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Influence of stage lactation on quality and protein compositions of Kazakh mare milk and koumiss

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ABSTRACT

Limited studies have examined the effects of geography, climate, and lactation on mare's milk in Kazakhstan. The study aimed to assess the protein components and quality of mare's milk and koumiss from 24 mares in southern Kazakhstan. Milk samples were collected monthly between July and December 2023. The soluble protein fraction was analysed via SDS-PAGE. Casein fractions were examined using SDS-PAGE polyacrylamide electrophoresis. The results indicated the presence of α-, β-, and κ-caseins, along with whey proteins such as α-lactalbumin and β-lactoglobulin in the milk and fermented products. The milk contained 9.02% total solids, 1.62% protein and 1.22% casein on average. The mare milk fat content was 0.71% in Almaty, and in the Zhambyl region, it was significantly higher - 1.24%. The fermented koumiss products had a fat content of 1.22% in Almaty, while in Zhambyl, it was significantly higher at 1.94%. Similarly, the casein content in the Zhambyl region was 1.38%, compared to 0.81% in Almaty. The results indicate that different zones significantly affect mare's milk's fat and protein composition.

Keywords: mare milk, koumiss, protein profile, fat, electrophoresis, SDS-PAGE

INTRODUCTION

Mare's milk represents a highly esteemed alimentary product attributable to its chemical composition, which resembles human milk **[1]**, **[2]**. It is distinguished by its elevated biological value and digestibility, a characteristic with a longstanding historical precedent, particularly in the Central Asian regions where it has been ingested for centuries **[3]**, **[4]**. Recently, a global trend has emerged regarding the consumption of mare milk **[5]**. This increasing demand can be ascribed to its advantageous health properties **[6]**. Mare's milk is highly regarded for its nutritional value and distinctive attributes **[7]**. Furthermore, it is frequently consumed in its fermented variant, known as koumiss (or kumis). This beverage is characterised by its low alcohol content (below 5%) and slightly acidic profile derived from mare's milk **[8]**. Historically, this drink was predominantly consumed by nomadic populations. Traditionally, its production entails the fed-batch fermentation of raw milk **[9]**.

Mare's milk exhibits a markedly lower concentration of casein than cow's milk. Furthermore, including mare's milk in the dietary regimen of individuals afflicted with inflammatory bowel diseases has been shown to mitigate discomfort **[10]**, **[11]**. The therapeutic advantages associated with mare's milk within the realm of gastroenterology may be attributed to the presence of lactoferrin, lysozyme, and additional proteins that possess bactericidal characteristics and enhance the proliferation of bifidobacteria **[12]**, **[13]**. Notably, lactoferrin assumes a pivotal function in the attenuation of inflammation and the stimulation of humoral immunity **[14]**, **[15]**. Owing to its elevated whey protein composition, mare's milk, akin to human milk, is categorised as an albumin type **[16]**.

The lacteal secretion of the mare is often utilised in conventional medicinal practices to aid in mitigating gastrointestinal disorders and lactose intolerance, owing to its enzymatic components that promote the

hydrolysis of lactose **[17]**. Moreover, its immunomodulatory properties may confer therapeutic advantages for chronic conditions, infectious illnesses, and immunological deficiencies, as well as in the therapeutic management of various pathologies, including chronic hepatitis and tuberculosis **[18]**. An array of scholarly investigations suggests that mare's milk may exhibit anti-inflammatory characteristics and be advantageous in treating conditions such as eczema and other dermatological issues **[19]**.

This study aimed to determine Kazakh mare's milk's physicochemical characteristics and protein fractions during lactation.

Scientific Hypothesis

This study focused on changes in contents of physical and chemical quality contents of mare milk and fermented koumiss. In this study, an analysis was undertaken regarding the physicochemical characteristics of mare's milk and koumiss throughout the lactation period. Consequently, it was determined that the chemical compositions and whey protein of the milk and koumiss can be affected by several variables: breed, stage of lactation, and seasonal variations. Furthermore, in addition to these determinants, the composition of mare's milk, particularly the whey protein content, can be altered, notably concerning protein and fat concentrations.

MATERIAL AND METHODOLOGY

Samples

Mare's milk was obtained from 24 Kazakh mares of ages ranging from 4 to 7 years, which were housed at a horse dairy farm located in the Almaty (n=12) and Zhambyl (n=12) regions (Southern Kazakhstan). These mares had given birth between March and June. The milk collection took place from July to December. Over six months, each month 24 samples of mare's milk were collected from each mare at two farms in the Almaty and Zhambyl regions of Kazakhstan. Each sample was at least 500 ml. The milk was collected by hand milking after cleaning the udder and stored in sterilised plastic bottles. The milk products were frozen at -20°C post-harvest for further processing. Before use, samples were thawed overnight at 4°C, heated in a water bath at 37°C, under mixing and cooled at room temperature.

