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Effect of Ratios of Linoleic and Alpha-Linoleic Fatty Acids in Diet of Lactating Dairy Ewes on Milk Composition and Quantity, Fatty Acid Profile and Some Plasma Metabolites

Samadi MS[1]; Chashnidel Y[1]; Deldar H[2*]; Fouladi-Nashta AA[3]

[1]Department of Animal Science, Sari Agricultural Sciences and Natural Resources University, Iran

[2]Department of Animal Sciences, University of Guilan, Iran

[3]Reproduction Research Group, the Royal Veterinary College, UK

***Corresponding author: Hamid Delder**

Department of Animal Sciences, University of Guilan, Iran.

Tel: +981155272268; Fax; +981333690282

Email: hamiddeldar@guilan.ac.ir

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Abstract

The current trial aimed to investigate the effects of three ratios of Omega-6 to Omega-3 Fatty Acids (FAs) in experimental diets in milk composition, milk yield, and FA profile and plasma metabolites in Zel dairy ewes. Sources of omega-6 and Omega-3 FAs in experimental diets consist of extruded soybean and extruded linseed, respectively. Multiparous lactating dairy ewes were divided into four experimental groups to receive the experimental diets: Control (without FA sources), Linoleic Acid (LA) to Alpha-Linolenic (ALA) ratio 1:1, 5:1 and 10:1. Diets showed no effect on lactose and Solid Non-Fat (SNF) concentration in secreted milk, while milk yield, protein and fat content of milk had influenced by FA supplementations in diets ($P < 0.05$). Dairy ewes fed FA profile sources showed greater portions of all investigated FA profiles except (Caproic acid) C6:0. Regarding plasma metabolites, High-Density Lipoprotein (HDL) and Very Low-Density Lipoprotein (VLDL) concentrations were higher in the Control group; however, HDL and thyroxin (T4) concentration in plasma in 1:1 experimental group showed highest concentration among experimental groups. Non-Esterified Fatty Acids (NEFA), β-Hydroxybutyric Acid (BHBA), and T3 had not changed significantly between experimental treatments ($P > 0.05$). Overall and according to obtained data, including different omega-3 to omega-6 FAs in different ratios in diets is effective for enhancing the portions of healthy Fas in produced milk, with no reverse effect on milk yield.

Keywords: Fatty acids; Omega-3; Omega-6; Milk composition; Dairy ewe

Introduction

The world's population is increasing at an alarming rate, leading to a growing demand for dairy products and their by-products. In 2015, the per capita milk consumption reached 111.3 kg, so it is expected to increase by 12.5% by 2025, according to IDF [35]. As per the estimates of FAO [26], the global production of fresh sheep's milk in 2016 was 10,366,980 tons, with most of it being produced in the Asian region, accounting for 44.6% of the total production Compared to cow's milk, sheep's milk is highly nutritious and contains higher levels of vitamins A, B, and E, calcium, and phosphorus. Sheep's milk possesses approximately 33% more energy compared to cow or goat milk [62]. The advantages of sheep's milk are vast, as it serves as a significant reservoir of bioactive compounds that contribute to promoting overall health [70]. In terms of human nutrition, two crucial fatty acids are LA (C18:2 omega-6) and ALA (C18:3 omega-3) (Kaur, 2014). Since, the human body lacks the ability to synthesize essential fatty acids, it becomes crucial to obtain these vital compounds solely through dietary sources. Consequently, enriching food products with higher levels of essential fatty acids may significantly enhance the supply of these crucial compounds within the body of human [53]. Over the past century and a half, there has been a significant shift in the levels of omega-6 and omega-3 fatty acids found in Western societies for food supply. This shift has resulted in the Western diet adopting a ratio of 20:1 for omega-6 to omega-3 fatty acids [22]. Such high omega-6: omega-3 ratio has been linked to pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases and decline in human fertility [64]. Moreover, Balić et al. (2020) illustrated one possible dietary modification involves the reduction of omega-6 fatty acid consumption and boost omega-3 FA intake. This adjustment aims to lower the dietary omega-6 to omega-3 ratio. It has been established that the chemical composition of milk can differ based on the diet of ruminant animals [8]. This variation influences the levels of fat, protein, lactose, and the fatty acid profile of the milk.

