

## Donkey Milk Decoded: Biochemical Insights into Protein, Fat, and Lactose Variability from Field to Farm

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### ABSTRACT:

This study explores the milk yield and biochemical profile of donkey milk from diverse sources, including field conditions and organized farms. The milk's composition is characterized by factors such as lactation stage, genetics, environment, and milking practices. Remarkably, donkey milk is rich in lysozyme, which enhances its shelf life, making immediate refrigeration vital during transport to maintain quality. By analyzing samples from the same animals under consistent lactation periods, the study reveals notable differences in protein and fat content between farm and field milk. Farm milk showed higher levels of protein ( $3.38 \pm 0.04$  g%) and fat ( $1.03 \pm 0.10$  g%) compared to field milk during early lactation. Meanwhile, the average SNF ( $8.46 \pm 0.57\%$ ) and total solid ( $8.6 \pm 0.57\%$ ) content were consistent with previous research in spite of Fat content that was varied from 1.0% to 2.0% in the first three months of lactation. These findings highlight the critical role of environmental factors and management practices in shaping the nutritional profile of donkey milk, emphasizing the importance of source location in assessing its quality and health benefits.

### Keywords:

Donkey Milk, Farm And Field Samples, Lactation Stage, Protein Content, Fat Content, Lactose Content

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## INTRODUCTION

Over the past five decades, the Indian dairy sector has experienced extraordinary growth, with milk production soaring from 21.2 million tonnes in 1970-71 to 221.06 million tonnes in 2021-2022. This increase has significantly boosted per capita milk availability from 112g/day to 444g/day (source: dahd.nic.in). While cows and buffaloes remain the primary contributors, accounting for nearly 95% of the nation's total milk output, other non-bovine

species, such as goats, sheep, camels, mares, donkeys, and reindeer, also play a role. Milk from these species is renowned for its therapeutic properties and potential health benefits. However, despite their unique chemical and microbial profiles, these non-bovine milk sources have been largely overlooked in India's dairy industry (Park and Haenlein, 2006). Milk from non-bovine species rich in functionally active compounds including lipids, lactose, immunoglobulins, immune proteins, peptides, nucleotides,

oligosaccharides, and metabolites, offers a wealth of bioactive ingredients that can be harnessed to promote human health.

Non-ruminant species such as camels, donkeys, yaks, and reindeer have been increasingly studied for their milk production, revealing both economic potential and unique health benefits. Among these, donkey milk (DM) stands out for its lactose content of 5.8% to 7.4%, giving it a naturally sweet flavor that appeals to children (Carminati and Tidona, 2014). Studies by Arecoet *al.*, 2015 and Cao *et al.*, 2018 demonstrate that the high lactose concentration in donkey milk enhances the absorption of calcium and phosphorus, aiding bone formation and preventing osteoporosis through improved mineral deposition. Additionally, DM has a slightly alkaline to neutral pH (7.0–7.2), attributed to its low phosphate and casein content (Montiet *al.*, 2012; Markiewicz-Kęszycka *et al.*, 2013). With a low protein content of 1.5%–1.8%, comparable to human milk (0.9%–1.7%), DM offers advantages by potentially reducing the risk of early adiposity rebound, a condition associated with high protein intake in formula-fed infants (Michaelsen and Greer, 2014).

Recent research by Akan, 2021 revealed that the peptide concentration in the *in-vitro* gastrointestinal digested (GIS) casein fraction of DM ( $4.94 \pm 0.06$  mg/mL) is significantly higher than in the whey fraction ( $3.90 \pm 0.4$  mg/mL). Notably, the peptides in the casein fraction show promising potential as a therapeutic agent in managing Type 2 diabetes (Akan, 2021).

## MATERIAL AND METHODS

### Collection of samples

In this study, donkey milk samples were collected at various intervals over two consecutive years (2020–2022) from different breeds, across two seasons: summer (March to June) and winter (November to February). The samples were obtained at three distinct stages of lactation: early (0–30 days), mid (31–60 days), and late (61 days onwards).

