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Impact of Age, Parity and Milking Frequency on Dromedary Camels' Susceptibility to Subclinical Mastitis

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ABSTRACT

This study investigates the impact of age, parity, and frequency of milking on the susceptibility of three dromedary camel breeds to subclinical mastitis (SCM). The study involved 133 animals from the Al-Qassim region of Saudi Arabia, assessing systemic, milk, immunological, and bacterial characteristics, and determining the likelihood of SCM through somatic cell count (SCC) and bacterial count of milk. Blood and milk samples were analyzed for bacterial counts, somatic cell count, and immunoglobulin G (IgG), lactoferrin (LTF), and lactoperoxidase (LPO) concentrations. ELISA kits were used to quantify serum Cam-TNF-α and Cam-IL-6 Cam-IL-10 concentrations. SCC and milk examination were used to determine the tendency to form SCM. The study found a significant increase in SCC in Majahem and Shaele camels ($P<0.001$) after TAD milking and age >7 years ($P<0.001$), while a decline in Wadha camels ($P<0.05$). All breeds showed an increase in SCC at parity >2, with Majahem and Shaele most affected by factors like increased SCC and bacteriological examination. The study examined the impact of factors on serum IgG, LTF, and LPO in different breeds of camels. Results presented significant ($P<0.05$) elevations in serum IgG and LPO in Majahem, Wadha, and Shaele camels ($P<0.01$), while milk LTF increased significantly ($P<0.05$) in Shaele camels. The study found that after TAD milking, Majahem camels showed increased TNF-α ($P<0.05$), IL-6 ($P<0.001$) and IL-10 ($P<0.05$) levels, while Shaele camels showed a decrease in TNF-α ($P<0.001$) and a decline in IL-10 ($P<0.05$), indicating excellent indicator of udder status. The study highlights the importance of understanding microbiology, SCC, and immune parameters of milk camel breeds for hygienic practices. It highlights the need for early detection of SCM in camel milk, promoting further research to improve detection performance.

Key words: Lactating Dromedary Camels, Lactoferrin, Antioxidants, Immunity, Camel milk, Subclinical mastitis.

INTRODUCTION

For those living in arid and semi-arid areas, camel milk is an essential source of nutrition (Seligsohn et al. 2020; 2021; Seligsohn 2021). Mastitis is a common and expensive disease that affects dairy camels and has a major impact on household economy, milk yield, quality and hygiene. Mastitis can be classified as subclinical if there are no clinical symptoms at all, or clinical if symptoms are obvious to owners and veterinarians. To identify the infected camels and stop the disease from spreading throughout the herd, the detection of subclinical mastitis (SCM) necessitates additional diagnostic testing (Carvalho-Sombra et al. 2021).

SCM may have an unfavorable impact on the milk's nutritional value and composition, making it less suitable for processing and of worse quality (Iraguha et al. 2015). SCM has a major financial impact on the dairy business as a result of production and processing losses (Krishnamoorthy et al. 2021). Since there are no outward signs of abnormality in the milk, SCM cannot be detected without specialized diagnostic testing. Knuth et al. (2019) shown that there are no indications of SCM in milk or in animals who are in distress. Because changes in the udder tissue occur before they become noticeable, early identification of SCM is essential. Aljumaah et al. (2019) have demonstrated that the somatic cell count (SCC) is a valid and dependable diagnostic for detecting SCM in

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dromedary camels. Somatic cells proliferate because a defense mechanism against invading pathogens is produced by mammary epithelial cells, which sense their ligands and trigger the proper immune responses (Mohsin et al. 2022). The majority of somatic cells are composed of leukocytes and milk-secreting epithelial cells. Jadhav et al. (2018) determined a cut-off of $310x10^3$ cells/mL of milk for camels and $200x10^3$ cells/mL for cattle to distinguish SCM animals from healthy ones (Mohamed et al. 2022). In another opinion, the best dependable approach for diagnosing SCM is through milk bacterial screening (Seligsohn et al. 2020; Meçaj et al. 2023).

There is a broad range in the quantities of immuno-protective proteins found in camel milk, such as immunoglobulin G (IgG), lactoferrin (LTF), and lactoperoxidase (LPO) (Mohamed et al. 2022). Both the unique functional single-chain antibodies (IgG2 and IgG3) and the conventional heterotetrameric antibodies (IgG1) are present in camel serum. Research has shown that camels secrete both IgG1 and HCAbs in their milk (Mann and Ndung 2020; Mohamed et al. 2022). Additionally, camel milk has been shown to contain higher amounts of immunoglobulin G than milk from goats, cows, sheep, buffalo, and humans (Kowalczyk et al. 2022). The LTF of camel milk was ten times higher than that of cow's milk (Mahala et al. 2022). A good measure of the health of the udder is the release of cytokines, which can vary in milk in response to pathological or physiological changes (Akhtar et al. 2020; Vitenberga-Verza et al. 2022). TNF-α and IL-6 are two examples of Th1 cytokines that are critical for initiating the innate immune system's defense against intramammary infection. As a result, in both clinical and SCM infections of the mammary glands, these cytokines have been reported to be significantly increased (Akhtar et al. 2020; Serdal et al. 2021). Another cytokine present in milk is called Th2 (IL-10), which is known to limit the immune response to infections, safeguard host tissues, inhibit the synthesis of Th1 cytokines and inhibit T cell activation and effector functions (Šerstņova et al. 2022).

Three camel breeds totaling 133 animals (Majahem n=43, Shaele n=43, and Waddah n=47) were used in this study. The animals were taken from various sites within the Al-Qassim region of Saudi Arabia. Age, parity, and variations in milking frequency were assessed in relation to systemic, milk, immunological, and bacterial characteristics, as well as SCC. To determine the likelihood of forming SCM, we employed SCC and bacteriological analysis of milk. We next looked at another parameter to determine the presence of SCM.

MATERIALS AND METHODS

Ethical Approval

The Animal Ethics Committee at Qassim University in Saudi Arabia authorized all of the experimental methods utilized in this work (23-32-04).

Study Area and Animals

A total of 133 lactating camels between the ages of 4 and 10 years from various places in the Qassim region of the Kingdom of Saudi Arabia (KSA) were used in this study (Fig. 1). This area experiences an arid environment, with summer temperatures typically ranging from 40 to 45°C. The months of November through February are when it rains. Pastures in the area are regarded as arid during the remainder of the year. The time frame for this study was November 2021–August 2022. The animals were housed in grazing and supplement farming systems after being selected at random. From dawn until midday, the camels graze in the open spaces around the farm; after that, they were housed inside for milking and offered additional feed. The used feed consisted of dry hay 3-4kg/day (depending on the farm) and concentrates which consisted of the same ration of barley and cottonseed meal *ad libitum*. The animals received regular watering. Most calving takes place in the winter. She-camels without systemic diseases or abnormalities were classified into three distinct subspecies (Abdallah and Faye 2012): Majahem (black) n=43, Shaele (yellow) n=43, and Wadha (white) n=47. The farms' approaches to management and feeding were identical. Animal similarity in the housing, feeding system, and nutrition sources; they only differ in the milking times (OAD: once a day and TAD: twice a day); age (<7 years and >7 years); and parity (<2 and >2).