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Triglycerides, phospholipids, or glycolipids are the most abundant forms of fat sources found in vegetables [63]. These compounds can undergo hydrolysis by ruminal bacteria, resulting in the liberation of fatty acids and glycerol [37]. It is crucial to be aware of and control the rate at which Unsaturated Fatty Acids (UFAs) are biohydrogenated in the rumen. This is because the absorption of UFAs by ruminant animals can have an impact on milk production and composition, as highlighted in previous investigation [13]. The inclusion of fatty acids sources without heat processing in ruminant diets has been found to interfere with the microbial population in the rumen and caused reduction in the level of dry matter intake [36].

Hence, we designed the experimental diets by incorporating varying ratios of a mixture of extruded linseed (ALA source) and extruded soybean (LA source). In fact, our previous research led us to the finding that the extrusion processing of linseed and soybean resulted in the highest level of resistance to ruminal biohydrogenation of UFAs [36].

The primary objective of this study was to examine the effects of incorporating three different ratios (1:1, 5:1, and 10:1) of ALA to ALA into the diet of lactating ewes on milk characteristics and also some plasma metabolites and hormones. Additionally, our study also aimed to enhance the production of healthier milk for consumers by enrichment with essential fatty acids.

Materials and Methods

Animals, Experimental Groups, and Experimental Design

A total of 120 multiparous lactating dairy ewes (breed: Zel; Calving: 2nd; body weight: approximately 48 ± 2 kg), were divided into four experimental groups, with 30 ewes in each group. The allocation of the experimental ewes to the different treatments was randomized. The four experimental groups consisted of: Group 1, the Control group (without a source of fatty acids); Group 2, a ratio of 1:1 LA to ALA; Group 3, a ratio of 5:1 LA to ALA; and Group 4, a ratio of 10:1 LA to ALA.

Diets Formulation and Food Management

The experimental diets were supplemented with extruded soybean and extruded linseed as sources of LA and ALA, respectively. Feeding of these isoenergetic and isonitrogenous experimental diets lasted for a total of six weeks, with the first week dedicated to diet adaptation. In order to accurately determine the concentration of fatty acids in the diet, the fatty acid profile of the diet sources was measured separately in a different experiment (Tables 1-2) [63].

Based on the determined fatty acid profile, experimental diets were formulated using three different ratios of LA to ALA. The experimental diets contained approximately a total of 20 grams for LA and ALA. The formulation of the diets was performed using Small Ruminant Nutrition Software (SRNS) version 1.13.8656.18296, based on the requirements of a 48 ± 2 kg lactating dairy ewe. The ewes were fed a total mixed ration twice a day *ad libitum* (Table 3). The dry matter intake for the ewes was estimated to be around 1.8 kilograms per day, based on the daily milk production.

Milk and Blood Collection and Chemical Analysis

The ewes were milked once a day at 07:00 a.m. Milk production was recorded weekly (days 0, 7, 14, 21, 28, 35 and 42) individually for each lactating ewe. For analyzing of milk lactose, fat, protein and Solid Non-Fat (SNF), Fourier infrared analysis with a MilkoScan 4000 (FOSS, Hillerod, Denmark) was used.