### Initiation of process

To ensure the highest standards of hygiene, the udders were thoroughly cleaned with 70% ethanol or a spirit-soaked cotton swab, and handlers wore sanitized gloves. The initial milk stream was discarded to obtain uncontaminated samples, which were then collected in sterile vials. Immediately after collection, the samples were kept in an ice basket to maintain freshness during transport to the laboratory. Fresh milk samples were promptly analyzed for bacterial cultures, while the remaining portions were stored at  $-20^{\circ}\text{C}$  in a laboratory freezer for further analysis.

### Analysis of Biochemical Properties in Donkey Milk Samples

The biochemical properties of the donkey milk samples including solid non-fat (SNF), fat content, density, corrected lactometer reading (CLR), and protein levels were assessed using the Ultra Scan Swift Twinsonic Milk Analyzer from Hindustan Thermostatics, India.

This analysis followed the established protocol described by Aspriet *al.*, 2017.

## RESULTS

### Analysis of Biochemical Properties in Donkey Milk Samples

The biochemical properties of freshly collected donkey milk samples were examined at each lactation stage. During the early lactation stage (0–7 days), the milk was observed to be slightly pale yellow and exhibited the highest fat content, lactose concentration, and density compared to other lactation stages. The overall composition of donkey milk across all lactation stages is detailed in Table 1.

Notably, DM samples were characterized by a high lactose concentration ( $5.84 \pm 0.18\%$ ) and a low fat content ( $2.0 \pm 0.3\%$ ). The protein content was relatively modest ( $3.90 \pm 0.60\%$ ), while the pH remained within an optimal range ( $7.1 \pm 0.2$ ). The average density was measured at  $40 \pm 0.5$ , and the total solids and solid-not-fat (SNF) were recorded as  $8.06 \pm 0.57$  and  $8.46 \pm 0.22$ , respectively.

**Table 1: The statistical overview of nutritional components of 20 different donkey milk samples collected from different sites**

Composition	Range	Mean ± SD
Lactose (%)	5.75 - 5.95	5.84± 0.18
Density	42.5-37.5	40.0±0.50
SNF	7.78-8.35	8.06±0.57
Fats (%)	0.5-1.80	1.15±1.30
Total solids	8.35-8.57	8.46±0.22
Proteins (%)	3.6-4.2	3.9±0.60
Ph	7.0-7.2	7.1±0.2

SNF= Solid not fats; SD= Standard Deviation

**Protein Content Analysis**

The average protein content of farm and field donkey milk samples at different lactation stages is graphically presented in Figures 1-3. The comparison of mean protein levels across lactation stages is summarized in Table 2. During the early lactation phase, farm milk had a mean protein content of 3.38 ± 0.04 g%, slightly lower than the 3.42 ± 0.03 g% observed in field milk samples. However, this difference was not statistically significant.

In mid-lactation, farm milk showed a lower mean protein content (3.22 ± 0.05 g%) compared to field milk (3.40 ± 0.03 g%), with a statistically significant difference (p < 0.05) as detailed in Table 2 and Figure 10. By late lactation, farm milk had a higher mean protein content (3.40 ± 0.03 g%) as compared to field milk (3.18 ± 0.04 g%), with this difference also being statistically significant (p < 0.05). These findings indicate that farm management practices or environmental factors significantly influence the protein content in donkey milk.