Milk Samples

Prior to sampling, researchers explained the goals of the study and the methods of sampling to animal owners and herders and they got their verbal consent to participate. The study's anonymous participation policy and the option to leave at any moment were explained to the participants. After water washing, disinfecting the teats with alcohol (70°C), and removing the first streams, the samples were collected early in the morning and placed into sterilized tubes. These were then promptly labeled. The angle of the test tube was set at approximately 45 degrees. Furthermore, the samples were placed in ice bags inside a special box and transported to the lab in a matter of two to four hours. After thorough mixing, 500mL were taken out of each milking for analysis. After being transported, the samples were sorted and placed in a tiny 2mL tube, which was centrifuged for 10min at 10,000rpm in order to extract the milk and fat. Skim milk samples had their fat removed and were kept cold until further analysis.

Blood Samples

Venipuncture tubes (10mL) were used to draw blood from camels, and the samples were stored at room temperature. The serum from the collected blood samples was recovered by centrifugation at 3000rpm. for 15min. The samples were then split and kept at -20°C for the assessment of immunological and cytokine parameters.

Bacteriological Analysis

After being transported to the laboratory, the milk samples were promptly refrigerated at 4°C until the analysis procedure started. Sterile peptone water was used to serially dilute the milk samples, and 1.0mL aliquots were applied to each Petri plate that was repeated. A volume of 15-20mL agar was added to each Petri dish. The resultant plates were properly mixed, allowed to harden, and then incubated for 24 hours at 32˚C.

Total Plate Count (TPC) was carried out using Plate Count Agar (PCA, Oxoid) in accordance with the ISO4833-1 formulation, and *Enterobacteriaceae* were counted using Violet Red Bile Glucose Agar (VRBG,

Neogen) in accordance with the ISO21528-2 formulation. Violet, red bile lactose agar (VRBL, Neogen) is used to count total *coliform* (TCC) in accordance with the ISO 4832 formulation. Tryptone bile x-glucuronide agar (TBX, Neogen) was used to count *E. Coli* in accordance with the ISO 16649-2 formulation (Fig. 2). Colony counter was used to count the plates, and the result was reported as cfu/mL. Everything was assembled inside a biological safety cabinet and incubated for 48 hours ±2.0 at 37°C for *Enterobacteriaceae* and TCC and 44°C for *E. coli.*

Somatic Cell Count

An automated cell counter and a direct microscopic approach were used to count the somatic cells in a maximum of three hours. A total volume of 1.0mL of raw milk was centrifuged for 10min at 10,000rpm. After removing the creamy component and supernatant, the pellet was resuspended in 1.0mL of phosphate-buffered saline (PBS) to reconstitute the volume. Ten µL of the cellular suspension were diluted with 125µL of Turk's solution, which is methylene blue in one to two percent acetic acid and distilled water. Somatic cells were counted using a hemocytometer in an extent of 10mL of the diluted pattern.

Quantification of Cytokines and Bioactive Proteins

The quantification of serum Cam-TNF-α and Cam-IL-6 concentrations was done by using the commercial enzyme-linked immunosorbent assay (ELISA) Kits (Sunlog Biotech, Hangzhou, Zhejiang, China, Cat. No. SL0030cm and SL0032cm for CamTNF-α and Cam-IL-6 respectively) following the manufacturer's instructions. The sensitivity of the test was 0.5pg/mL and 0.1pg/mL and the intra - assay <10% and inter - assay variability CV was <12%. The detection range was 3–200pg/mL and 1–70pg/mL for CamTNF-α and CamIL-6 respectively. The concentration Cam-IL-10 was determined by using a commercial ELISA kit (Wuhan Fine Biotech Co., Ltd, Optics Valley Biomedical Industrial Park, Fine Biotech Co., Ltd, Optics Valley Biomedical Industrial Park, Wuhan, China; Cat. No. ECM0010 following the manufacturer's instructions. The intra-and inter assay was <8% and <10% respectively. Sensitivity was 9.375pg/mL with the detection range of 15.625-1000pg/mL. The concentration of IgG, LTF and LPO was determined using a commercial ELISA kit (Sunlog Biotech, Hangzhou, Zhejiang, China; kits, Cat. Nos. SL0050cm, SL0051cm, and SL0039cm, respectively) following the manufacturer's instructions. The sensitivity of the assay was set at 0.06 µg/mL in IgG, 0.05ng/mL in LTF and 6pg/mL in LPO and the accuracy (intra-assay variance) The CV of the assay was set as <10%, the intra-assay variance was lower from 12%. The detection range was 0.3-20µg/mL in IgG, 0.3-20ng/mL in LTF and 30-2000pg/mL in LPO.

Statistical Analysis

Values were represented as mean±SE. To detect significant differences, a one-way ANOVA will be performed for the various factors and breeds. The Mann-Whitney test was used in post hoc analysis to compare the groups. Analyses were done with GraphPad 7. The level of significance used was $P<0.05$; $P<0.01$ and $P<0.001$.

RESULTS

The impact of milking frequency on various camel breeds was examined in this study (Table 1). It was discovered that after TAD milking, Majahem and Shaele camels' SCC significantly ($P<0.001$) increased in comparison to that after OAD milking. On the other hand, SCC revealed a significant ($P<0.05$) decrease in Wadha camels following TAD milking. Following TAD milking, Majahem ($P<0.05$) and Shaele ($P<0.01$) camels had considerably greater levels of *Enterobacteriaceae*. The findings showed that TCC in Majahem camels was significantly ($P<0.05$) higher following TAD.

The frequency of milking had an impact on the serum and milk IgG, LTF, and LPO for various camel breeds (Table 1). The results demonstrated that Wadha and Shaele camels' serum IgG levels were considerably higher ($P<0.01$) after TAD milking compared to after OAD milking. In Wadha camels, serum LTF was significantly higher ($P<0.01$). However, serum LPO was significantly lower ($P<0.001$) following TAD milking. Nevertheless, following TAD milking, there was a significant ($P<0.05$) increase in serum LPO in Shaele camels. Following TAD milking, milk IgG significantly increased in Wadha ($P<0.001$) and Shaele ($P<0.01$) camels. Nevertheless, milk LTF in Wadha camels dramatically decreased ($P<0.01$). In contrast, following TAD milking, Shaele camels' milk LTF considerably increased ($P<0.001$). Additionally, compared to OAD milking, milk LPO significantly increased in Wadha ($P<0.001$) and Shaele ($P<0.05$) camels following TAD milking (Table 1).