Blood samples were obtained from the jugular vein using EDTA vacutainer tubes following a 12-hour fasting period at 06:00 a.m. on days 0 (commencement of fat supplementation), 7, 14, 21, 28, 35, and 42. After centrifugation for 15 minutes at 2000g, the plasma was separated and stored at -20°C for future analysis. Various metabolites and hormones such as LDL, HDL, VLDL, NEFA, BHBA, Triiodothyronine (T_3), and Thyroxin (T_4) were quantified in the plasma. The levels of NEFA and BHBA were determined using an ELISA kit provided by RANDOX Laboratories Ltd., located in London, UK. LDL and HDL levels were enzymatically measured using a spectrophotometer (Shimadzu 2100, Kyoto, Japan). Plasma T3 concentrations were determined using a competitive enzyme immunoassay kit (Padtan Elm Co., Tehran, Iran). Plasma T_4 concentrations were measured using a competitive enzyme immunoassay kit (Mono bind Inc., CA, and USA).

Fatty Acid Profile of Milk Chemical Analysis

The fat content of milk samples was assessed using the Folch method (1957). In accordance with this procedure, the total lipids present in 0.5 mL of raw milk were extracted twice using a mixture of chloroform and methanol (2:1, v/v). The resulting organic phase was then transferred to a vial and dried under a stream of nitrogen gas. Subsequently, 2.4 mL of a derivatization reagent containing 0.2 M KOH in methanol was added to the sample, which was then incubated at 50°C for 20 minutes. After cooling, 1 mL of water was introduced, and the resulting Fatty Acid Methyl Esters (FAMEs) were extracted into 1 mL of n-hexane for further analysis using gas chromatography with flame ionization detection (GC-FID).

The chromatographic analyses were conducted utilizing an Agilent 7890B gas chromatograph (Agilent, Santa Clara, CA, USA), which was equipped with a Flame Ionization Detector (FID), split/splitless injector, and multipurpose auto-sampler. A SP-2380 column (Supelco, Bellefonte, PA, USA) with dimensions of 30 m × 0.25 mm × 0.2 µm was employed, and helium gas was used as the carrier gas at a constant flow rate of 1.0 mL/min. The injector port was maintained at a temperature of 230°C and operated in the split mode with a split ratio of 10:1. Injection volumes of 1 µL were used. The detector temperature was set at 250°C. The oven temperature program initiated at 50°C, held for 2 minutes, and then increased at a rate of 4 °C/min until reaching 220°C, where it was held for 15 minutes.

Statistical Analysis

Data were tested for normal distribution of the residuals using SAS software by the PROC UNIVARIATE procedure (SAS Institute, 2006). Data were analyzed as repeated measurements using PROC MIXED of SAS software the following model:

$$Yijk = \mu + \alpha i + \tau j + (\alpha\tau)ij + Bk + eijk$$

Where µ is the population mean, αi is the treatment effect, τ*j* is the effect of sampling day or time, $(\alpha\tau)ij$ is the interaction effects of treatment and sampling day or time, B*k* is a random effect of animal, and eijk is the residual error. Significance was declared at $P<0.05$. The binomially distributed data were analyzed using the logistic procedure of SAS.

Result and Discussion

Significant differences were observed in milk yield; milk pro-

tein, Solid-Not-Fat (SNF), and fat percentage between the various experimental treatments and the control group (P<0.05) (Table 4).

Notably, the control group illustrated the lowest milk yield, while the other experimental groups showed higher milk yields (P<0.05). Our research aligns with previous studies conducted on small ruminants, where the inclusion of diverse plant-based fat sources in their diets did not negatively impact milk production or interfere with rumen metabolism, even when employing different methods to process the fat sources [6,48,50]. In ruminants, augmenting lactation performance may be attributed to the enhanced dietary energy content and also providing sufficient amino acids involved in milk production in the mammary glands through the addition of various processed fat sources [29,68]. It has been observed that the decrease in milk production when different sources of fat are added to the diet can be attributed to the presence of Polyunsaturated Fatty Acids (PUFAs) in the fat content of the fat sources. This type of fatty acid has been found to reduce dry matter intake and also interfere with ruminal digestion, resulting in a decrease in milk production [19,44].

