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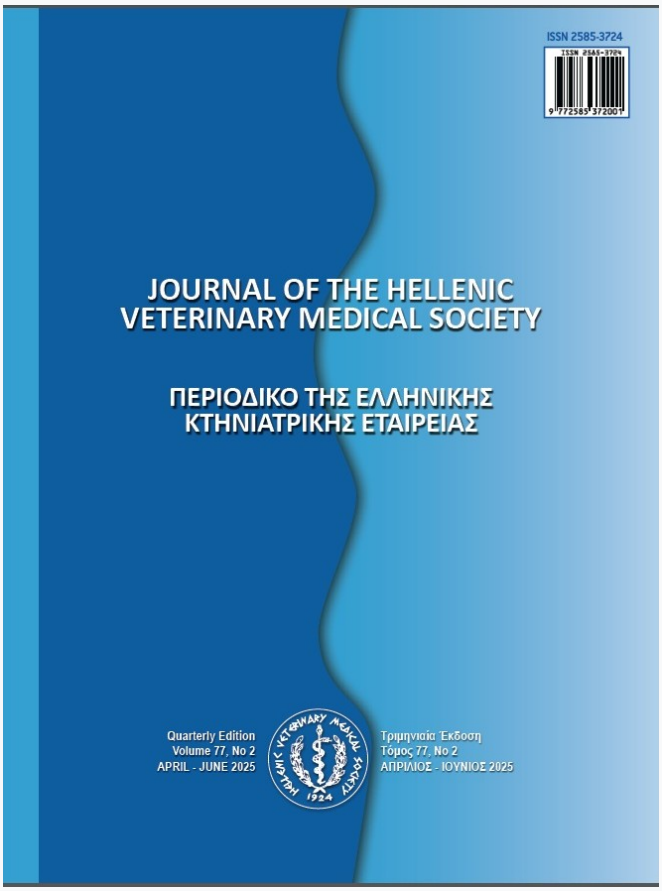

Detection of miRNA-15a, miRNA-29b, miRNA-34a and miRNA-223 in Cow, Sheep, Goat, Buffalo, and Donkey Milk and Investigation of Their Relationship with Milk Composition

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Detection of miRNA-15a, miRNA-29b, miRNA-34a and miRNA-223 in Cow, Sheep, Goat, Buffalo, and Donkey Milk and Investigation of Their Relationship with Milk Composition

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ABSTRACT: The objective of this study was to ascertain the expression levels of miRNA-15a, miRNA-29b, miRNA-34a, and miRNA-223 in milk from cows, buffaloes, sheep, goats, and donkeys and to examine their relationship with milk composition. The miRNA expression levels were quantified by RT-qPCR, and their correlation with the physicochemical data of the milk was examined. The expression of miRNAs was observed in all milk samples and there was a correlation between miRNA expression and milk fat, protein, dry matter and pH. A positive correlation was observed between fat and miRNA-34a in cows'milk, a positive correlation between fat and miRNA-29b in donkey milk ($P<0.05$), and a negative and significant correlation between fat and miRNA-29b in sheep milk ($P<0.05$). A positive and significant correlation was identified between dry matter and miRNA-34a in sheep milk ($P<0.05$), whereas a negative and significant correlation was observed between dry matter and miRNA-223 in goat milk ($P<0.05$). Additionally, a negative and significant correlation was found between dry matter and miRNA-29b in sheeps'milk ($P<0.05$). A positive and significant relation ship was identified between miRNA-34a expression and pH in sheep milk ($P<0.05$). It can be concluded that miRNAs play an important role in milk composition, hygiene, health and technology. Further studies are recommended to gain a deeper understanding of these aspects.

Keyword: Donkey milk; milk composition; miRNA; miRNA-15a; miRNA-34a.