TAD milking resulted in a significant ($P<0.05$) rise in TNF-α of Majahem camels compared to OAD milking. However, following TAD milking, there was a significant ($P<0.05$) drop in the TNF-α of Shaele camels. The results showed that Majahem camels' IL-6 was significantly higher ($P<0.001$) after TAD milking than it was after OAD milking. Additionally, following TAD milking, IL-10 showed a substantial elevation ($P<0.05$) in Majahem camels, but a large drop ($P<0.001$) in Shaele and Wadha camels compared to the groups after OAD milking (Table 1).

Comparing to that at age <7 years, it was found that SCC showed a significant increase ($P<0.001$) in Majahem and Shaele camels at age >7 years. However, SCC ($P<0.05$) and TPC ($P<0.001$) showed a significant decrease in Wadha camels at age >7 years. *Enterobacteriaceae* was significantly higher ($P<0.001$) in Majahem camels at age >7 years. However, *Enterobacteriaceae* was significantly lower ($P<0.001$) in Wadha camels at age >7 years. TCC was significantly declined ($P<0.05$) in Majahem camels at age >7 years. The results showed that *E. coli* count was significantly elevated ($P<0.05$) in Majahem, Wadha and Shaele camels at age >7 years (Table 2).

The results showed that serum IgG of Majahem and Shaele camels at age >7 years was significantly higher ($P<0.05$). However, serum IgG of Wadha camels at age >7 years showed significant decline ($P<0.05$). Serum LPO was significantly higher ($P<0.05$) in Shaele camels at age >7 years. Milk IgG showed significant decline ($P<0.01$) in Wadha camels at age >7 years. A significant elevation was recorded in milk IgG ($P<0.05$) and LTF ($P<0.001$) of Shaele camels at age > 7 years. Milk LPO showed a

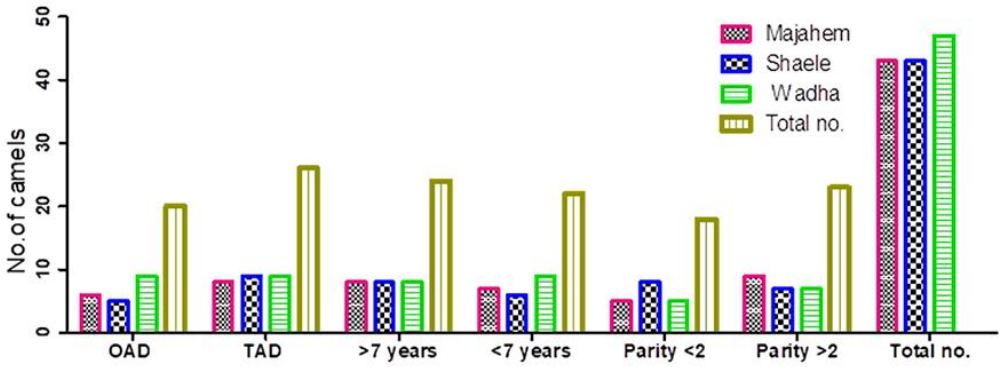

Fig. 1: Number of dromedary camels with variability of age, parity, and milking frequency. OAD: once-a-day milking; TAD: twice-a-day milking.

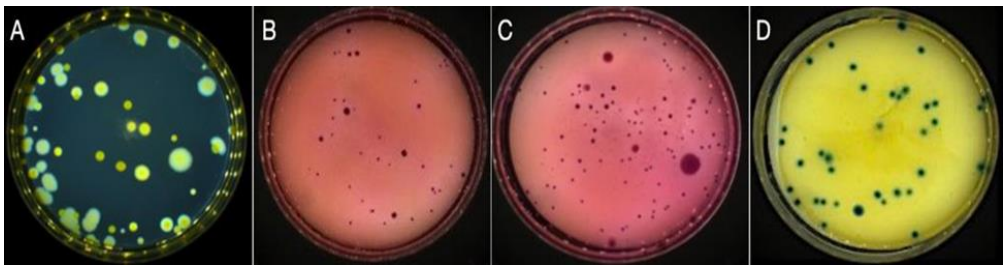

Fig. 2: Petri dishes showing TPC (A) *ENTB* (B) *Coliform* (C) *E. coli* (D) of camel's milk.

Table 1: The effect of milking frequency on milk and serum parameters of different camel breeds

Breeds	Majahem		Wadha		Shaele	
Milking frequency	OAD	TAD	OAD	TAD	OAD	TAD
SCC (cells/mL)	120.0±25.7	306.7±18.2c	292.2±62.8	255.0±10.7a	143.7±118	442.4±50.4c
TPC (cfu/mL)	2.925±0.559	2.229±0.669	2.167±0.525	2.200±0.311	0.307±0.239	3.313±1.420
Enterobacteriaceae (cfu/mL)	0.150±0.018	0.407±0.069a	0.210±0.074	0.330±0.129	0.181±0.121	0.433±0.079b
TCC (cfu/mL)	0.140±0.015	0.310±0.080a	0.100±0.024	0.173±0.073	0.400±0.078	0.533±0.215
E. coli (cfu/mL)	0.710±0.131	0.526±0.198	0.267±0.033	0.300±0.000	0.503±0.192	0.610±0.220
Serum IgG (µg/mL)	10.06±2.22	9.24±1.51	8.082±0.378	16.78±3.14b	7.158±0.524	14.18±2.08b
Serum LTF (ng/mL)	3.60±1.49	3.499±0.767	2.485±0.822	6.706±0.897c	4.545±0.164	5.848±0.960
Serum LPO (pg/mL)	6.309±0.955	6.65±2.05	6.188±0.780	1.735±0.728c	7.66±1.03	15.26±4.00a
Milk IgG (µg/ mL)	8.303±1.684	11.267±2.669	6.48±1.23	14.476±0.536c	3.926±0.509	7.267±0.396b
Milk LTF (ng/mL)	4.05±1.44	3.127±0.826	3.323±0.368	1.5176±0.066b	5.99±1.45	15.49±1.32c
Milk LPO (pg/mL)	8.173±0.909	6.52±1.69	3.108±0.528	8.749±0.346c	8.819±0.815	11.965±0.915a
Milk TNF α (pg/mL)	2.602±0.826	6.481±0.771a	2.850±0.759	4.14±1.15	6.05±1.55	2.608±0.283a
Milk IL-6 (pg/mL)	1.694±0.429	7.69±2.34c	3.061±0.810	3.765±0.994	2.092±0.654	1.841±0.145
Milk IL-10 (pg/mL)	15.38±4.94	27.7±3.8a	17.25±2.64	8.04±1.36a	27.21±3.65	8.445±0.736c

Data (Mean±SE) in the same row within one species, values with a, b and c are significantly different at P<0.05, P<0.01 and P<0.001, respectively. OAD: once-a-day; TAD: twice-a-day; TPC: total plate count; TCC: total coliform count; cfu: colony forming unit; IgG: immunoglobulin; LTF: lactoferrin; LPO: lactoperoxidase; SCC: somatic cells count.

significant elevation (P<0.05) in Wadha and Shaele camels at age >7 years. In this study, the effect of age on milk cytokines levels of different breed of camels was determined comparing to their corresponding groups at age <7 years. The obtained results showed a significant increase (P<0.001) in TNF-α level of Majahem and Wadha camels at age >7 years. However, a significant decline (P<0.01) was recorded in TNF-α level of Shaele camels at age >7 years. In addition, data revealed a significant elevation (P<0.05) in the level of IL-6 of Majahem at age >7 years. In contrast, the level of IL-6 was significantly declined (P<0.05) in Wadha and Shaele camels at age >7 years. Furthermore, a significant elevation was recorded in the level of IL-10 (P<0.05) in Majahem camels at age >7 years. However, IL-10 level was significantly lower (P<0.01) in Shaele camels at age >7 years.

