Effect of Fish Oil and Linseed Oil on Intake, Milk Yield and Milk Fatty Acid Profile in Goats

Lam Phuoc Thanh *, Juan Jose Loor *, Duong Tran Tuyet Mai , Tran Thi Thuy Hang

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Article

Effect of Fish Oil and Linseed Oil on intake, Milk Yield and Milk Fatty Acid Profile in Goats

Lam Phuoc Thanh [1,*], Duong Tran Tuyet Mai [1], Tran Thi Thuy Hang [2] and Juan J Loor [3,*]

[1] Department of Animal Sciences, Can Tho University, Ninh Kieu, Can Tho 94000, Viet Nam
[2] Department of Agricultural Technology, Can Tho University, Phung Hiep, Hau Giang 95000, Viet Nam
[3] Department of Animal Sciences, Division of Nutritional Sciences, University of Illinoi, Urbana Champaign, Urbana, IL 61801, USA
* Correspondence: author: phuocthanh@ctu.edu.vn; jloor@illinois.edu

Simple Summary: Improvements in milk healthy fatty acids by feeding oil mixtures have been reported in dairy cows. However, in some cases, the oil addition has been reported to reduce milk yield and milk fat in ruminants. It is unknown whether the inclusion of linseed oil and fish oil at the high level in goat diet increases milk healthy fatty acids without affecting milk production. The objective of this study was to investigate effect of linseed oil added alone at 2.5% or in combination with tuna fish oil at 2.5% and 4.16% in goat diet on intake, milk yield, and milk fatty acid profiles. Compared with control without oil addition, feeding linseed oil and fish oil at 4.16% markedly increased the levels of milk healthy fatty acids such as *c*9,*t*11 conjugated linoleic acid and docosahexaenoic acid, but decreased milk total saturated fatty acids, atherogenicity, and thrombogenicity indexes. Oil addition did not have a negative effect on intake, milk yield, and milk fat. Therefore, supplementing linseed oil and fish oil at 4.16% in the diet of lactating goats could have a positive impact on human health without any adverse effect on animal performance.

Abstract: This study aimed to evaluate the effect of incorporating linseed oil and fish oil in the diet on intake, ruminal fermentation milk yield and milk fatty acid profile in dairy goats. Four crossbred Saanen lactating goats in mid-lactation and milking 1.30±0.28 g/day were used in a 4 × 4 Latin square design. The basal diet consisted of concentrate and Para grass (C:F 40:60). Treatments included the basal diet without oil supplement (Ctrl) or with 2.5% linseed oil ($LO_{2.5}$), 2.5% linseed oil and fish oil (3:2, w/w, $LFO_{2.5}$), and 4.16% linseed oil and fish oil (3:2, w/w, $LFO_{4.16}$). Diets had no effect on intake, milk yield, milk composition and ruminal fermentation patterns ($P > 0.05$). The lower ($P < 0.05$) proportions of C10:0-C14:0 in milk fat were observed with $LFO_{4.16}$ compared with Ctrl. Compared with the Ctrl and linseed oil added alone, feeding $LFO_{4.16}$ resulted in a 589% and 303% increase in C18:1 *t*11, respectively. Milk *c*9,*t*11 CLA was markedly increased with the $LFO_{4.16}$ diet, accounting for 4.53 and 2.94 times greater concentrations than those in Ctrl and $LO_{2.5}$ diets, respectively ($P < 0.01$). Goats fed $LFO_{2.5}$ and $LFO_{4.16}$ had greater levels of C22:6*n*-3 (0.63% and 0.87%; $P < 0.001$) compared with those fed Ctrl and $LO_{2.5}$ diets (0.06% and 0.08%). Overall, the combined data suggested that including 4.16% linseed oil and fish oil in the diet of dairy goats is effective for improving the concentrations of healthy fatty acids in milk, without affecting milk production.

Keywords: dairy goats; intake; milk fatty acids; milk yield; rumen fermentation

1. Introduction

Over the past few years, there has been a surge in global milk consumption, primarily driven by the steady growth in the world population. [1] reported that between 2007 to 2017, the global goat population increased by 21.5%. As of now, Asia is ahead of other regions with the largest goat population, currently accounting for 56% of the world's total. Furthermore, the rise in income and better living standards among people has led to a growing trend of consuming premium goat milk products in place of cow's milk. Small size of milk fat particles, high digestibility and low allergy potential are the main reasons goat milk is a preferred choice for infants [2].

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The fat in goat milk contains a ratio of *n*-6 to *n*-3 fatty acids (FA) of 5:1, which is similar to the recommended ratio for the prevention of cardiovascular disease in humans [3]. The potential advantages offered by goat milk products have resulted in an increase in their consumption by humans. Recent development in dairy goat production has focused on enhancing the nutritional value of milk and dairy products, as well as improving the functionality of milk and milk products [4]. In order to improve the quantity and quality of milk, changes have been made in the goat production diet.