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INTRODUCTION

In the early stages of mammalian life, milk is the primary source of nutrition, providing the essential nutrients required for growth and development. Furthermore, milk is of public health significance for minerals such as calcium and phosphorus, proteins, some B-group vitamins, and organic and inorganic nutrients. It is established that milk proteins are involved in many important biological processes, including development, growth, and tissue differentiation. Additionally, they have beneficial effects on the immüne system and calcium absorption. It has been documented that milk consumption is linked to a reduction in blood pressure and cancer risk, as well as an effective means of controlling body weight (Black et al., 2002). In addition to its protein-based components, milk contains RNA, miRNAs, and a plethora of organic and inorganic substances (Zhou et al., 2012). miRNAs are non-protein-coding RNAs that play pivotal roles in gene expression, comprising 17-25 nucleotides (Carrillo-Lozano et al., 2020). In a study by Lee et al. (1993), the lin-4 gene in the round worm *Caenorhabditis elegans* transcribed a small RNA of 22 nucleotides, despite the absence of any protein-encoding capacity. Reinhart et al. (2000) identified a distinct miRNA (22 nucleotides in length, designated let-7) in *C. elegans*. In subsequent studies, numerous RNA molecules with analogous properties to let-4 and let-7 were identified and designated as microRNA (miRNA) (Lagos-Quintana et al., 2001). Additionally, extracellular miRNAs are present in diverse body fluids, including tears, saliva, blood, and milk (Shen et al., 2013).Circulating miRNAs are located in extracellular vesicles with lipid structures, such as exosomes, to protect them from the negative effects of enzymes, such as ribonuclease (RNase), which are present in some body fluids. To protect against these harmful effects, some miRNAs associate with lipoproteins, such as HDL, forming various complex structures (Etheridge et al., 2011). miRNAs act by suppressing or activating gene expression during the development al period and in cellular processes. These properties enable miRNAs to perform a multitude of functions across a diverse range of areas, including influencing DNA repair, cell division, and proliferation, tissue development, apoptosis, the immunesystem, and infections (Bertoli et al., 2015). It has been established that miRNAs, which are believed to function as post-transcriptional regulators of gene expression, exert influence across a range of biological processes, including carcinogenesis and embryogenesis (Pieters et al., 2015).

The objective of this study was to as certain the presence and expression levels of miRNA-15 and miRNA-34a, which have been demonstrated to play a role in cancer formation and have been linked to reduced cancer risk. Additionally, the study investigated the expression of miRNA-223, which influences immunesystem development. Furthermore, the study aimed to determine the presence and expression rates of miRNA-15 and miRNA-34a, which are effective in the mechanism of cancer formation and have been reported to reduce cancer risk. Additionally, the studys ought to identify the presence of miRNA-29b, which plays a role in osteoblast development by increasing bone mineralization, in cow, buffalo, sheep, goat, and donkey milk and to reveal its relationship with milk composition.

METHOD

Milk collection

The material of this study consisted of five Holstein cows, five sheep, five hair goats, five Anatolian buffaloes, and five Anatolian donkeys of similar ages that were fed with similar rations. Milk was collected from healthy mid-lactation animals under aseptic conditions by visiting different farms on different dates and transported to the laboratory under cold chain protection.

Quality analysis of the milk

The somatic cell count (Aydın et al., 2022), total mesophilic aerobic bacteri acount (Harrigan, 1998), pH value (Case et al., 1985), fat (Turkish Standards Institute, 2015), dry matter (Metin, 2001), and protein content (Hatipoğlu et al., 2021) were determined using classical methods. The milk was stored at -80°C until miRNA analysis.

Preparation of milk for miRNA analysis

Milk is a highly complex, comprising many different substances. To isolate miRNAs from this structure of milk and eliminate potential disadvantages that may arise from compositional variations in different animal milks, a series of centrifugation and filtration procedures were applied to the milk prior to RNA extraction. In order to achieve this, the milk was thawed at 4°C for 12 h in a refrigerator set to a temperature of -80°C. A total of 1.5 mL of the milk sample was subjected to a series of centrifugation steps, with the following parameters: 3000 g for 10 min, 6000 g for 10 min, and 2000 g for 30 min. Subsequently, the supernatant was transferred from the original tube to a new Eppendorf tube. Subsequently,

the tubes were centrifuged at 20,000 g for 60 min at 4°C. After centrifugation, the pellet at the bottom of the tube was removed and suspended in 300 µl of a phosphate-buffered saline (PBS) solution. Subsequently, the tubes were subjected to centrifugation at 20,000 g for 60 min, after which the supernatant was removed and transferred to another Eppendorf tube. The samples from the Eppendorf tubes were filtered through a 0.45 µM PVDF syringe filter and subsequently resuspended in PBS. The samples were then filtered through a 0.22 µM PVDF syringe filter. The resulting filtrate was stored at -80 °C until RNA extraction (Hata et al., 2010).