In the current study, comparing to that of corresponding breed at parity <2, SCC showed a significant increase in Majahem, (P<0.001) Wadha (P<0.05) and Shaele (P<0.001) camels at parity >2. The results showed that TPC was significantly higher (P<0.05) in Shaele camels at parity >2. However, *Enterobacteriaceae* was significantly higher (P<0.05) in Majahem and Wadha camels at parity >2. Moreover, *E. coli* was significantly higher (P<0.01) in Wadha (P<0.01) and Shaele (P<0.05) camels at parity >2.

Serum and milk IgG, LTF and LPO of different breeds camels were affected by parity (Table 3). The results showed that serum IgG of Wadha camels at parity >2 was significantly lower (P<0.05). However, serum IgG of Shaele camels at parity >2 was significantly higher (P<0.001). In addition, serum LPO was significantly higher (P<0.05) in Shaele camels at parity >2.

Table 2: The effect of age on milk and serum parameters of different camel breeds

Breeds	Majahem		Wadha		Shaele	
Age	<7	>7	<7	>7	<7	>7
SCC (cells/mL)	295.00±7.72	760.8±229 c	292.1±38.9	218.2±16.9a	615.2±125	1053.1±280c
TPC (cfu/mL)	1.828±0.620	2.11±1.10	0.368±0.175	2.40±0.693 b	1.862±1.021	1.450±0.621
Enterobacteriaceae(cfu/mL)	0.027±0.011	0.413±0.099 c	0.220±0.085	0.053±0.027c	0.470±0.065	0.388±0.161
TCC (cfu/mL)	0.333±0.108	0.118±0.071a	0.152±0.034	0.115±0.015	0.450±0.085	0.236±0.202
E. coli (cfu/mL)	0.310±0.104	0.617±0.100a	0.044±0.014	0.137± 0.035b	0.388±0.167	0.610±0.124b
Serum IgG (µg/mL)	8.356±0.970	11.83±1.81a	11.07±1.83	8.890±0.977a	7.158±0.524	12.49±2.08a
Serum LTF (ng/mL)	3.646±0.767	3.02±1.38	3.761±0.996	4.15±2.26	4.545±0.164	6.57±1.09
Serum LPO (pg/mL)	6.40±1.70	7.18±5.26	4.28±1.10	5.55±1.89	7.66±1.03	15.26±4.00a
Milk IgG (µg/mL)	9.963±0.667	10.93±1.25	11.37±1.95	6.32±2.16a	3.926±0.509	6.838±0.487a
Milk LTF (ng/mL)	3.152±0.698	4.56±1.57	2.575±0.489	2.663±0.558	5.916±0.843	15.49±4.32c
Milk LPO (pg/mL)	6.64±1.39	8.03±3.08	3.938±0.871	6.81±2.24a	8.819±0.815	10.88±1.20a
Milk TNF α(pg/mL)	6.806±0.316	14.608±0.560 c	3.24±1.21	11.39±2.48c	6.65±1.44	2.608±0.283b
Milk IL-6 (pg/mL)	2.302±0.679	5.55±1.33a	4.50±1.70	2.627±0.651a	4.89±2.66	1.523±0.329a
Milk IL-10 (pg/mL)	12.153±1.86	18.76±2.68a	11.09±1.69	15.213±0.393	17.21±3.65	8.445±3.736b

Data (Mean±SE) in the same row within one species, values with a, b and c are significantly different at P<0.05, P<0.01 and P<0.001, respectively. OAD: once-a-day; TAD: twice-a-day; TPC: total plate count; TCC: total coliform count; cfu: colony forming unit; IgG: immunoglobulin; LTF: lactoferrin; LPO: lactoperoxidase; SCC: somatic cells count.

Table 3: The effect of parity milk and serum parameters of different camel breeds

Breeds	Majahem		Wadha		Shaele	
Parity	<2	>2	<2	>2	<2	>2
SCC (cells/mL)	281.38±7.72	760.1±22.6c	215.7±16.91	292.1±28.90 a	634.8±125	1042.2±280c
TPC (cfu/mL)	1.828±0.620	2.71±1.35	1.157±0.355	2.400±0.693	0.763±0.184	1.450±0.321a
Enterobacteriaceae (cfu/mL)	0.193±0.093	0.413±0.099a	0.062±0.027	0.220±0.085a	0.480±0.090	0.388±0.161
TCC (cfu/mL)	0.418±0.126	0.237±0.081	0.152±0.034	0.060±0.032	0.450±0.085	0.236±0.202
E. coli (cfu/mL)	0.505±0.100	0.312±0.104	0.061±0.014	0.124±0.035b	0.411±0.167	0.722±0.124a
Serum IgG (µg/mL)	8.356±0.970	10.14±2.60	13.48±2.84	8.890±0.977a	7.158±0.524	14.49±1.24c
Serum LTF(ng/mL)	3.646±0.767	3.02±1.38	3.761±0.996	4.15±2.26	4.545±0.164	6.57±1.09
Serum LPO(pg/mL)	6.40±1.70	7.18±5.26	4.28±1.10	5.55±1.89	7.23±1.03	17.03±4.00a
Milk IgG (µg/mL)	9.963±0.667	10.91±1.76	9.74±1.75	9.05±3.86	3.926±0.509	6.357±0.939a
Milk LTF (ng/mL)	3.152±0.698	4.56±1.57	2.575±0.489	2.663±0.558	7.332±0.843	16.27±4.32c
Milk LPO (pg/mL)	5.79±1.24	5.03±2.65	4.57±1.11	3.363±0.998	6.278±0.815	11.965±0.91a
Milk TNF α(pg/mL)	1.625±0.447	4.120±0.652 b	2.927±0.917	3.84±1.27	3.019±0.443	6.10±1.42a
Milk IL-6 (pg/mL)	2.128±0.679	5.72±1.33b	2.829±0.523	2.427 ±0.628	4.89±2.66	6.86±5.57
Milk IL-10 (pg/mL)	18.530±1.868	24.43±2.87a	11.27±1.69	18.111±2.393a	25.01±3.65	10.52±2.736b

Data (Mean±SE) in the same row within one species, values with a, b and c are significantly different at P<0.05, P<0.01 and P<0.001, respectively. OAD: once-a-day; TAD: twice-a-day; TPC: total plate count; TCC: total coliform count; cfu: colony forming unit; IgG: immunoglobulin; LTF: lactoferrin; LPO: lactoperoxidase; SCC: somatic cells count.