**Table 2: Comparison of Donkey on different lactation stages between farm and field milk samples.**

Mean ± S.E. of donkey milk constituents from farm and field locations		
	Farm	Field
Protein g%		
Early Lactation	3.38 ± 0.04 <sup>a</sup>	3.42 ± 0.03 <sup>a</sup>
Mid Lactation	3.22 ± 0.05 <sup>b</sup>	3.40 ± 0.03 <sup>bc**</sup>
Late lactation	3.40 ± 0.03 <sup>a</sup>	3.18 ± 0.04 <sup>c**</sup>
Lactose g%		
Early Lactation	5.51 ± 0.04 <sup>a</sup>	5.35 ± 0.04 <sup>a**</sup>
Mid Lactation	5.40 ± 0.04 <sup>b</sup>	5.24 ± 0.03 <sup>b**</sup>
Late lactation	5.48 ± 0.04 <sup>a</sup>	5.31 ± 0.04 <sup>a**</sup>
Fat g%		
Early Lactation	1.03 ± 0.10 <sup>a</sup>	0.37 ± 0.02 <sup>a**</sup>
Mid Lactation	0.37 ± 0.02 <sup>b</sup>	0.21 ± 0.01 <sup>b**</sup>
Late lactation	0.23 ± 0.18 <sup>b</sup>	0.08 ± 0.01 <sup>c**</sup>

a,b,c,: values within a column (effect of lactation stage) with different superscripts differ significantly (p<0.05)with each other  
 \*\*: values differ significantly (p<0.01) between the two farms within the same stage of lactation.

**Lactose Content Analysis**

The lactose content in donkey milk samples from both farm and field sources across different lactation stages, is illustrated in Figures 4-6. The

comparison of mean lactose levels at each stage is summarized in Table 2.

In the early lactation stage, farm milk had a slightly higher mean lactose content (5.51 ± 0.04

g%) compared to field milk ( $5.35 \pm 0.04$  g%), with this difference being statistically significant ( $p < 0.05$ ). This trend continued into mid-lactation, where farm milk again showed a higher mean lactose content ( $5.40 \pm 0.04$  g%) than field milk ( $5.24 \pm 0.03$  g%), as noted in

Table 2 and Figure 11. By late lactation, farm milk consistently maintained a higher mean lactose content ( $5.48 \pm 0.04$  g%) compared to field milk ( $5.31 \pm 0.04$  g%), with the difference remaining statistically significant ( $p < 0.05$ ).

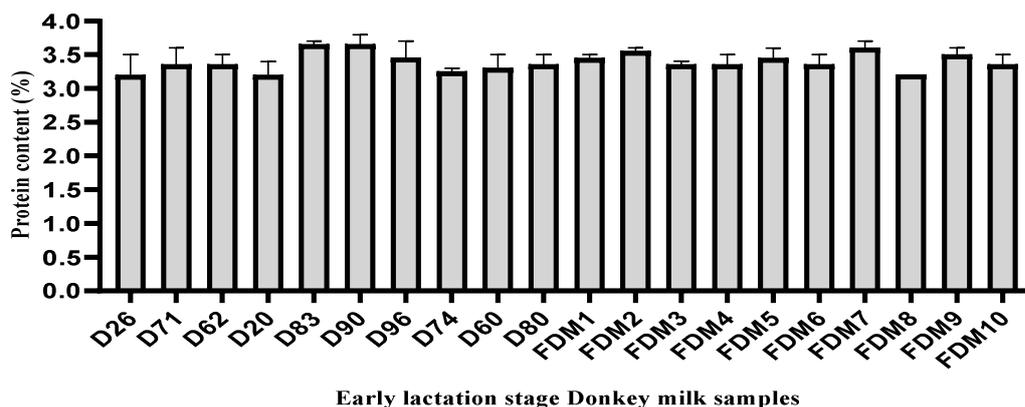


Figure 1: Relative analysis of protein content in farm donkey milk samples (DM) and field donkey milk samples (FDM) at early lactation stage

