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Identifying the Influence of the Dietary Polyunsaturated Fatty Acid Content on Milk Quality Traits in Najdi Sheep at Different Stages of Lactation

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Abstract: The quality of milk is fundamental to producing safe milk and dairy products that are suitable for consumption. The objective of this study was to determine the influence that the content of dietary polyunsaturated fatty acids (PUFA) has on the total mixed ration (TMR) and lactation stage of milk's composition and the fatty acid profile (FA) of the dairy Najdi breed. During the lactation period, 48 Najdi ewes used the following four treatments: traditional feed (TR) and three total mixed rations (TMR1, TMR2, and TMR3) with different PUFA content. Milk samples were taken from whole milk in the morning. The chemical composition and FA profile were analyzed. The results show that the ewes fed TMR1 with a PUFA content (25.20%) produced high-quality milk as they contained high levels of polyunsaturated fatty acids (PUSF), linoleic acid (LA), α-linolenic acid (ALA) and omega-3 (n3). On the other hand, the milk of ewes fed TMR2 with PUFA (15.06%) contained high levels of unsaturated fatty acids (USF) and monounsaturated fatty acids (MUFA) and had significantly reduced hypercholesterolemia (HFA). It can be concluded that milk fat, protein, and total solids in the milk of Najdi sheep increased as lactation progressed, while lactose remained constant. Furthermore, as lactation progressed, saturated fatty acids (SFA), stearic acids, and oleic acids (OA) increased while USF and MUFA decreased. TMR diets can directly benefit the producer and livestock by producing high-quality milk while reducing its economic value.

Keywords: TMR; unsaturated fatty acid feed; stage of lactation; milk quality; Najdi sheep

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1. Introduction

Dairy production faces a major challenge in meeting the demand for high-quality foods with health benefits, especially given the increasing number of people affected by the obesity and diabetes epidemic worldwide, particularly in the Gulf region [1]. Milk quality is critical to producing safe and healthy milk and dairy products. To achieve this quality, appropriate feeding practices must be used at all stages of lactation. However, for milk to meet these nutritional expectations, it must meet a series of requirements, which are summarized as follows: quality, physicochemical composition, organoleptic properties, and the number of microorganisms present [2].

The current global livestock situation requires improved management that can enable the efficient use of available sustainable resources to meet the nutritional needs of most dairy animals [3]. To improve production parameters and profitability, nutrition must be optimized since it accounts for 60–70% of total costs. The introduction of pelleted feeds into the animal industry has become increasingly important in recent decades, as studies indicate that this leads to better feed efficiency [4]. In a study by Schingoethe [5], they found that TMR silage is an effective method for simplifying feed management on dairy farms. Furthermore, Ref. [6] found that TMR-fed sheep had higher concentrations of volatile fatty acids (VFAs) in the rumen after 2 to 4 h of feeding, with these VFAs related to the fat content in milk.

The addition of lipids with a high PUFA content from 2 to 5% in the diet of ruminants plays a crucial role as an energy source, reduces the risk of rumen acidosis, alters milk fatty acids, and reduces nutritional costs [7]. By contrast, the intake of large amounts of lipids in the diet of ruminants can influence the microbial population in the rumen and, thus, the fermentation parameters in the rumen and the digestion of feed components [8]. It has also been observed that adding fat to the diet reduces methane production [9]. Therefore, fermentation processes in the rumen are influenced by diet, especially oil supplementation, and the use of this strategy can improve milk quality [10]. Furthermore, Nudda et al. [11] mentioned that the rumen is considered the central axis for the control of PUFA components, especially conjugated linoleic acid (CLA), in milk and meat, as it is mainly responsible for converting feed nutrients into high-quality products.

Several studies have examined the effect of adding unsaturated fats of plant origin in the diet of sheep on the fatty acid composition of milk fat [12–14]. However, they were unable to determine the connection between the fatty acids in milk and the consumption of feed. On the other hand, the amounts of unsaturated lipids supplied to dairy sheep to meet their requirements are around 60 to 165 g/day [14].

Dairy sheep products are subject to several factors that negatively impact their productivity, efficiency, sustainability, and product quality. The main factors that stand out include management strategies, the quality of diet, and the stage of lactation [15]. The important physiological factor is the stage of lactation, as milk properties in sheep improve as lactation progresses, and sheep reach their maximum production a few weeks after birth. In addition, the FA profile of fatty milk is influenced by the physiological state of the animal; e.g., long-chain fatty acids (LCFA) were higher in ewe milk after lambing. As part of the negative energy balance (NEB), fatty acids were mobilized from the tissue and converted into milk fat [16].

Dairy fat and human health are related topics that are well-known to consumers. In addition, the consumption of dairy products containing PUFA, especially -ALA, n3, and CLA, has beneficial effects on human health and exhibits properties associated with anticancer, antiatherogenic, antidiabetic, and antiadipogenic effects [17,18]. By contrast, sheep's milk fat contains a higher proportion of SFA [19].

Changing milk composition and fatty acids is extremely effective through feeding strategies during the lactation stage. This study proposes highlighting the benefits of using a TMR as an important complete feed for livestock with high nutritional value through different amounts of PUFA. The aim of the study is to investigate the influence of the TMR PUFA content and lactation stage on the milk components and fatty acid profile of Najdi sheep in semi-intensive breeding.