Regarding milk protein content, lactating ewes fed the control diet had lowest levels compared to the other experimental groups (P<0.05). Similarly, these data were consistent with some of the previous studies [9,34,42]. In contrast, some studies have shown a decrease in the percentage of milk protein in response to fatty acid sources [33,52]. Thermal processing of the fat supplements in the experimental diet can modify the site of protein digestion in the digestive system of ruminants, moving it away from the rumen and toward the lower parts of the digestive tract [31]. The SNF content of the milk was lowest in ewes fed a diet with a ratio of 10:1 and the control group. Conversely, the experimental diet with a ratio of 5:1 demonstrated the highest solid non-fat content in the milk (P<0.05). Almeida et al. (2019) and Thanh et al. (2023) discovered that the SNF concentration of milk was not significantly impacted by the addition of fatty acid supplements to the diet.

There were variations in the milk fat percentage among the experimental groups. The lowest fat content was observed in Control, but the experimental group with ratio 10:1 showed highest (P<0.05). This was in agreement with the findings of other studies, which showed an increase in the fat content of milk when linseed was added to the diet [48,58]. Additionally, an investigation found that the addition of different levels of encapsulated flaxseed oil in the diet led to a reduction in the fat content of milk due to microbial digestion in the rumen, which could influence the availability of fatty acid profiles in the added fats [47]. Additionally, a study by Abuelfatah et al. (2016) hypothesized that the inclusion of linseed in the diet might augment the molar proportion of acetate concentration in the rumen. This elevation in the levels of acetate in the rumen fluid could also be a crucial factor in the increased fat content of the milk.

The effects of different ratio of LA: ALA on milk fatty acid profile is displayed in Table 5. Based on the obtained results, except for Caproic acid (C6:0) (P>0.05), significant differences were observed in the concentrations of other fatty acids analyzed in milk samples (P<0.05). Results from our trial illustrated that adding fat supplements into the diet of lactating dairy ewes increased the proportion of butyric acid (C4:0) in milk. Likewise, supplementation with sunflower oil and a calcium salt of sunflower oil (2.3 % of DM) was found to be associated with an increase in the butyric acid (C4:0) level of milk [42]. Increasing the proportion of butyric acid (C4:0) in milk can potentially have beneficial effects on human health, such as supporting digestion performance, inhibiting inflammation, and reducing the risk of disease exposure [9].

The lower content of medium-chain fatty acids (C10:0, C12:0, C14:0, and C16:0) in the milk produced, as compared to the non-supplemented group were shown in experimental groups [27,29,45]. This suggests that the addition of vegetable fat sources in the diet can impact the fatty acid composition of milk, potentially influencing its nutritional properties. The lower proportions of C12:0 and C14:0 fatty acids can be beneficial for human health, such as reduction in risk of cardiovascular issues and heart attacks in humans [22,56]. The majority of fatty acids produced within the body are saturated, with chain lengths varying from C4:0 to C16:0, because the delta-9 desaturase enzyme has low activity for fatty acids shorter than 18 carbon chain length [38]. Also, long-chain fatty acids directly inhibit the activity of the enzyme Acetyl-CoA Carboxylase (ACC), which is responsible for producing fatty acids in the mammary gland [14]. As a result, when long-chain fatty acids are present in the diet (such as vegetable oils), a decrease in the percentage of medium-chain fatty acids (C8:0 to C14:0 or C16:0) in the milk fat is observed [18]. This change is further exacerbated by the reduced availability of acetate and 3-Hydroxybutyrate (3-HB) for mammary fatty acid synthesis, caused by the decreased voluntary feed intake due to increased dietary fat consumption, as well as the decreased acetate: propionate ratio in the rumen [19]. The ruminant mammary gland Fatty Acid Synthase (FAS) displays transacylase enzyme activity with both loading and releasing capability for acyl chains with carbon chain lengths ranging from two to twelve [49].

