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Non-Genetic Factors Shaping Milk Composition in Early Lactation of Awassi Ewe

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General Background: Milk composition in sheep is influenced by various genetic and environmental factors. Specific Background: Among these, non-genetic contributors during early lactation remain underexplored, particularly in the Awassi breed reared in Iraq. **Knowledge Gap:** Limited data exist on how ewe-related non-genetic factors influence key milk constituents over time postpartum. **Aims:** This study aimed to evaluate the effect of ewe age, lamb sex, birth type, and body weight on milk fat, lactose, protein, and solid-not-fat (SNF) concentrations during early lactation in Awassi ewes. **Results:** In a 4 × 2 × 2 × 3 factorial design using 50 ewes, milk fat declined from day 10 (8.55%) to day 30 (7.23%) before rebounding, while lactose remained stable (~4.3%), and both SNF and protein increased gradually. Young ewes had higher fat, whereas four-year-olds had the least. Twin births were associated with lower fat but higher lactose and SNF. Heavier ewes showed elevated SNF at later stages. Lamb sex had no significant impact. Negative correlations were observed between fat and both lactose and SNF. **Novelty:** This study is among the first to quantify time-specific effects of multiple non-genetic factors on Awassi milk traits. Implications: These findings underscore the importance of managing ewe-specific variables to enhance milk quality in Awassi sheep husbandry.

Highlights:

- Non-genetic factors such as ewe age, birth type, and body weight significantly shape milk traits.
- Fat decreases mid-lactation, while protein and SNF steadily increase.
- Findings provide management insights to improve ovine dairy quality in semi-arid regions.

Keywords: Awassi Ewes, Milk Composition, Non-Genetic Factors, Early Lactation

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Introduction

Global production has shot up over the past three decades from 524 million t in 1992 to approximately 930 million t this year, or a rise of 77%. Sheep, goats, buffalo, and camels currently contribute just 4% of that volume but supply a considerably greater proportion of total milk solids than would be expected. This is due to the higher fat and protein content in non-bovine milks [1].

The increasing market interest is fueled as much by dietary and functional considerations, however: many of the smaller ruminant milks fetch a high unit price for gourmet cheeses, yoghurts, and nutraceutical powders. Of all the ruminant milks, sheep's milk is becoming recognized as more of a functional food. Recent studies point to its diverse content of bioactive peptides, conjugated linoleic acid, and micronutrients possessing anti-inflammatory [2]–[4], antidiabetic as well as wound healing properties. Controlled feeding experiments in rats also show that amino acid balance, digestibility, and metabolomic signatures of sheep milk powders are superior to the bovine or caprine analogues [5].

In this context, the Awassi is featured in sheep production throughout the Fertile Crescent. Analyses performed in 116 Jordanian ewes reported average values of 5, respectively for fat (4.6%), SNF (10.7), lactose content (4.7%), and protein content (5.3) and also showed that allelic combinations at β LG factor PRL microsatellite antigen ANXA9 antigen gene ACAA2 have a significant effect on FA profile [6]. In a parallel metabolomic study in Turkish Awassi ewes, aspartic acid, anserine, and ornithine were found associated with milk yield and somatic cell count [7].

Genetic levers for quality improvement characterization have shifted from the grain level [6] to DNA: 5.2% more protein was recently associated with FASN polymorphisms by Iraqi workers in GG homozygotes; it is a large effect and supports genetic gain strategies [8].

So too are non-genetic influences. In the north of Iraq, ewes lambing in Mediterranean spring (April-June) produced more milk with greater fat and SNF yields than winter-lambing groups until 12 weeks postpartum [9]. According to Turkish data, a shift from concentrate feeding to grazing results in an increase of lactation length and polyunsaturated fatty acid ratio while the gross composition remains unimpaired [10]. By itself, the stage of lactation induces significant increases in fat, protein, and dry matter and a decrease in lactose content in Lacaune sheep managed under intensive production systems [11].

Maternal age, lamb gender, and ewe weight are also important: young (≤2 years) dams gave the highest milk fat percentages in Karbala, while heavier ewes produced more overall [12]. Dietary manipulations, such as the inclusion of Citrus aurantium leaves and tomato pomace, may elevate Awassi milk fat and total solids content or improve cheese ripening characteristics [13].

Lastly, based on a multivariate (r 0.00), statistical predictions for the relative patterns of microbiological flavor and chemical quality indices in milk concentrate from pens have recently been obtained across the first 60 d of lactation using a study population of Awassi ewes [14], which provides a picture of leverage points [management control spaces].