Goat milk produced through traditional dairy farming methods typically contains negligible amounts of conjugated linoleic acid (CLA), with alpha linolenic acid (ALA) and docosahexaenoic acid (DHA) being barely detectable. [5]. Previous studies have demonstrated that supplementing the diet with linseed oil significantly enhanced the quantity of unsaturated fatty acids (UFA) present in goat and cow milk, with particular emphasis on CLA and ALA [6-8]. However, when linseed oil is added alone in the diet, it undergoes biohydrogenation (BH) by rumen microorganisms, leading to the conversion of most of the ALA and CLA to stearic acid (SA, C18:0). This results in a substantial loss of ALA and CLA and an amplified accumulation of SA in milk.

In an *in vitro* study, the replacement of linseed oil with fish oil significantly elevated contents of ALA and DHA in rumen fluid of Saanen goats [9]. A study on dairy cows by [10] showed that incorporating a blend of tuna fish oil and linseed oil in the diet simultaneously increased CLA, ALA and DHA in milk. As far as we know, there is no published research assessing the potential impact of a combination of fish oil and linseed oil on milk yield, composition, and fatty acid profile in dairy goats. The main objective of this study was to investigate the impact of supplementing linseed oil either alone or in combination with fish oil on milk production, composition, and fatty acid profiles. We hypothesized that supplementing a combination of linseed oil and fish oil would increase healthy FA in milk with minimal effects on intake and ruminal fermentation.

2. Materials and Methods

The study was performed at Experimental Farm, Can Tho University, Vietnam. All procedures were carried out in compliance with the ethical standards stated in the Helsinki Declaration of 1975, revised in 2000, in addition to following the national laws.

2.1. Animals

Four F3 crossbred lactating Saanen goats (♂Saanen × ♀Bach Thao), mid-lactation, 2nd parity, 1.30±0.28 kg of milk and 36.6±1.65 kg of body weight, were housed in individual wooden cages (1.2 m×0.6 m×1.2 m, L×W×H) and offered daily rations as equal meals at 7:00 and 17:00 h. The animals had free access to water and a mineral block, and had enough space to exercise. Prior to conducting the experiment, goats were fed freely a basal diet for 1 week to determine the maximum feed intake.

2.2. Experimental Design and Diets

The animals were assigned to a 4×4 Latin square, each period consisted of 16 days for adjustment and 5 days for sampling. During the experimental period, all goats were fed a basic diet consisting of 40% fresh Para grass (*Brachiaria mutica*) and 60% pelleted concentrate (dry matter basis). Treatments were 1) basic diet without oil inclusion as a control (Ctrl), 2.5% linseed oil ($LO_{2.5}$), 2.5% linseed oil and tuna oil (3:2 w:w; $LFO_{2.5}$) and 4.16% linseed oil and tuna oil (3:2 w:w; $LFO_{4.16}$). The ratio (3:2 w:w) of linseed oil and tuna oil used was 2.5% DM in this study according to the findings of [9]. Oil blend was added at 4.16% in $LFO_{4.16}$ diet such that this diet contained the same amount of linseed oil as the $LO_{2.5}$ diet. Concentrate was mixed and pelleted once a week. Oil was mixed daily with concentrate before feeding the animals. They then had *ad libitum* access to Para grass. Diets were monitored daily to ensure that the goats consumed exactly the ratios as they were designed. Feed ingredients and chemical composition of the diets are shown in Table 1.

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Table 1. Ingredients and chemical compositions of experimental diets.

Item	Diet[1]			
	Ctrl	$LO_{2.5}$	$LFO_{2.5}$	$LFO_{4.16}$
Ingredient, % DM				
Soybean meal	15.2	15.7	15.6	15.9
Ground corn	15.5	9.46	9.03	-
Rice bran	6.85	11.45	12.0	20.2
Para grass	60.0	58.5	58.5	57.5
NaCl	0.30	0.30	0.30	0.30
[2]Premix	0.50	0.50	0.50	0.50
$CaCO_3$	1.74	1.62	1.53	1.43
Linseed oil	-	2.50	1.50	2.50
Tuna fish oil	-	-	1.00	1.66
Chemical composition, % of DM unless otherwise noted				
DM	45.6	46.7	46.7	47.8
Ash	9.80	10.2	10.2	11.2
OM	90.2	89.8	89.8	88.8
CP	17.9	17.9	17.9	18.3
NDF	46.8	50.7	50.7	50.1
ADF	25.4	26.4	26.4	29.4
CF	23.2	23.9	23.9	25.9
NFE	46.9	43.3	43.3	38.4
EE	2.23	4.74	4.74	6.56
ME, Mcal/kg DM	2.33	2.55	2.55	2.61

DM: dry matter, Ash: total minerals, OM: organic matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, CF: crude fiber, NFE: nitrogen - free extract, EE: ether extract, ME: metabolizable energy. [1]Ctrl: control; $LO_{2.5}$: 2.5% linseed oil; $LFO_{2.5}$: 2.5% linseed oil + fish oil (3:2 wt:wt); $LFO_{4.16}$: 4.16% linseed oil + fish oil (3:2 wt:wt). [2]Content in 1 kg premix included: 290-350 g Ca, P: 62, 35 mg Mg, 450000 UI vitamin A, 70.000 UI vitamin D, 1.800 UI vitamin E and others.