RNA isolation

RNA isolation was conducted to obtain miRNAs. The Zymo Research Direct-zol RNA Mini Prep Plus (Cat. no.: R2072, Lot no.: ZRC201547) miRNA isolation kit was used for RNA isolation.

RNA measurement

The obtained RNA samples were subjected to analysis using a Thermo Nano Drop Nd 2000 analyzer. For each sample, a volume of 2 µl of the obtained RNA was introduced in to the device and subjected to measurement.

Complementary DNA (cDNA) synthesis

The RNAs obtained after extraction were converted into cDNA, which is more stable than miRNA, to prevent the degradation of miRNAs and the negative consequences that may arise. In order to achieve this, all samples were converted to cDNA in an Applied Biosystems Veriti Thermal Cycler using a Gene All Hyper Script First Strand Synthesis Kit (Catno: 601-005).

Primer Design

Primers compatible with the five animal species (cow, buffalo, sheep, goat, donkey) used in the study were selected (Table 1). miRNA-92a gene was used as a reference gene for relative assessment of miRNA levels. The selection of miRNA-92a as a reference gene was based on its demonstrated stability in previous studies. Lai et al. (2017) identified miRNA-92a as the most stable housekeeping gene for miRNA analysis in bovine milk. Jadhav et al. (2024) successfully used miRNA-92a as a reference in buffalo milk miRNA expression studies. Again, Smulski et al. (2023) and Yoshitha et al. (2025) used miRNA-92a as a reference gene in their normalization procedures.

Real-time PCR protocol

The real-time PCR assay was conducted using an Applied Biosystems 7500 Fast System and a FAST Real-Time PCR System Device. Promega Go-Taq RT-Master Mix (Cat. No. A6002) was used during the study. The Ct (cycle threshold) value was obtained for each miRNA in the evaluation of the

Table 1. Gene sequences of the primers used in the study

miRNA	Primer Sequence	miRBase Accession	GC	TM
chi-miR-15a-5p RT primer	5'GTCGTATCCAGTGCAGGGTCCGAGGAGGTATTCGCGACTGGATACGACCCACAA	MIMAT0035990	56%	66
chi-miR-15a-5p Forward	AACCGGTAGTAGCAGCACATAATG		48%	
bta-miR-34a RTprimer	5'GTCGTATATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACAACC	MIMAT0004340	56%	68
bta-miR-34a Forward	AACCGGTGGCAGTGTCTTAG		55%	
chi-miR-29b-5p RT primer	5'GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCGCACTGGATACGACTCTAAG	MIMAT0036114	54%	67
chi-miR-29b-5p Forward	AACACGCCTGGTTTCACATG		50%	
bta-miR-223 RT primer	5'GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCGACTGGATACGACTGGGGGGT	MIMAT0009270	56%	68
bta-miR-223 Forward	AACGGCTGTCAGTTTGTCAA		55%	

real-time PCR data. Subsequently, the ΔCt values were determined by subtracting the Ct value of the reference gene (miRNA-92a) from that of the related gene for normalization. The discrepancies between the ΔCt values of each miRNA across species were examined. In terms of expression, high ΔCt values indicate low gene expression, where a slow ΔCt values indicate high gene expression.

Statistical analysis

The Ct values of each microRNA were normalized to the reference gene, and the ΔCt values were calculated as the difference between the target microRNA and the reference gene Ct values. Subsequently, the ΔCt values were employed to assess the correlation with protein, fat, dry matter, and pH in milk via Spearman correlation coefficient analysis. This statistical approach was selected to directly evaluate the relationship between the expression of microRNAs and the composition of milk. All analyses were performed using SPSS 22.0 software. (Wenqing et al., 2022).

RESULTS

Somatic Cell Count in Milk

The somatic cell count, total mesophilic aerobic bacteria (TMAB) count, and physico-chemical results of the milk samples under analysis are presented in Tables 2, 3, and 4.