2. Materials and Methods

2.1. Experimental Design

This study was conducted at Al-Ammariyah Farm, affiliated with the Animal Production Department of the College of Food and Agricultural Sciences, King Saud University, under the supervision of veterinarians, where the animals were observed during pregnancy and birth.

A total of 48 multiparous Najdi breed ewes were used before lambing and divided into four homogeneous groups of four replicates (with 3 ewes in the replicate) based on live weight (46.2 $\pm$ 5.8 kg). The total duration of the experiment was 90 days for sampling plus 30 days for pre-lambing. Feed given to the animals consisted of traditional feed (TR = alfalfa 60% and barley 40%) and a three total mixed ration (TMR) in the barn, which contained the following as a basis: barley (12%), alfalfa (4%), maize (57%), soybean meal (18%), palm oil (2.5%), molasses (6.25%), minerals, vitamins and salt (3.75%). Four experimental diets were formulated on a dry matter (DM) basis for smaller ruminants according to NRC [20] to meet animals' requirements at different stages of production. The feeds used differed in their fat content for PUFA (TR = 57.59; TMR1 = 25.20; TMR2 = 15.06; TMR3 = 10.14), as shown in Table 1. The animals were fed twice daily at 8:00 a.m. and 4:00 p.m. via feeding

troughs, and water was freely accessible for a longer period of time. The entire daily ration was offered at 4–5% of body weight (BW).

Table 1. An analysis of the chemical composition and FA profile (g/100 g of FA) for traditional feed (TR) (barley and alfalfa hay) and total mixed ration (TMR1, TMR2 and TMR3) on a dry matter basis.

Diets	TR	TMR1	TMR2	TMR3
Chemical components %				
Dry matter %	90.97	93.16	92.94	93.26
Crude protein %	14.68	12.70	12.2	12.95
Fat %	0.71	2.43	2.36	2.31
EE %	2.88	2.48	2.63	2.28
NDF %	37.23	40.77	40.25	41.81
ADF %	20.15	26.47	26.25	26.65
Ash %	6.06	10.35	9.89	10.01
Calcium (%)	0.70	2.24	2.16	2.12
Phosphorus (%)	0.29	0.57	0.4	0.64
Fatty acids composition %				
C6:0; caproic acid	0.02	Nd	0.07	Nd
C8:0; caprylic acid	0.21	0.34	0.27	0.38
C10:0; capric acid	Nd	0.50	0.35	0.60
C12:0; lauric acid	1.67	9.11	6.53	11.26
C14:0; myristic acid	2.05	3.74	2.76	4.61
C16:0; palmitic acid	20.43	15.25	14.33	14.89
C16:1 cis 9; palmitoleic acid	Nd	0.28	0.31	0.27
C17:0; margaric acid	0.43	0.31	Nd	Nd
C18:0; stearic acid	6.12	4.13	4.02	3.425
C18:1 trans 9	1.66	0.81	Nd	Nd
C18:1 cis 9; oleic acid	8.65	33.96	33.68	32.12
C18:2 cis 9, 12 (LA)	34.46	23.69	14.04	9.645
C18:3 cis 9, 12, 15 (ALA)	21.45	1.51	1.01	0.49
C20:0; arachidic acid	2.43	0.59	0.62	0.515
C22:0; heneicosylic acid	0.97	0.36	0.40	0.265
C24:0; lignoceric acid	0.615	0.36	0.39	0.31
SFA; saturated fatty acid	30.70	34.69	29.78	36.27
UFA; unsaturated fatty acid	72.02	60.84	49.06	42.53
MUFA; monounsaturated fatty acid	14.44	35.64	34.00	32.40
PUFA; polyunsaturated fatty acid	57.59	25.20	15.06	10.14

EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; LA = linolenic acid; ALA = α-linoleic acid; Nd = not detected.

2.2. Dietary Analysis

Samples (300 g) were collected three times per month during the lactation period. To analyze the chemical composition, all samples were dried in an air oven at 60–65 °C for 48 h and then ground with a grinder to a mesh size of 1 mm. Each sample was analyzed in triplicate for dry matter (DM), crude protein (CP), and ether extract (EE) using methods described by the AOAC [21]. The neutral detergent fibers (NDF) and acidic detergent fibers (ADF) to the crude fiber were determined according to Van Soest et al. [22] and were analyzed using the sequential method. The crude protein (CP) was analyzed using the micro-Kjeldahl technique, and the nitrogen N obtained was converted into the crude protein by a factor of 6.25. Fat extraction from the feed with the Auto Soxhlet fat extractor using Hexane 95% determined the crude fat concentration in the feed. The ash was determined by complete combustion in a muffle furnace [21]. Fatty acids (FA) in fat-containing diets were measured using gas chromatography-mass spectrometry (GC mass) and methylation, as described by Sbihi et al. [23].

2.3. Analysis of Milk Composition

The milk samples were placed into 50 mL tubes at 8:00 a.m., and then two subsamples per animal were formed for each sampling time. One of them was stored at minus 4 °C

for the analysis of the fat, protein, total solids, solids not fat (SNF), and freezing point depression (FPD) using Milko_Scan technology and the Fossomatic Minor (Fossomatic 90, FOSS Electric, Hillerød, Denmark) to calculate the number of somatic cells (SCC) as the number/mL. Another sample was stored at minus 20 °C for the subsequent analysis of FA using gas chromatography-mass spectrometry.