In the current investigation, the stearic acid (C18:0) fraction in milk fat was found to be higher in the experimental groups. Using similar approaches, dairy ruminants received diets enriched with soybean or linseed oil, showed higher proportion of stearic acid (C18:0) in milk [9,42,70] (Thanth et al., 2020). Due to the fact that stearic acid (C18:0) is the end product of the rumen biohydrogenation of LA and ALA [18], the higher portions of stearic acid (C18:0) in milk fat in the present study can be attributed to the inclusion of extruded soybean and linseed in experimental rations (LA and ALA sources, respectively). Although stearic acid (C18:0) is not known to increase plasma cholesterol concentration in human nutrition [43], a reduction in the ratio of C18:0 to C18:1 in milk is considered a positive factor for human health [70].

The milk secreted from ewes fed with a 1:1 ratio of LA to ALA showed a significantly higher concentration of C18:1, *cis*-9 (oleic acid) fatty acid compared to the control group (P<0.05). Similar findings reported in studies involving ewes received diets added extruded linseeds [27] and cows fed diets supplemented with flaxseed [17]. Since stearic acid (C18:0) is the end product of the biohydrogenation of UFAs in the rumen, which can be converted into oleic acid through the action of delta-9 desaturase in the mammary gland. The rise in the concentration of oleic acid in milk may also be a result of desaturation [42] The concentration of vaccenic acid in diets enriched with varying proportions of omega-6 and omega-3 fatty acids had been found to be elevated as previously studied for diets containing linseed oil in ewes [42]. In this line, the highest vaccenic acid portions were observed in diets that included soybean and linseed oils [9]. In this context, it demonstrated that supplementation of ruminant

Table 1: Fat and selected FA contents in the experimental diets (% Dry Matter).

	FA profiles (%)					
FA sources	**Fat**	**C16:0 Palmitic acid**	**C18:0 Stearic acid**	**C18:1 Oleic acid**	**C18:2 LA**	**C18:3 ALA**
Extruded Linseed	36.71	7.64	5.22	20.05	14.81	51.28
Extruded Soybean	18.88	9.65	4.43	21.64	50.21	4.90

Table 2: The exact amounts (gram) of ALA and LA included in experimental diets except Control group.

	Experimental diets (except Control group)		
FA profiles	**Ratio 1:1**	**Ratio 5:1**	**Ratio 10:1**
Extruded soybean added to experimental diet (g)	70	145	155
Extruded linseed added to experimental diet (g)	46	8	0
Total amount of FA in extruded soybean (g)	13.21	27.37	29.26
Total amount of FA in extruded linseed (g)	16.88	2.93	0
Total amount of FA in added FA sources	30.09	30.31	29.26
Total LA in extruded soybean added to experimental diet (g)	9.13	14.18	14.69
Total ALA in extruded linseed added to experimental diet (g)	9.30	2.84	1.43
Considered ratios of LA to ALA in FA sources	~1	~5	~10

Table 3: Ingredient and chemical composition of experimental diets from week's one to six.

Item	**Experimental diets***			
	Control	**Ratio 1:1**	**Ratio 5:1**	**Ratio 10:1**
Ingredient, g/kg of DM				
Alfalfa Hay	20.67	19.22	18.44	19.64
Wheat – Straw	20.88	22.05	23.74	23.85
Barley Grain	13.86	13.57	14.88	14.59
Corn Dry	8.61	8.48	5.79	5.61
Soybean Meal – 44	4.92	2.83	2.23	1.12
Wheat Bran	10.59	10.18	12.01	12.06
Rice Bran	18.13	15.83	13.13	13.19
Soybean – Extruded	0.00	3.70	7.58	8.14
Linseed - Extruded	0.00	2.34	0.41	0.00
Calcium – Carbonate	1.34	1.40	1.40	1.40
Salt	0.4	0.4	0.39	0.39
Chemical Components, % DM				
Protein	15.0	15.1	15.1	15.1
Neutral Detergent Fiber (NDF)	41.11	41.38	41.43	41.62
Fat	5.1	5.2	5.2	5.1
Ash	7.91	7.95	8.12	8.2

