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The relationship between milk components, immunoglobulins, and cytokine content at the end of lactation in Kyrgyz mares

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Abstract

The aim of the present study was to determine the relationship of immunoglobulins and cytokine levels in the milk of Kyrgyz mares during the last period of lactation. For this purpose, seven Kyrgyz mares were used. During the last week of lactation, milk samples were obtained from mares four times at intervals of two days. Physicochemical tests were performed on all collected milk samples, and pH, fat, ash, titratable acidity, and dry matter were measured. Interferon-gamma (IFN-γ), interleukin -2 (IL-2), tumour necrosis factor-alpha (TNF-α), and immunoglobulin G (IgG) levels were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits. Based on the measurements, the lowest IgG level was determined on the last day of lactation (10.23 ± 13.13 µg/mL) and the TNF-α level was determined to be the highest on the last day lactation (61.10 ± 75.69 pg/mL). However, no statistical differences between the days in terms of IFN-γ, IL-2, pH, titratable acidity, dry matter, ash, and fat were found. Varying degrees of positive correlations between the parameters existed and were more pronounced between IgG levels and pH values. As a result, it was concluded that in the last period of lactation in Kyrgyz mares, IgG concentrations decreased and TNF-α levels increased in their milk, but substantial changes in milk composition, immunoglobulins, and cytokine levels in milk did not show positive correlations with the physicochemical properties of milk.

Keywords: cytokine, immunoglobulin, Kyrgyz mare, mare's milk
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Introduction

Mare's milk is used by the people of Central Asia to make koumiss, which is both a food and a fermented beverage (Danków *et al.,* 2009; Abdel-Salam *et al.,* 2010). Consumption of mare's milk and koumiss is also increasing in European countries (Sheng and Fang, 2009; Barłowska *et al.,* 2023). Among the milk from many mammalian species, mare's milk, which is similar to human milk in terms of its chemical composition, is seen as a food that can be used for feeding infants (Jastrzębska *et al.,* 2017). In addition, mare's milk is considered an alternative food for infants and children that have allergies to cow's milk proteins (Pieszka *et al.,* 2016; Businco *et al.,* 2000; Alipour *et al.,* 2023). Colostrum content in mares changes rapidly within the first 12 h after birth (Włodarczyk-Szydłowska *et al.,* 2005). The first three weeks after the foal is weaned is a transitional period in which the milk content stabilizes. The lactation peak is observed two months after the foal is weaned (Oftedal *et al.,* 1983). Lactation usually lasts for

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5–8 months and the estimated milk production is between 2000 and 3000 L per lactation (Salamon *et al.,* 2009). Mare's milk is affected by many factors, such as season, lactation, milk yield, and lactation period (Centoducati *et al.,* 2012; Markiewicz-Kęszycka *et al.,* 2015). Both the amount and composition of produced milk vary according to the mare's breed, age, and ration (Pieszka *et al.,* 2004; Markiewicz-Kęszycka *et al.,* 2015).
The concentration of mare milk components varies throughout lactation. In the first 25 d of lactation, the amount of dry matter and protein decreases, after which the composition stabilizes (Pecka *et al.,* 2012). At the end of lactation, the amount of protein may decrease slightly, whereas the amount of dry matter and fat may increase or remain the same. The amount of lactose may increase or remain the same throughout lactation (Oftedal *et al.,* 1983). The average composition of whole mare milk in the middle of the lactation period consists of dry matter, 10.5%; fat, 1.25–1.3%; protein, 1.93–2.1%; and lactose, 6.4–6.91% (Salimei and Fantuz, 2012; Pieszka and Łuszczyński, 2013). The main immunoglobulin found in mare colostrum is IgG, but the content of IgA is also high. The immunoglobulin ratio of mare milk is substantially higher than that found in cow's milk (Uniacke-Lowe *et al.,* 2010).

In the present study, we aimed to determine the relationship between immunoglobulin and cytokine content in different milk components of Kyrgyz mare's milk during the last period of lactation.