Real-time PCR Results

The ΔCt values of miRNAs in raw milk from different animal species are presented in Table 5. The resulting graph, created according to the above normalization procedure, is presented in Figure 1. Upon examination of the normalization values, it became evident that the miRNAs under investigation were expressed in the milk of all species. miRNA-15a was more highly expressed in sheep milk, where as miRNA-34a exhibited higher expression in donkey milk. miRNA-223 was expressed higher levels in cow and sheep milk than in other species.

Statistical Evaluation

Table 6 presents the correlation coefficients and sig-

Table 2. Somatic cell counts in milk.

samples	Cow milk	Sheep milk	Goat milk	Buffalo milk	Donkey milk
1	$2,37x10^5$	$2,7\ x10^5$	$<9\ x10^4$	$<9\ x10^4$	$<9\ x10^4$
2	$2,03\ x10^5$	$1,78\ x10^5$	$<9\ x10^4$	$1,06\ x10^5$	$<9\ x10^4$
3	$2,12\ x10^5$	$1,7\ x10^5$	$<9\ x10^4$	$1,11\ x10^5$	$<9\ x10^4$
4	$1,98\ x10^5$	$2,83\ x10^5$	$<9\ x10^4$	$1,48\ x10^5$	$<9\ x10^4$
5	$1,87\ x10^5$	$4,6\ x10^5$	$<9\ x10^4$	$<9\ x10^4$	$<9\ x10^4$

Table 3. TMAB counts in milk (log10cfu/mL)

samples	Cow milk	Sheep milk	Goat milk	Buffalo milk	Donkey milk
1	6,04	5,04	4,90	6,63	4,89
2	5,78	4,60	4,49	6,41	5,32
3	5,63	4,30	4,29	6,49	4,75
4	4,69	5,07	5,32	6,23	5,50
5	5,57	5,64	5,20	6,27	5,44

Table 4. Physicochemical properties of milk from the species

	Cow milk	Sheep milk	Goat milk	buffalo milk	Donkey milk
Drymatter	11,45 ± 0,49	15,8 ± 0,67	9,4 ± 1,03	13,4 ± 0,74	5,86 ± 0,41
Protein	3,16 ± 0,53	8,84 ± 0,45	4,05 ± 0,8	5,24 ± 0,63	0,72 ± 0,43
Fat	3,28 ± 0,11	7,12 ± 0,51	4,29 ± 0,1	7,06 ± 1,32	3,3 ± 0,39
pH	6,61 ± 0,10	6,6 ± 0,18	6,44 ± 0,04	6,7 ± 0,1	7,46 ± 0,07

Table 5. Mean normalization values by species

	ΔCtValues				
Samples	**Cow milk**	**Sheep milk**	**Goat milk**	**Buffalo milk**	**Donkey milk**
miRNA-15a	4,84	1,89	6,58	6,83	5,97
miRNA-29b	-7,94	-10,71	-6,77	-10,71	-9,07
miRNA-34a	-5,55	-5,57	-4,93	-6,29	-1,93
miRNA-223	3,31	3,55	6,46	6,65	5,37

nificance levels between protein, fat, dry matter, pH, and miRNA (miRNA-15a, miRNA-29b, miRNA-34a and miRNA-223) levels in milk according to animal species. A positive correlation was observed between fat and miRNA-34a in cows'milk, where as a positive correlation between fat and miRNA-29b was noted in donkey milk ($P<0.05$). Conversely, a negative and significant correlation between fat and miRNA-29b was identified in sheep's milk ($P<0.05$). A positive and significant relationship was identified between dry matter and miRNA-34a in sheep milk ($P<0.05$), whereas a negative and significant relationship was observed between dry matter and miRNA-223 in goat milk ($P<0.05$). Additionally, a negative and significant relationship was found between dry matter and miRNA-29b in sheep milk ($P<0.05$). A positive and statistically significant correlation was observed between miRNA-34a levels and pH in sheep milk ($P<0.05$).

