	1.10	0.95	1.18	0.86	0.29	0.87	0.71	0.76	0.49
C17:0	0.96 ^{a}	0.69 ^{b}	0.66 ^{b}	0.62 ^{b}	0.07	0.004	0.001	0.10	0.45
C17:1	0.40 ^{a}	0.29 ^{ab}	0.35 ^{ab}	0.27 ^{b}	0.03	0.03	0.02	0.76	0.03
C18:0	9.58 ^{a}	8.59 ^{a}	9.61 ^{a}	7.45 ^{b}	0.29	<0.0001	<0.0001	0.04	0.002
C18:1cis-9	19.11 ^{c}	23.36 ^{ab}	20.44 ^{bc}	27.02 ^{a}	1.09	<0.0001	<0.0001	0.28	0.0009
C18:1 1n9t	0.07	0.08	0.06	0.09	0.01	0.20	0.41	0.17	0.14
C18:2n6c	9.74 ^{b}	10.86 ^{b}	15.31 ^{ab}	16.95 ^{a}	1.48	0.001	0.0002	0.85	0.35
C18:2n6t	2.42	1.97	2.45	2.55	0.28	0.46	0.48	0.33	0.29
C20:0	0.19 ^{ab}	0.21 ^{ab}	0.17 ^{b}	0.24 ^{a}	0.01	0.01	0.08	0.09	0.03

Table 7. *Cont.*

Fatty Acid (g/100 g Fatty Acids)	% Olive Oil					Contrast			
	0	2	4	6	SE	*p*-Value	L	Q	C
C18:3n6	0.21 ^{a}	0.17 ^{ab}	0.16 ^{b}	0.16 ^{b}	0.01	0.01	0.003	0.19	0.92
C20:1	0.13	0.11	0.08	0.11	0.01	0.17	0.23	0.11	0.26
C18:3n3	11.45 ^{a}	12.67 ^{a}	8.87 ^{ab}	10.73 ^{b}	0.54	<0.0001	0.01	0.55	<0.0001
C21:0	1.33	0.68	0.86	1.63	0.43	0.35	0.60	0.08	0.90
C20:2	0.11	0.10	0.11	0.12	0.01	0.74	0.57	0.39	0.69
C22:0	0.16	0.19	0.16	0.22	0.02	0.24	0.19	0.45	0.16
C20:3n6	0.13	0.11	0.11	0.14	0.01	0.46	0.67	0.12	0.91
C22:1n9	0.19	0.22	0.20	0.27	0.03	0.35	0.13	0.62	0.39
C20:3n3	0.07	0.06	0.05	0.07	0.007	0.25	0.77	0.11	0.20
C23:0	0.07	0.06	0.05	0.08	0.008	0.14	0.74	0.07	0.14
C20:4n6	0.08 ^{b}	0.10 ^{b}	0.09 ^{b}	0.14 ^{a}	0.008	0.0001	0.0002	0.05	0.03
C22:2	0.07	0.06	0.05	0.07	0.007	0.17	0.51	0.08	0.19
C24:0	0.35	0.48	0.32	0.89	0.17	0.07	0.06	0.20	0.17
C20:5n3	0.15 ^{b}	0.18 ^{ab}	0.22 ^{ab}	0.27 ^{a}	0.03	0.04	0.004	0.76	0.91
C24:1	0.03	0.03	0.02	0.03	0.004	0.36	0.54	0.42	0.13
∑ SFA	56.72 ^{a}	53.02 ^{ab}	50.13 ^{b}	43.61 ^{c}	1.73	<0.0001	<0.0001	0.42	0.56
∑ MUFA	13.28 ^{a}	11.70 ^{ab}	13.13 ^{a}	11.51 ^{b}	0.44	0.001	0.01	0.65	0.001
∑PUFA	29.77 ^{c}	35.08 ^{bc}	36.60 ^{b}	45.06 ^{a}	1.81	<0.0001	<0.0001	0.39	0.18
AI	0.09 ^{ab}	0.09 ^{b}	0.13 ^{a}	0.06 ^{b}	0.01	0.0003	0.16	0.004	0.001
TI	0.17 ^{a}	0.14 ^{ab}	0.18 ^{a}	0.10 ^{b}	0.01	0.006	0.06	0.11	0.007
Δ 9-desaturase index	0.21 ^{a}	0.19 ^{a}	0.12 ^{b}	0.20 ^{a}	0.01	0.005	0.21	0.01	0.01