2.3. Sampling and Measurements

Feed offered and refused were recorded daily for each goat during a 5-d period (d15-d19). Feed samples were dried in a forced-air oven (FD 53, Binder, Germany) at 60°C for 72 h. After this, the samples were stored at −20°C until analyses of chemical composition.

The dairy goats were milked daily at 7:30 and 17:30 h and milk yields were recorded at each milking. Milk was sampled in 2 consecutive days (d19-d20) to analyze milk composition. To measure milk FA composition, pooled milk samples from 2-day samplings were stored at –20°C until further analysis. To count somatic cells in milk, samples were taken twice (morning and afternoon) at the beginning and the end day of each period.

On d21, rumen fluid samples were collected at 0 and 3 h post morning feeding using a 100-mL syringe and pH was immediately determined using a digital pH meter (HI-5522, Hanna Instruments, Inc., US). The subsample was then filtered through a clean double layer cotton cloth, and the liquid fraction was acidified with 1M H_2SO_4 (9:1 v/v), centrifuged at 10,000×g for 15 min and stored at −20°C for the analyses of volatile fatty acids (VFA) and NH_3-N concentrations.

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2.4. Chemical Analysis

Samples were ground through a 1-mm mesh (Cutting Mill SM100, Retsch, Germany) and subjected to proximate analysis. Feed samples were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), ether extract (EE) and Ash using standard methods [11]. Analyses of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were done using methods of [12]. Concentration of NH_3-N was analyzed by the micro-Kjeldahl method. Milk composition including total solid, lactose, protein, fat and solid not fat was analyzed with a MilkoScan infrared automatic analyzer (MilkoScan Mars, Foss, Denmark). The milk samples were warmed at 40°C in a shaking incubator (ISS-4075R, Jeiotech, Korea) prior to the analysis of milk composition. Milk samples were kept in Eppendorf at 1°C and immediately analyzed for somatic cell counts using a milk somatic cell analyzer (Adam-SCC, Nano Entek Inc, Korea).

Concentrations of individual VFA were analyzed using a Thermo Trace 1310 GC system (Thermo Scientific, Waltham, MA, USA) equipped with a flame ionization detector. Aliquots (1 µL) were injected with a split ratio of 10:1 into a 30 m × 0.25 mm × 0.25 µm Nukol fused-silica capillary column (Cat. No: 24107, Supelco, Sigma-Aldrich, St. Louis, MO, USA) with helium carrier gas set to a flow rate of 1 mL/min. Temperature program of the GC was set up following the method of [13]. Individual VFA peaks were identified based on their retention times, compared with external standards including acetic, propionic, butyric, valeric, iso-butyric and iso-valeric acids (Sigma-Aldrich, USA).

Lipids in feed samples (1 g) were extracted in chloroform:methanol solution (2:1 v:v) following the traditional Folch procedure [14], with minor modifications as described by [15]. Lipid content in milk samples (2 mL) were extracted with 25% ammonium solution, 95% ethanol, diethyl ether and petroleum ether, according to the method of Chouinard et al. (1997). One milliliter of internal standard (1 mg C13:0/mL chloroform) was added to all extracted lipids and evaporated to complete dryness under a N_2 stream. Dried lipids of feed and milk samples were then methylated with 3 mL of NaOH in methanol (0.5 M) followed by 2 mL of acetyl chloride in methanol (1:5 vol/vol). The FA methyl esters (FAME) were extracted twice with 2 mL of hexane and pooled extracts were evaporated under a N_2 stream until dryness. The residue was dissolved in 1 mL of hexane and analyzed using a gas chromatograph (Thermo Scientific Trace 1310 GC system, Waltham, MA) equipped with a flame ionization detector. Aliquots (1 µL) were injected at a split ratio of 50:1 into a 100 m × 0.25 mm × 0.25 µm high polar fused silica capillary column (Cat. No: 24056, Supelco Inc., Bellefonte, PA) with helium carrier gas set to a flow rate of 1 mL/min. Temperature program of the GC was set up following the method of [16]. Individual FAME were identified by comparison of retention times with 37 component FAME mix of standard (Cat. No: CRM47885, Supelco Inc, Bellefonte, PA), CLA mix of standard (Cat. No: O5632, Sigma–Aldrich, Louis, MO), and C18:1 *t*11 standard (Cat. No: CRM46905, Supelco Inc, Bellefonte, PA).

2.5. Calculations

Metabolizable energy intake (ME) was caculated following the equation of [17]. Atherogenicity index = [12:0+4(14:0)+16:0]/[MUFA+PUFA] and thrombogenicity index = (14:0+16:0+18:0)/[(MUFA+*n*-6 PUFA)/2+3(*n*-3 PUFA)+(*n*-3 PUFA/*n*-6 PUFA)] [18].