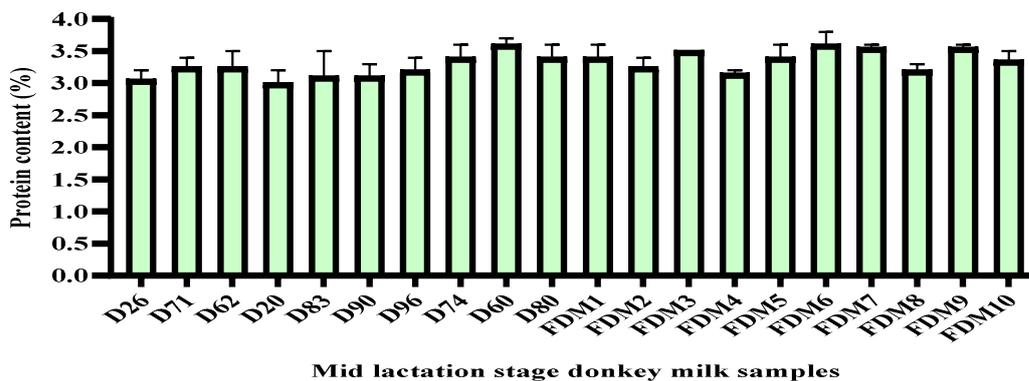


Figure 2: Relative analysis of protein content in farm donkey milk samples (DM) and field donkey milk samples (FDM) at mid lactation stage

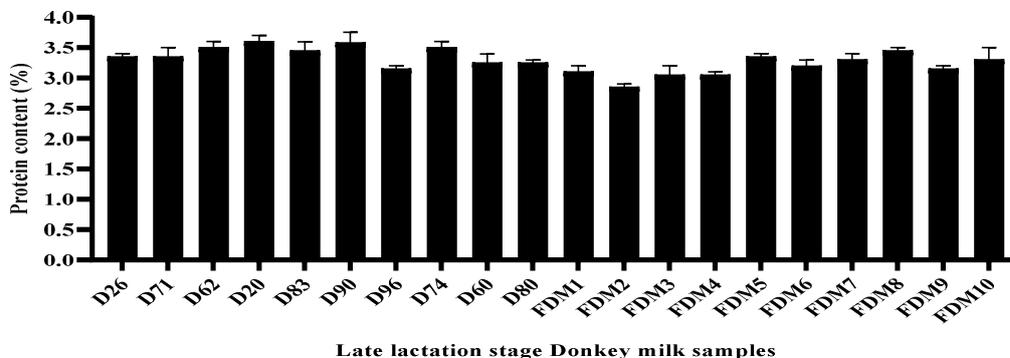


Figure 3: Relative analysis of protein content in farm donkey milk samples (DM) and field donkey milk samples (FDM) at late lactation stage

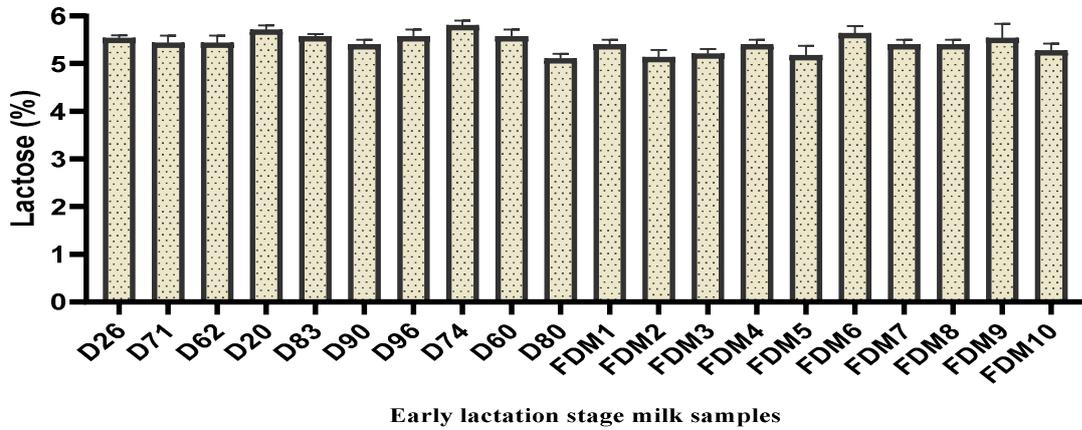


Figure 4: Relative analysis of lactose concentration in farm donkey milk samples (DM) and field donkey milk samples (FDM) at early lactation stage