Comparing to group at parity <2, milk IgG showed a significant decline (P<0.05) in Shaele camels at parity >2. Furthermore, a significant elevation was recorded in milk LTF (P<0.001) and LPO (P<0.05) of Shaele camels at parity >2.

In this study, the effect of parity >2 comparing to corresponding camels at parity <2 on milk cytokines in the different breeds of camels was determined. The obtained results showed a significant increase in TNF-α level in Majahem (P<0.01) and Shaele (P<0.05) camel at parity >2. A significant elevation (P<0.01) in IL-6 level was recorded in Majahem camels at parity >2. Furthermore, IL-10 recorded a significant elevation in Majahem, Wadha (P<0.05) and Shaele (P<0.01) camels at parity >2 (Table 3).

DISCUSSION

This study examined the effects of age, parity, and frequency of milking on eleven milk parameters and three blood parameters of different camel breeds. In order to determine the probability of forming SCM, we employed SCC and bacteriological analysis of milk. Afterwards, more SCM indications were looked into. The findings indicated that after TAD milking and at age >7 years, SCC showed a substantial increase in Majahem and Shaele camels and a significant drop in Wadha camels compared to that after OAD milking and at age <7 years. SCC showed a statistically significant increase when all camel breeds at parity >2 were compared to those at parity less than 2. It was observed that the three elements primarily affected the Majahem and Shaele breeds. Furthermore, the sole element that affected Wadha camels was parity. The SCC test has been demonstrated to be valid and reliable for diagnosing SCM in dromedary camels since it is widely used as a measure of udder health and milk quality (Qayyum et al. 2026; Jadhav et al. 2018; Aljumaah et al. 2019; Sumon et al. 2020 and Mohamed et al. 2022). According to Seligsohn et al. (2020), milk bacterial screening is the most reliable method for diagnosing SCM.

Results showed that *Enterobacteriaceae* was significantly higher in Majahem and Shaele camels after TAD milking. TCC and *Enterobacteriaceae* were significantly higher in Majahem camels after TAD milking and at age >7 years. However, *Enterobacteriaceae* was significantly lower in Wadha camels at age >7 years. In addition, the results showed that *E. coli* count was significantly elevated in Majahem, Wadha and Shaele camels at age >7 years more than their corresponding at the age <7 years. The results also showed that TPC was significantly higher in Shaele camels at parity >2 comparing

to that at parity <2. However, *Enterobacteriaceae* was significantly higher in Majahem and Wadha camels at parity >2 more than their corresponding groups at parity <2. Moreover, *E. coli* was significantly higher in Wadha and Shaele camels at parity >2. It was observed that Wadha camels were most impacted by the three elements, followed by Majahem and Shaele breeds. Overall, the study showed that camel milk samples obtained from the udder under several circumstances contained Enterobacteriaceae in Majahem, TPC in Shaele, and E. coli in the three breeds. Different sites got the mean TPC reading of milk samples from the analyzed breeds (Abera et al. 2016 and Bashir and Ahmed 2018 and Hassen and Amentie 2022). These discrepancies may result from various herd health management strategies used at farms, udder health, and other locations. Therefore, hygienic measures will need to be implemented in Majahem and Shaele in order to improve the quality and acceptability of camel milk for its intended usage. It is crucial to have knowledge about the microbiological purity of camel milk. This is due to the possibility that developmental organizations should pay attention to such information. Unfortunately, there is currently a lack of reliable information regarding the microbiological quality of milk from different camel breeds that considers the three parameters under investigation. The results from earlier reports (De Matteis et al. 2020; Alhussien et al. 2021 and Alhafiz et al. 2022) regarding the efficacy of bacteriological examination and SCC as diagnostic tools for monitoring mammary gland infections in camels were confirmed by the elevated SCC and bacteriological parameters in the current study. Furthermore, the immune system differences among the three breeds under study may be the cause of this discrepancy. The system and milk immunological characteristics were investigated to address these problems. Serum IgG, LTF and LPO of different camel breeds were affected by the milking frequency. The only change obtained in Majahem that at age >7 years showed that serum IgG was significantly higher than their corresponding groups at age<7 years that reflect the tendency of SCM characteristic of increased SCC and bacteriological examination. IgG is a part of the immune system that has several biological functions, including antibacterial and anti- inflammatory qualities (Dashe et al. 2020). According to Thorsteinsdottir et al. (2019), acute inflammation may cause the mammary gland's blood-to-milk permeability barriers to be destroyed, which increases the passive transfer of IgG into milk and causes an increase in IgG output.

The results of the current investigation demonstrated that the serum IgG of the Wadha and Shaele camels was considerably higher after TAD milking than it was after OAD milking. The obtained findings at an age greater than 7 years indicated that the Majahem and Shaele camels' blood IgG levels were significantly higher than those of their comparable groups at age less than 7 years. Serum IgG of Shaele camels at parity >2 was significantly higher comparing to that at parity <2. As far as we are aware, no studies have looked at IgG levels in different breeds of dromedary camels. The IgG levels in milk samples from the two different species of camels; *Camelus bactrianus* and *Camelus dromedarius* as well as their hybrids, however, did not differ significantly, according to Pou et al. (2019).

LTF is an effective iron scavenger and one of numerous molecules released by the immune system that bind transient metals to prevent bacterial infections. Iron is required for several essential biological processes, including DNA and ATP synthesis (Betelhem et al. 2022). Milk contains LTF, a naturally occurring, multifunctional glycoprotein that binds iron and is mostly produced by mammary epithelial cells (Shimazaki and Kawai 2017). The main immune system is strengthened and unwanted microbial growth in the gastrointestinal tract is prevented by camel milk LTF (Mohammadabadi and Husain 2021).

LTF is found in animal fluids like milk, saliva, tears, and semen, as well as in certain leukocytes (Asfour et al. 2022). It has been demonstrated that LTF helps control invasive viruses, fungi, bacteria, and parasites (Dierick et al. 2021). There hasn't been any research done on how different dromedary camel breeds' serum and milk LTF concentrations differ from one another.

As a result, following TAD milking, serum LTF was considerably greater in Wadha camels in the current study that had a low inclination to SCM. However, milk LTF in Wadha camels drastically decreased following TAD milking. In contrast, milk LTF from Shaele camels exhibited a considerable rise following TAD milking. Nevertheless, when LTF concentrations were examined in milk samples from Bactrian, Dromedary, and their hybrids, it was discovered that the season, not the species, determined LTF concentrations in milk (Pou et al. 2019).

These results are parallel with other studies that discovered that dromedary camel LTF concentrations were quite varied (Rainard 1993). Another study found that the antibacterial enzyme activity of individual camels varied widely, and their summertime milk had larger levels of lactotransferrin and other bioactive proteins (Salhi et al. 2015). Furthermore, LTF possesses potent antiviral and antifungal properties to provide further protection against infections (Habib et al. 2021). Based on the current results, LTF, like in ovine species, may be a good predictor of SCM in Shaele camels (Schukken et al. 2003).