Instruments

FOSS Milkoscan FT1 (FOSS, Denmark), Electrophoresis (Mini-PROTEAN® Tetra Cell (Bio-Rad) USA)

Laboratory Methods

Mare milk quality analysis

The samples were analysed using the reference infrared absorption method on the FOSS Milkoscan FT1 equipment (Denmark) to determine their chemical composition. Each sample was placed in a 50 cm^3 laboratory beaker, heated to (40±2)°C, thoroughly mixed, and immediately measured. This quantitative instrumental express method was used to determine the mass fractions of milk protein, fat, moisture, dry matter, and casein content, utilising an infrared analyser and infrared spectroscopy. All analyses were conducted following ISO standards: ISO 9622 IDF 141: 201, ISO 8196 IDF 128-3:2009.

Determination of mare milk protein fraction by SDS-PAGE (Sodium dodecyl-sulfate polyacrylamide gel electrophoresis)

Acrylamide Solution 30%: Exactly 29 g of acrylamide (Sigma-Aldrich) and 1 g of bisacrylamide (Sigma-Aldrich) were dissolved in double distilled water, and the volume was 100 mL. It was stored at 4°C in the amber colour bottle. Electrode (running) buffer: Exactly 3.03 g of Tris base (Sigma-Aldrich), 1 g of SDS (Sigma-Aldrich) and 14.4 g of Glycine (Sigma-Aldrich) were added to water in 1000 mL. 10% SDS (Sigma-Aldrich) Solution: Exactly 10 g of SDS (Sigma-Aldrich) was dissolved in 75 mL double distilled water, and a volume made up to 100 mL. It was stored at room temperature in a plastic container till further use. 0.5M Tris (Sigma-Aldrich) HCl (standard 31118-77 RU) (pH6.8): Exactly 6.05g of Tris base (Sigma-Aldrich) was dissolved in 60 mL double distilled water, and pH was adjusted to 6.8 with concentrated HCl (standard 31118-77 RU). Volume was made to 100 mL and stored at 4ºC. 2M Tris (Sigma-Aldrich) HCl (standard 31118-77 RU (pH8.8): Exactly 24.2g of Tris base (Sigma-Aldrich) was dissolved in 80 mL double distilled water, and pH was adjusted to 8.8 with concentrated HCl (standard 31118-77 RU). Volume was made to 100 mL and stored at 4ºC. Laemmli buffer solution adds 1 ml of 1 % bromophenol blue (Sigma-Aldrich) to 4 ml of 1.5 M tris (Sigma-Aldrich) HCI (standard 31118-77 RU) pH=6.8 add 10 of glycerol (standard 6259-75 RU) and 2 g of SDS (Sigma-Aldrich) and 5 ml of β- mercaptoethanol (Sigma-Aldrich) mix and store at -20 °C. 10% W/V Ammonium Per Sulphate (standard 20478-75 RU) (APS): APS solution was always prepared fresh by dissolving 100 mg of APS (standard 20478-75 RU) in 1mL double distilled water. 2X Sample Buffer: The sample buffer was prepared by dissolving the substances given below, and the final volume was made to 20 mL with double distilled water and stored at 4 °C till further use.

Table 1 Composition of Sample buffer.

Sl No	Components	Resolving gel 15%	Stacking gel 6%
1	Distilled water (mL)	3.09	2.4
2	Acrylamide: 30% (mL)	6	0.75
3	Tris HCl: 0.5 M 6.8 pH (µl)	-	495 µl
4	Tris HCl: 2M, 8.8 pH, (mL)	2.7 ml	-
5	SDS:10% (µL)	75	42
6	APS:10% (µL)	75	38
7	TEMED (µL)	9	5

Staining Solution: Exactly 0.2 g of Coomassie Brilliant Blue – G250 250 (Sigma-Aldrich) (0.2%) was dissolved in a solvent mixture containing ethanol, acetic acid (Sigma-Aldrich) and water in the ratio of 5:1:5. The staining solution was filtered and stored at room temperature. Destaining Solution – 10% Acetic Acid (Sigma-Aldrich): Exactly 10 mL of glacial acetic acid (Sigma-Aldrich) was made up to 100 mL with double distilled water just before use.