DISCUSSION

Milk is a highly complex, comprising many physical and chemical components. Altough milk from different animal species contains a similar range of components, the quantities of these components vary significantly. These differences are contingent on the genetic factors of the animal in question (Walstra et al., 2006). In this study, the expression differences of miRNA-34a, miRNA-29b, miRNA-15a and miRNA-223 in cow, sheep, goat, buffalo and donkey milk and their relationships with milk composition were investigated. Chen et al. (2010) reported that raw cow milk contains large amounts of miRNAs and that milk miRNAs may serve as a new marker and possible new standard for quality control of raw milk. The findings of this study highlight significant correlations between specific miRNAs and milk composition parameters such as fat, dry matter and pH. These correlations indicate that miRNAs may play a regulatory role in milk biosynthesis and composition and have the potential to serve as biomarkers to assess milk quality. However, further studies are needed to validate these findings across larger sample sizes and various conditions.

Cow Milk

In their study on miRNA levels in colostrum and lactation period milk in cattle, Izumi et al. (2012)

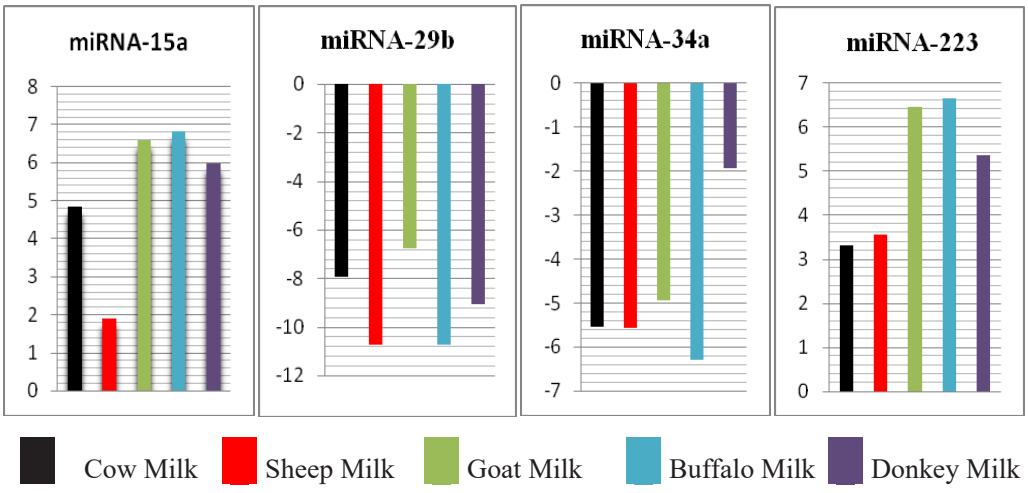

Figure 1. Average normalization values by species.

Table 6. Correlation between miRNAs expression and the physicochemical properties of milk

Type	miRNA	Dry matter	Fat	Protein	pH
Cow Milk	**miRNA 34a**	-0,20	0,90	-0,70	-0,20
		0,74	0,03	0,18	0,74
	miRNA 29b	-0,15	-0,50	0,50	-0,70
		0,80	0,39	0,47	0,20
	miRNA 15a	-0,13	0,20	-0,60	0,50
		0,70	0,74	0,28	0,39
	miRNA 223	0,15	0,70	-0,50	0,40
		0,80	0,18	0,25	0,50
Buffalo Milk	**miRNA 34a**	-0,41	-0,10	-0,66	0,56
		0,49	0,87	0,21	0,32
	miRNA 29b	-0,35	-0,20	-0,15	0,66
		0,55	0,65	0,80	0,21
	miRNA 15a	0,82	0,50	0,97	-0,60
		0,08	0,39	0,05	0,20
	miRNA 223	0,20	-0,10	0,15	-0,67
		0,74	0,87	0,80	0,21
Sheep Milk	**miRNA 34a**	-0,94	-0,70	-0,10	0,90
		0,01	0,18	0,87	0,03
	miRNA 29b	-0,90	-0,90	0,05	0,80
		0,01	0,03	0,93	0,10
	miRNA 15a	0,10	0,10	-0,70	-0,20
		0,86	0,87	0,17	0,74
	miRNA 223	0,79	0,80	0,20	-0,60
		0,11	0,10	0,74	0,28
Goat Milk	**miRNA 34a**	0,20	0,30	0	-0,56
		0,74	0,61	1	0,32
	miRNA 29b	0,10	0,20	-0,3	-0,60
		0,87	0,74	0,62	0,21
	miRNA 15a	0,70	0,66	0,90	0,20
		0,18	0,21	0,37	0,74
	miRNA 223	0,90	0,82	0,70	-0,20
		0,03	0,08	0,18	0,74
Donkey Milk	**miRNA 34a**	0,10	0,20	0,66	-0,10
		0,87	0,74	0,21	0,87
	miRNA 29b	-0,66	0,90	-0,51	0,35
		0,21	0,03	0,93	0,55
	miRNA 15a	-0,56	0,30	-0,61	0,20
		0,32	0,62	0,26	0,74
	miRNA 223	0,35	-0,50	-0,56	0,15
		0,55	0,39	0,32	0,80