2.4. Analysis of FA in Milk

The analysis of FA in the milk was carried out using 0.5 g of milk fat, which was converted to a fatty acid methyl ester (FAME) by adding 1 mL of hexane at 95%, then 1 mL of HCl and 1 mL of $NaOH_2$ before being placed in a water bath and mixed at 50 °C for 15 s, as described by Sibhi et al. [23]. After sampling, the precipitate formed 1 mL on the top layer of the sample and was added to the GC–MASS test tube, and 1 mL of 99% pure hexane was added. Instrumental analysis using GC-MS (gas chromatography-mass spectrometry) _GC_MSQP2010-Shimadzu, Kyoto, Japan) was performed on an Agilent_6890 gas chromatograph coupled to 5973-MSD, which operated in the electron impact mode at 70_eV of ion source energy, a wavelength an m/z range of 50 to 500, and a speed of 1666 scans/min. The thickness of the capillary column film DB_5MS (Agilent) was (100 m × 0.25 mm × 0.20 m). The temperature for the injector and detector was between 220 °C and 280 °C. The carrier gas was helium and was maintained at a stable flow of 1.0 mL/minute. One liter of the sample was injected at a ratio of 1:100. The temperature of the column was held at 60 °C for 1 min, then increased from 60 °C to 165 °C at a rate of 13 °C/min and held for 10 min. Finally, the temperature increased from 165 °C to 280 °C at a rate of 4 °C/min and was held for 30 min. The GC-MS Chem_Station data system was used to acquire and process mass spectrometric data (Riyadh-Saudi Arabia). The total amount of fatty acids was expressed as a percentage. The fatty acid indices were calculated as follows: atherogenic index = (C12:0 + (4 C14:0) + C16:0)/(MUFA + PUFA); Thrombogenicity index = (C14:0 + C16:0 + C18:0)/(0.5 MUFA) + (0.5 × n − 6) + (3 × n − 3) + (n − 3:n − 6), according to Chilliard et al. [24] and hypercholesterolemic FA (HFA) = the sum of C12:0, C14:0 and C16:0 according to Ulbricht and Southgate [25]. The transfer rate of essential fatty acids was measured using the FA output in milk divided by dietary fatty acid intake, as described in [26].

2.5. Statistical Analysis

Data were analyzed using the SAS program (SAS Institute, version 9.4). The completely randomized design using the Proc mixed procedure was used to analyze the results with animals included as a random effect.

The model equation can be described as follows: $Y_{ij} = \mu + TMR_{si} + LS_{ej} + Error_{ij}$ where μ is the overall mean, TMR_{si} is the effect of the treatment diet (TR, TMR1, TMR2, and TMR3), LS_{ej} is the effect the lactation stage (days 30, 60, and 90), and $Error_{ij}$ is the experimental error. The effects of diets and lactation stage were considered as fixed effects. Differences between means were compared using Tukey's test and reported at a significance level of $p < 0.05$.

3. Results

3.1. Diet Composition

The chemical composition of the experimental diets is shown in Table 1. The nine samples (three replicates of all diets each month) were analyzed. The TR diet had a higher protein content, 14.03%, compared to the TMR diets. By contrast, the proportions of NDF and ADF were lower in the TR diet than in the TMR diets, while the energy content of the diets was similar. Furthermore, the percentage of fat extraction was 70.78%, which was higher in TMR diets than TR diets. Based on the fatty acid profile of the diets, the ratio of LA and ALA was higher in TR diets (34.46 and 21.45, respectively) than in TMR diets. By contrast, the TMR diets contained high amounts of OA. Additionally, UFA and PUFA

levels were 25 to 1 and 2.5 to 1 higher in the TR diet than in the TMR diets, respectively. By contrast, MUFA was 2.2 to 1 higher in TMR diets than TR diets.

3.2. Influence of Diets on Milk Quality

Milk components were influenced by the different PUFA content of the feeds (Table 2). The TR diet showed a significant effect ($p < 0.05$) on the milk's fat and total solids (5.25% and 14.42%, respectively), whereas TMR3 showed a significant effect ($p < 0.05$) on the milk's proteins and SNF (4.67% and 9.75%, respectively), as shown in Table 2.

Table 2. Influence of dietary PUFA content on the milk components of Najdi ewes fed traditional and TMR diets.

Components	Dietary Treatments [1]				SEM	*p* Value
	TR	TMR1	TMR2	TMR3		
Fat %	5.25 [a]	4.18 [c]	4.95 [b]	4.13 [c]	0.35	0.024
Protein %	3.99 [c]	4.14 [b]	4.18 [b]	4.67 [a]	0.13	0.003
Lactose %	4.42	4.50	4.13	4.52	0.17	0.39
Total soled	14.42 [a]	13.13 [c]	13.18 [bc]	13.68 [b]	0.38	0.048
SNF	9.23 [b]	9.22 [b]	8.81 [c]	9.75 [a]	0.19	0.014
FPD	0.57	0.58	0.53	0.56	0.02	0.25
SCC	600.62	549.65	646.07	538.92	107.17	0.93

[a–c] within a row, the means with and without a common superscript differ ($p < 0.05$). [1] Dietary treatments are as follows: TR = a traditional feed (basal diet alfalfa and barley) PUFA = 57.59 g/100 g); TMR1 = TMR diet with PUFA = 25.20 g/100 g); TMR2 = TMR diet with PUFA = 15.06 g/100 g; TMR3 = TMR diet with PUFA = 10.14 g/100 g. SEM = standard error of mean; SNF = solids not fat; FPD = freezing point depression; SCC = somatic cell count.