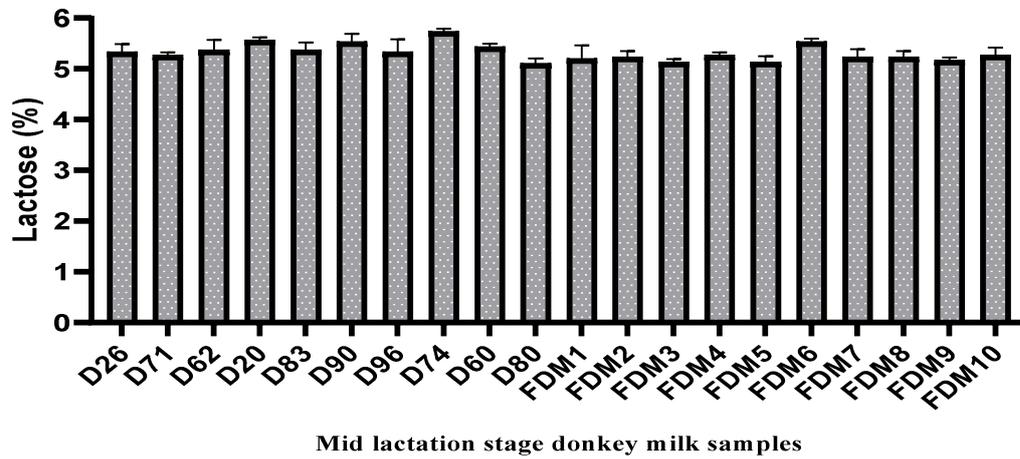


Figure 5: Relative analysis of lactose concentration in farm donkey milk samples (DM) and field donkey milk samples (FDM) at mid lactation stage

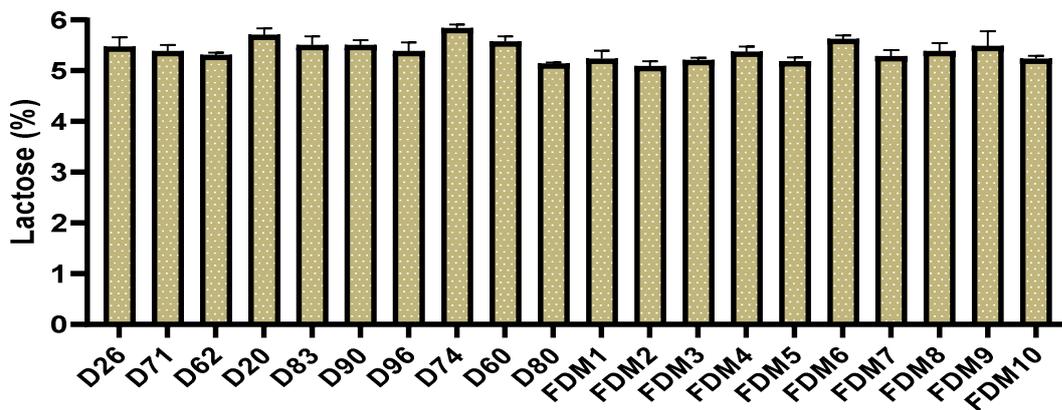


Figure 6: Relative analysis of lactose concentration in farm donkey milk samples (DM) and field donkey milk samples (FDM) at late lactation stage