reported that miRNA-27b, miRNA-15b, miRNA-106b, miRNA-29b, miRNA-29b, miRNA-130a, miRNA-155, miRNA-34a and miRNA-223 exhibited elevated expression in colostrum. The researchers employed miRNA-39a for normalization and observed the expression of miRNA-15a, miRNA-34a, miRNA-29b and miRNA-223 in cow's milk. Although these results are in agreement with our findings, it is not feasible to conduct a comprehensive comparison in terms of expression levels when miRNA-92a was employed for normalization. It is plausible that discrepanciesmay be attributed to this circumstance. Wang et al. (2014) demonstrated that miRNA-152 plays a pivotal role in the development and lactation processes of the mammary glands of cows, with its expression being higher in high-milk-yielding cows. Shen et al. (2016) identified miRNA-152 in cows'milk, whereas Melnik et al. (2017) discovered miRNA-148a in cows'milk, both of which were shown to regulate lipid metabolism. Chen et al. (2019) observed that the concentration of miRNA-16 in cows at distinct lactation stages exhibited fluctuations across each period and that there was a negative correlation between miRNA-16 and milk fat.

In this study, the relationships between the dry matter, protein, and fat contents and the pH values of cows'milk and miRNA-15a, miRNA-34a, miRNA-29b, and miRNA-223 were analyzed. A strong positive correlation ($r = 0.9$) was observed between miRNA-34a and fat content ($P<0.05$). This data was identified as being of particular significance, given that they represented one of the first studies to investigate the relationship between miRNA-34a and fat in cows'milk.

Buffalo milk

In a previous study, Guelfi et al. (2017) demonstrated that microRNAs (miRNAs) can be employed as biomarkers for the detection of pregnancy in buffalo. Cai et al. (2017) employed high-throughput sequencing technology (Illumina-Solexa) to investigate the mammary gland miRNA profiles of lactating and dry period buffaloes. They provided an overview of the miRNA expression profiles of buffaloes and the interactions between their target genes. Chen et al. (2020) analyzed the exosomal miRNA profiles in milk from buffalo, cow, pig, panda, and human milk. The researchers reported that 558 different miRNAs were identified across all species. Additionally, the first 10 highly expressed miRNAs were found to be similar across all studied milk species. The authors posit that buffalo milk consumption may have significant physiological implications. In a study conducted to identify and characterize miRNAs in buffalo milk exosomes, Rani et al. (2020) identified 351 and 17 previously undescribed miRNAs.

This present study revealed the expression of miRNA-15a, miRNA-29b, miRNA-34a, and miRNA-223 in buffalo milk. The miRNAs that we examined are among those identified in milk exosomes in studies conducted by Rani et al. (2020) and Chen et al. (2020). The discrepancies in miRNAs employed for normalization, along with variations in buffalo feding and breeding characteristics, preclude a comprehensive comparison of expression levels. The degree of association between these miRNAs and dry matter, protein, fat content, and pH was also investigated. Although a strong positive correlation ($r:0.82$) was identified between miRNA-15a expression and dry matter and fat content in buffalo milk, this correlation was not statistically significant ($P > 0.05$).