2.6. Statistical Analysis

Data were analyzed using GLM in trình SAS OnDemand for Academics 2021 (SAS Institute Inc., Cary, NC, USA) for a completely randomized design with the statistical model $Y_{ij} = \mu + D_i + \varepsilon_{ij}$, where Y_{ij} = the dependent variable, μ = the overall mean, D_i = the effect of diet, and ε_{ij} = the random residual error. Significant differences among treatment means were compared using Tukey. Statistical tests were performed using SAS OnDemand for Academics 2021 (SAS Institute Inc., Cary, NC, USA). A significant effect of treatment was declared at $P < 0.05$ and tendencies were declared at $0.05 \leq P < 0.10$.

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3. Results

3.1. Intake

Diet had no effect on the intake of DM, CP and ME (Table 3). Except for C12:0, C16:0, C18:0 and C18:2 *c*9,*c*12, the inclusion of oil resulted in an increased intake of all FA (Table 3). As expected, compared with other diets, the addition of LO alone or in combination with FO at 4.16% resulted in a greater consumption of C18:3*n*-3 (21.2 g/d and 23.4 g/d; $P < 0.05$), which is the predominant FA in linseed oil (4.40-14.6 g/d). Intakes of C20:5*n*-3 and C22:6*n*-3 were greatest with $LFO_{4.16}$ (3.49 g/d and 1.73 g/d) compared with $LFO_{2.5}$ (1.88 g/d and 0.93 g/d) and other diets (undetected). The total FA intake with $LFO_{4.16}$ was 2.52 and 1.42 times greater ($P < 0.05$) than those in the Ctrl and other diets with added oil, respectively.

Table 2. Fatty acid composition in the diet.

Fatty acid	Feed				Diet[1]			
(g/100 g FA)	Fish oil	Linseed oil	Para grass	Concentrate	Ctrl	$LO_{2.5}$	$LFO_{2.5}$	$LFO_{4.16}$
C12:0	0.08	0.01	0.69	0.03	0.43	0.42	0.42	0.42
C14:0	6.33	0.06	0.84	0.17	0.57	0.55	0.63	0.70
C16:0	21.6	5.52	47.2	19.5	36.1	38.3	38.3	39.1
C18:0	2.03	3.22	10.1	5.34	8.20	8.04	8.67	7.54
C18:1 *c*9	13.9	17.9	3.63	35.6	16.4	18.7	18.1	20.2
C18:2 *c*9,*c*12	2.39	16.5	11.4	26.1	17.3	16.6	16.3	13.9
C18:3*n*-3	0.29	55.8	21.5	0.68	13.2	14.2	13.7	14.1
C20:5*n*-3	14.9	nd[2]	nd	nd	nd	nd	0.15	0.25
C22:6*n*-3	7.37	nd	nd	nd	nd	nd	0.07	0.12
SFA	45.2	9.06	62.3	37.0	52.2	49.7	50.6	50.3
UFA	54.8	90.9	37.7	63.0	47.8	50.3	49.4	49.7
MUFA	29.0	18.0	4.65	36.1	17.2	19.4	19.0	21.2
PUFA	25.8	72.9	33	26.9	30.6	30.9	30.3	28.5
n-3 PUFA	22.9	56.1	21.5	0.76	13.2	14.2	13.9	14.5
n-6 PUFA	2.75	16.5	11.5	26.1	17.3	16.7	16.4	14.0

[1]Ctrl: control; $LO_{2.5}$: 2.5% linseed oil; $LFO_{2.5}$: 2.5% linseed oil + fish oil (3:2 wt:wt); $LFO_{4.16}$: 4.16% linseed oil + fish oil (3:2 wt:wt). [2]nd: Not detectable.

Table 3. Intakes in dairy goats fed a basal diet without supplement or added mixture of linseed oil and fish oil.

Item	Diet[1]				SEM	P
	Ctrl	$LO_{2.5}$	$LFO_{2.5}$	$LFO_{4.16}$		
Main components						
DM, g/day	1,635	1,517	1,514	1,414	112	0.190
CP, g/day	297	274	274	262	24,0	0.300
ME, Mcal/d	3.89	3.94	3.94	3.73	0,31	0.768
Fatty acids[b], g/d						
C12:0	0.14	0.14	0.14	0.14	0.01	0.896
C14:0	0.20[b]	0.20[b]	0.99[a]	1.67[a]	0.15	0.001
C16:0	12.7	13.5	15.5	18.2	1.24	0.074