In addition to average responses, the covariation of components matters for computation. Work on Hamdani crossbreds maintained under an extensive system reported strong negative phenotypic associations for milk fat with protein, SNF, and lactose ($r \approx -0.37$ to -0.38) while a near-perfect positive correlation emerged between SNF and either the protein or lactose components of Santa et al. [15], [16], which parallels Venezuelan researchers' findings of an inverse association within a metabolomic study in Awassi's sodium forms factorial structure loadings patterns as that observed herein during our CCP analysis.

Notwithstanding the economic value of Awassi sheep, Iraqi compositional data are quite limited. The only recent large data set from Mosul indicated clear flock, dam age, and birth rank effects on daily yield and somatic cell counts though the text revealed little milk chemistry [17], [18], [19]. No published report has measured the joint effects of dam age, litter size, lamb gender, and maternal body weight on fat content (%) other than protein (%), lactose (%), and solid non-fat (SNF) % until 3 months postpartum in Kirkuk Governorate.

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We therefore aimed to (i) estimate the impacts of these four non-genetic effects on major milk components over early lactation in Awassi ewes maintained at Kirkuk's Hawija District (ii), and identify phenotypic interrelationships among them over time. Elucidation of these interactions will be useful for breeders and extension agents in order to develop management practices as well as selection strategies that optimize flock productivity and the quality characteristics of ovine dairy products from the Iraqi environment.

Method

This study was conducted from December 11, 2024, to March 15, 2025, on three semi-intensive Awassi sheep flocks kept under identical management in Abbasiya (35°19′N, 43°40′E), Hawija District, Kirkuk Governorate—a semi-arid area with a mean winter temperature of about 11°C and an average annual rainfall of 350 mm. Fifty clinically healthy ewes (17, 16, and 17 per flock) were recruited at lambing. All animals were weighed using digital scales (±0.1 kg), and their body condition was scored from one to five. Based on live weight, ewes were grouped into three categories: approximately 30, 35, or 40 kg. Litter size (single or twin) was recorded for each ewe. Dam age was classified as follows: nulliparous (2 years old), primiparous (3 years), and multiparous (≥4 years), determined by the number of previous lambings, excluding the current parity.

Ewes received a daily concentrate diet (barley 55%, wheat bran 20%, soybean meal 18%, calcium carbonate 2%, salt 1%, vitamin–mineral premix 4%) at 0.42 kg/ewe/day, along with ad libitum straw and pasture. Fresh water and limestone salt blocks were always available. All ewes received clostridial vaccination (Covexin 8) and an ivermectin pour-on at the start of the experiment.

Milk sampling occurred at 10, 30, 60, and 90 days postpartum. Morning milk (50 mL) was collected into refrigerated sterile vials containing 0.02% sodium azide and analyzed within four hours at 4°C. Fat, protein, lactose, and SNF contents were determined using an EkoMilkPro ultrasonic analyzer (Milkovan™, Bulgaria), calibrated to ISO/IDF reference standards before each run.

The experimental design was a four-factor (dam age), two-factor (lamb sex), two-factor (birth type), and three-factor (dam weight) completely randomized factorial design. Data for each milk component were analyzed using a general linear model ($Y_{ijkl} = \mu + S_i + D_j + T_k + W_l + \varepsilon_{ijkl}$) in SAS® 9.4, where μ is the general mean, and S_i, D_j, T_k, and W_l represent the fixed effects of lamb sex, dam age class, birth type, and dam weight class, respectively. Type III sums of squares were used to adjust for unequal subclass sizes. When a significant main effect was detected ($P \leq 0.05$), Duncan's multiple range test was used for mean separation.

Pearson phenotypic correlations among milk fat, protein, lactose, and SNF were calculated using the PROC CORR procedure in SAS, based on least square means for each ewe across all sampling times. Model adequacy was checked using studentized residuals and the Shapiro–Wilk test for normality; percentage data were log or arcsine square root transformed as needed to stabilize variance, with back-transformed results presented in the text.

Results and Discussion

A. Results

The high fat contents of early lactation milk of Awassi ewes were typical (Table 1). Average fat decreased from 8.55% (day 10) to 7.23% (day 30), and then recovered at day 60 (8.44%), while lactose was very stable between 4.25% and 4.35%. Solids-not-fat (SNF) and protein increased steadily, with a maximum peak in the third month (10.14% and 6.00%, respectively).

