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How the milk chemical composition and fatty acid profile are influenced by physiological factors in Najdi dairy sheep

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Abstract: Sheep milk production is a complex process that is influenced by various factors. This study aims to investigate how the litter size (single vs. twins), birth type (male vs. female), age of the ewes and body weight affect the milk composition and fatty acid profile of milk fat. Milk samples were collected from 119 Najdi ewes that were the subject of this study. Gas chromatography-mass spectrometry (GC-MASS) was used to quantify the fatty acids (FAs). The results showed that the twins birth type (female/female) has a significant influence ($P < 0.05$) on the concentration of linoleic acid (LA), alpha-linolenic acid (ALA), behenic acid (C22:0) and polyunsaturated fatty acid (PUFA). On the other hand, saturated fatty acids (SFA), docosahexaenoic acid (C22:4; DHA), and odd-chain fatty acids (OCFA), such as C15:0-antiso and C19:1-*cis* 10, increased significantly ($P < 0.05$) with the increasing age and body weight of the ewes, while the ALA and unsaturated fatty acids (UFA) significantly decreased ($P < 0.05$). The principal component analysis (PCA) revealed a positive association between the age and the OCFA, ALA and small-chain fatty acids (C6:0 and C8:0). In addition, the type of birth showed a positive association with the fat, lactose and palmitoleic acid C16:1 cis9. Conversely, there is a negative association between the UFA, monounsaturated fatty acids (MUFA) and PUFA. In addition, the body weight (BW) and litter size were negatively associated with the protein, SFA and medium-chain fatty acids (C10:0, C12:0, C14:0 and C16:0). The physiological factors generally suggested that the milk quality and essential FA, such as ALA, were influenced by the type of the lamb's birth and the age of the ewes.

Keywords: birth type; ewes age; ewes body weight; fatty acids; litter size; Najdi ewes

Sheep's milk and sheep's milk products play an important role in the nutrition of the population and in a sustainable economy in many countries around the world, especially in Saudi Arabia. The connection between human health and nutrition has been the subject of extensive research

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in recent years, partly due to consumer concerns about food security, but also because diet can be used to control the nutrient intake, which has been shown to have a positive impact on disease prevention (Martini et al. 2023). From this point of view, milk and dairy products provide 25% to 65% of the consumed saturated lipids, which brings milk fat under criticism (Revilla et al. 2017). However, in the last decade, this negative perception has changed following the discovery that some saturated fatty acids, such as steric acid (C18:0), are not necessarily atherogenic and some of them are naturally unsaturated, such as rumenic acid (C18:1), which have positive properties for human health (Chilliard et al. 2006).

The quality of sheep's milk is a crucial factor in obtaining good dairy products. Various factors influence both the productivity and the composition of milk. These can be divided into intrinsic factors (individual dependent and difficult to modify), such as the breed, genetics, lactation stage, age, parity and type of birth, and extrinsic factors (independent of the individual and easily modifiable), such as nutrition and management (Park et al. 2007). Not all the factors have the same influence on the production and composition of the milk. Not all of these are equally controllable or selectable, but in an improvement programme, it is important to be clear about which ones add the most value to the herd's productivity.

Several studies have shown that milk production is more dependent on the number of lambs suckled, as it has been shown that ewes with two or more lambs can produce up to 9.8% more milk than ewes with a single lamb (Arias et al. 2012; Dhaoui et al. 2019). The physiological interpretation of the performance difference in multiple births (twins or more lambs) took into account the possibility that a larger placental surface area created in multiple pregnancies lead to higher hormone levels (prolactin and oxytocin) and therefore to a better developed udder (Othmane et al. 2002), while, at the same time,the influence of the type of birth (gender) remains unclear (Wohlt et al. 1981).

On the other hand, there is limited information on the physiological factors that influence the variations in the fatty acid (FA) content in the milk of dairy sheep, as reported by De La Fuente et al. (2009), where the ewe age, lactation stage and season significantly influenced the variations in the FAs. In contrast, no studies have been conducted on the physiological factors affecting the milk FA content in Najdi ewe flocks. The Najdi breed, native to Saudi Arabia, represents a fundamental genetic stock that must be preserved due to its high production potential and the animal's ability to adapt to very difficult environmental conditions, as they are widespread in areas of northern Saudi Arabia (Matar et al. 2023).

Given the increasing demand for dairy products and the increasing quality requirements of the market, farmers are forced to improve their products through process production and final quality in order to enter the market at more favorable conditions and thereby become more competitive. For this purpose, it would be useful to study the main factors affecting the technological parameters, physicochemical composition and milk production of the herd. This study aimed to investigate effect of the litter size (single vs twins), birth type (male vs female), age of the ewes and body weight on the milk composition and the fatty acid profile of Najdi breed sheep milk.

MATERIAL AND METHODS

The experimental procedures were carried out in strict accordance with the guidelines of the Saudi Arabia Regulations for the Use and Care of Animals in Research and were approved by the Research Ethics Committee of King Saud University (KSUSE2019).