*Ratios 1:1, 5:1, and 10:1 consist of three different ratios of LA: ALA, respectively.

diets with LA and ALA leads to an augmentation in the proportion of vaccenic acid present in milk [16,28]. Vaccenic acid also serves as an intermediary fatty acid in the ruminal biohydrogenation pathway and is discernible in substantial quantities within ruminant adipose tissue [18]. In humans, rumenic acid (C18:2, *cis*-9 *trans*-11) can fulfill a plethora of functions, including anti-tumor, anti-diabetic, anti-inflammatory, immunomodulatory, and anti-Alzheimer diseases [30].

Table 4: Effects of different ratios of LA to ALA supplemented in lactating dairy ewes' diet on milk yield and milk composition.

Item	**Control**	**1:1**	**5:1**	**10:1**	**SEM**	**D**	**T**	**D×T**
Milk Yield (g/d)	0.266^{b}	0.321^{a}	0.301^{a}	0.310^{a}	0.007	<0.0001	0.278	0.498
Lactose (%)	4.63	4.82	4.61	4.68	0.561	0.061	0.091	0.367
Protein (%)	5.87^{c}	6.85^{a}	6.28^{b}	6.39^{b}	0.106	<0.0001	0.160	0.711
Solid non-fat (SNF) (%)	9.68	9.71	9.75	9.62	0.132	0.084	0.173	0.209
Fat (%)	7.64^{b}	7.86^{c}	7.97^{c}	8.25^{a}	0.078	<0.0001	0.012	0.890

^{a,b,c}In same rows means bearing different superscripts differ significantly (P<0.05).

Ewes fed with ratio 1:1 (LA to ALA) showed the highest LA proportion in milk. Previous studies have shown that including vegetable oils in the diet can also raise the content of LA in milk [32]. However, study involving cows had consumed encapsulated flaxseed oil illustrated no significant change in the LA profile of their milk [71]. A marked proportion of dietary LA is biohydrogenated via bacteria in the rumen environment [69]. Nonetheless, the processing of oilseeds (extrusion) could avoid this process completely in the rumen environment [27], leading to a part of the dietary LA being directed to the lower digestive tract and eventually observed in milk fat. Eventually, the UFAs reach the mammary gland, thereby increasing the concentration of UFAs in milk [54]. LA have showed a critical role in human nutrition as it has the potential to reduce plasma cholesterol and LDL concentrations, and it is also a precursor of 20-carbon fatty acids such as arachidonic and eicosapentaenoic acids [24].

The concentration of LA in milk tended to increase when ewes were fed higher levels of linseed (treatment 1:1; 1.8 g/100g milk). The explanation for the higher ALA levels in the milk in the 1:1 treatment compared to other groups might be due to the supplementation of the diet with higher levels of extruded linseed (a source of omega-3). A high percentage of dietary ALA is converted into intermediate and Saturated Fatty Acids (SFAs) in the rumen via biohydrogenation. According to previous reports, enriching rations with linseed is one of the best methods for higher ALA potrion in milk [16]. ALA in human nutrition has anti-inflammatory, neuroprotective, and antidepressant effects, and can reduce plasma LDL concentration [11,15].

Regarding the human diet, the recommended ideal ratio of omega-6 to omega-3 fatty acids ranges from 1-4:1 [55]. Hence, milk derived from ewes fed a diet containing a balanced ratio of omega-6 to omega-3 fatty acids, specifically a 1:1 ratio of LA: ALA seems considerably more beneficial for human consumption. It is necessary to incorporate sources of these essential fatty acids into the animals' diet for the aim of higher proportions in dairy products [70]. To achieve a lower ratio of omega-6 to omega-3 fatty acids in dairy products, various origins of omega-3 fatty acids (linseed or fish oil) can be supplemented in the livestock ration. In accordance with the findings of our review, Oliveira et al. (2021) reported the lowest ratio of omega-6 to omega-3 fatty acids (2.7:1) compared to other experimental groups by adding linseed oil to the diet of lactating dairy cows.