The individual FA profile and total FA for the milk fat of ewes fed TR and TMR diets are shown in Tables 3 and 4. The TMR1 diet with a PUFA content of 25.20% showed a significant effect ($p < 0.05$) on AL, Rumenic acid (conjugated linoleic acid-CLA), PUFA, and total omega-3 (n3), while the TMR2 diet with a PUFA content of 15.06% showed a significant increase ($p < 0.05$) in UFA, MUFA and the atherogenicity index (AI). Furthermore, the TMR3 diet with a PUFA content of 10.14% showed a significant increase ($p < 0.05$) in small-chain fatty acids (SCFA: C6:0, C8:0, C12:0, and C14:0), C16:1 cis9, vaccenic acid (VA), SFA and HFA. By contrast, the TR diet with a higher PUFA content of 57.59% showed a significant effect ($p < 0.05$) on stearic acid and OA, as shown in Table 3.

A summary of the intake, production in milk, and transfer rates of essential fatty acids (EFA) and UFA from the feed to milk is shown in Table 5. As found, OA transfer rates were highest in ewes fed TR diets ($p < 0.05$) by rates of (227.9%) compared to TMR diets (57.51%, 73.87, and 58.29, respectively). On the other hand, the transfer rates of LA, ALA, and UFA were higher ($p < 0.05$) in ewes fed TMR diets than in the TR diet, as shown in Table 5.

3.3. Influence of Lactation Stage on Milk Quality

In the current study, the stage of lactation showed no effect on milk composition ($p > 0.05$), except for the protein content, which increased significantly ($p < 0.05$) as lactation progressed, as shown in Table 6. The fatty acid profile in milk fat changed with the stage of lactation, as shown in Table 7. Medium chain fatty acids (MCFA) such as myristic acid; C14:0, myristoleic acid; C14:1, isopentadecylic acid; C15:0 iso, palmitic

acid; C16:0, palmitoleic acid; and C16:1 cis9 increased significantly ($p < 0.05$) from early to late lactation. On the other hand, long chain fatty acids (LCFA), including stearic acid, C18:1 trans8, C18:1 trans13, oleic acid, and C18:2 trans9 trans12, were significantly reduced ($p < 0.05$) as lactation progressed; this is shown in Table 7. In addition, Table 8 shows the total fatty acid profile, such as UFA, MUFA, and PUFA, which were affected by the lactation stage and significantly decreased ($p < 0.05$) from early to late lactation. By contrast, AI increased significantly ($p < 0.05$) with continued lactation.

Table 3. Influence of dietary PUFA content on the fatty acid profile (g/100 g FA) of milk fat from Najdi ewes fed traditional and TMR diets.

Fatty Acid Profiles	Dietary Treatments [1]				SEM	*p* Value [2]
	TR	TMR1	TMR2	TMR3		
C6:0	1.46 ^{a}	1.20 ^{b}	1.21 ^{b}	1.46 ^{a}	0.08	0.02
C8:0	1.82 ^{ab}	1.34 ^{c}	1.52 ^{b}	1.90 ^{a}	0.18	0.01
C10:0	5.92	5.98	5.13	6.92	0.48	0.09
C11:0	0.15	0.22	-	0.15	0.02	0.06
C12:0	3.77 ^{c}	6.22 ^{ab}	5.41 ^{b}	7.33 ^{a}	0.45	<0.0001
C14:0	10.68 ^{c}	13.11 ^{ab}	12.58 ^{b}	14.04 ^{a}	0.64	0.01
C14:1	0.12 ^{b}	0.20 ^{a}	-	0.21 ^{b}	0.01	0.03
C15:0 iso	0.32 ^{b}	0.44 ^{a}	0.29 ^{c}	0.40 ^{ab}	0.03	0.002
C15:0	0.82 ^{ab}	0.88 ^{a}	0.49 ^{c}	0.76 ^{b}	0.08	0.005
C16:0	31.31	29.85	29.47	29.99	1.23	0.51
C16:1 cis7	0.25	0.22	0.24	0.23	0.02	0.35
C16:1 cis9	1.31 ^{c}	1.56 ^{b}	1.50 ^{bc}	1.64 ^{a}	0.06	0.005
C17:0 iso	0.35 ^{a}	0.24 ^{bc}	0.28 ^{b}	0.19 ^{c}	0.02	<0.0001
C17:0	0.84 ^{a}	0.59 ^{bc}	0.68 ^{b}	0.41 ^{c}	0.07	0.006
C17:1	0.36	0.34	0.37	0.21	0.04	0.05
C18:0	11.09 ^{a}	9.01 ^{bc}	10.34 ^{b}	7.27 ^{c}	1.27	0.001
C18:1 trans 8	0.30	0.37	0.31	0.40	0.04	0.27
C18:1 trans 11(VA)	0.66 ^{c}	1.89 ^{b}	1.76 ^{bc}	2.70 ^{a}	0.26	<0.0001
C18:1 trans 13	0.27 ^{c}	0.33 ^{b}	0.46 ^{a}	0.27 ^{c}	0.04	0.003
C18:1 trans 16	0.50 ^{b}	0.68 ^{a}	0.38 ^{c}	0.52 ^{b}	0.06	0.002
C18:1 cis 9 (OA)	25.49 ^{a}	19.50 ^{bc}	24.39 ^{b}	18.73 ^{c}	1.34	<0.0001
C18:1 cis11	0.48 ^{c}	0.76 ^{b}	0.91 ^{a}	0.65 ^{bc}	0.04	<0.0001
C18:1 cis13	0.19 ^{c}	0.49 ^{a}	0.23 ^{bc}	0.29 ^{b}	0.03	<0.0001
C18:1 cis14	0.27 ^{a}	0.17 ^{b}	0.16 ^{bc}	0.14 ^{c}	0.03	0.01
C18:2 trans9 trans12	0.24	0.20	0.25	0.20	0.04	0.06
C18:2 cis9 cis 12 (LA)	2.65 ^{bc}	3.35 ^{a}	2.93 ^{b}	2.60 ^{c}	0.20	0.01
C18:3 cis9 cis12 cis15 (ALA)	1.05	1.29	1.17	1.20	0.13	0.44
C18:2 cis9 trans11 (CLA)	0.43 ^{bc}	0.50 ^{a}	0.47 ^{b}	0.29 ^{c}	0.04	0.02
C20:0	0.20	0.19	0.17	0.14	0.02	0.06
C20:4 cis5 cis8 cis11 cis14	0.26	0.28	0.29	0.29	0.03	0.82