$^{a, b, c}$ Different literals in the same column indicate statistical differences ($p < 0.05$). SE: standard error of the mean. L, linear contrast; Q, quadratic linear; C: cubic contrast. ∑SFA = (C4:0 + C6:0 + C10:0 + C8:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:20 + C23:0 + C24:0); ∑MUFA= (C14:1 + C15:1 + C16:1 + C17:1+ C18:1 1n9t + C18:1cis-9 + C20:1 + C22:1n9 + C24:1); ∑PUFA = (C18:2n6t + C18:2n6c + c18:3n6 + C18:3n3 + C20:3n6 + C22:1n9 + C20:3n3 + C20:4n6 + C22:2 + C20:5n3) [22]; AI: atherogenic index, (12:0 + 4(C14:0) + C16:0/(n − 6 + n − 3) + C18:1 + ∑MUFA); TI: thrombogenicity index, (∑C14:0 + C16:0 + C18:0)/(0.5 × C18:1) + (0.5 × ∑MUFA) + (0.5 × n − 6) + (3 × n − 3) + (n − 3/n − 6) [28]; Δ 9-desaturase index: C14:1/(C14:0 + C14:1) [28].

4. Discussion

4.1. Voluntary Intake

Voluntary intake in animals is one of the most important variables in productive behavior and can be affected by multiple factors. In this sense, feed intake is related to the success of the mother and lamb production (pre-weaning weight gain) [29,30]. In sheep fed diets with canola oil, DMI was higher when using 5%DM, and the crude protein passage rate was lower. Similar results were observed in this experiment when 4% olive oil was used. This reduction was also observed with flaxseed oil at up to 9% DM, which could be explained by a reduction in rumen fiber digestibility [31].

Fiorentini et al. [32] evaluated different lipid sources (palm oil, linseed oil, protected fat, and soybean oil) in Nellore steers with 4.5%, where the highest intakes of DM, OM, and CP were recorded in animals fed an oil-free diet. However, in this study, the intake of DM, OM, and CP decreased as the amount of olive oil increased or had a linear increase in EE, which could indicate that the reduction in DMI was probably due to fiber digestion problems [31]. This coincided with what was reported by Vargas-Bello-Pérez et al. [9], which indicated that dry matter intake and digestibility decreased as olive oil increased; this could be due to a regulatory response indicating a higher energy intake, which reduces voluntary intake.

4.2. Productive Behavior of Sheep and Lambs

In this study, it was observed that the levels of oil used did not affect the productive parameters in the sheep and their offspring, which was similar to other studies carried out on sheep in which different oils were used in the diets; in some cases, over 6% of the DM was offered [11,33–35]. However, there are reports in sheep and goats in which levels greater than 6% of the DM of oil were added to the diet without generating changes in productive behavior [36,37], as observed in dairy sheep of the Spanish Assaf breed that had

6% soybean oil added to their diets; this was similar to what happened in this trial for hair sheep in a tropical region.

Martínez-Marín et al. [38] added 4.8% sunflower oil to the diets of dairy goats of the Malagueña breed without observing an effect on voluntary intake. In this regard, Parente et al. [39] evaluated various oils at 3% inclusion in the diet (canola, sunflower, and castor oils) and reported a lower feed intake in the groups that had oil without finding effects on lambs, while the sheep showed a weight gain of over 1 kg during the experiment; these findings were similar to the results found in this study in sheep and lambs. This is because a decrease was found in the DMI without affecting the productive indicators, which are related to the amount of energy added to the ration, because the lactation stage requires a greater energy demand to avoid weight loss during this period, which would ensure post-weaning ovarian reactivation.

4.3. Production and Chemical Composition of Milk

In this study, the addition of olive oil to the sheep diet did not affect the production or chemical composition of milk at the different levels offered (0, 2, 4, and 6% olive oil); this finding is different from that of Gómez-Cortés et al. [17], who observed an increase in milk production in dairy sheep of the Assaf breed with 233 g/d with the addition of 6% olive oil, with reductions in the percentage of fat and protein at this level. By contrast, Gallardo et al. [40] did not report changes in milk production or protein content; these findings were similar to those observed in this study, except for the reduction in fat content. Antongiovanni et al. [15], using the technique of calcium salts of free fatty acids obtained from olive oil, observed that the fat and protein content was increased without modifying milk production. However, Vargas-Bello-Pérez et al. [9] developed diets for lactating sheep (cross with Finnish Landrace, Border Leicester, Poll Dorset, and Merino) with 36 and 88 g/d of lampante olive oil; no effects of these dietary treatments on milk production and milk chemical composition were reported, which agrees with the results obtained in this study. These changes found in sheep in milk production and fat and protein yield may depend on the genetic potential of the sheep to increase milk production as they consume greater amounts of nutrients because studies have been conducted in milk-producing sheep [41].