Blood Metabolites

Table 6 presents the impact of supplementation diets containing different ratios of LA: ALA on ewes' plasma metabolites. Our findings indicate significant difference (P<0.05) in HDL, LDL,

Table 5: Milk fatty acid profile in lactating dairy ewes fed diets with different ratio of LA to ALA.

Parameter (g/100 g milk fatty acid)	Experimental Diets[1]				SEM	Trt	Time	Trt×Time
	Control	1:1	5:1	10:1				
C4:0	2.4[b]	2.7[a]	2.6[a]	2.6[a]	0.036	<0.0001	0.09	0.01
C6:0	2.5	2.6	2.4	2.5	0.066	0.432	0.37	0.50
C8:0	2.8[a]	2.6[a]	2.3[b]	2.4[b]	0.042	<0.0001	0.57	0.69
C10:0	9.1[a]	7.1[b]	6.8[c]	6.9[c]	0.068	<0.0001	0.62	0.70
C12:0	5.1[a]	4.6[b]	4.3[c]	4.3[c]	0.056	<0.0001	0.93	0.44
C14:0	12.3[a]	10.2[b]	10.6[b]	10.5[b]	0.247	0.022	0.24	0.23
C16:0	25.4[a]	20.2[b]	19.9[b]	20.1[b]	0.253	<0.0001	0.27	0.49
C18:0	9.3[b]	10.4[a]	10.6[a]	10.5[a]	0.284	<0.0001	0.47	0.93
C18:1, *cis*-9	24.3[b]	26.4[a]	26.1[ab]	25.9[ab]	0.274	<0.0001	0.23	0.65
C18:1, *trans*-11	2.1[c]	4.7[a]	4.2[b]	4.3[b]	0.068	<0.0001	0.37	0.67
C18:2 (n-6)	1.8[b]	5.1[a]	5.0[a]	4.9[a]	0.049	<0.0001	0.61	0.74
C18:2, *cis*-9, *trans*-11	1.2[c]	1.4[b]	1.6[a]	1.7[a]	0.0499	<0.0001	0.81	0.86
C18:2 *trans*-10, *cis*-12	0.4[c]	0.6[a]	0.5[b]	0.6[a]	0.0312	0.0001	0.64	0.16
Total CLA[2]	1.6[c]	2[ab]	2.1[ab]	2.3[a]	0.0512	<0.0001	0.92	0.60
C18:3 (n-3)	1.1[b]	2.7[a]	2.6[a]	2.4[a]	0.070	<0.0001	0.14	0.52
Total saturated	68[a]	50.8[b]	50.7[b]	49.5[b]	0.875	<0.0001	0.29	0.87
Total unsaturated	31.8[b]	49.1[a]	49.2[a]	50.2[a]	0.821	<0.0001	0.42	0.25
MUFA[3]	18.7[b]	21.7[a]	20.9[ab]	21.2[a]	0.311	<0.0001	0.72	0.12
PUFA[4]	6.2[b]	8.3[ab]	8.4[ab]	9.2[a]	0.171	<0.0001	0.07	0.90
n-6/n-3 fatty acid ratio	7.41[a]	1.94[c]	2.48[c]	4.18[b]	0.218	<0.0001	0.87	0.71

[a,b,c]In same rows means bearing different superscripts differ significantly (P<0.05)
[1]Control = diet with no fatty acid resource supplementation. 1:1, 5:1, and 10:1 = experimental diets with ratios 1:1, 5:1, and 10:1 LA to ALA FAs, respectively.
[2]Conjugated LA
[3]Monosaturated FAs
[4]Polysaturated FAs

Table 6: Effects of different ratios of LA to ALA supplemented in lactating dairy ewes' diet on the concentrations of some plasma metabolites.