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Item	Diet[1]				SEM	P
	Ctrl	$LO_{2.5}$	$LFO_{2.5}$	$LFO_{4.16}$		
C18:0	2.92	3.71	3.56	4.21	0.33	0.138
C18:1 *c*9	6.74[b]	11.8[ab]	11.4[ab]	16.4[a]	1.54	0.025
C18:2 *c*9,*c*12	6.68	11.3	9.6	12.8	1.26	0.059
C18:3*n*-3	4.40[b]	21.2[ab]	14.6[ab]	23.4[a]	3.29	0.024
C20:5*n*-3	n.d.	n.d.	1.88[b]	3.49[a]	0.35	0.001
C22:6*n*-3	n.d.	n.d.	0.93[b]	1.73[a]	0.17	0.001
SFA	18.7[b]	20.2[ab]	24.7[ab]	30.5[a]	2.16	0.030
UFA	18.2[b]	44.8[ab]	40.8[ab]	62.1[a]	6.58	0.018
MUFA	7.02[b]	12.1[ab]	13.6[ab]	20.2[a]	1.80	0.012
PUFA	11.1[b]	32.7[ab]	27.2[ab]	41.9[a]	4.83	0.021
n-3 PUFA	4.42[b]	21.3[ab]	17.5[ab]	28.8[a]	3.59	0.016
n-6 PUFA	6.70[b]	11.3[ab]	9.65[ab]	12.9[a]	1.26	0.058
Total FA	36.8[b]	65.1[ab]	65.5[ab]	92.7[b]	8.51	0.021

[1]Ctrl: control; $LO_{2.5}$: 2.5% linseed oil; $LFO_{2.5}$: 2.5% linseed oil + fish oil (3:2 wt:wt); $LFO_{4.16}$: 4.16% linseed oil + fish oil (3:2 wt:wt). [b]n.d. indicates proportions of FA in feed ingredients not detected or below 0.01% of total FA.

3.2. Milk Yield and Composition

Milk yield of the experimental goats ranged from 1.34 to 1.44 kg/day and did not differ among the diets ($P > 0.05$; Table 4). The milk composition and somatic cell count remained unchanged ($P > 0.05$) regardless of the different source and levels of oil inclusion in the diet.

Table 4. Milk yield and composition in dairy goats fed a basal diet without supplement or added mixture of linseed oil and fish oil.

Item	Diet[1]				SEM	P
	Ctrl	$LO_{2.5}$	$LFO_{2.5}$	$LFO_{4.16}$		
Milk yield, kg/day	1.44	1.34	1.36	1.44	0.15	0.687
Milk composition, %						
Fat	2.78	3.18	2.83	3.03	0.57	0.743
Protein	3.08	3.02	3.04	2.92	0.12	0.363
Lactose	4.26	4.35	4.38	4.41	0.66	0.508
Solid not fat	8.14	8.18	7.71	7.96	0.42	0.451
Total solid	10.5	10.7	10.6	10.9	0.89	0.716
Somatic cell count, $\times 10^3$/mL						
Initial	1,032	1,007	715	658	538	0.696
Final	692	720	435	1,046	357	0.221
Difference	-340	-287	-279	388	622	0.377

[1]Ctrl: control; $LO_{2.5}$: 2.5% linseed oil; $LFO_{2.5}$: 2.5% linseed oil + fish oil (3:2 wt:wt); $LFO_{4.16}$: 4.16% linseed oil + fish oil (3:2 wt:wt).

3.3. Ruminal Fermentation Patterns

The diets did not affect ruminal fermentation patterns such as pH, NH_3-N and total VFA ($P > 0.05$; Table 5). Total VFA concentration was higher at 3 h post feeding (59.8-69.1 mM) compared with before feeding (53.1-57.8 mM). Compared with the Ctrl goats fed the basal diet without oil inclusion,

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there was no change in percentage of individual VFA in goats that were fed 4.16% oil ($P > 0.05$; Table 5).

Table 5. Ruminal fermentation patterns in dairy goats fed a basal diet without supplement or added mixture of linseed oil and fish oil.

Item	Diet[1]				SEM	P
	Ctrl	$LO_{2.5}$	$LFO_{2.5}$	$LFO_{4.16}$		
0 h						
pH	6.80	6.90	6.88	6.81	0.10	0.490
NH_3-N, mg/dL	32.2	37.8	31.5	30.1	4.50	0.182
Total VFA, mM	54.9	57.8	53.5	53.1	4.39	0.476
Acetate,%	67.0	66.4	65.3	64.7	1.62	0.254
Probionate, %	17.7	18.8	19.3	20.1	1.57	0.286
Acetate/propionate	3.78	3.54	3.38	3.22	0.38	0.254
Iso-butyrate, %	3.81	3.60	3.39	3.23	0.13	0.608
Butyrate, %	1.85	1.82	1.78	1.73	0.49	0.466
Iso-valerate, %	8.59	8.26	8.80	8.73	0.17	0.734
Valerate, %	2.53	2.55	2.52	2.43	0.17	0.572
3 h						
pH	6.52	6.56	6.63	6.74	0.12	0.128
NH_3-N, mg/dL	37.1	32.6	37.8	29.4	5.35	0.190
Total VFA, mM	64.5	63.0	69.1	59.8	6.47	0.321
Acetate,%	66.7	66.4	67.1	66.0	2.66	0.941
Probionate, %	19.0	19.5	19.0	19.7	1.70	0.318
Acetate/propionate	3.50	3.40	3.53	3.34	0.71	0.953
Iso-butyrate, %	3.62	3.49	3.62	3.36	0.13	0.585
Butyrate, %	1.60	1.62	1.58	1.70	0.52	0.916
Iso-valerate, %	8.14	8.21	8.23	8.17	0.16	0.612
Valerate, %	2.19	2.22	2.16	2.31	0.15	0.403

[1]Ctrl: control; $LO_{2.5}$: 2.5% linseed oil; $LFO_{2.5}$: 2.5% linseed oil + fish oil (3:2 wt:wt); $LFO_{4.16}$: 4.16% linseed oil + fish oil (3:2 wt:wt).