Animals and management

A total of 119 Najdi ewes, selected from a total of around 650 ewes were sampled on semi-extensive farms. All the ewes were milked once a day (at 8:00 a.m.) before the lambs were isolated at 6 p.m. on the first day. The lambs were fed by their mothers for three months and then weaned. Sampling was carried out once during 30 to 40 days of lactation in the winter season (December 2021 to February 2022). The considered physiological factors: age of the ewe (age 1 = 1.8 to 3 years; age 2 = 3.3 to 4 years; age 3 = > 4 years); litter size (single or twin), type of birth (F = female; FF = female/female; FM = female/male; M = male; MM =male/male) and weight of the ewes (W 1 = 45 kg to 55 kg; W 2 = 56 kg to 65 kg; W 3 = 66 kg to77 kg).

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All the ewes in the flock received the same ration throughout lactation. The lactating ewes received a mixed ration of concentrate (55% corn, 17% barley, 23% soybean meal, molasses and minerals) and forage (alfalfa hay) in a ratio 70 : 30 as shown in Table 1. After the morning milking, the ration was distributed *ad libitum* once daily into a double-walled feeding trough that allowed the simultaneous access to all the animals in the group. In addition, clean, fresh water was provided to the animals *ad libitum*.

Milk analysis

A milk sample was collected from each ewe in sterile 50 ml bottles at mid-lactation. The collected samples (three subsamples from each ewe) were stored in an ice box and then transported to the laboratory of the Department of Animal Production, Faculty of Food Science and Agriculture, King Saud University for analysis of the milk components and then stored at −20 °C until the further analysis of the FA. The chemical analysis of the milk samples was performed using infrared spectroscopy (Milko-Scan FT120; Foss Electric) to determine the proportion of fat, protein, lactose, and solids.

Determination of the fatty acid profile

The first step of the fat extraction was to blend the sample in a water bath at 42 °C for 20 min with gentle stirring. Then, 10 ml of milk was transferred to a 12 ml tube. The homogenate sample was centrifuged in a 12 ml Heraeus Labofuge 400 tube (Kendro Laboratory Products, Germany) at −4 °C and 3 500 rpm for 10 min until the fat separation was performed according to (Matar et al. 2023). After this time, and after the separation of the fat was observed, an aliquot of this fat layer was collected into micro tubes without disturbing the floating layers.

Table 1. Fatty acid composition of the feed concentrate and alfalfa hay

Fatty acids composition (%)	Alfalfa hay 30%	Concentrate 70%
C14:0 pentadecylic acid	1.83	0.12
C16:0 palmitic acid	22.66	15.04
C16:1 palmitoleic acid	1.29	0.18
C18:0 stearic acid	6.26	2.29
C18:1 oleic acid	10.20	23.70
C18:2 linolenic acid	17.42	51.43
C20:0 arachidic acid	3.72	0.39
C18:3 alpha-linoleic acid	25.32	4.93
C22:0 tricosylic acid	3.92	0.29
C20:4 arachidonic acid	1.87	0.10

The methylation procedure according to Matar et al. (2023) was followed, as briefly described: 0.5 g of fat (total lipids) was weighed, previously extracted and placed in a 10 ml glass tube to which 1.5 ml of solvent (95-hexane) was added, and the tube was carefully stirred for a minute until the fat was completely dissolved. Next, 0.2 m of 1 N sodium hydroxide (NaOH) was added, the tubes were sealed and placed in a water bath at 45 °C for 30 s, followed by gentle shaking to promote the reaction. In the final step, 0.2 ml of 1 N HCL was added and then mixed for 1 min, keeping the tubes closed during this process. At the end of processing time, 1 ml of the top layer was placed into glass vials, 1 ml of 99-hexane was added, and then injected into the gas chromatography mass equipment (GC-MASS).

The fatty acid profile was analysed as fatty acid methyl esters (FAMEs). Chromatographic analysis, to identify and quantify the methyl esters of the total fatty acids of milk, was performed using gas chromatography mass spectrometry (GC-MASS-MSQP2010; Shimadzu, Kyoto, Japan). An Agilent 122-5532 DB-5MS capillary column (30 mm, 0.25 mm, 0.25 mm) was used to separate the different fatty acids. In the used chromatography column, an injection volume of 1.0 l of the methylated sample was used and the working conditions were as follows: carrier gas flow (helium); Injection volume: 1 l with distribution: 10 : 1; programme temperature: 230 °C; as reported in a previous study (Matar et al. 2023). Identification of the 32 fatty acids was undertaken by comparing the R-TIME of the external standards and a later confirmation with the mass spectra of the peaks containing the fatty acids in the database. The results for fatty acids are given in g/100 g of total fatty acids.