LPO is a non-immunoglobulin protective glycoprotein that is present in milk and other human and animal fluids (Hao et al. 2019). It is believed that the LPO plays a crucial role in the host's innate defense system (Hayajneh 2018). The LPO can affect both Gram-positive and Gram-negative bacteria to varying degrees, and antiviral effects have also been observed (Hu et al. 2020). Activation of LPO has been studied to preserve milk safety by eliminating hazardous microbes (Khan et al. 2022).

In the present study, milk LPO showed a significant elevation in Wadha and Shaele camels after TAD milking compared to that after OAD milking. Serum LPO was significantly higher in Shaele camels at age >7 years comparing to that at age >7 years. In addition, serum LPO was significantly higher in Shaele camels at parity >2 comparing to camels at parity <2. The results of a recent investigation in cattle that examined milk samples from healthy and mastitis-affected animals found that there was a direct and positive link between LPO levels and the number of somatic cells, which supports the findings of the present study (Shao et al. 2018). LPO has been suggested as a potential biomarker for the diagnosis of SCM in cattle in recent years (Silva et al. 2022). There was no significant change by the three factors in Magahim milk IgG, LTF, and

LPO. Following TAD milking, Wadha and Shaele camels exhibited a notable increase in milk IgG and LPO levels. On the other hand, following TAD milking, milk LTF in Shaele camels was much higher. The acquired results demonstrated that the Shaele camels' serum levels of IgG and LPO were significantly greater than those of the equivalent groups.

The release of cytokines, which varies in milk in response to pathological or physiological changes, is an excellent indicator of the health of the udder (Akhtar et al. 2020; Vitenberga-Verza et al. 2022). Two measured Th1 cytokines that are essential for starting the innate immune system's response against intramammary infection are TNF-α and IL-6. Consequently, these cytokines have been shown to be markedly elevated in both clinical and SCM infections of the mammary glands (Akhtar et al. 2020; Serdal et al. 2021). Th2 (IL-10) is another cytokine found in milk that is known to protect host tissues from infections, restrict the immune response, prevent the creation of Th1 cytokines, and prevent T cell activation and effector functions (Šerstņova et al. 2022). In this study, the impact of studied factors including a significant increase in TNF-α, IL-6 and IL-10 of Majahem camels after TAD milking. However, a significant decrease was recorded in TNF-α of Shaele camels and IL-10 of Wadha and Shaele camels after TAD milking. Expression of Th1 cytokines such as TNF-α and Il-6 has been shown to be upregulated in bovine mastitis (Akhtar et al. 2020). However, the milk contents of bioactive molecules including Th1 cytokines can be influenced by several factors including diets, environment, and exposure to infectious diseases. The obtained results showed a significant increase in TNF-α level of Majahem and Wadha camels and elevation of IL-6 of Majahem at age >7 years. In contrast, the level of IL-6 was significantly declined in Wadha and Shaele camels at age >7 years. Furthermore, a significant elevation was recorded in the level of IL-10 in Majahem camels at age >7 years. However, IL-10 level was significantly lower in Shaele camels at age >7 years. The obtained results showed a significant increase in TNF-α level in Majahem and Shaele camels at parity >2. A significant elevation in IL-6 level was recorded in Majahem camels at parity >2 comparing to that at parity <2. Furthermore, IL-10 recorded a significant elevation in Majahem, Wadha and Shaele camels at parity >2. This investigation effectively induced a systemic innate immune response in Majahem and Shaele due to the notable rise in SCC and bacterial proliferation. This result was indicated by the notable rise in Th1 cytokine (TNF-α, IL-6) concentrations. Th1 cytokine increase has been seen in dromedaries with inflammatory and bacterial illnesses (Hussen and Schuberth 2021), as well as in clinical and SCM conditions in dairy cows and ewes (Katsafadou et al. 2019; Akhtar et al. 2020; Ji et al. 2020; Sadat et al. 2023). On the other hand, IL-10 inhibits the inflammatory immune response by preventing it from being overly exaggerated (Shaheen et al. 2020; Takashima et al. 2021).

Conclusion

Documented information on the microbiology, SCC, and immune parameters of milk camel breeds is crucial for governmental, nongovernmental and developmental organizations to focus on hygienic practices for safe milk production and handling. The study reveals that there is limited information on indicators of detrimental mastitis SCM in camel milk from different breeds. However, elevated SCC, microorganism presence, and altered milk indicators can be observed. Early detection is crucial for dairy farmers and veterinarians to ensure animal health and milk quality. Considering the limited numbers of herds sampled in this study, our findings regarding herd management and risk factors, the feasibility and efficiency of different interventions should be studied in low-prevalence herds, in comparison with high-prevalence herds. The discussion of these new risk factors opens the way to a sequence of new studies aiming to improve the performance of SCM detection.

Author's Contribution

F.S.A. and A.A.Z. contributed to the study conception and design. F.S.A. collected and prepared the milk and blood samples, performed the bacteriological analysis and somatic cell count. S.M.A. contributed to the quantification of cytokines and bioactive Proteins. F.A.M.A. and A.A.Z. performed the statistical analysis. F.S.A. and A.A.Z. prepared the first manuscript draft. F.A.M.A. and S.M.A. revised and reviewed the manuscript draft for publication. All authors had read and approved the final manuscript.

REFERENCES

Abdallah HR and Faye B, 2012. Phenotypic classification of Saudi Arabian camel (Camelus dromedarius) by their body measurements. Emirates Journal of Food and Agriculture 24 (3): 272-280.

Abera T, Legesse Y, Mummed B and Urga B, 2016. Bacteriological Quality of Raw Camel Milk along the Market Value Chain in Fafen Zone, Ethiopian Somali Regional State. BMC Research Notes (9): 285. https://doi.org/10.1186/s13104-016-2088-1

Akhtar M, Guo S, Guo YF, Zahoor A, Shaukat A, Chen Y, Umar T, Deng G and Guo M, 2020. Upregulated-gene expression of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) via TLRs following NF-κB and MAPKs in bovine mastitis. Acta Tropica 207: 105458. https://doi.org/10.1016/j.actatropica.2020.105458

Alhafiz GA, Alghatam FH, Almohammed H and Hussen J, 2022. Milk Immune Cell Composition in Dromedary Camels with Subclinical Mastitis. Front Veterinary Science 9: 885523. https://doi.org/10.3389/fvets.2022.885523

Alhussien MN, Panda BSK and Dang AK, 2021. A comparative study on changes in total and differential milk cell counts, activity and expression of milk phagocytes of healthy and mastitic indigenous Sahiwal cows. Frontiers in Veterinary Science 8: 670811. https://doi.org/10.3389/fvets.2021.670811

Aljumaah RS, Almutairi F, Ayadi M, Alshaikh MA, Al-Haidary AA and Samara EM, 2019. Practicability of somatic cell count and electrical conductivity as subclinical mastitis diagnostic tests in camels (Camelus dromedarius). Scientia Agricola 77(4): 1-12. https://doi.org/10.1590/1678-992X-2018-0373