3.4. Milk Fatty Acids

Supplementation of oils altered the proportions of some medium- and long-chain SFA in milk, including C10:0, C11:0, C12:0 and C14:0 (Table 6). Compared with the Ctrl group, goats fed $LFO_{4.16}$ had the lowest proportions ($P < 0.05$) of C10:0 and C11:0 (5.75% and 0.14%, respectively) (9.30% and 0.24%, respectively). Compared with Ctrl (4.45% and 15.2%), the inclusion of oils at 2.5% led to a lower proportion of C12:0 (3.16-3.18%) and C14:0 (11.0-11.4%), but a greater proportion of these FA in $LFO_{4.16}$ (2.25% and 8.59%) ($P < 0.001$). As expected, the proportions of beneficial FA including C18:1 *t*11, C18:1 *c*9, *c*9,*t*11 CLA and C22:6*n*-3 were remarkably increased ($P < 0.05$; Table 6) in goats receiving $LFO_{4.16}$. Supplementing the combination of LO and FO at 4.16% resulted in a 589% and 303% increase in C18:1 *t*11 compared with the Ctrl and SO added alone, respectively. Milk *c*9,*t*11 CLA was impressively increased with the $LFO_{4.16}$ diet,, accounting for 4.53 and 2.94 times greater concentrations than those in the Ctrl and $LO_{2.5}$ diets, respectively ($P < 0.01$). Animals fed $LFO_{2.5}$ and $LFO_{4.16}$ diets had greater levels of C22:6*n*-3 (0.63% and 0.87%; $P < 0.001$) compared with those fed the Ctrl and $LO_{2.5}$ diets (0.06% and 0.08%).

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Table 6. Milk individual fatty acid composition in dairy goats fed a basal diet without supplement or added mixture of linseed oil and fish oil.

Fatty acid (g/100 g FA)	Diet[1]				SEM	P
	Ctrl	$LO_{2.5}$	$LFO_{2.5}$	$LFO_{4.16}$		
Saturated FA						
C4:0	0.44	0.81	0.40	0.56	0.28	0.255
C6:0	1.30	1.74	1.14	1.35	0.39	0.255
C8:0	1.69	1.93	1.39	1.43	0.45	0.371
C10:0	9.30^{a}	7.47^{ab}	6.64^{ab}	5.75^{b}	1.16	0.024
C11:0	0.24^{a}	0.21^{ab}	0.17^{ab}	0.14^{b}	0.03	0.021
C12:0	4.45^{a}	3.18^{b}	3.16^{b}	2.25^{c}	0.23	<0.001
C14:0	15.2^{a}	11.0^{b}	11.4^{b}	8.59^{c}	0.66	<0.001
C15:0	0.99	0.96	1.08	1.07	0.32	0.499
C16:0	36.2	29.1	40.0	35.8	5.23	0.120
C17:0	0.66	0.59	0.68	0.65	0.08	0.472
C18:0	6.49	10.2	8.52	7.94	2.73	0.366
C20:0	0.03	0.04	0.09	0.12	0.05	0.106
C21:0	0.01	0.25	0.01	0.21	0.34	0.653
C22:0	0.03	0.04	0.09	0.12	0.05	0.105
C23:0	0.02	0.02	0.02	0.03	0.01	0.422
C24:0	0.02	0.02	0.03	0.04	0.01	0.096
Unsaturated FA						
C14:1	0.29	0.20	0.20	0.15	0.06	0.080
C15:1	0.01	0.14	0.00	0.12	0.20	0.667
C16:1	0.65	0.56	0.60	0.69	0.13	0.525
C17:1	0.06	0.05	0.05	0.06	0.08	0.990
C18:1 *t*9	0.07	0.14	0.20	1.99	1.74	0.406
C18:1 *t*11	0.82^{b}	1.40^{b}	2.70^{ab}	5.65^{a}	1.25	0.006
C18:1 *c*9	18.4^{ab}	26.2^{a}	16.8^{b}	18.6^{ab}	3.79	0.047
C18:2 *t*9,*t*12	0.12^{b}	0.29^{ab}	0.38^{a}	0.50^{a}	0.10	0.008
C18:2 *c*9,*c*12	1.13	1.58	1.07	1.30	0.21	0.054
*c*9,*t*11 CLA	0.51^{b}	0.79^{b}	1.38^{ab}	2.32^{a}	0.49	0.007
*c*12,*c*12 CLA	0.01	0.01	0.02	0.02	0.02	0.413
*t*10,*c*12 CLA	0.02^{b}	0.02^{b}	0.02^{b}	0.09^{a}	0.02	0.012
C18:3*n*-6	0.01	0.01	0.01	0.01	0.01	0.454
C18:3*n*-3	0.68	0.78	0.80	0.91	0.25	0.644
C20:1*n*-9	0.02^{b}	0.02^{b}	0.06^{ab}	0.14^{a}	0.04	0.021
C20:2	0.01	0.01	0.01	0.02	0.01	0.075
C20:3*n*-6	0.01	0.01	0.01	0.01	0.01	0.517
C20:3*n*-3	0.01	0.01	0.01	0.03	0.01	0.090
C20:4*n*-6	0.04	0.04	0.04	0.04	0.02	0.985
C20:5*n*-3	0.03	0.04	0.03	0.07	0.03	0.273
C22:1*n*-9	0.01	0.01	0.01	0.03	0.01	0.090