Sheep Milk

In a study conducted by Galio et al. (2013), the levels of miRNA-21, miRNA-205, and miRNA-200 were examined in the mammary gland at different stages of pregnancy and during estrus in sheep. These miRNAs exert an influence on epithelial tissue development and milk secretion in the mammary gland. Wang et al. (2021) employed RNA sequencing to examine the expression profiles of miRNAs in the mammary gland of sheep during lactation and the dry period and reported that a total of 147 mature miRNAs were expressed during both periods. Quan et al. (2020) proposed that miRNAs in milk EVs facilitate intercellular communication, particularly between mothers and neonates. They characterized the miRNA profile in sheep EVs and compared it with that of cows'milk. When they compared the most expressed miRNAs in cows' and sheep's milk, they identified four common miRNAs. Duman et al. (2022) conducted a study to determine the expression of certain pivotal lactation-related miRNAs in sheep milk between high and low lactation yield groups in Akkaraman sheep. In addition to miRNA expression results obtained through experimental analysis, the researchers identified miRNA target genes through bioinformatic analysis to determine relevant biological pathways. As a result, the researchers identified five miRNAs (miRNA-181a, miRNA-23a, miRNA-27a, miRNA-16b and miRNA-374) that exhibited not able differences in expression levels between

the two groups. Additionally, a negative correlation was observed between miR-27a and milk protein and lactose content, and between miR-16b and high milk yield. Hao et al. (2021) observed differential expression of miRNA-432 in mammary gland tissue during the dry and lactation periods. The researchers observed a negative correlation between miRNA-432 and milk fat. Wang et al. (2020) reported a negative correlation between miRNA-199a-3p expression and milk fat content in mammary gland cells of sheep.

In the this study, miRNA-15a, miRNA-34a, miRNA-29b, and miRNA-223 were identified as being expressed in sheep milk. Notably, the expression of miRNA-15a, which suppresses cancer, was markedly elevated compared to other species. A paucity of studies in the scientific literature has addressed the negative correlation between miRNA-34a and drymatter, as well as the positive correlation between miRNA-34a and pH and the negative correlation between miRNA-29b and drymatter, as well as fat. This study is important because it represents one of the first investigations into the relationship between miRNA-34a, miRNA-29b, and sheep milk composition.

Goat Milk

In a study conducted by Li et al. (2012), the miRNA profiles of mammary gland tissues from goats during the peak lactation and dry periods were examined. In total, 441 miRNAs were identified, including 346 known and 95 novel miRNAs. The authors posit that these data will be invaluable in elucidating the roles of miRNAs in the biological processes of lactation, target gene expression, and regulatory mechanisms. Lin et al. (2013a) reported that miRNA-27a suppressed triglyceride accumulation in dairy goat mammary gland epithelial cells and affected fat metabolism-related gene expression. Lin et al. (2013b) reported that miRNA-103 increased the transcription of genes associated with milk fat synthesis in mammary gland cells, resulting in triglyceride accumulation. Chen et al. (2018) reported that miRNA-183 regulates milk fat metabolism in goat mammary epithelialcells. Ma et al. (2018) asserted that miRNAs play a pivotal role in the lipid metabolism of goat milk, with miRNA-25 exhibiting deleterious effects by reducing the expression of genes involved in lipid metabolism.

In a study examining the expression of 11 miRNAs, including miRNA-223, Lin et al. (2013c) investigated lipid metabolism in mammary gland tissue in goats. The results indicated that there was no change in miRNA-223 levels. In a further study, Na et al. (2015) compared the expression levels of miRNA-146, miRNA-155, miRNA-181a, miRNA-150, and miRNA-223 in goats, humans and dairy cattle. The researchers observed that miRNA-223 was expressed at a higher level in bovine milk than in goat milk. Na et al. (2015) observed miRNA-223 expression in both cows and goats. Altough not statistically significant, miRNA-223 expression was higher in goats than in cows. Golan-Gerstl et al. (2017) conducted a study using next-generation sequencing and RT-PCR in humans, cow, and goat milk. The researchers reported that approximately 91-92% of the miRNA profile expressed in human milk was also expressed in cow and goat milk. Furthermore, it was reported that 89% and 83% of the miRNAs expressed in bovine and goat milk, respectively, were also identified in human breast milk. Yun et al. (2021) conducted a comparative analysis of exosom al miRNAs in humans, cow, and goat milk, including colostrum and raw milk samples. The miRNAs that exhibited the highest expression levels in human milk exhibited a similar degree of conservation across species. A review of the literature revealed that miRNAs play a significant role in regulating the composition of goat milk. Additionally, there is a growing body of research examining the role of fat metabolism. The examination of all miRNAs in this study revealed their expression in goat milk. In particular, miRNA-29b, which has been demonstrated to play a role in bone mineralization in humans, was expressed at a higher level in goat milk than in other species. A strong positive correlation ($r = 0.9$) was observed between miRNA-223 and dry matter in goat milk ($P<0.05$). This data was identified as being of particular significance because they represented one of the first studies to investigate the relationship between miRNA-223 and dry matter in goat milk.