Gel Preparation: The separating gel was 15%, and the stacking gel was 6%. All solutions were stored at 4°C. The electrophoresis was performed in Bio-Rad Mini-PROTEAN electrophoresis system gel electrophoresis unit. The gel mixtures were gently poured into the casting modules. After filling, the separating gel (8 cm deep) was carefully overlaid with a 1-2 mm deep layer of distilled water to allow a flat surface and protect the top of the gel mixture from atmospheric oxygen. After polymerisation, the distilled water was replaced by the spacer gel (2 cm deep). The stacking gel was added to about 3 cm deep, and soon after adding, the combs were inserted. After the polymerisation of the stacking gel, the comb was removed, and wells were rinsed with cathode buffer.

The molecular weight distribution of proteins was evaluated using protein electrophoresis following the Laemmli method **[20]**. Proteins were separated in a denaturing polyacrylamide gel (15% separating gel and 6% stacking gel) using SDS-PAGE. Electrophoresis was conducted in a single buffer system with SDS-PAGE at 15 mA. The gel was stained with 0.2% Coomassie R-250 dye (Sigma-Aldrich) (prepared with 10% glacial acetic acid) and washed thrice with distilled water. Before electrophoresis, milk and fermented products (mare's milk and kumiss) were incubated with sodium dodecyl sulfate to form negatively charged complexes with milk proteins. Additionally, treating milk proteins with mercaptan, which reduces disulfide bonds, resulted in the complete dissociation of protein complexes. The mobility of a protein in polyacrylamide gel is influenced by its molecular weight.

The gel was calibrated with protein markers consisting of seven highly purified recombinant proteins, ranging in molecular weights from 10 to 250 kDa Thermo Scientific. These markers form distinct bands after electrophoresis in polyacrylamide gel and subsequent dye fixation. The resulting data were then processed using standard statistical methods.

The Quantitative determination of protein was then performed using ImageJ and Imagelab software, which digitally processed the band images.

Description of the Experiment

Sample preparation: Samples were diluted by adding an equal volume of 2X sample buffer (1:1), heated at 100°C for 3 min, centrifuged, and immediately stored at 4°C. Wide range molecular weight standards from 10 kDa to 250 kDa Thermo Scientific were used as standards. Samples and standards were applied under the cathode buffer.

Run Conditions: Electrophoresis was performed at room temperature using constant voltage. Voltage was kept constant at 100V until the samples completely left the stacking gel, and in the separating gel, it was increased to 150V, and then voltage was maintained constant (100-150V) until the tracking dye reached the bottom of the gel. Staining with Coomassie Brilliant Blue R-250 (Sigma-Aldrich): Immediately after the end of electrophoresis, gels were removed from the plates, and the gel containing the marker and samples was cut and placed in a fixative solution containing 50% ethanol and 10% acetic acid (Sigma-Aldrich). After 30 minutes, the fixative solution was replaced by a staining solution containing 0.2% Coomassie Brilliant Blue R-250 (Sigma-Aldrich), where the gels were left for 30 min. After staining, the gels were transferred to the de-staining solute ion at room temperature. De-staining was done till the bands appeared and the background became clear.

Statistical Analysis

The influence of physical-chemical indicators on the milk was evaluated by one-way ANOVA followed by Tukey's HSD post hoc test for multiple comparisons when significant differences ($p < 0.05$) between the mean values were found. All statistical analyses were done using JMP 17 Pro (JMP Statistical Discovery LLC, Cary, NC, USA).

RESULTS AND DISCUSSION

Our task was to evaluate the chemical composition of mare's milk from different regions of Kazakhstan. To achieve this, we studied mare milk samples from various climatic regions, specifically Almaty and Zhambyl.

Our analysis revealed several distinctive features in the physicochemical parameters of mare's milk, which varied significantly based on the region, season, and animal husbandry conditions. It is well-known that milk production and quality are influenced by breed, lactation stage, age, feeding level, season, and animal care conditions.

Mare's milk can be consumed fresh and is particularly beneficial for young children, serving as an excellent substitute for mother's milk. However, preserving fresh mare's milk is challenging due to its high sugar content and lack of a fat film, which cause it to sour quickly.

Table 2 Chemical composition of Almaty and Zhambyl regions Kazakh mare milk during lactation.