Asfour HAE, Eid RH, EL-Wakeel SA, Behour TS and Darwish SF, 2022. Phenotypic and genotypic investigation of yeast species associated with bovine subclinical mastitis with a special reference to their virulence characteristics. International Journal of Veterinary Science 11(1): 49-58. https://doi.org/10.47278/journal.ijvs/2021.071

Bashir AM and Ahmed AF, 2018. Assessment of Bacteriological Quality Major Bacterial Pathogens and Handling Practices of Raw Camel Milk in Ab'ala Woreda of Afar National

Regional State, Ethiopia. A Review. International Journal of Current Research and Academic Review (6): 71-86. https://doi.org/ 10.20546/ijcrar.2018.610.008

Betelhem T, Shubisa AL and Bari FD, 2022. Isolation, identification and antimicrobial resistance of Staphylococcus aureus isolates from mastitis cases of lactating dairy cows found in Sululta and Holleta Towns, Oromia, Ethiopia. Agrobiological Records 8: 27-34. https://doi.org/10.47278/journal.abr/2022.006

Carvalho-Sombra TCF, Fernandes DD, Bezerra BMO and Nunes-Pinheiro DCS, 2021. Systemic inflammatory biomarkers and somatic cell count in dairy cows with subclinical mastitis. Veterinary and Animal Science 11(3): 100165. https://doi.org/10.1016/j.vas.2021.100165

Dashe D, Hansen EB, Kurtu MY, Berhe T, Eshetu M, Hailu Y, Waktola A and Shegaw A, 2020. Preservation of Raw Camel Milk by Lactoperoxidase System Using Hydrogen Peroxide Producing Lactic Acid Bacteria. Open Journal of Animal Sciences 10(03): 387. https://doi.org/ 10.4236/ojas.2020.103024

De Matteis G, Grandoni F, Scata MC, Catillo G, Moioli B and Buttazzoni L, 2020. Flow cytometry-detected immunological markers and on farm recorded parameters in composite cow milk as related to udder health status. Veterinary Science 7(3):114. https://doi.org/10.3390/vetsci7030114

Dierick M, Vanrompay D, Devriendt B and Cox E, 2021. Lactoferrin, a versatile natural antimicrobial glycoprotein that modulates the host's innate immunity. Biochemistry and Cell Biology 99(1): 61-65. https://doi.org/10.1139/bcb2020-0080

Habib HM, Ibrahim S, Zaim A and Ibrahim WH, 2021. The role of iron in the pathogenesis of COVID-19 and possible treatment with lactoferrin and other iron chelators. Biomedicine & Pharmacotherapy 136: 111228. https://doi.org/10.1016/j.biopha.2021.111228

Hao L, Shan Q, Wei J, Ma F and Sun P, 2019. Lactoferrin: major physiological functions and applications. Current Protein and Peptide Science 20(2): 139-44. https://doi.org/10.2174/1389203719666180514150921

Hassen M and Amentie T, 2022. Microbiological Quality of Raw Camel Milk in Degahbour District of Jarar Zone, Somali Regional State, Ethiopia Open Journal of Animal Sciences (12): 226-238. https://www.scirp.org/journal/ojas

Hayajneh FM, 2018. The effect of subclinical mastitis on the concentration of immunoglobulins A, G, and M, total antioxidant capacity, zinc, iron, total protein and calcium in she-camel blood in relation with pathogens present in the udder. Tropical Animal Health and Production 50(6): 1373-1377. https://doi.org/10.1007/s11250-018-1570-2

Hu P, Zhao F, Wang J and Zhu W, 2020. Lactoferrin attenuates lipopolysaccharide-stimulated inflammatory responses and barrier impairment through the modulation of NFκB/MAPK/Nrf2 pathways in IPEC-J2 cells. Food & Function 11(10): 8516-8526. https://doi.org/10.1039/D0FO01570A

Hussen J and Schuberth HJ, 2021. Recent advances in camel immunology. Frontiers in Immunology 11:614150. https://doi.org/10.3389/fimmu.2020.614150

Ji Y, Xiao F, Zhu W, Liu SS, Feng X, Sun C, Lei L, Dong J, Khan A, Han W and Gu J, 2020. LysGH15 effectively control murine mastitis caused by *Staphylococcus aureus*. Pakistan Veterinary Journal 40(4): 519-522. http://dx.doi.org/10.29261/pakvetj/2020.056

Iraguha B, Hamudikuwanda H and Mushonga B, 2015. Bovine mastitis prevalence and associated risk factors in dairy cows in Nyagatare District, Rwanda. Journal of the South African Veterinary Association 86(1): 1-6. https://doi.org/10.4102/jsava.v86i1.1228

Jadhav PV, Das DN, Suresh KP and Shome BR, 2018. Threshold somatic cell count for delineation of subclinical mastitis cases. Veterinary World 11(6):789. https://doi.org/10.14202/vetworld.2018.789-793

Katsafadou AI, Politis AP, Mavrogianni VS, Barbagianni MS, Vasileiou NGC and Fthenakis GC, 2019. Mammary defences and immunity against mastitis in Sheep. Animals 9(10):726. https://doi.org/10.3390/ani9100726

Khan MZ, Ma Y, Xiao J, Chen T, Ma J, Liu S, Wang Y, Khan A, Gibson Alugongo M and Cao Z, 2022. Role of selenium and vitamins e and b9 in the alleviation of bovine mastitis during the periparturient period. Antioxidants (Basel) 11(4): 657. https://doi.org/10.3390/antiox11040657

Knuth RM, Stewart WC, Taylor JB, Yeoman CJ, Bisha B, Page CM, Rowley CM, Lindsey BC, Van Emon ML and Murphy TW, 2019. Subclinical mastitis in sheep: etiology and association with milk somatic cell count and ewe productivity in three research flocks in the Western United States. Translational Animal Science 3(1): 1739-1743. https://doi.org/ 10.1093/tas/txz078

Kowalczyk P, Kaczyńska K, Kleczkowska P, Bukowska-Ośko I, Kramkowski K, and Sulejczak D, 2022. The lactoferrin phenomenon—a miracle molecule. Molecules 27(9):2941. https://doi.org/10.3390/molecules27092941

Krishnamoorthy P, Suresh KP, Jayamma KS, Shome BR, Patil SS and Amachawadi RG, 2021. An understanding of the global status of major bacterial pathogens of milk concerning bovine mastitis: A systematic review and meta-analysis (scientometrics). Pathogens 10(5): 545. https://doi.org/10.3390/pathogens10050545

Mahala N, Mittal A and Dubey US, 2022. Medicinal Potential of Camel Milk Lactoferrin Dairy Processing - From Basics to Advances Medicinal Potential of Camel Milk Lactoferrin November. https://doi.org/10.5772/intechopen.108316