$^{a–c}$ within a row, the means with and without a common superscript differ ($p < 0.05$). [1] Dietary treatments are as follows: TR = a traditional feed (basal diet alfalfa and barley) PUFA = 57.59 g/100 g; TMR1 = TMR diet with PUFA = 25.20 g/100 g; TMR2 = TMR diet with PUFA = 15.06 g/100 g; TMR3 = TMR diet with PUFA = 10.14 g/100 g. SEM = standard error of mean; OA = oleic acid; VA = vaccenic acid; LA = linolenic acid; ALA = alpha linoleic acid; CLA = conjugated fatty acid. [2] dietary treatment effect ($p < 0.05$).

Table 4. Influence of dietary PUFA content on the total fatty acid profile (g/100 g FA) of milk fat from Najdi ewes fed traditional and TMR diets.

FA Groups [1]	Dietary Treatments [2]				SEM	*p* Value [3]
	TR	TMR1	TMR2	TMR3		
SFA	67.46 ^{b}	69.67 ^{ab}	67.11 ^{c}	71.25 ^{a}	2.98	0.03
UFA	32.50 ^{ab}	29.15 ^{b}	33.97 ^{a}	28.63 ^{c}	1.38	0.04
MUFA	28.83 ^{ab}	24.30 ^{c}	30.39 ^{a}	25.32 ^{b}	1.54	0.04
PUFA	4.20 ^{ab}	4.45 ^{a}	3.91 ^{b}	3.31 ^{c}	0.29	0.05
n3	2.83 ^{c}	3.65 ^{a}	3.17 ^{b}	2.75 ^{c}	0.17	0.01
n6	1.11	1.43	1.27	1.31	0.13	0.19
n6/n3	2.88	2.96	2.62	2.21	0.23	0.13
OCFA	3.73	3.19	3.33	2.77	0.27	0.08
AI	2.52 ^{c}	3.47 ^{ab}	3.51 ^{a}	3.34 ^{b}	0.28	0.02
T1	1.50	1.56	1.49	1.48	0.21	0.50
HFA	43.16 ^{b}	49.27 ^{ab}	42.40 ^{c}	51.41 ^{a}	2.40	0.03

$^{a–c}$ within a row, the means with and without a common superscript differ ($p < 0.05$). [1] = Values are FA; [2] Dietary treatments: TR = a traditional feed (basal diet alfalfa and barley) PUFA = 57.59 g/100 g; TMR1 = TMR diet with PUFA = 25.20 g/100 g; TMR2 = TMR diet with PUFA = 15.06 g/100 g; TMR3 = TMR diet with PUFA = 10.14 g/100 g; SEM = standard error of mean; SFA = saturated fatty acid; UFA = unsaturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; n3 = omega-3; n6 = omega-6; OCFA: odd chain fatty acid; AI = Atherogenicity index; TI = Thrombogenicity index; HFA = hypercholesterolemic FA. [3] dietary treatment effect ($p < 0.05$).

Table 5. Influence of dietary PUFA content on the transfer rate of essential fatty acids (OA, LA, ALA and UFA) into the milk of Najdi ewes that fed traditional (TR) and TMR diets.