Donkey Milk

The existing literature on miRNA expression and characterization in donkey milk is limited. In a study designed to elucidate the role of epigenetic regulation in the donkey genome and its karyotype, Huang et al. (2015) analyzed differentially expanded miRNA families in the donkey and other mammalian species, identifying 1198 miRNAs in the donkey genome. In a study investigating the use of miRNAs as biomarkers for the diagnosis of sarcoid disease in single-hoofed animals, Unger et al. (2021) compared serum miRNA-331, miRNA-100 and miRNA-1 expression levels in horses and donkeys. They

found that miRNA-331 was more highly expressed in diseased tissues, that this miRNA could be utilized in disease diagnosis. In a separate study, Li et al. (2022) selected the psoas major muscle and biceps femoris muscle in donkeys and conducted a systematic comparison of the mRNA and miRNA transcriptomes of these muscles using RNA-seq. The authors indicated that miRNAs, particularly miRNA-193a-5p and miRNA-370, predominantly influence actin binding and may regulate myofibril composition in donkeys. It has been stated that these miRNAs can be employed to enhance meat quality traits in donkeys. In a study conducted by Fei et al. (2022), the authors investigated the expression of mRNAs and miRNAs in the mammary gland tissues of three donkeys during the lactation and dry periods. In particular, miRNA 874-3p was identified as a key miRNA targeting mRNAs involved in immunity and lipid, protein, and vitamin metabolism of milk in the mammary gland of donkeys. The miRNAs regulated the metabolism of bioactive milk components in mammary glands and may enhance milk production in donkeys. Mecoci et al. (2021) conducted a transcriptomic analysis of mRNA and miRNA libraries to characterize the RNA content of extracelluler vesicles from cows, donkey and goat. The researchers reported that miRNAs comprised the most prevalent class of small RNAs across all species, and that 10 miRNAs with a significant impact on immuno regulatory function were highly expressed in all three species (cow, goat and donkey).

In this study, miRNA-15a, miRNA-223, miRNA-34a, and miRNA-29b were expressed in donkey milk. A strong positive correlation ($r = 0.9$) was observed between miRNA-29b levels and fat content in donkey milk ($P<0.05$). Accordingly, these data wasidentified as a significant contribution to the field because they represente done of the first studies on donkey milk.

CONCLUSION

The aim of this study was to investigate the presence and amounts of miRNA-15a, miRNA-29b, miRNA-34a and miRNA-223 in the milk of cows, buffaloes, sheep, goats, and donkeys. Furthermore, the relationships between miRNAs and milk composition were analyzed. The study revealed that the miRNAs under examination were expressed in all animal species. A positive correlation was identified between miRNA-34a expression and fat content in cows'milk. In sheep milk, a negative correlation was observed between miRNA-34a and drymatter, whereas a positive correlation was noted between pH and a negative correlation between miRNA-29b and dry matter and fat. In goat milk, a positive correlation was observed between miRNA-223 and drymatter. In donkey milk, a robust positive correlation was observed between miRNA-29b levels and fat content. In studies conducted in the literature examining the relationship between milk composition and miRNAs, it has been reported that miRNAsplay an active role in milk composition. The findings of this study indicate that miRNA-15a, miRNA-29b, miRNA-34a, and miRNA-223 are associated with milk composition in the analyzed milk. To fully elucidate this relationship, further research is required in this field. Further research in this area may facilitate the utilization of these miRNAs to enhance milk yield and quality. The revelation of the effects of the miRNAs examined on nutrition and health will be beneficial to public health and the consumption of the analyzed milk.

CONFLICT OF INTEREST

None declared

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