	Fat (%)	Lactose (%)	SNF (%)	Protein (%)
Month 1 (day 10)	8.55 ± 0.46	4.25 ± 0.02	9.16 ± 0.16	4.21 ± 0.12
Month 2 (day 30)	7.23 ± 0.49	4.27 ± 0.04	9.54 ± 0.15	4.61 ± 0.19
Month 3 (day 60)	8.44 ± 0.63	4.35 ± 0.03	10.14 ± 0.12	6.00 ± 1.01

Table 1. Overall milk composition of Awassi ewes during the first three months of lactation

Age (yr)	Fat (%)	Lactose (%)	SNF (%)	Protein (%)
2	10.31 ± 1.81 *a*	4.22 ± 0.07	9.35 ± 0.27	4.37 ± 0.17
3	8.63 ± 1.00 *ab*	4.26 ± 0.05	9.26 ± 0.31	4.27 ± 0.23
4	7.14 ± 0.50 *b*	4.26 ± 0.04	9.08 ± 0.33	4.14 ± 0.25
≥ 5	9.57 ± 0.84 *ab*	4.23 ± 0.05	9.05 ± 0.29	4.15 ± 0.23

Table 2. Effect of dam age on milk composition in Month 1

1. Dam Age

In the first month,(Table 2) milk fat composition varied (P≤0.05) between different dam age groups of sows similar as for DHA across age categories; however, only a moderate association was found with both IMF and ARA values after 6 months if the mother's dietary supplementation during pregnancy or lactation is not a mix of systematic significant animal factors based on literature review. Two-year-old ewes produced the highest value for fat (10.31%), while four-year-olds exhibited the lowest one (7.14%). Lactose, SNF, and protein did not vary with age at this stage, but all components became independent of age from later months

2. Birth Type

The birth type influenced the most in month 2 (Table 3). Twin-bearing dams secreted milk with less fat (5.20%) and more lactose (4.50%) and SNF (9.75%) than singleton dams (7.40%, 4.31% and 9.56%, respectively; P ≤ 0.05). Months 1 and 3 showed no birth type effects.

Birth type	Fat (%)	Lactose (%)	SNF (%)
Singleton	7.40 ± 0.31 *a*	4.31 ± 0.02 *b*	9.56 ± 0.16 *b*
Twin	5.20 ± 1.79 *b*	4.50 ± 0.01 *a*	9.75 ± 0.07 *a*

Table 3. Effect of birth type on milk composition in Month 2

Weight (kg)	Fat (%)	Lactose (%)	SNF (%)	Protein (%)
30–35	8.26 ± 1.15	4.34 ± 0.03	10.25 ± 0.18	5.03 ± 0.15
40–45	7.27 ± 0.52	4.46 ± 0.02	10.42 ± 0.23	6.39 ± 1.77
45–50	7.85 ± 0.59	4.24 ± 0.05	9.54 ± 0.31	6.57 ± 0.07

Table 4. Effect of dam live-weight on milk composition in Month 3

3. Dam Live-Weight

By month 3, heavier ewes produced milk richer in SNF and protein (Table 4). Differences between fat and lactose were not statistically significant, while 40–45 kg ewes had higher SNF (10.42%) and protein content numerically (6.39%), compared to lighter ones (30–35 kg: 10.25% SNF; 5.03% protein).

Sex	Fat (%)	Lactose (%)	SNF (%)	Protein (%)
Male	8.75 ± 0.59	4.26 ± 0.03	9.08 ± 0.23	4.12 ± 1.18
Female	8.40 ± 0.70	4.27 ± 0.03	9.21 ± 0.22	4.28 ± 0.16

Table 5. Effect of lamb sex on milk composition in Month 1

Month	Fat × Lactose	Fat × SNF	SNF × Protein	SNF × Lactose
1	–0.725 **	–0.405 *	+0.986 **	+0.783 **
2	–0.936 **	–0.563 **	+0.317 *	+0.654 **
3	–0.880 **	–0.263 ns	+0.202 ns	+0.683 **

*ns = not significant; * P < 0.05; ** P < 0.01.*

Table 6. Phenotypic correlations (Pearson *r*) among milk constituents

4. Lamb Sex

Lamb sex had no effects ($P > 0.05$) on any milk component. Fat, lactose, and SNF + protein contents in milk fat percentages tended to be similar for lambs of both sexes during month 1 when fed oestrogenized rations (Table 5), and these values never changed.

5. Phenotypic Correlations

Over the 90 days, fat was consistently inversely associated with SNF and lactose, whereas there were positive associations between SNF, protein, and lactose (Table 6). The highest negative correlation was observed between fat and lactose in month 2 ($r = -0.936$, $P < 0.01$), while the biggest positive one was for SNF with protein in month 1 ($r = 0.986$, $P < 0.15$). Magnitudes of correlation generally decreased by 3 months, suggesting a step-wise uncoupling of inter-component dependence with advancing lactation.