	Almaty mare milk samples by month						p-values by regions
	July (n=12)	August (n=12)	September (n=12)	October (n=12)	November (n=12)	December (n=12)	
Fat, %	1.23±0.2	0.95±0.24	0.56±0.18	0.49±0.23	0.50±0.22	0.51±0.04	0.0011
Protein, %	1.73±0.11	1.69±0.13	1.63±0.11	1.67±0.13	1.59±0.15	1.65±0.03	0.0027
Casein, %	1.44±0.09	1.51±0.07	1.08±0.08	1.04±0.09	1.24±0.08	1.17±0.02	0.0019
SNF, %	8.99±0.14	8.06±0.11	9.45±0.21	8.72±0.88	8.78±0.84	8.74±0.03	0.0222
TS, %	10.21±0.18	9.22±0.23	8.72±0.17	8.71±0.71	8.75±0.7	8.64±0.02	0.0001

	Zhambyl mare milk samples by month					
	July (n=12)	August (n=12)	September (n=12)	October (n=12)	November (n=12)	December (n=12)
Fat, %	1.71±0.31	1.69±0.16	1.32±0.14	1.00±0.36	0.84±0.25	0.89±0.02
Protein, %	1.61±0.13	1.72±0.11	1.48±0.07	1.57±0.08	1.51±0.07	1.58±0.01
Casein, %	1.36±0.13	1.53±0.12	0.95±0.03	1.00±0.11	1.17±0.1	1.11±0.08
SNF, %	8.41±0.36	8.63±0.31	8.43±0.47	8.54±0.23	8.65±0.54	8.50±0.11
TS, %	10.57±0.47	9.24±0.33	9.02±0.51	8.79±0.48	8.24±0.66	8.15±0.05

Note: SNF – solid not fat, TS – total solid.

Table 3 Chemical composition of Almaty and Zhambyl regions koumiss during lactation.

	Almaty koumiss samples by month						p-values by regions
	July (n=12)	August (n=12)	September (n=12)	October (n=12)	November (n=12)	December (n=12)	
Fat, %	1.28±0.02	0.0011	1.17±0.01	1.18±0.42	1.21±0.17	1.14±0.02	0.0011
Protein, %	1.72±0.01	0.0027	1.50±0.02	1.65±0.06	1.64±0.03	1.34±0.02	0.0027
Casein, %	1.50±0.02	0.0019	0.43±0.03	0.62±0.03	0.64±0.08	0.38±0.04	0.0019
SNF, %	7.70±0.01	0.0222	9.31±0.05	7.45±0.21	8.09±0.06	8.2±0.03	0.0222
TS, %	8.58±0.04	0.0001	8.17±0.12	6.26±0.13	9.87±0.11	9.67±0.27	0.0001

	Zhambyl koumiss samples by month					
	July (n=12)	August (n=12)	September (n=12)	October (n=12)	November (n=12)	December (n=12)
Fat, %	2.02±0.02	2.01±0.01	1.99±0.01	1.78±0.05	1.89±0.02	1.69±0.41
Protein, %	1.71±0.02	1.72±0.02	1.12±0.01	1.61±0.02	1.58±0.01	0.72±0.02
Casein, %	1.46±0.05	1.51±0.04	1.02±0.01	1.65±0.11	1.27±0.01	1.23±0.05
SNF, %	7.07±0.13	7.28±0.07	7.70±0.01	10.77±0.03	8.58±0.08	8.51±0.11
TS, %	6.43±0.07	6.60±0.05	5.13±0.01	8.22±0.05	7.38±0.24	7.13±0.04

Note: SNF – solid not fat, TS – total solid.

When examining the dynamics of individual components in mare's milk during lactation, we observed changes in its chemical composition. Notably, the fat content initially increased and then slightly decreased, with the highest fat content occurring in the early months of lactation. Specifically, the fat content was 1.23% and 1.17% in the first months at both regions (p=0.0011), respectively, and decreased to 0.51% and 0.84% by the end of lactation, as shown in Table 2. The peak lactation period for mares is typically in the second to third months, after which milk yield gradually declines due to the physiological characteristics of horses. The fat content of koumiss in the Almaty region ranged from 1.14 to 1.35% during lactation (Table 3). Concurrently, the fat percentage in the Zhambyl region exhibited higher values, recorded between 1.69 and 2.02%. In comparing the two regions concerning the fat content in koumiss, it is evident that the steppe Zhambyl region demonstrates significantly elevated levels, particularly noted during the summer months when increased fat concentrations in koumiss. The mean fat content of mare milk (Table 2) was similar to that of mares' milk **[21]**, especially steppe region fat contents demonstrated reflecting the differences in pasture botanical diversity. We observed a significant regional variation in fat content. The present study revealed variations in fat content, which aligns with previous research findings **[22]**. Chen et al. reported high-fat content in koumiss in their research, similar to our findings, where fat content varied from 1.14% to 2.02% depending on the lactation period **[23]**.

The transformation of mare's milk into koumiss involves significant changes in its protein content and composition, influenced by fermentation. The protein content also varied month to month, influenced by the lactation period (p=0.0027). The protein content of mare's milk ranged from 1.51% to 1.73% (p=0.0019), with a high coefficient of variation in casein content (52.47%). The protein content in koumiss was similar to mare milk protein percentage during the lactation. The content showed statistically significant results for the region, where Mariani et al. also observed the same trend **[24]**. Other researchers have also reported a decrease in protein during this fermentation process **[25]**.