Statistical analysis

The statistical analysis performed in this experiment was conducted with a dual approach, univariate and multivariate. The univariate analysis was

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performed using an analysis of variance (ANOVA) model with the general linear model (GLM) procedure (SAS v9.4). The general model was as follows:

$$Y_{ijklm} = \mu + TB_i + LS_j + AGE_k + WH_l + e_{ijklm} \quad (1)$$

where:

Y_{ijklm} – dependent variable;

μ – mean;

TB_i – effect associated with the type of birth (F = female; FF = female/female; FM = female/male; M = male; MM = male/male);

LS_j – effect associated with the litter size including (single and twin lamb);

AGE_k – effect of the age of ewes (including Age 1 = 1.8 to 3 years; Age 2 = 3.3 to 4 years; Age 3 = < 4 years);

WH_l – effect of the ewes weight at lambing (including W1= 45 kg to 55 kg; W2 = 56 kg to 65 kg; W3 = 66 kg to 77 kg);

e_{ijklm} – residual random effect.

If a significant effect was found ($P < 0.05$), Tukey's test was used to compare the means.

The multivariate analysis was performed using principal component analyses (PCAs) with the milk composition analyses and 32 milk fatty acids, using the OriginPro software version according to (Correddu et al. 2021).

RESULTS

Influence of the litter size and type of birth

In the current study, the proportion of twins born in the Najdi breed was 20.2%, where the number of females (74 lambs) was higher than that of males (65 lambs). The litter size result showed a numerical increase in the daily milk production in ewes raising twin lambs (Table 2). In contrast, the milk fat percentage of the ewes raising single lambs increased numerically compared to the ewe's raising twins. In general, the litter size showed no significant influence on the milk components and fatty acid profile in Najdi sheep.

The influence of the type of birth (gender) on the composition of the milk and fatty acids is shown in (Table 3). The results showed that the type of birth of the lambs (female/female) had a significant ($P < 0.05$) influence on the concentration of the linolenic acid (C18:2), alpha-linoleic acid (C18:3), behenic acid (C22:0), and polyunsaturated fatty acid (PUFA).

On the other hand, the type of birth had no influence on the milk components and other fatty acid profiles in Najdi dairy sheep. A numerical increase in the milk fat percentage was only observed in ewes rearing twin (male/male) lambs.

Influence of the age and body weight of the ewes

The results on the influence of the age and body weight of the ewes on the milk components and fatty acid profile are summarised in (Tables 4 and 5). The results show a trend, although not significant, towards an increase in the milk production in the middle age and body weight (3–4 years and 56–65 kg). It is worth noting that neither the age nor the weight of Najdi ewes had a significant effect ($P > 0.05$) on the milk composition. Regarding the composition of the milk fatty acids, the influence of the ewe age was significantly increased ($P < 0.05$) for the odd-chain fatty acids, such as C15:0-antiso, and C19:1-cis 10, while C16:0-iso, LA and ALA decreased significantly ($P < 0.05$). Furthermore, the concentration of DHA (C22:4) increased significantly in older ewes ($P < 0.05$). It has been observed that with the increasing age, the concentration of the saturated fatty acids (SFAs) increases and the UFA decreases. In addition, the concentration of short- and medium-chain fatty acids increases significantly with the increasing body weight ($P < 0.05$), including myristic acid (C14:0), C15:0-*anteiso* and C16:0-*iso*. In contrast, the heptadecanoic acid (C17:0), ginkgolic acid (C17:1 *cis*10), stearic acid (C18:0), oleic acid (18:1 *cis*9) and C19:1-*cis*10 significantly decreased ($P < 0.05$) with the increasing body weight. Regarding the total fatty acids, a significant increase ($P < 0.05$), was observed for the SFA, while the UFA and MUFA decreased significantly with the increasing body weight.

Correlation matrix by the principal component analysis (PCA)

The principal components describe 36.64% of the total difference in the milk components and FA profile in the Najdi dairy milk as shown (Figure 1). The milk FA and components were

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Table 2. Influence of the litter size on the chemical components (%) and fatty acid profile (g/100 g FA) of the Najdi dairy breed milk