FA Parameters	Dietary Treatments [1]				SEM	*p* Value [2]
	TR	TMR1	TMR2	TMR3		Diets
Oleic acid (OA)						
Intake g/100 g	8.65 ^{c}	33.95 ^{a}	33.68 ^{ab}	32.12 ^{b}	0.26	<0.0001
Output g/100 g	19.72 ^{b}	19.53 ^{b}	24.88 a	18.73 ^{c}	1.08	<0.0001
Transfer rate %	227.9 ^{a}	57.51 ^{c}	73.87 ^{b}	58.29 ^{c}	6.21	<0.0001
Linoleic acid (LA)						
Intake g/100 g	34.46 ^{a}	23.69 ^{ab}	14.04 ^{b}	9.64 ^{c}	1.27	<0.0001
Output g/100 g	2.58 ^{c}	3.34 ^{a}	2.91 ^{b}	2.57 ^{c}	0.20	0.01
Transfer rate %	7.512 ^{c}	14.07 ^{bc}	20.54 ^{b}	26.43 ^{a}	1.04	<0.0001
α-linolenic acid (ALA)						
Intake g/100 g	21.45 ^{a}	1.51 ^{b}	1.02 ^{b}	0.49 ^{c}	0.70	<0.0001
Output g/100 g	1.06 ^{c}	1. 29 ^{a}	1.17 ^{b}	1.20 ^{b}	0.09	0.02
Transfer rate %	5.59 ^{c}	59.95 ^{a}	11.65 ^{c}	31.92 ^{b}	3.31	<0.0001
Unsaturated fatty acid (UFA)						
Intake g/100 g	72.02 ^{a}	60.84 ^{b}	49.06 ^{c}	42.53 ^{c}	1.91	<0.0001
Output g/100 g	32.98 ^{ab}	29.25 ^{b}	33.88 ^{a}	28.65 ^{c}	1.68	0.002
Transfer rate %	45.89 ^{c}	46.80 ^{b}	68.93 ^{a}	67.35 ^{ab}	2.83	<0.0001

$^{a–c}$ within a row, the means with and without a common superscript differ ($p < 0.05$). [1] Dietary treatments are as follows: TR = a traditional feed (basal diet alfalfa and barley) PUFA = 57.59 g/100 g; TMR1 = TMR diet with PUFA = 25.20 g/100 g; TMR2 = TMR diet with PUFA = 15.06 g/100 g; TMR3 = TMR diet with PUFA = 10.14 g/100 g. SEM = standard error of mean; OA = oleic acid; LA = linolenic acid; ALA = α linoleic acid; UFA = unsaturated fatty acid; [2] dietary treatment effect ($p < 0.05$).

Table 6. Influence of lactation stage on the milk components of Najdi ewes fed traditional and TMR diets.

Components [1]	Stage of Lactation [2]			SEM	*p* Value [3]
	Frist	Mid	Late		
Fat %	4.46	4.81	4.63	0.32	0.656
Protein %	3.97 [c]	4.09 [b]	4.69 [a]	0.12	<0.0001
Lactose %	4.51	4.43	4.24	0.16	0.45
Total soled	13.53	13.52	13.75	0.37	0.88
SNF	9.14	9.11	9.52	0.18	0.17
FPD	0.57	0.55	0.55	0.02	0.59
SCC	531.39	686.49	533.57	86.29	0.54

[a–c] within a row, the means with and without a common superscript differ ($p < 0.05$). [1] milk components value, [2] stages of lactation (first = 30 days, mid = 60 days and late = 90 days). SEM = standard error of mean; SNF = solids not fat; FPD = freezing point depression; SCC = somatic cell count. [3] Stage of lactation effect ($p < 0.05$).

Table 7. Effect of lactation stage on the fatty acid profile (g/100 g FA) of milk fat in Najdi ewes fed traditional and TMR diets.

Fatty Acid Profile [1]	Stage of Lactation [2]			SEM	*p* Value [3]
	Frist	Mid	Late		
C6:0	1.34	1.37	1.29	0.06	0.71
C8:0	1.66	1.72	1.54	0.12	0.49
C10:0	5.47	6.06	6.43	0.39	0.24
C11:0	0.15	0.17	0.18	0.02	0.68
C12:0	5.16	5.84	6.05	0.45	0.22
C14:0	11.30 [c]	13.26 [a]	13.25 [a]	0.62	0.04
C14:1	0.10 [b]	0.22 [a]	0.22 [a]	0.01	0.001
C15:0 iso	0.31 [c]	0.38 [b]	0.41 [a]	0.02	0.02
C15:0	0.71	0.70	0.81	0.07	0.44
C16:0	28.65 [c]	30.49 [b]	31.35 [a]	0.79	0.05
C16:1 cis7	0.21 [c]	0.25 a	0.24 [b]	0.01	0.04
C16:1 cis9	1.34 [c]	1.57 [b]	1.59 [a]	0.05	0.001
C17:0 iso	0.28	0.24	0.27	0.02	0.16
C17:0	0.65	0.63	0.61	0.06	0.93
C17:1	0.28	0.35	0.34	0.04	0.38
C18:0	10.34 [a]	9.65 [b]	8.25 [c]	0.71	0.03
C18:1 trans 8	0.40 [a]	0.30 [c]	0.33 [b]	0.04	0.04
C18:1 trans 11(VA)	1.89	1.64	1.74	0.21	0.74
C18:1 trans 13	0.30 [b]	0.44 [a]	0.26 [c]	0.03	0.003
C18:1 trans 16	0.55	0.46	0.55	0.05	0.20
C18:1 cis 9 (OA)	25.23 [a]	22.04 [b]	19.81 [c]	1.01	0.001
C18:1 cis11	0.70	0.69	0.72	0.03	0.83
C18:1 cis13	0.31	0.27	0.32	0.03	0.46
C18:1 cis14	0.21	0.16	0.19	0.02	0.26
C18:2 trans9 trans12	0.23 [b]	0.19 [c]	0.25 [a]	0.03	0.01
C18:2 cis9 cis 12 (LA)	3.08	2.87	2.70	0.17	0.20
C18:3 cis9 cis12 cis15 ALA	1.13	1.23	1.18	0.11	0.78
C18:2 cis9 trans11 CLA	0.45	0.42	0.39	0.04	0.53
C20:0	0.18 [b]	0.15 [c]	0.20 [a]	0.01	0.05
C20:4 cis5 cis8 cis11 cis14	0.32	0.27	0.25	0.02	0.14