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C22:2	0.01	0.02	0.09	0.19	0.16	0.421
C22:6*n*-3	0.06^{b}	0.08^{b}	0.63^{a}	0.87^{a}	0.07	<0.001
C24:1*n*-9	0.06	0.04	0.08	0.11	0.05	0.406

[1]Ctrl: control; $LO_{2.5}$: 2.5% linseed oil; $LFO_{2.5}$: 2.5% linseed oil + fish oil (3:2 wt:wt); $LFO_{4.16}$: 4.16% linseed oil + fish oil (3:2 wt:wt).

Feeding LO alone or in combination with FO at 4.16% markedly increased ($P < 0.01$; Table 7) the proportion of C18 UFA compared with other diets. Feeding $LO_{2.5}$ and $LFO_{4.16}$ reduced SFA and increased UFA, especially MUFA, in milk fat ($P < 0.01$; Table 7). As expected, PUFA was highest in $LFO_{4.16}$ (6.39%) compared with the lower values (2.64% and 3.70%) detected in the Ctrl and $LO_{2.5}$ ($P < 0.05$). Additionally, percentage of total CLA increased ($P < 0.01$) from 0.54% and 0.81% with the Ctrl and $LO_{2.5}$ diets to 2.43% in the $LFO_{4.16}$. Goats fed $LFO_{4.16}$ diet exhibited a tendency to increase MUFA/SFA ($P = 0.062$) and increased PUFA/SFA ($P < 0.05$) in comparison with those fed the Ctrl diet. As the result of decreased proportion of SFA and increased proportions of MUFA and PUFA with the $LFO_{4.16}$ diet, atherogenicity and thrombogenicity indices in milk fat decreased by 2.09- and 1.69-fold ($P < 0.05$; Table 7) relative to the Ctrl diet.

Table 7. Milk fatty acid group composition in dairy goats fed a basal diet without supplement or added mixture of linseed oil and fish oil.

Fatty acid (g/100 g FA)	Diet[1]				SEM	P
	Ctrl	$LO_{2.5}$	$LFO_{2.5}$	$LFO_{4.16}$		
FA groups						
C18 UFA	21.7^{b}	31.2^{a}	23.4^{b}	31.4^{a}	0.94	0.001
SFA	77.0^{a}	67.6^{b}	74.8^{a}	66.1^{b}	2.25	0.001
UFA	23.0^{b}	32.4^{a}	25.2^{b}	33.9^{a}	2.25	0.001
MUFA	20.4^{b}	28.7^{a}	20.7^{b}	27.5^{a}	1.45	0.003
PUFA	2.64^{b}	3.70^{b}	4.48^{ab}	6.39^{a}	1.31	0.032
n-3 PUFA	0.78	0.91	1.47	1.89	0.74	0.229
n-6 PUFA	1.31	1.95	1.50	1.86	0.28	0.055
Total CLA	0.54^{b}	0.81^{b}	1.42^{ab}	2.43^{a}	0.25	0.007
Indices						
MUFA/SFA	1.13	1.13	1.22	1.23	0.03	0.062
PUFA/SFA	0.03^{b}	0.05^{ab}	0.06^{ab}	0.09^{a}	0.01	0.027
Atherogenecity index	4.61^{a}	2.37^{b}	3.59^{ab}	2.21^{b}	0.72	0.010
Thrombogenicity index	4.32^{a}	2.74^{ab}	3.72^{ab}	2.56^{b}	0.71	0.038

[1]Ctrl: control; $LO_{2.5}$: 2.5% linseed oil; $LFO_{2.5}$: 2.5% linseed oil + fish oil (3:2 wt:wt); $LFO_{4.16}$: 4.16% linseed oil + fish oil (3:2 wt:wt).