Parameters	Litter size		SEM	*P*-value
	single (n = 99)	twin (n = 20)		
MY (kg/day)	1.39	1.71	0.25	0.35
C6:0	1.08	1.13	0.12	0.47
C8:0	1.40	1.48	0.18	0.46
C10:0	4.76	5.05	0.65	0.63
C12:0	3.19	3.26	0.41	0.85
C14:0	8.98	9.06	0.60	0.87
C15:0 *iso*	0.29	0.28	0.03	0.68
C15:0 *anteiso*	0.49	0.49	0.05	0.55
C15:0	0.97	0.94	0.07	0.49
C16:0	26.7	26.7	1.04	0.87
C17:0 *iso*	0.53	0.50	0.03	0.16
C16:1 *cis*7	0.30	0.30	0.02	0.27
C16:1 *cis*9	0.72	0.70	0.07	0.38
C17:0 *anteiso*	0.71	0.68	0.03	0.17
C17:0	1.03	0.98	0.06	0.64
C17:1	0.31	0.29	0.03	0.38
C18:0	13.6	13.7	0.85	0.99
C18:1 *cis*9 (OA)	26.4	25.8	1.71	0.81
C18:1 *cis*11	0.50	0.47	0.04	0.27
C18:1 *cis*13	0.31	0.32	0.03	0.95
C18:1 *cis*14	0.29	0.30	0.03	0.91
C19:0	0.15	0.13	0.01	0.35
C18:2 *trans*9, *trans*12	0.23	0.23	0.02	0.67
C18:2 *cis*9, *cis*12 (LA)	3.91	4.09	0.27	0.26
C19:1 *cis*10	0.10	0.08	0.02	0.64
C20:0	0.30	0.30	0.02	0.49
C18:3 *cis*9, *cis*12, *cis*15 (ALA)	0.81	0.77	0.14	0.45
C18:2 *cis*9, *trans*11 (CLA)	0.76	0.74	0.06	0.39
C21:0	0.08	0.07	0.01	0.70
C22:0	0.13	0.16	0.03	0.80
C20:4 *cis*5, *cis*8, *cis*11, *cis*14 (DHA)	0.33	0.32	0.04	0.30
C22:4 *cis*7, *cis*10, *cis*13, *cis*16	0.05	0.05	0.01	0.34
C22:5 *cis*4, *cis*7, *cis*10, *cis*13, *cis*16	0.17	0.17	0.02	0.83
SFA	64.7	65.2	1.84	0.88
UFA	35.2	34.6	1.85	0.86
MUFA	28.8	28.2	1.77	0.74
PUFA	6.33	6.38	0.36	0.48
OCFA	4.56	4.34	0.22	0.25
Fat	3.29	3.17	0.62	0.07
Protein	4.49	4.56	0.30	0.42
Lactose	4.62	5.04	0.58	0.53
Total solid	13.5	13.4	0.92	0.25

ALA = alpha-linoleic acid; CLA = conjugated linoleic acid; DHA = docosahexaenoic acid; LA = linolenic acid; MUFAs = monounsaturated fatty acids; MY = milk yield; OA = oleic acid; OCFA = odd chain fatty acid; PUFAs = polyunsaturated fatty acids; *P*-value = significance level, different letters in the same row indicate significant differences ($P < 0.05$); SEM = standard error of means; SFAs = saturated fatty acids (SCFA: C4:0–C10:0; MCFA: C12:0–C15:0; LCFA: C16:0–C24:0); UFAs = unsaturated fatty acids

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Table 3. Influence of the type of birth (gender) on the chemical components (%) and fatty acid profile (g/100 g FA) of the Najdi dairy breed

Parameters	Type of birth (gender)					SEM	*P*-value
	F/F (*n* = 6)	F/M (*n* = 6)	F (*n* = 56)	M/M (*n* = 8)	M (*n* = 43)		
MY (kg/day)	1.38	1.85	1.38	1.83	1.41	0.15	0.65
C6:0	1.13	1.10	1.11	1.16	1.04	0.13	0.59
C8:0	1.54	1.37	1.45	1.50	1.34	0.22	0.55
C10:0	5.43	4.79	4.94	5.00	4.52	0.77	0.60
C12:0	3.52	3.06	3.28	3.26	3.07	0.51	0.69
C14:0	8.80	9.32	9.10	9.11	8.83	0.71	0.75
C15:0 *iso*	0.25	0.29	0.30	0.31	0.29	0.03	0.46
C15:0 *anteiso*	0.47	0.51	0.50	0.51	0.48	0.06	0.77
C15:0	0.90	0.93	0.97	0.99	0.96	0.11	0.79
C16:0	26.2	27.5	26.7	26.5	26.7	1.23	0.77
C17:0 *iso*	0.50	0.51	0.53	0.51	0.53	0.04	0.82
C16:1 *cis*7	0.28	0.30	0.30	0.33	0.30	0.02	0.15
C16:1 *cis*9	0.64	0.78	0.71	0.70	0.74	0.08	0.47
C17:0 *anteiso*	0.69	0.67	0.70	0.69	0.72	0.04	0.48
C17:0	0.97	0.94	1.03	1.02	1.03	0.09	0.58
C17:1	0.27	0.27	0.31	0.30	0.32	0.03	0.50
C18:0	13.7	13.6	13.4	13.7	13.8	1.06	0.84
C18:1 *cis*9 (OA)	25.2	25.7	25.9	26.1	27.1	2.03	0.52
C18:1 *cis*11	0.49	0.51	0.49	0.44	0.50	0.05	0.54
C18:1 *cis*13	0.34	0.31	0.31	0.30	0.30	0.03	0.60
C18:1 *cis*14	0.33	0.31	0.30	0.27	0.29	0.04	0.81
C19:0	0.14	0.13	0.15	0.13	0.14	0.02	0.85
C18:2 *trans*9; *trans*12	0.22	0.24	0.23	0.23	0.23	0.03	0.93
C18:2 *cis*9; *cis*12 (LA)	4.54	3.97	4.05	3.79	3.75	0.33	**0.03**
C19:1 *cis*10	0.10	0.04	0.10	0.10	0.09	0.02	**0.04**
C20:0	0.31	0.29	0.30	0.30	0.30	0.03	0.96
C18:3 *cis*9, *cis*12, *cis*15 (ALA)	0.88	0.71	0.86	0.73	0.74	0.12	**0.05**
C18:2 *cis*9, *trans*11 (CLA)	0.78	0.74	0.77	0.71	0.75	0.07	0.66
C21:0	0.08	0.06	0.08	0.07	0.08	0.01	0.82
C22:0	0.27	0.10	0.13	0.12	0.13	0.04	**0.01**
C20:4 *cis*5, *cis*8, *cis*11, *cis*14 (DHA)	0.32	0.29	0.32	0.35	0.34	0.04	0.27
C22:4 *cis*7, *cis*10, *cis*13, *cis*16	0.05	0.04	0.05	0.05	0.05	0.01	0.71
C22:5 *cis*4, *cis*7, *cis*10, *cis*13, *cis*16	0.18	0.14	0.17	0.19	0.17	0.03	0.52
SFA	65.2	65.5	65.1	65.2	64.2	2.3	0.83
UFA	34.6	34.3	34.8	34.6	35.7	2.29	0.82
MUFA	27.6	28.2	28.3	28.5	29.5	2.19	0.54
PUFA	7.00	6.08	6.50	6.11	6.11	0.42	**0.03**
OCFA	4.22	4.27	4.57	4.52	4.55	0.27	0.57
Fat	2.62	2.99	3.25	3.96	3.29	0.73	0.22
Protein	4.33	4.91	4.52	4.56	4.45	0.36	0.56
Lactose	5.02	5.02	4.75	5.05	4.46	0.69	0.83
Total solid	12.7	13.2	13.5	14.4	13.4	1.08	0.71