In the analysis of mare's milk, it was determined that, on average, the concentration of TS exhibited values of 10.57% and 10.21%, reflecting a notable quantitative presence of these components within the fluid. The measurement of TS revealed its lowest recorded percentages, specifically 8.75% and 8.24%, during the fifth month of lactation. In contrast, the peak concentrations were observed to occur in the 1th and 2th month of lactation, where they reached maximum values of 10.57% and 10.21%. Furthermore, it was noted that in the initial month of lactation, there were instances where the appearance of an average greater quantity of substances was recorded compared to the following lactation period. The substance of interest, SNF, presented values ranging from 8.99% to 8.78%. In the Almaty region, this percentage experienced a decline by the conclusion of the lactation period. Conversely, in the Zhambyl region, the SNF content increased, manifesting values between 8.41% and 8.65%. After the lactation period, the availability of pasture grasses diminishes as primary nutritional sources for lactating organisms, subsequently leading to a reduction in both SNF and TS **[26]**. Another study observed that the TS content of mare milk decreased from 12.5% to 10.2% at the end of lactation, which aligns with the results of our study **[27]**. Access to pasture significantly impacted the quality parameters profile in the milk of Kyrgyz mares. In contrast to our study, the climatic conditions in the Almaty region closely resemble those described by Mazhitova et al. **[28]**, with similar results in fat (2.20%) and dry matter (10.93%) indices.

The SDS-PAGE analysis of the whey protein fraction revealed the most abundant whey proteins, as shown in the representative results of Figure 1 and Figure 2. Electrophoresis in SDS-PAGE allowed us to clearly identify the heterogeneity of casein and detect fluctuations in casein fractions in the studied samples of milk and koumiss.