[a–c] within a row, the means with and without a common superscript differ ($p < 0.05$). [1] Values of FA at different, [2] lactation stages (first = 30 days, mid = 60 days and late = 90 days). SEM = standard error of mean; VA = vaccenic acid; OA = oleic acid; LA = linolenic acid; ALA = alpha linoleic acid; CLA = conjugated fatty acid. [3] Stage of lactation effect ($p < 0.05$).

Table 8. Effect of lactation stage on the total fatty acid profile (g/100 g FA) of milk fat from Najdi ewes fed traditional and TMR diets.

FA Groups [1]	Stage of Lactation [2]			SEM	*p* Value [3]
	Frist	Mid	Late		
SFA	65.34	70.20	69.09	2.26	0.26
UFA	34.69 [a]	30.63 [b]	27.37 [c]	1.33	0.002
MUFA	30.17 [a]	26.21 [b]	23.71 [c]	1.42	0.001
PUFA	4.58 [a]	3.75 [b]	3.55 [c]	0.24	0.004
n6	3.34	2.98	2.97	0.14	0.09
n3	1.28	1.33	1.24	0.08	0.81
n6/n3	2.94	5.61	2.45	0.23	0.20
OCFA	3.42	3.16	3.13	0.23	0.60
AI	2.66 [c]	3.62 [a]	3.35 [b]	0.22	0.01
TI	1.34	1.53	1.60	0.12	0.37
HFA	45.07	46.36	48.25	2.17	0.54

[a–c] within a row, the means with and without a common superscript differ ($p < 0.05$). [1] = Values are the sum of FA; [2] Stages of lactation, including (first = 30 days, mid = 60 days and late = 90 days). SEM = standard error of mean; SFA = saturated fatty acid; UFA = unsaturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; n3 = omega-3; n6 = omega-6; OCFA: odd chain fatty acid; AI: Atherogenicity index; TI: Thrombogenicity index; HFA: hypercholesterolemic FA. [3] Stage of lactation effect ($p < 0.05$).

4. Discussion

The increased content of essential fatty acids (EFA) such as ALA, CLA, and n3 fatty acids in the fat of sheep's milk improves the FA profile and makes it healthier by reducing SFA and increasing UFA. Among the alternatives considered, supplementing animal diets with oils rich in MUFA and PUFA is one of the strategies that has yielded the most interesting results. From this point on, the central theme of this work is to determine the change in milk's fatty acid profile due to the dietary content of different PUFA levels during the lactation stage [19]. However, studies in sheep [27–29] and cows [30] when fed diets high in LA showed no adverse changes in rumen microbial activity, milk yield, and milk composition.

In the current study, a trend toward the higher production of fat and total solids ($p < 0.05$) was observed in the milk composition of TR-fed ewes. This increase in productivity could be due to the higher fiber content of the fed ration, which provides an optimal environment for digestion in the rumen (higher pH values), resulting in higher acetic acid production, promoting lipogenic metabolic pathways, and resulting in a higher milk fat content [11]. These results are similar to those of Angeles-Hernandez et al. [31], where high forage feeding in dairy sheep had a positive influence on the fat content of the milk compared to concentrated feed. By contrast, the protein content of Najdi ewe's milk increased significantly ($p < 0.05$) when fed the TMR3 diet and decreased when fed the TR diet. This result is consistent with results reported in dairy sheep, where reduced protein content ($p < 0.05$) in the milk is associated with increased forage feed intake [31]. This fact has been described in animal nutrition, possibly due to the rapid fermentation of carbohydrates and the production of propionate and microbial proteins. This leads to an increasing insulin concentration, milk, and milk protein [32]. Another result was found in the study of Najdi sheep, where milk proteins were higher in ewes that consumed more forage feed [33].

It is worth noting that the SCC was below the natural range of 800,000/mL, meaning that the SCC decreased, and the ewes were free of udder mastitis. This result reported by [34] shows that an oil-containing diet in sheep and goat's milk showed promising results when reducing SCC.

As a result of feeding, the main changes in the milk lipid FA profile of Najdi dairy sheep fed with TMR1 increased ($p < 0.05$) in LA, CLA, C15:0, C15: iso, n3, and PUFA. By contrast, the content of long-chain fatty acids (LCFA), such as stearic acid and oleic acid, increased ($p < 0.05$) in Najdi dairy sheep fed TR.