Materials and Methods

Ethical approval for this study was granted by the Kyrgyzstan–Turkey Manas University Animal Experiments Local Ethics Committee (Approval no: 17.01.2022- 2022/01). The study was conducted in the autumn of 2022 using the Kyrgyz breed and maiden mares aged 3–5 years on a private farm in Bishkek, the capital city of Kyrgyzstan.
Seven mares during the last period of lactation were examined. The animals included in the study were selected from clinically healthy mares. The mares were kept in individual, large, straw-bed boxes. They were fed hay *ad libitum* hay and concentrate twice a day and allowed to go out to pasture (clover and weeds) throughout the day. All mares were continuously observed by veterinarians throughout the study.
During the last week of lactation, milk samples were obtained from mares four times at 2-d intervals (0-d, 2^{nd}, 4^{th}, and 6^{th} days). After the last milk sample was obtained, the animals were not milked again.
Before milk was obtained from the mares, the California Mastitis Test (CMT) was applied to the udders and milk was obtained from CMT negative animals. Therefore, the animals were clinically healthy animals without subclinical mastitis. The animals included in the study were selected from 100 mares.
Last lactation (180 d of lactation) in addition to -2^{nd} (178 d of lactation), -4^{th} (176 d of lactation), and -6^{th} (174 d of lactation) end of the lactation samples were obtained from both teats on the same day. One of the milk samples was brought to the laboratory (preserving the cold chain) for milk component analysis. Milk samples taken for cytokine and immunoglobulin analyses were stored in a deep freezer at −80 ˚C until measurements were obtained.

Cytokine and Immunoglobulin Analysis: IFN-γ, IL-2, TNF-α, and IgG contents in the collected milk samples were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Can-Şahna and Rişvanlı, 2015). The ELISA test procedures were conducted in accordance with the manufacturer's instructions, as outlined in the booklet provided with the commercial kits. After all steps were completed, cytokine levels were measured by reading the plates in an ELISA reader (Bio-Tek Instruments, USA) at 450 nm. The pH, titratable acidity, fat, dry matter, and ash amounts in milk were determined. The pH was determined using a pH meter (Thermo Scientific Orion 3-star benchtop, USA) as described by Tekinsen *et al.,* (2001). Titratable acidity in the milk samples was determined as a percentage (%) of lactic acid, according to the Association of Official Agricultural Chemists (AOAC) 947.05 (2000). The amount of fat was determined using the Gerber method (2000). Dry matter was determined according to AOAC 942.05 (2000). Ash was determined according to AOAC 948.12 (2000).

All data obtained from milk samples were compared among themselves and the variables were loaded into the SPSS 22.0 for the Windows program and descriptive statistics were determined. Whether the data showed normal distribution was checked; nonparametric tests were used because data did not meet the normal distribution assumptions. A Friedman Two-Way Analysis of Variance method was used for nonparametric, dependent samples in the analysis of differences between days, and the Bonferroni test was used to compare subgroups. Correlations between milk components and the variables examined on different days were evaluated using a Spearman's correlation analysis.

Results and Discussion

The data from the final lactation period of Kyrgyz mares obtained on the corresponding day of the week are summarized in Table 1. The lowest IgG content was found on the last day of lactation (10.23 ± 13.13 µg/mL) and the highest content (45.28 ± 38.06 µg/mL) was found on the 6th (174 days of lactation) post-lactation day (P = 0.043). The highest TNF-α content (61.10 ± 75.69 pg/mL) was determined on the last day of lactation (P = 0.018). However, no statistical differences between the days in terms of IFN-γ, IL-2, pH, titratable acidity, dry matter, ash, and fat were found.

When the correlations between the analysed features according to the days were examined, it was found that IFN-γ levels were positively correlated with IgG, fat ratio was positively correlated with TNF-α, and the dry matter ratio was positively correlated with pH on the last day of lactation.

On the -2nd (178 days of lactation) post-lactation day, it was determined that a positive correlation between daily fat rates and IgG levels existed. On the -4th (176 days of lactation) post-lactation day, it was determined that a positive correlation between IgG and pH values, and dry matter ratios and TNF-α per day existed. On the -6th (174 days of lactation) post-lactation day, it was determined that a positive correlation between IgG and pH values existed.

Table 1. Distribution of analysed parameters by day

	-6th (174 d of lactation)	-4th (176 d of lactation)	-2 nd (178 d of lactation)	Last day of lactation (180 d of lactation)	P-value
IgG (µg/mL)	45.28^{a} ± 38.06	22.25^{ab} ± 15.40	53.38 ^{ab} ± 42.10	10.23^{b} ± 13.13	0.043
IL-2 (pg/mL)	0	0	0	0	-
TNF-α (pg/mL)	27.52^{ab} ± 40.12	0^{b}	6.51^{ab} ± 8.29	61.10^{a} ± 75.69	0.018
IFN-γ (pg/mL)	20.76 ± 21.19	14.88 ± 20.38	17.56 ± 29.00	1.59 ± 2.73	0.199
pH	7.25 ± 0.05	7.25 ± 0.05	7.22 ± 0.05	7.23 ± 0.05	0.745
Titratable acidity (% L.A.)	0.066 ± 0.012	0.063 ± 0.007	0.070 ± 0.010	0.064 ± 0.010	0.682
Dry matter (%)	10.31 ± 0.46	10.18 ± 0.31	9.86 ± 0.38	10.05 ± 0.17	0.156
Ash (%)	0.35 ± 0.03	0.36 ± 0.03	0.30 ± 0.03	0.35 ± 0.04	0.062
Fat (%)	0.76 ± 0.10	0.76 ± 0.05	0.70 ± 0.04	0.77 ± 0.05	0.440

$^{a, b, c:}$ The difference between groups with different letters on the same line is statistically significant.