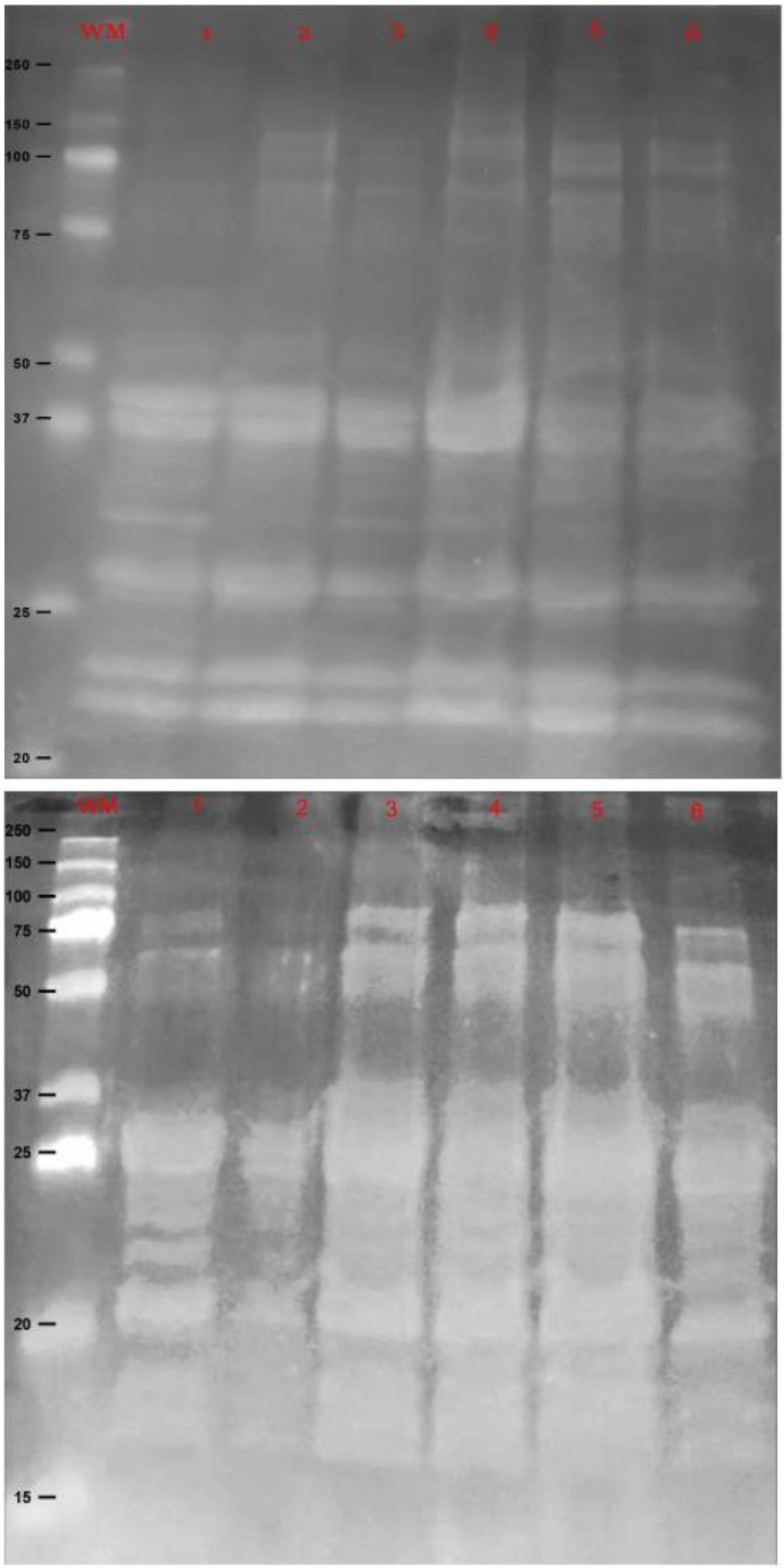

Figure 1 Electrogram mare's milk Zhambyl (upper) and Almaty (lower) region.
Note: Electrophoretic separation of mares' milk protein by month (July – December).

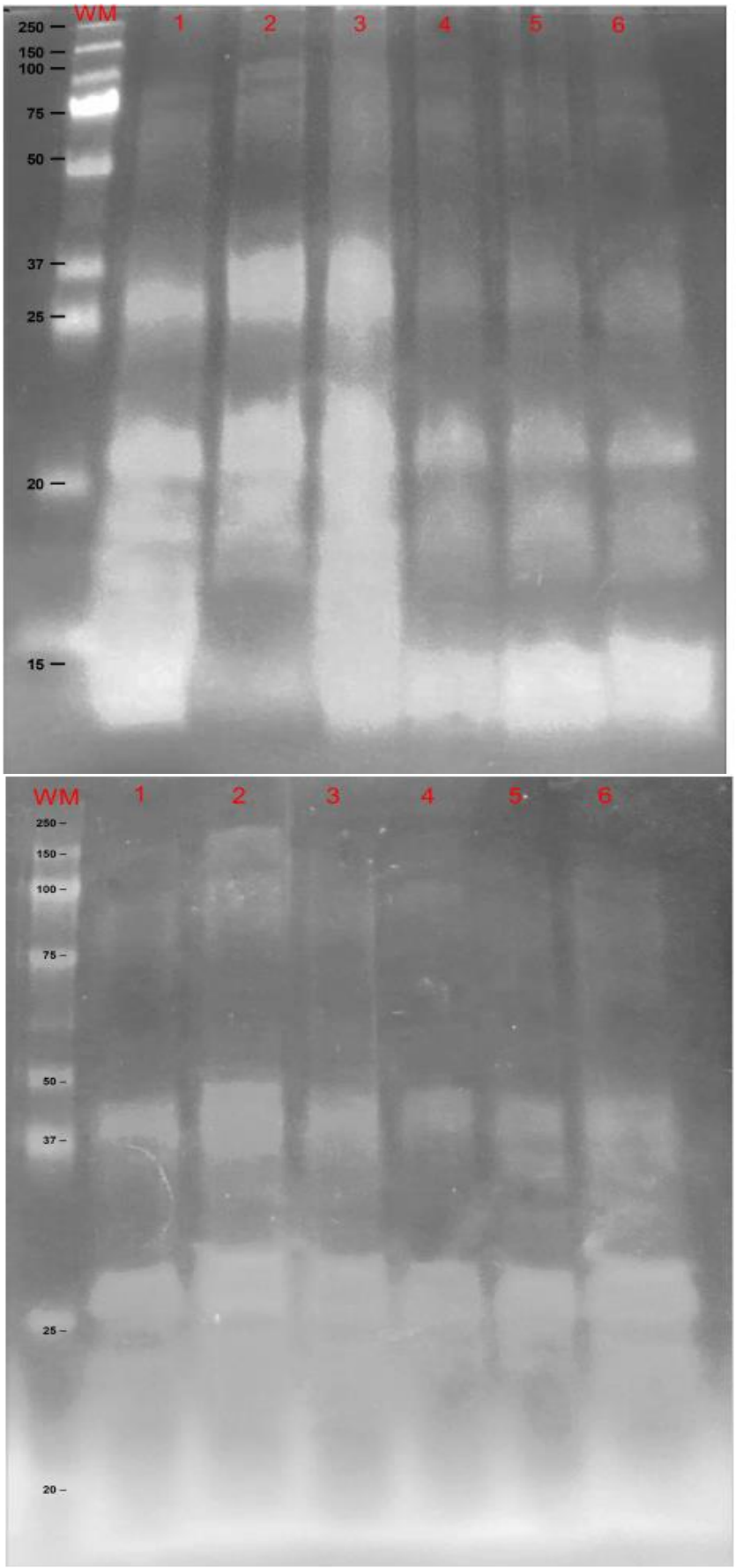

Figure 2 Electrogram koumiss Zhambyl (upper) and Almaty (lower) region.
Note: Electrophoretic separation of koumiss protein by month.