ALA = alpha-linoleic acid; CLA = conjugated linoleic acid; DHA = docosahexaenoic acid; F = female; F/F = female/female; F/M= female/male; LA = linolenic acid; MUFAs = monounsaturated fatty acids; M = male; M/M = male/male; MY = milk yield; OA = oleic acid; OCFA = odd chain fatty acid; PUFAs = polyunsaturated fatty acids; *P*-value: significance level, different letters in the same row indicate significant differences ($P < 0.05$); SEM = standard error of means; SFAs = saturated fatty acids (SCFA: C4:0–C10:0; MCFA: C12:0–C15:0; LCFA: C16:0–C24:0); UFAs = unsaturated fatty acids

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Table 4. Influence of the age of the ewes on the chemical components (%) and fatty acid profile (g/100 g FA) of the Najdi dairy breed

Parameters	Age			SEM	*P*-value
	Age 1 (*n* = 45)	Age 2 (*n* = 43)	Age 3 (*n* = 31)		
MY (kg/day)	1.30	1.61	1.44	0.12	0.08
C6:0	1.11	1.08	1.08	0.06	0.61
C8:0	1.45	1.39	1.41	0.09	0.59
C10:0	4.76	4.82	4.86	0.33	0.96
C12:0	3.19	3.17	3.27	0.21	0.85
C14:0	8.68	9.30	9.04	0.30	0.19
C15:0 *iso*	0.27	0.30	0.31	0.01	0.07
C15:0 *anteiso*	0.45	0.51	0.53	0.02	**0.01**
C15:0	0.92	0.98	1.01	0.04	0.09
C16:0 *iso*	0.32	0.36	0.36	0.01	0.03
C16:0	26.1	27.3	26.7	0.59	0.09
C17:0 *iso*	0.52	0.52	0.55	0.01	0.14
C16:1 *cis*7	0.30	0.30	0.31	0.01	0.44
C16:1 *cis*9	0.69	0.73	0.74	0.03	0.25
C17:0 *anteiso*	0.69	0.70	0.73	0.02	0.19
C17:0	1.06	0.98	1.02	0.03	0.06
C17:1	0.32	0.30	0.31	0.01	0.32
C18:0	13.6	13.4	13.8	0.43	0.59
C18:1 *cis*9 (OA)	27.1	25.7	25.9	0.90	0.33
C18:1 *cis*11	0.50	0.48	0.49	0.01	0.82
C18:1 *cis*13	0.32	0.31	0.29	0.02	0.37
C18:1 *cis*14	0.30	0.29	0.29	0.02	0.67
C19:0	0.15	0.14	0.15	0.01	0.34
C18:2 *trans*9, *trans*12	0.24	0.23	0.22	0.01	0.67
C18:2 *cis*9, *cis*2 (LA)	4.04	3.98	3.75	0.15	0.17
C19:1 *cis*10	0.10	0.08	0.10	0.01	0.04
C20:0	0.29	0.31	0.31	0.01	0.09
C18:3 *cis*9, *cis*12, *cis*15 (ALA)	0.90	0.74	0.74	0.05	0.01
C18:2 *cis*9, *trans*11 (CLA)	0.75	0.77	0.75	0.03	0.63
C21:0	0.08	0.07	0.09	0.01	0.40
C22:0	0.13	0.14	0.13	0.02	0.62
C20:4 *cis*5, *cis*8, *cis*11, *cis*14 (DHA)	0.31	0.33	0.35	0.02	0.09
C22:4 *cis*7, *cis*10, *cis*13, *cis*16	0.04	0.04	0.06	0.01	**0.02**
C22:5 *cis*4, *cis*7, *cis*10, *cis*13, *cis*16	0.17	0.17	0.17	0.01	0.98
SFA	63.8	65.5	65.4	0.92	0.23
UFA	36.1	34.4	34.4	0.92	0.22
MUFA	29.6	28.1	28.3	0.93	0.36
PUFA	6.52	6.33	6.09	0.20	0.11
OCFA	4.45	4.49	4.68	0.11	0.14
Fat	3.17	3.41	3.51	0.31	0.10
Protein	4.40	4.59	4.53	0.15	0.40
Lactose	4.92	4.74	4.29	0.30	0.14
Total solid	13.2	13.9	13.2	0.46	0.25