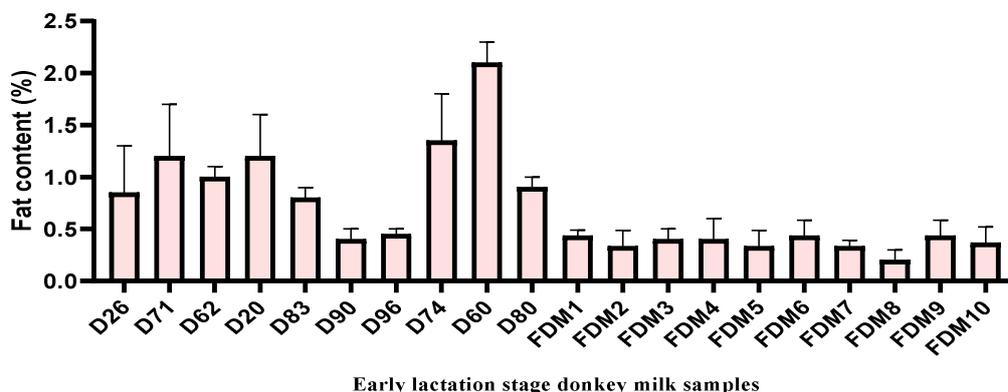


Figure 7: Relative analysis of fat concentration in farm donkey milk samples (DM) and field donkey milk samples (FDM) at early lactation stage

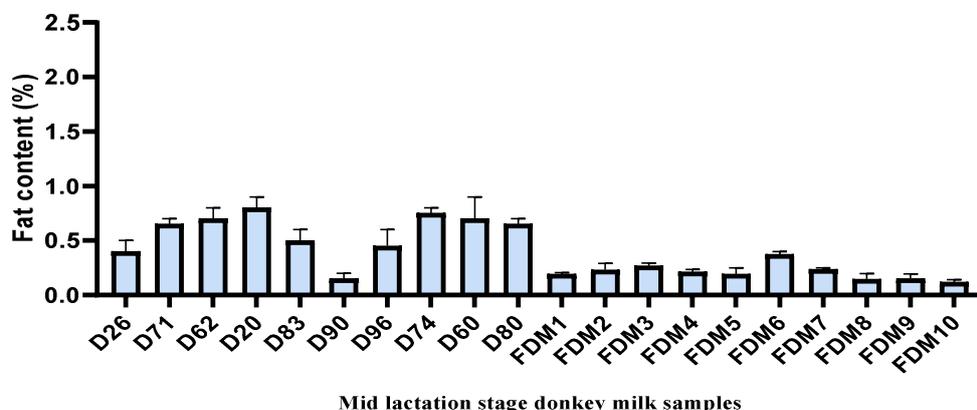


Figure 8: Relative analysis of fat concentration in farm donkey milk samples (DM) and field donkey milk samples (FDM) at mid lactation stage

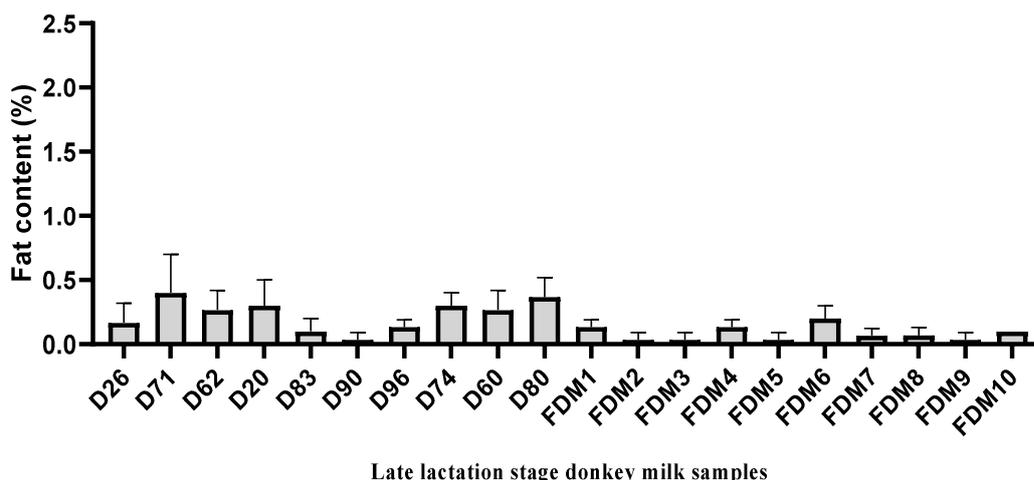


Figure 9: Relative analysis of fat concentration in farm donkey milk samples (DM) and field donkey milk samples (FDM) at late lactation stage