4. Discussion

4.1. Intake

The lack of effect of oil supplementation on total DM intake in the current study supports some previous studies in which dairy goats were fed diets containing 3% DM linseed oil [7] and 2.5% soybean oil [19]. In a recent study conducted with dairy cows, [6] detected a decreased tendency in total intake when animals were fed a 3% mixture of linseed, sunflower, and tuna oil. [20] reported that inclusion of either 2.2% FO or 5.3% sunflower oil in the diet had no effect on total DM intake in dairy goats, but decreased total DM intake in dairy cows. This finding revealed that dairy goats have

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a lower sensitivity towards intake in the presence of oils added to the diet. [21] recommended limiting total fat intake in the diet to 6-7% DM, as higher concentrations may result in a decrease in DM intake. In the present study, the highest concentration of EE in the diets was 6.56%

4.2. Milk Yield and Composition

The goats used in this research had a relatively low body weight and a moderate milk production, which could be attributed to the tropical conditions they were managed in. As the air temperature, temperature-humidity index, and rectal temperature rise above critical thresholds, a decline in DM intake and a decrease in milk yield occur in tropical ruminants. Additionally, this can lead to a reduction in the efficiency of milk yield [22].

There have been inconsistent results in ruminants fed oil supplements. Milk fat depression (MFD) was found when oils were supplemented in cows [6,23,24], but milk fat content remained unchanged when cows were fed 4% linseed oil [25,26] and goats fed a 2.5% oil blend [19]. The negative impact of adding oil to the diet of dairy ruminants, which leads to a reduction in milk fat content, is more commonly detected when the lipid sources used are high in polyunsaturated fatty acids (PUFA).

In most of the experimental conditions that have been studied, MFD is partially linked to a change in ruminal biohydrogenation. This leads to the production of various ruminal intermediates such as *t*10 18:1 and *t*10,*c*12 CLA, which may adversely affect the mRNA abundance of lipogenic enzymes [27]. When dairy cows were fed milk fat-depressing diets, it resulted in the inhibition of mRNA abundance of mammary lipogenic enzymes. Additionally, supraphysiological concentrations of *t*10,*c*12 CLA were originally associated with MFD [28]. In the current study, the milk fat content remained unchanged despite the detection of a higher concentrations of *t*10,*c*12 CLA in the $LFO_{4.16}$ diet. It is worth noting that goats are less responsive to fat supplements compared with cows [29], which may explain the lack of differences in milk composition observed in this study.

Milk somatic cell counts for goats in this study fluctuated within the standard range for goat milk, which is 1,000 × 10^3/mL [30], ranging from 658 to 1,032 × 10^3/mL at the beginning and 435-1,046 × 10^3/mL at the end of experiment. The $LFO_{4.16}$ diet had an opposite effect on milk somatic cell counts compared to other diets as it showed an increase in this parameter towards the end of the experiment. It is imperative to consider this aspect while conducting future research involving a larger number of goats.

4.3. Milk Fatty Acid Composition

The reduction in C10:0-C14:0 content ($P < 0.01$) was partially attributed to slight alterations in the activity of acetyl-CoA carboxylase and other enzymes that involved in the *de novo* synthesis of SFA in the mammary gland [31]. The reduction in C12:0 and C14:0 concentrations in milk fat from goats supplemented with linseed oil and fish oil could have a favorable impact on human health since the consumption of these FA was reported to have an inverse correlation with the occurrence of heart attacks in humans [32].

That higher milk *c*9,*t*11 CLA in goats fed $LFO_{4.16}$ was in agreement with the finding of [33]. Milk that contains higher levels of *c*9,*t*11 CLA originates from the biohydrogenation of linoleic acid and alpha linolenic acid in the rumen as an intermediate or from endogenous synthesis in the mammary gland from vaccenic acid [34]. Compared to the results (0.098 g/100 g FA) obtained by [35], in this study, the goats fed with a diet containing fish oil had a greater DHA content in the milk (0.63-0.87 g/100 g FA), where goats were supplemented with extruded linseed and fish oil. The consumption of dairy products with lower atherogenic index values (e.g., DHA) leads to a decrease in total cholesterol in human plasma [36].

The current research was designed to include linseed oil and fish oil at 4.16%, resulting in an enhancement of milk concentrations of MUFA and PUFA. These findings align with previous studies [6,37]. In this study, inclusion of a high PUFA oil mixture in the diet led to a decrease in atherogenicity index and thrombogenicity index, which can effectively counteract the negative impact of high levels of SFA and *n*-6 FA present in milk. A notable decrease in atherogenicity and thrombogenicity indices

was also detected when cows were fed 3% linseed oil and fish oil [10] or 3% of linseed oil, sunflower oil and fish oil [6].

5. Conclusions

There was no impact on intake, ruminal fermentation patterns, milk yield, and milk composition when linseed oil and fish oil were incorporated at 4.16% in the diet of lactating goats. However, this diet effectively increased the levels of healthy milk fatty acids such as C18:1 *t*11, *c*9,*t*11 CLA, and C22:6n-3. Additionally, it decreased milk total SFA, atherogenicity, and thrombogenicity indexes. Thus, supplementing linseed oil and fish oil at 4.16% in the diet of lactating goats could have a positive impact on human health without any adverse effect on animal performance.

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