Age 1 = 1.8–3 years; Age 2 = 3.3–4 years; Age 3 = more than 4 years); ALA = alpha-linoleic acid; CLA = conjugated linoleic acid; DHA = docosahexaenoic acid; LA = linolenic acid; MUFAs = monounsaturated fatty acids; MY = milk yield; OA = oleic acid; OCFA = odd chain fatty acid; PUFAs = polyunsaturated fatty acids; *P*-value: significance level, different letters in the same row indicate significant differences ($P < 0.05$); SEM = stander error of means; SFAs = saturated fatty acids (SCFA: C4:0–C10:0; MCFA: C12:0–C15:0; LCFA: C16:0–C24:0); UFAs = unsaturated fatty acids

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Table 5. Influence of the body weight of the ewes on the chemical components (%) and fatty acid profile (g/100 g FA) of the Najdi dairy breed

Parameters	Body weight			SEM	*P*-value
	BW 1 (*n* = 27)	BW 2 (*n* = 67)	BW 3 (*n* = 31)		
MY (kg/day)	1.21	1.54	1.46	0.14	0.07
C6:0	1.06	1.08	1.15	0.07	0.29
C8:0	1.36	1.40	1.50	0.11	0.38
C10:0	4.50	4.82	5.11	0.30	0.32
C12:0	3.01	3.24	3.32	0.18	0.47
C14:0	8.40	9.12	9.30	0.27	**0.03**
C15:0 *iso*	0.27	0.29	0.32	0.01	0.06
C15:0 *anteiso*	0.44	0.50	0.54	0.03	0.01
C15:0	0.90	0.97	1.02	0.04	0.07
C16:0 *iso*	0.31	0.35	0.37	0.02	0.01
C16:0	25.7	27.0	26.9	0.45	0.06
C17:0 *iso*	0.55	0.52	0.53	0.01	0.10
C16:1 *cis*7	0.31	0.30	0.30	0.01	0.51
C16:1 *cis*9	0.67	0.74	0.72	0.03	0.22
C17:0 *anteiso*	0.70	0.70	0.72	0.02	0.48
C17:0	1.11	1.01	0.98	0.04	0.01
C17:1	0.33	0.31	0.28	0.02	0.02
C18:0	14.2	13.2	13.9	0.38	0.04
C18:1 *cis*9 (OA)	27.9	26.2	24.8	1.07	0.02
C18:1 *cis*11	0.50	0.49	0.48	0.02	0.65
C18:1 *cis*13	0.31	0.31	0.31	0.02	0.92
C18:1 *cis*14	0.30	0.29	0.31	0.02	0.91
C19:0	0.16	0.14	0.15	0.01	0.11
C18:2 *trans*9, *trans*12	0.23	0.23	0.22	0.01	0.88
C18:2 *cis*9, *cis*12 (LA)	3.95	3.93	3.95	0.17	0.78
C19:1 *cis*10	0.11	0.09	0.07	0.01	0.01
C20:0	0.29	0.30	0.32	0.01	0.12
C18:3 *cis*9, *cis*12, *cis*15 (ALA)	0.82	0.81	0.77	0.06	0.76
C18:2 *cis*9, *trans*11 (CLA)	0.73	0.77	0.76	0.04	0.37
C21:0	0.08	0.08	0.08	0.01	0.85
C22:0	0.13	0.12	0.17	0.02	0.31
C20:4 *cis*5, *cis*8, *cis*11, *cis*14 (DHA)	0.33	0.33	0.33	0.02	0.30
C22:4 *cis*7, *cis*10, *cis*13, *cis*16	0.04	0.05	0.05	0.01	0.64
C22:5 *cis*4, *cis*7, *cis*10, *cis*13, *cis*16	0.17	0.17	0.16	0.01	0.74
SFA	63.2	64.9	66.4	1.15	0.03
UFA	36.7	34.9	33.4	1.05	0.03
MUFA	30.3	28.6	27.2	1.11	0.02
PUFA	6.36	6.35	6.28	0.22	0.69
OCFA	4.52	4.50	4.60	0.14	0.52
Fat	3.04	3.44	3.08	0.38	0.58
Protein	4.45	4.54	4.45	0.19	0.82
Lactose	4.67	4.67	4.77	0.36	0.83
Total solid	13.2	13.7	13.1	0.57	0.42