This result could be caused by the entry of large amounts of 18-carbon fatty acids into the mammary gland, as reported by Bionaz et al. [35], where LCFA could inhibit the enzymatic activities involved in the de novo synthesis of short and medium-chain fatty acids, particularly acetyl-CoA carboxylase. On the other hand, the high content of stearic acid in milk fat could be a consequence of rumen hydrogenation for OA contained in the rations, depending on the physiological conditions of the rumen [12]. Furthermore, the increased content of OA in the milk fat of sheep fed the TR diet with a high transfer ratio of 227.9%; a lower content of this fatty acid in the diet may be due to a combination of factors through biohydrogenation in the rumen and the activity of the enzyme Δ-9-desaturase in the mammary gland, leading to the desaturation of stearic acid [36]. In cattle, it has been documented that approximately 40% of stearic acid entering the mammary gland is desaturated to OA by the action of the enzyme Δ-9 desaturase [37]. In this study, the TMR1 diet resulted in superior milk in terms of nutraceutical quality due to a higher concentration of PUFA and n3.

This study shows the negative effect of high levels of ALA, LA, and PUFA in the TR diet, which resulted in lower VA, LA, ALA, and PUFA levels in ewes' milk when fed a TR diet in comparison to TMR diets. This result explains that PUFA in the TR diet is not available for milk fat synthesis, as well as the effects of PUFA uptake on the function of membranes and cell signaling, as well as the regulation of gene expression via various transcription factors. As a result, PUFA affects virtually every cell in the body [38]. Other studies reported that a higher ALA content in the milk of cows and sheep was due to a feed high in PUFA [12,36]. This could be attributed to the influence of the rumen environment and pH; as mentioned by Tsiplakou et al. [39], the biohydrogenation process occurs in the rumen to convert ALA into VA. The other process was the conversion of VA to CLA via delta-9 desaturase in the mammary gland. Furthermore, fat supplementation with LA in the diet of lactating animals influences microbial activity in the rumen, and this process influences the CLA and ALA content in milk [40].

Based on human health, AI, TI, and HFA levels in ewes' milk were in the natural range for all diets during the lactation period. However, ewes fed TMR2 produced healthier milk as HFA was 42.40% lower which, in turn, led to a high MUFA content in milk fat.

Depending on the stage of lactation, milk production in Najdi ewes' peaks in the third week of lactation and gradually decreases continuously thereafter [41]. In general, peak milk secretion occurs between the 2nd and 4th week of lactation in various sheep breeds (17). The chemical composition of milk from Najdi ewes showed that the proportions of fat, protein, and total solids increased at the end of the lactation period. This is logical because, in general, the concentration of total solids and fat increases as milk production decreases; this was reported in different breeds [42], including breeds that specialize in milk production [42,43], and in goats [44], which increased over time and can be related to the quality of milk [45]. It is noteworthy that in the present study, the proportion of lactose remained constant over time in contrast to an increase in other components over the stage of lactation. This could represent an important advantage for the nutritional properties of milk from Najdi breeds. However, further research is recommended to confirm this finding.

The distribution of FA content in milk fat at each stage of lactation showed that C14:0, C16:0 and SFA increased as lactation progressed. This result is consistent with the report by Salari et al. [46] in Assaf ewes, [47] Wallachian sheep and goats [44]. On the other hand, the proportion of stearic acid, OA, UFA, PUFA, and MUFA decreased significantly as lactation progressed. Studies in Wallachian sheep [47] and Tisgay ewes [48] were identical to our results and found that UFA and MUFA decreased as lactation progressed. In contrast to the results obtained, Mel'uchová et al. [49] in the dairy sheep of the Valachian, Tsigai and Lacaune breeds and in goats by Currò et al. [44] found an increase in USF and MUFA as lactation progressed. On the other hand, Ref. [46] found no differences in the milk content of USF and MUFA as lactation progressed in Assaf ewes. Based on OA, the same results were reported by De La Fuente et al. [47], who observed no change in the OA concentration of Wallachian sheep milk during the lactation period. In general, FA concentration and

milk components were influenced by animal physiology at different stages of lactation and were related to milk yield due to dilution.

One of the biggest problems we faced was that the ewes did not accept machine milking and were forced to milk manually, making it impossible to determine the milk yield. In addition, the weaning period was 90 days. In addition, the elimination **of rumen** samples to estimate biological processes and volatile fatty acids, which would have been ineffective, provides a great impetus to this work.

5. Conclusions

The TMR diets with a higher PUFA content can be explained by the high content of essential FAs such as LA, CLA, n3, and PUFA in the milk fat of the Najdi breed. The TMR1 diet contained high amounts of n3 and PUFA in milk fat, while the proportion of HFA was lower in the TMR2 diet. Furthermore, the milk content of OA, UFA, MUFA, and PUFA decreased as lactation progressed. TMR diets can directly benefit the producer and livestock by producing high-quality milk while reducing production costs. For this reason, it is recommended to mix all feed ingredients in the form of pellets with a high-fat content in PUFA during the different stages of lactation in order to improve the properties of the milk while promoting its production.

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