Electrophoresis results indicated that the casein content in mare's milk from the Zhambyl region increased slightly in July, August, and September but decreased in October, November, and December. Specifically, the amount of αS1-casein was 21.1 kDa in July, 21.0 kDa in August, 20.1 kDa in September, 19.9 kDa in October, 20.0 kDa in November, and 19.1 kDa in December. For β-casein, the values were 24.3 kDa in July, 24.2 kDa in August, 23.2 kDa in September, 23.8 kDa in October, 22.9 kDa in November, and 22.4 kDa in December. Additionally, the presence of lactalbumin and whey proteins was noted. The amount of β-lactoglobulin was 18.9 kDa in July, 18.7 kDa in August, 18.1 kDa in September, 18.0 kDa in October, 17.8 kDa in November, and 18.0 kDa in December. The α-lactoalbumin content was 14.5 kDa in July, 14.9 kDa in August, 14.2 kDa in September, 13.7 kDa in October, 13.0 kDa in November, and 12.8 kDa in December.

The electrogram illustrates the protein fractions of mare's milk from the Almaty region. The amount of αS1-casein was 20.0 kDa in July and August, 20.5 kDa in September, 21.6 kDa in October, 20.8 kDa in November, and 19.6 kDa in December. For β-casein, the values were 21.9 kDa in July, 22.2 kDa in August, 22.6 kDa in September, 23.4 kDa in October, and 22.9 kDa in November and December. The β-lactoglobulin content was 18.7 kDa in July, 18.2 kDa in August, 18.1 kDa in September, 18.5 kDa in October, 17.8 kDa in November, and 16.9 kDa in December.

Based on protein separation using SDS-PAGE, the most abundant proteins in mare milk samples were between 10 and 50 kDa. Our research results on protein fractions align with similar trends observed in studies by other researchers **[29]**, **[30]**. Our findings align with those of other researchers **[31]**, who reported molecular weights of 28 kDa and 22.9 kDa for α- and κ-casein, respectively. In our studies, we observed molecular weights of 27.6 kDa and 22.4 kDa, with an average casein weight of 21.0 kDa depending on the lactation period. Our results also indicate a tendency for decreasing casein content in Kazakh mare milk during lactation. Doval et al. reported that casein and whey proteins in mare milk are influenced by horse breed. Their study also indicated that these proteins remain fairly stable concerning factors such as mare care and lactation stage, although some proteins exhibited alterations **[32]**.

The electrophoresis results for koumiss from the Zhambyl region showed that the amount of αS1-casein was 20.0 kDa in July and August, 20.5 kDa in September, 21.6 kDa in October, 20.8 kDa in November, and 19.6 kDa in December. For β-casein, the values were 21.9 kDa in July, 22.2 kDa in August, 22.6 kDa in September, 23.4 kDa in October, 22.9 kDa in November, and 22.4 kDa in December. Denaturing electrophoresis in polyacrylamide gel with mercaptan allowed us to separate the casein fraction into αS1 and β-caseins, as well as the fractions of α-lactalbumin and β-lactoglobulin. The amount of β-lactoglobulin was 18.9 kDa in July, 18.7 kDa in August, 18.1 kDa in September, 18.0 kDa in October, 17.8 kDa in November, and 18.0 kDa in December. The α-lactalbumin content was 14.5 kDa in July, 14.9 kDa in August, 14.2 kDa in September, 13.7 kDa in October, 13.0 kDa in November, and 12.8 kDa in December.