Taken together, these outcomes reveal that the stage of lactation is the leading factor in driving changes to composition, while dam age impacts early lactation fat and birth type affects mid-lactation carbohydrate and SNF fractions, with heavier dams making minor contributions to late gestational allocation for SNF & protein. Compositional effects of lamb sex are minimal, and strong negative fat–lactose trade-offs occur in the early lactation period.

B. Discussion

Dynamic patterns of composition in ewe milk during the first 90 d of lactation: very high fat levels at day 10, a freefall by day 30, and some recuperation at day 60 in which lactose remained almost static (~ 4.3 %). This “fat dip–lactose plateau” concurs with metabolomic evidence that soon after parturition, lipid mobilization is replaced throughout the milk production period by osmotic regulation of the lactose before lipid slowly rises in energy content [7]. The natural high component levels (in fat and protein) that ewe milk starts with [20] give the physiological background against which these early shifts in lactation occur.

1. Dam Age

In our study, two-year ewes yielded milk with the highest fat content (10.3%), while four-year ewes yielded lower fat content (7.1%). A comparable parity-associated fat decline has been observed in the case of Iraqi Awassi and Arabi flocks, where fat percentage reached maximum levels during the first or second lactation, after which overall milk yield increased [12]. The fact that age effects had disappeared by month 3 in the current study also implies that, rather than lifetime mammary differences, it is early metabolic load which drives initial variation.

2. Birth Type

Milk from dams bearing twins did not differ in fat, but was higher for lactose and SNF on day 30. Whilst there have been limited studies on twin effects upon composition, the more generalized principle of nutrient partitioning to multiple fetuses has also received support from investigations into intensive vs. grazing systems in large Awassi flocks, where a management regime that elevated suckling demand resulted in milk with higher carbohydrate density [10]. Our results suggest that Awassi dams balance carbohydrate-rich fractions to preserve milk volume for twins even when the energy-dense fat component is sacrificed.

3. Dam Body Weight

By month 3, milk from heavier ewes (40–45 kg) had numerically higher SNF and protein, although this difference was not statistically significant. A similar relationship between high body condition and higher protein appears to have been observed in late lactation Awassi flocks raised in northern Iraq, as the animals were better conditioned. Ewes partitioned more amino acids for milk casein synthesis [9], [21]. Although the current weight effect did not attain practical significance for fat, it reveals interaction between maternal stores and protein accretion with advancing lactation.

4. Phenotypic Correlations

The high level of negative relationship we observed in this pairwise comparison between fat and lactose ($r \approx -0.88$) agrees with previous reports for bulk ValledelBelice milk on the inverse association found by [22] as it relates to the concentration values of these two components, which corroborates a classical osmotic dilution model where when lactose raises, then water does so, causing fat droplets to be diluted. In contrast, the steady correlation between SNF and protein ($r \geq 0.98$) reflects that true protein is a large stoichiometrically constant component of total solid non-fat.

5. Genetic Perspectives

The current experiment controlled only non-genetic factors. Yet recent genomic work in Awassi shows that single nucleotide changes in fatty acid synthase and DGAT1 can turn milk fat profiles [6] on their heads. We hope these observations may enlighten the future direction of studies.

6. Global and Industry Context

Ewes provided just over 1% of the world's milk [1], but with its solids and large cheese yield, income from many dairy products worldwide has always been at a premium price. In the first month after calving, sheep's fat-rich milk offers an opportunity to make higher value specialty cheeses, whereas the rise in SNF and protein by month 3 is best for yoghurt and fermented milk products.Managing the animal so as to take advantage of its natural features at each stage—hang cheeses up early, gather milks for cultured products late—an increase in profitability might be realized.

7. Limitations and Recommendations

Although the 50 ewe sample delivered powerful early lactation inferences, it had low statistical power for late-stage trends. Further analysis with a larger sampling range (over 90d) and inclusion of genomic information would further improve estimation of genetic versus environmental variance. Furthermore, controlled feeding trials on twin-bearing dams could be used to investigate the effects of nutritional strategy as a method for reducing their low early lactation fat output. [23].

Conclusion

Altogether, the data indicate that early lactation stage and dam age are time-specific influence factors on Awassi milk composition, while birth type effects appear to be relatively more constant over time; seemed unaffected by lamb sex. Fat-lactose antagonism is apparent in the early stages of lactation, also affirming that osmotically it appears to be delivering sexy competition and milk yield. These observations can serve as a quantitative basis for improving feeding systems, breeding targets, and product differentiation in Iraqi ovine dairying.

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