4.4. Milk Fatty Acid Profile

Short-chain fatty acids are related to low-density cholesterol levels in the blood, causing cardiovascular problems in humans [5,6]. Due to this, new mechanisms are being sought to modify the proportion of fatty acids in milk and meat, which is why different vegetable oils, such as soybean, linseed, sunflower, and safflower oils, have been used to change milk fatty acid profiles [10,22,42–44], as they can increase the milk contents of MUFA and PUFA.

The high concentration of C18:1 in olive oil was reflected in sheep milk as concentrations increased; in this sense, other PUFAs (C18:2n6c, C18:3n3, and C18:1cis-9) increased with 2% olive oil inclusion. This could be explained by the fact that when olive oil fatty acids were biohydrogenated, the increased fatty acid elongation led to an increased production of polyunsaturated fatty acids.

These effects were observed in this study when adding extra virgin olive oil. For their part, Martini et al. [16], when supplementing Massese sheep with calcium soaps of olive oil, observed less than five percent reductions in saturated fatty acids, while unsaturated fatty acids increased, with considerable amounts of CLA observed in the milk. In this study, CLA precursors, such as stearic acid, were observed. Gomez-Cortez et al. (41), by including three percent olive, flaxseed, and soybean oils, observed a decrease in saturated fatty acids and an increase in the concentration of unsaturated acids. In this study, we observed that with small concentrations of olive oil, changes could be generated in the fatty profile of the milk; the inclusion of 2% of olive oil in the diet of lactating hair sheep decreased saturated fatty acids and increased polyunsaturated acids.

So far, studies related to supplementation strategies with vegetable oils have used less than 5% inclusion in the diet; this is because it can affect voluntary consumption, which could reduce the production, composition, and portion of milk fatty acids in ruminants [45,46]. There have been a few studies with levels greater than 5%, such as a study performed by Gómez-Cortes et al. [17] that obtained results similar to those of this study, with the addition of 6% oil resulting in a reduction in unsaturated fatty acids. The reduction in saturated fatty acids in milk may be related to the negative effects of high oil levels on de novo synthesis because saturated fatty acids are synthesized in the mammary glands [11,47,48], and most MUFAs and PUFAs, such as linoleic acid, linolenic acid, and eicosapentaenoic acid, are generated in the rumen through incomplete desaturation, which occurs by the biohydrogenation process.

Similarly, it has been observed that when the bioavailability of medium- and long-chain fatty acids increases because of higher dietary intake, de novo synthesis decreases the concentrations of short-chain fatty acids in milk [17]. This is similar to this work because there were increases in polyunsaturated fatty acids and a decrease in Δ 9-desaturase activity with extra virgin olive oil. However, a diet rich in oleic acid should have increased C18:0 concentrations due to biohydrogenation and increased Δ^9-desaturase activity [6,7,17].

According to Vargas-Bello-Pérez et al. [9], AI was reduced as lampante olive oil was added to the diet; [17] conducted tests with 6% olive oil in the diet, yielding similar effects to those observed when 6% extra virgin olive oil was added in this study. Vargas-Bello-Pérez et al. [9] used lampante olive oil in smaller quantities than those used in this study, and no differences in IT were found; in this study, this index was lower with 2 and 6% extra virgin olive oil. C14:0 was the most atherogenic one, with approximately four times more potential to increase cholesterol than 16:0 [28]. In this study, with C14:0, it was possible to decrease by 7% when 6% extra-virgin olive oil was added to the diet.

5. Conclusions

The addition of extra virgin olive oil did not generate negative effects on the productive behavior, milk production, or chemical composition of lactating sheep, but it did generate changes in the fatty acid profile. Therefore, it is feasible to add extra virgin olive oil to reduce saturated fatty acids; increase the levels of monounsaturated and polyunsaturated fatty acids; increase the contents of acid linoleic, linolenic, and eicosapentaenoic acids; and reduce the atherogenicity index.

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