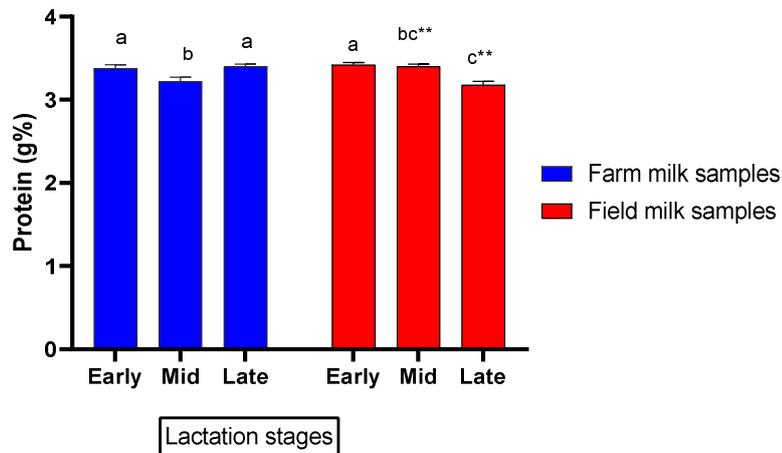


Figure 10: Relative analysis of protein content in farm milk samples (DM) and field milk samples (FDM) throughout the lactation period

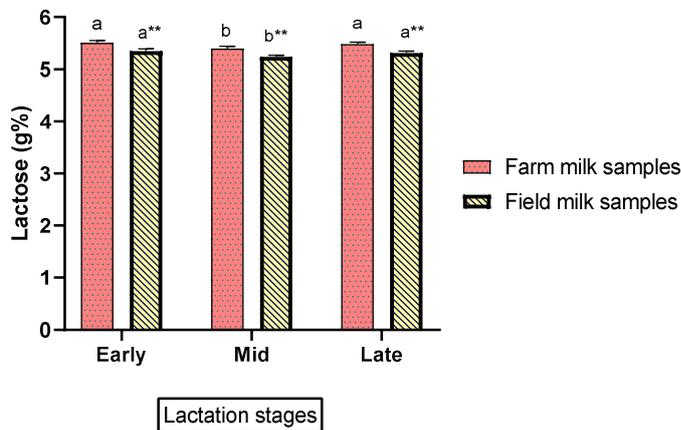


Figure 11: Relative analysis of lactose concentration in farm milk samples (DM) and field milk samples (FDM) throughout the lactation period

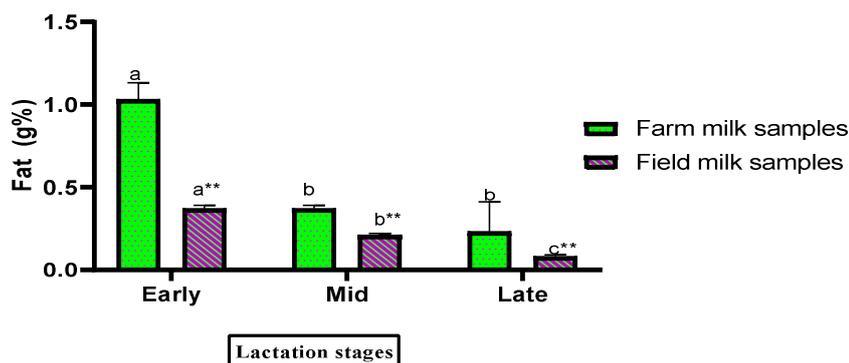


Figure 12: Relative analysis of fat concentration in farm milk samples (DM) and field milk samples (FDM) throughout the lactation period

a,b,c,: values within a column (effect of lactation stage) with different superscripts differ significantly ( $p < 0.05$ ) with each other

\*\*,: values differ significantly ( $p < 0.01$ ) between the two farms within the same stage of lactation.