ALA = alpha-linoleic acid; BW 1 = 45–55 kg; BW 2 = 56–65 kg; BW 3 = 66–77 kg); CLA = conjugated linoleic acid; DHA = docosahexaenoic acid; LA = linolenic acid; MUFAs = monounsaturated fatty acids; MY = milk yield; OA = oleic acid; OCFA = odd chain fatty acid; PUFAs = polyunsaturated fatty acids; *P*-value = significance level, different letters in the same row indicate significant differences ($P < 0.05$); SEM = stander error of means; SFAs = saturated fatty acids (SCFA: C4:0–C10:0; MCFA: C12:0–C15:0; LCFA: C16:0–C24:0); UFAs = unsaturated fatty acids

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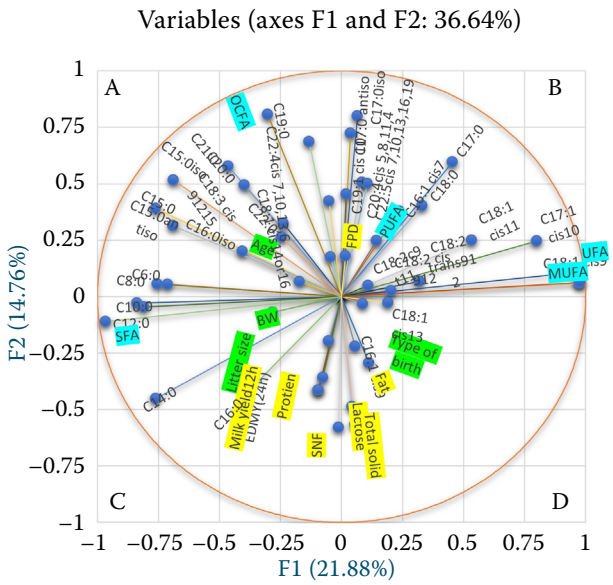

Figure 1. A diagram illustrating the relationship between the physiological factors (age, weight, litter size and type of birth) with the milk components and fatty acids derived from a principal component analysis in Najdi dairy milk

distributed into 4 groups as shown in plot 1 including quadrant (A); The age is associated with the OCFA, DHA, C20:0, C21:0, C22:0, ALA, C18:2, C15:0, C15:0-*anteiso*, C8:0 and C6:0; while in quadrant (B); UFA, MUFA and PUFA, shown with C16:1 7-*cis*, C18:0, C18:1-11-*cis*, C18:2-*cis*9,11(LA), C22:5, DHA, C17:0, C17:0-*iso* and C17:0-*anteiso*; also in quadrant (C); showed that the body weight and type of birth correlate with the SFA, C10:0, C12:0, C14:0, C16:0 and the protein content finally in quadrant (D). The gender correlates with the fat content, total solids, C18:1-*cis*13 and C16:1-*cis*9.

Quadrant A showed a positive loading for the age and for most OCFAs, ALAs and small chain fatty acids (C6:0 and C8:0), also quadrant D showed positive loading for the type of birth, fat, lactose and palmitoleic acid C16:1-cis9. In contrast, quadrants C and B showed a negative loading for UFA, MUFA, and PUFA, while the body weight (BW) and litter size showed a negative loading for the protein, SFA, and medium chain fatty acids (C10:0, C12:0, C14:0, and C16:0).

DISCUSSION

The chemical components of ruminant milk, especially fat, are among the most complex due to their fatty acid content and the influence of various factors on their constitution (Bauman et al. 1999). The age and weight of the ewe, as well as the number of lambs or sex, are crucial factors that are related and influence the milk quality (Othmane et al. 2002). This is the first study that aimed to identify physiological factors for the milk fatty acid profile in Najdi dairy sheep.

In the Najdi breed, the litter size does not have a significant impact on the milk components and fatty acid profile. These results were similar to those of Ayadi et al. (2014) for milk components of the Najdi breed and (Regmi et al. 2021) for Boer goats. In contrast, the various studies by Manuel Gonzalez-Ronquillo et al. (2021) on the Churra breed, Dhaoui et al. (2019) on the Dman breed and by Ochoa-Cordero et al. (2007) on Rambouillet ewes reported that the litter size had a significant influence on the milk production, protein and fat contents. In another study by Oravcova et al. (2007) in Tsigai and Valachian dairy sheep, ewes raising two or more lambs had the highest milk protein content. The results showed that litter size directly influences the blood flow through the mammary gland, alters all the metabolic products, especially the energy balance, and the efficiency increases as the lactation progresses (Gonzalez-Garcia et al. 2015).