Item	Control	1:1	5:1	10:1	SEM	Trt	Time	Trt × Time
HDL, mg/d	55.59[b]	62.35[a]	56.19[b]	58.90[ab]	2.301	0.17	0.01	0.01
LDL, mg/d	25.69[a]	24.95[ab]	24.88[b]	25.09[ab]	0.919	0.01	0.04	0.35
VLDL, mg/d	4.26[a]	3.66[b]	3.86[ab]	3.77[b]	0.156	0.10	0.01	0.23
NEFA, mmol/L	0.39	0.38	0.37	0.38	0.023	0.94	0.01	0.99
BHBA, mmol/L	0.22	0.24	0.24	0.25	0.011	0.56	0.25	0.99
Triiodothyronine, mmol/dl	2.29	2.59	2.51	2.37	0.151	0.50	0.27	0.87
Thyroxin, µg/dl	10.87[b]	12.09[a]	11.63[ab]	12.58[a]	0.541	0.17	0.01	0.096

[a,b,c]In same rows means bearing different superscripts differ significantly (P<0.05).

VLDL, and T_4 levels among the different experimental treatments. Conversely, there was no difference between NEFA, BHBA, and T_3 plasma concentrations in experimental treatments (P>0.05). The plasma HDL portion was higher with the addition of protected fats against the Control [23,41]. In contrast, the effect of omega-3 fatty acid sources in the ration on plasma lipoprotein concentration did not indicate a remarkable change [10,20]. An increase in the intestinal secretion of lipoproteins with higher amounts of triglycerides caused a higher content in lipid metabolite of plasma concentration; therefore, adding fat supplements to the diet of ruminants has the possibility of stimulating the release of lipoprotein cholesterol content through the small intestine and increasing the circulation of plasma cholesterol, HDL, and LDL concentrations [40]. This mechanism can be related to the decline in levels of lipogenic enzymes in both liver and adipose tissue related to dietary fatty acid supplementation [51]. During Negative Energy Balance (NEB), VLDL plays a crucial role in ruminants by facilitating the transport of triglycerides from the intestine to the lymph and bloodstreams [2]. The regulation of fatty acid oxidation is influenced by the presence of malonyl-CoA (inhibitor of carnitine acyltransferase), preventing fat oxidation [57]. Triglycerides, after being esterified in the liver, have two possible outcomes: storage within the liver tissue (leading to fatty liver) or entry into peripheral tissues as VLDL particles. In the context of Negative Energy Balance (NEB), the increase in plasma NEFA concentration results in the accumulation of NEFA in liver tissue. Once in the liver, NEFA can undergo esterification or oxidation [4]. Esterification leads to the storage of triglycerides within the liver, while oxidation promotes the release of triglycerides from the liver through VLDL particles. Consequently, elevated plasma NEFA levels can contribute to an increased concentration of plasma VLDL. Diets enriched with fatty acids exhibited a linear effect on the concentration of T_4 hormone. The quantity and quality of the consumed feed play a significant role in determining the concentration of this thyroid hormone in ruminants [67]. Consequently, supplementing diets with sources of fatty acids led to an increment in plasma T_4 concentration in ewes [65]. The overall effects include an enhancement in basal metabolic rate, availability of more glucose to the cells, stimulation of protein synthesis, heightened lipid metabolism, and stimulation of cardiac and neural functions [60].

Conclusions

According to the obtained data, added protected fat sources (extruded linseed and soybean) into diets could have optimal effects on milk yield, lactose, protein, and fat concentrations. Adding sources of essential fatty acids to the diet illustrated positive effects on the content of essential fatty acids in milk (beneficial in human nutrition). In addition, compliance with different ratios of omega-6 to omega-3 fatty acids previously proven on human health, showed relatively optimal effects on some blood parameters (HDL, LDL, VLDL concentration) in ewes' diets. However, it seems that supplementation runinant ration can be considered the best method to boost the quality of livestock products (milk & and meat).

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