### Fat Content Analysis

The fat content of donkey milk samples from both farm and field sources across various lactation stages, was found to be relatively low. The mean  $\pm$  standard deviation values for individual samples are graphically presented in Figures 7-9.

A comparison of mean fat content at each lactation stage (Table 2) revealed that farm milk consistently had significantly higher fat content ( $p < 0.05$ ) compared to field milk at all stages of lactation. This trend is further illustrated in Figure 12.

### DISCUSSION

The study found that the average milk yield from Jennies was  $0.709 \pm 0.34$  L, which aligns with the general range of 3 to 12 mL per kg body weight for donkey milk production (Miraglia *et al.*, 2020). The composition of donkey milk is influenced by various factors including lactation stage, genetic background, environmental conditions, breed, individual animal characteristics, maternal age, diet, health, and milking practices (Bhardwaj *et al.*, 2020). Previous research has highlighted that donkey milk contains high levels of lysozyme throughout lactation, which helps extend its shelf life (Martini *et al.*, 2019). To preserve milk quality during transport, immediate refrigeration is essential; failure to do so risks undesired proteolysis, which can skew analytical results (Kumari *et al.*, 2022).

In this study, milk samples were collected from the same animals during consistent lactation months, allowing for accurate comparisons between milk from different Jennies on field and organized farms. This approach also provided insights into the average age, milk yield, and litter size of the population. The biochemical profile of donkey milk, including fat content, solid non-fat (SNF), density, protein levels, pH, and total solids, were assessed. The average SNF ( $8.46 \pm 0.57\%$ ) and total solids ( $8.6 \pm 0.57\%$ ) were similar to those reported for Halaridonkey milk (SNF:  $8.81 \pm 0.14\%$ , total solids:  $9.61 \pm 0.08\%$ ) (Garhwalet *et al.*, 2023). These values also align with Guoet *al.* (2007), who found total solids in donkey milk (8.8–11.7 g/100 g) to be lower than

in human milk (10.9–13.0 g/100 g).

Farm milk consistently exhibited higher protein and fat levels compared to field milk as shown in study that was early lactation farm milk had a protein content of  $3.38 \pm 0.04$  g% and fat content of  $1.03 \pm 0.10$  g%, while field milk had  $3.42 \pm 0.03$  g% protein and  $0.37 \pm 0.02$  g% fat. Overall protein content ( $3.90 \pm 0.60\%$ ) was slightly higher than reported values for Halari donkey milk (2.03%) (Garhwalet *et al.*, 2023) and Sardinian donkey milk (1.5%) (Murgia *et al.*, 2016), but lower than the protein content of cow, buffalo, and goat milk (Guoet *al.*, 2007; Sunarićet *al.*, 2016).

Lactose content varied slightly, with farm milk generally showing higher levels across all stages. The lactose concentration in donkey milk ( $5.84 \pm 0.18\%$ ) remained consistent throughout lactation, comparable to human milk (6.87%) (Ballard *et al.*, 2013; Markiewicz-Kęszycka *et al.*, 2013).

The fat content findings were consistent with previous research, showing a range of 1.0% to 2.0% during the first three months of lactation (Aspriet *et al.*, 2017; Martini *et al.*, 2019; Kumari *et al.*, 2021; Garhwalet *et al.*, 2023). This pattern reflects the natural progression of lactation, as noted by Martini *et al.* (2014), who reported a subsequent decrease in fat content as lactation progresses.

These observations underscore the influence of environmental factors or management practices on the nutritional composition of donkey milk. They also highlight the importance of considering the source location when evaluating milk quality and its potential health benefits. Moreover, the similarities between donkey and human milk suggest that donkey milk could serve as a close alternative to human milk.

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