Wohlt et al. (1981) reported that the sex of the lambs had no influence on the milk composition of the Dorset breed. Even in goats (Brito et al. 2011), the type of birth has no influence on the fat, protein and lactose content of the milk. In contrast to our results, a significant difference in the milk lactose content was found between ewes nursing female lambs and ewes nursing male lambs (Ochoa-Cordero et al. 2007). It is worth noting that in this study, the birth type female/female showed a significant influence on the essential fatty acids, including the LA, ALA and PUFA. In general, the type of lamb and the number of lambs raised primarily influence the production and quality of the sheep's milk through the number of lambs suckled. This is because it allows for the easier emptying of the udder and stimulates udder development, resulting in increased milk production. This increase is mediated by the high concentration of oxygen and placental lactogen. Furthermore, ewes that raise multiple lambs maintain their maximum production for a longer period of time (Dhaoui et al. 2019).

This study found that middle-aged ewes and ewes weighing between 56 and 65 kg tended to produce

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more milk compared to other ewes. While the age of the ewe and body weight at lambing do not have a significant influence on the milk components. According to Wathes et al. (2007), the older ewes produced more milk than the younger ewes due to their higher body weight and ability to control the reserve mobilisation. On the other hand, a study conducted on the Churra breed has shown that the fat content remains constant in younger sheep and increases significantly beyond the age of 3 years (Othmane 2002). Other studies (Pugliese et al. 1999) reported that the proportion of protein, fat and caseins does not increase continuously with the number of lactations. In fact, there is a decline in these components, in particular, the fat content drops by around 0.2% from the 5th lactation. This could be due to the deterioration of the udder, leading to the reduced production of these components (Rovai et al. 2004). Another study showed that the increased milk production with age had a negative impact on the fat and protein content, possibly due to the dilution of the components (Libis-Marta et al. 2021).

Regarding milk fatty acids, the older ewes produce milk with a high SFA content, particularly short chain fatty acids (SCFAs) and medium chain fatty acids (MCFAs). Comparing our results with other reports (De La Fuente et al. 2009) on the Churra dairy breed, there is an increasing trend in the SFA contents and a decrease in the PUFA and MUFA contents with the increasing age, which is attributed to the increase in the *de novo* synthesised milk fatty acids, including SCFA and MCFA. This is an effect that is in contrast to other studies (O'Shea et al. 1998) which found that age has an influence on the distribution of CLA, particularly the *cis*-9-*trans*-11 isomer, that tends to become more pronounced with advanced age. On the other hand, Craninx et al. (2008) found no significant influence of the parity on the FA in dairy cows. However, despite the potential biological importance, there is limited information about the effects of these factors on dairy sheep. To confirm our results and determine their physiological or metabolic effects, further studies in other dairy sheep breeds are required.

A principal component analysis was used to determine the relationship between the milk composition, fatty acid profile and physiological factors. This analysis provides valuable insights into the synthesis and origin of these components. In this study, SCFA and OCFA were observed to have a positive association with each other and with the age of the ewes, while MCFA (C10:0, C12:0, C14:0 and C16:0) had a negative association with FA in quadrant C, as well as the body weight and type of birth. This result is consistent with previous studies by Fievez et al. (2003), on cows and (Correddu et al. 2021) on Sarda dairy sheep, which reported a similar trend for SCFA (C14:0 and C16:0) and OCFA. This illustrated the *de novo* synthesis of these fatty acids occurs through the process of biohydrogenation of acetate and hydroxybutyrate in the rumen, indicating their dietary origin.

According to another study (Arias et al. 2012), animal husbandry is the factor that most influences the physicochemical composition of milk. The factors of the litter size and lamb sex can have a small influence on the milk quality of Najdi sheep compared to the feeding or milking phase, but must be taken into account nevertheless. The number of studies on physiological factors affecting the fatty acid composition of dairy sheep is minimal. Most have nutrition-related goals and use different methods. In this sense, comparing our results with previously published results is somewhat complicated.

CONCLUSION

The physiological factors, such as the type of birth, age and body weight of ewes, had a significant influence on the FA content in the milk of Najdi sheep. The type of birth and the age of the ewes were the cause of differences in the essential FA such as LA, ALA, PUFA and arachidonic acid. This FA was higher in the milk of ewes that had female twins and were of an old age. While BW1 had significant effects on the stearic acid (C18:0), oleic acid, and MUFA. The PCA loading plots showed a positive association between the age with the ALA and OCFA and birth type with the fat, lactose and total solids. In contrast, the body weight and litter size factors had a negative association with the protein and SFA. As observed, factors such as the age and type of birth had a positive influence on the *de novo* synthesis of the milk fatty acids in the udder.

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Conflict of interest

The authors declare no conflict of interest.

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