Publications on cytokine concentrations and milk composition in other animal species are available, but very few publications on this subject in mares can be found. Mazhitova & Kulmyrzaev (2016) conducted a study in Kyrgyzstan and reported the pH value of mare's milk during the fourth month of lactation (August) as 6.59, titratable acidity as 5.03, dry matter content of 11.11%, protein content of 2.29%, and fat content of 1.60%. All parameters obtained in the current study were lower than the study conducted by Mazhitova & Kulmyrzaev (2016). It was concluded that the differences in pH, titratable acidity, dry matter, and fat quantity obtained in the current study could be attributed to conditions, such as season, animal breed, age of the mare, ration, and lactation period. Gregić *et al.* (2022) reported the amount of non-fat dry matter as 9.01%, fat content as 1.18%, lactose as 6.56%, and protein as 1.53% in mare's milk. Csapó-Kiss *et al.* (1995) reported that total dry matter and fat content of mare's milk was 4.25–26.28% and 2.85–2.93% on the first postpartum day, respectively. Within the same study from 2–5 postpartum days, these values were 12.15–12.78% and 2.05–2.17%, respectively; from postpartum days 8 to 45, these values were reported as 10.37–10.61% and 1.04–1.32%, respectively. Markiewicz-Kęszycka *et al.* (2013) found that the late lactation milk of Polish cold-blooded mares contained 9.5% total dry matter, which included 1.6% total protein, 0.4% fat, and 6.9% lactose. In the present study, no statistical differences between days in terms of pH, titratable acidity, dry matter, ash, and fat amounts could be found, and our data were similar to the data of previous studies.

Istanbullugil *et al.* (2023) evaluated cytokine levels in mare's milk over the first 20 d of lactation and reported that milk IL-2 levels were the highest on the 20th day (170.97 ± 24.88 pg/mL), whereas IFN-γ and TNF-α concentrations increased as lactation progressed. In the same study, when the correlations between cytokine levels in milk samples and milk composition were examined, a positive correlation between the IL-2 levels on the 20th day and the fat ratios was reported. The lack of a relevant study on the cytokine levels in mare's milk in the last period of lactation has prevented a comfortable interpretation of the data from present study. In the current study, the highest TNF-α content was determined on the last day of lactation but no statistical difference was found between the days in terms of IFN-γ and IL-2.

Markiewicz-Kęszycka *et al.* (2013) reported that IgG accounts for 15.8% of the total whey proteins in the last period of lactation in the milk of Polish cold-blooded mares. High IgG concentrations among whey proteins in the last period of lactation in mare milk were also observed by Summer *et al.* (2005) and Martuzzi & Doreau (2006). In the present study, IgG content was low on the last day of lactation, similar to previous studies.

Conclusions

In the present study, the IgG concentrations decreased and TNF-α levels increased in the last period of lactation in Kyrgyz mares, but no marked changes were observed in milk composition. Immunoglobulins and cytokines showed positive correlations with the physicochemical properties of milk. Since mare milk is traditionally consumed during the first months of lactation and, in other months, is used to make koumiss, a traditional fermented beverage, which is affected by the composition of the milk, this study provides important information and can be used in further studies.

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Authors' Contributions

FRI (ORCID: 0000-0001-9610-2797) conception of the work, drafted the manuscript, enrolled animals; AR (ORCID: 0000-0001-5653-0025), RS (ORCID: 0000-0001-8211-6813) responsible for follow-up visits; MB (ORCID: 0000-0001-7780-2982) performed statistical tests; UA (ORCID: 0000-0002-1533-4519) reviewed the manuscript; DAA (ORCID: 0000-0001-9230-6725) conception of the work, interpreted the data; SK (ORCID: 0000-0003-3032-8050), BY (ORCID: 0000-0002-7256-9189), MT (ORCID: 0000-0003-3526-0729), MU (ORCID: 0000-0003-4722-7061) performed laboratory analyses. All authors approved the final manuscript.

Conflict of Interest Declaration

The authors have not any financial or personal relationships that could inappropriately influence or bias the content of the manuscript.

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