Electrophoresis results for koumiss from the Almaty region showed that the amount of αS1-casein was 23.0 kDa in July, 24.8 kDa in August, 24.3 kDa in September, 24.9 kDa in October, 23.3 kDa in November, and 22.9 kDa in December. For β-casein, the values were 21.5 kDa in July, 21.9 kDa in August, 21.8 kDa in September, 21.7 kDa in October, 20.9 kDa in November, and 20.5 kDa in December. Additionally, lactalbumin and whey proteins were noted in mare's milk. Denaturing mercaptan-polyacrylamide gel electrophoresis allowed us to separate the casein fraction into αS1, αS2, β-, and κ-caseins, as well as the α-lactalbumin and β-lactoglobulin fractions. The amount of β-lactoglobulin was 16.0 kDa in July, 16.7 kDa in August, 16.5 kDa in September, 16.4 kDa in October and November, and 16.1 kDa in December. The α-lactalbumin content was 13.5 kDa in July, 14.3 kDa in August, 14.5 kDa in September, 14.6 kDa in October, 13.6 kDa in November, and 13.3 kDa in December.

The electrophoresis demonstrated elevated casein protein fractions in mare's milk compared to koumiss. Methodological advancements facilitated the separation of whey protein fractions, including α-lactalbumin and β-lactoglobulin. A 60 kDa band, indicative of whey protein, was characterised as immunoglobulins. The casein fraction, with a molecular weight of 27 to 30 kDa, exhibited different sensitivities in mare's milk **[33]**.

Ochirkhuyag et al. (2000) **[34]** reported the presence of caseins in mare milk. The observed percentage of 18 kDa β-lactoglobulin was significantly lower than in cow's milk, where 19 kDa β-lactoglobulin can constitute up to 17.5% and 16.6% of the total whey protein in mares, respectively, similar to findings by Schryver et al. (1986) **[35]**. The present study showed fractions of α-lactalbumin and β-lactoglobulin, 18.9 kDa and 14.5 kDa, respectively. Uniacke-Lowe et al. noted that the major whey proteins in equine milk are β-lactoglobulin and α-lactalbumin, which, similar to present research work, where β-lactoglobulin and α-lactalbumin fraction high profile showed in summer lactation period **[36]**. The results of other researchers reported that albumin contents increased during the lactation period **[37]**, **[38]**. β-lactoglobulin is a significant allergen in infants, whereas casein predominates in adult allergies **[39]**. Studies have suggested that goats **[40]**, mare **[41]**, donkeys **[42]**, and camel milk **[43]** can be suitable alternatives to human milk due to their hypoallergenic properties. However,

other studies indicate that cow milk may not always be a suitable alternative to breast milk, as they can cause allergies **[44]**, **[45]**. Fresh mares' milk and fermented koumiss have high nutritional value, whereas unripe koumiss has lower nutritional value with a shorter fermentation time **[46]**.

CONCLUSION

This study showed that the geographical conditions significantly affected mare milk and koumiss by quality parameters, especially protein, fat and TS. Kazakh mares were characterised by producing milk with the highest level of protein, casein. In the Almaty region, the molecular weight of αS1-casein and β-casein in mare caseins ranged from 20.0 to 19.6 kDa and 21.9 to 22.9 kDa, respectively. In the Zhambul region, the molecular weight of mare caseins ranged from 24,3 to 22,4 kDa and 21,1 to 19.1 kDa. In Almaty regions, the molecular weight of αS1-casein, and β-casein in mare caseins ranged from 23,0 to 22.9 kDa, 21.5 to 20.0 kDa. According to the study results, the number of cases in the Almaty region was less than in the Zhambyl region.

This study underscores the substantial influence of geographical and climatic factors on the quality characteristics of mare's milk and koumiss, notably affecting protein, fat, and total solids composition. Kazakh mares from different regions displayed significant variation in these parameters, with Zhambyl region samples generally exhibiting higher fat and protein content than those from Almaty. Such findings reinforce the importance of environmental conditions and pasture diversity, crucial in determining the nutritional profile of mare's milk and derived products.

The electrophoresis analysis further revealed distinct molecular weight patterns for casein and whey proteins, with differences evident between regions and throughout the lactation period. These protein profiles suggest the potential for optimising koumiss production based on regional characteristics, tailoring it to meet specific nutritional demands and health benefits. The differences in protein composition and molecular weight provide valuable insights for future research on therapeutic and dietary applications of mare's milk, especially in the context of functional foods and dietary supplements.

Given the findings, this study supports the continued exploration of mare milk products as a functional food with potential benefits for gastrointestinal health, immune support, and other therapeutic applications. The results advocate for more region-specific approaches to koumiss production, emphasising leveraging natural environmental factors to enhance product quality. Additionally, regulatory bodies and producers could consider establishing quality standards for mare milk and koumiss based on regional compositions, ensuring consistent quality while preserving the unique attributes associated with each area.

Future studies may expand upon this research by examining how other environmental factors, such as seasonal forage variations, impact mare milk composition. This could contribute to a more comprehensive understanding of how regional and seasonal conditions affect the bioactive compounds in mare's milk, potentially optimising its use in specialised nutrition and medicine.

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