Mann JK and Ndung'u T, 2020. The potential of lactoferrin, ovotransferrin and lysozyme as antiviral and immune-modulating agents in COVID-19. Future Virology 15(9): 609-624. https://doi.org/ 10.2217/fvl-2020-0170

Meçaj R, Muça G, Koleci X, Sulçe M, Turmalaj L, Zalla P, Koni A and Tafaj M, 2023. Bovine environmental mastitis and their control: an overview. International Journal of Agriculture and Biosciences 12(4) 216-221. https://doi.org/10.47248/journal.ijab/2023.067

Mohamed H, Ranasinghe M, Amir N, Nagy P, Gariballa S, Adem A and Kamal-Eldin A, 2022. A study on variability of bioactive proteins in camel (Camelus dromedarius) milk: Insulin, insulin-like growth factors, lactoferrin, immunoglobulin G, peptidoglycan recognition protein-1, lysozyme and lactoperoxidase. International Journal of Dairy Technology 75(2): 289-297. https://doi.org/10.1111/1471-0307.12836

Mohammadabadi T and Hussain T, 2021. Is Camel milk lactoferrin effective against COVID-19? World Journal of Pharmaceutical Sciences 9(2): 91-97.

Mohsin M, Swar SO, Imran M, Ali W, Sultan MD, Asrar R, Abbas RZ, Aleem MT, Aguilar-Marcelino L, Aslam A and Shahab A, 2022. Chronic mastitis: Leading cause of udder fibrosis and different means of its management. Agrobiological Records 8: 13-21. https://doi.org/10.47278/journal.abr/2022.004

Pou C, Nkulikiyimfura D, Henckel E, Olin A, Lakshmikanth T, Mikes J, Wang J, Chen Y, Bernhardsson AK, Gustafsson A, Bohlin K and Brodin P, 2019. The repertoire of maternal antiviral antibodies in human newborns. Nature Medicine 25(4): 591-596. https://doi.org/10.1038/s41591-019-0392-8

Rainard P, 1993. Activation of the classical pathway of complement by binding of bovine lactoferrin to unencapsulated Streptococcus agalactiae. Immunology 79(4): 648−652. https://doi.org/ 10.3389/fimmu.2017.01438

Sadat A, Farag AM, Elhanafi D, Awad A, Elmahallawy EK, Alsowayeh N, El-khadragy MF and Elshopakey GE, 2023. Immunological and oxidative biomarkers in bovine serum from healthy, clinical, and sub-clinical mastitis caused by *Escherichia coli* and *Staphylococcus aureus* infection. Animals 13(5): 892. https://doi.org/10.3390/ani13050892

Salhi I, Bessalah S, Mbarek SB, Chniter M, Seddik MM, Khorchani T and Hammadi M, 2015. Passive transfer of maternal immunity in the dromedary (Camelus dromedarius), involvement of heavy chain antibodies, Tropical Animal Health Production 47(3): 613-618. https://doi.org/10.1007/s11250-014-0751-x

Schukken YH, Wilson DJ, Welcome F, Garrison-Tikofsky L and Gonzalez RN, 2003. Monitoring udder health and milk quality using somatic cell counts. Veterinary Research 34(5): 579-596. https://doi.org/10.1051/vetres:2003028

Seligsohn D, 2021. Subclinical mastitis in dairy camels in Kenya with a special focus on Streptococcus agalactiae Doctoral Thesis No.:40 Faculty of Veterinary Medicine and Animal Science. http://doi.org/10.5772/intechopen.108316

Seligsohn D, Nyman AK, Younan M, Sake W, Persson Y, Bornstein S, Maichomo M, de Verdier K, Morrell JM and Chenais E, 2020. Subclinical mastitis in pastoralist dairy camel herds in Isiolo, Kenya: Prevalence, risk factors, and antimicrobial susceptibility. Journal of Dairy Science 103(5): 4717-4731. https://doi.org/10.3168/jds.2019-17701

Seligsohn lD, Younan M, Larsen T, Morrell JM, Chenais E and Nyman AK, 2021. Detection of subclinical mastitis in camels (*Camelus dromedarius)* using somatic cell count, N-acetyl-β-D-glucosaminidase and lactate dehydrogenase activity. Small Ruminant Research 204 (11): 106512. https://doi.org/ 10.1016/j.smallrumres.2021.106512

Serdal KU, Funda ES, Leyla MI and Demir PA, 2021. Evaluation of oxidative stress, immune system and mineral concentrations in milk and serum of cows with clinical and subclinical mastitis naturally infected by Staphylococcus aureus. KAFKAS Üniversitesi Veteriner Fakültesi Dergisi 27(6): 755-762. https://doi.org/10.9775/kvfd.2021.26281

Šerstņova K, Pilmane M, Vitenberga-Verza Z, Melderis I, Gontars L, Kochanski M, Drutowska A, Gergely M and Prieto-Simona B, 2022. Expression of anti-inflammatory markers IL-2, IL-10, TGF-β1, βDEF-2, βDEF-3 and Cathelicidin LL37 in dairy cattle milk with different health status of the udder. Polish Journal of Veterinary Science 25(2): 237-248. https://doi.org/10.24425/pjvs.2022.141808

Shaheen T, Ahmad SB, Rehman MU, Muzamil S, Bhat RR, Hussain I, Bashir N, Rahman MM, Paray BA and Dawood MAO, 2020. Investigations on cytokines and proteins in lactating cows with and without naturally occurring mastitis. Journal of King Saud University Science 32(6): 2863–2867. https://doi.org/10.1016/j.jksus.2020.07.009

Shao HY, Chen YC, Chung NH, Lu YJ, Chang CK, Yu SL, Liu CC and Chow YH, 2018. Maternal immunization with a recombinant adenovirus-expressing fusion protein protects neonatal cotton rats from respiratory syncytia virus infection by transferring antibodies via breast milk and placenta. Virology 521: 181-189. https://doi.org/10.1016/j.virol.2018.06.009

Shimazaki KI and Kawai K, 2017. Advances in lactoferrin research concerning bovine mastitis. Biochemical Cell Biology 95: 69–75. https://doi.org/ 10.1139/bcb-2016-0044

Silva EP, Moraes EP, Anaya K, Silva YM, Lopes HA, Andrade Neto JC, Oliveira JP, Oliveira JB and Rangel AH, 2022. Lactoperoxidase potential in diagnosing subclinical mastitis in cows via image processing. PloS One 17(2): e0263714. https://doi.org/10.1371/journal.pone.0263714

Sumon SMR, Parvin MS, Ehsan MA and Islam MT, 2020. Relationship between somatic cell counts and subclinical mastitis in lactating dairy cows. Veterinary World 13(8): 1709. https://doi.org/10.14202/vetworld.2020.1709-1713

Takashima M, Lalonde C, Olszanski LA and Zhao FQ, 2021. Localized and systemic inflammatory mediators in a murine acute mastitis model. Journal of Inflammatory Research 14: 4053-4067. https://doi.org/10.2147/JIR.S313799

Thorsteinsdottir L, Jonsdottir S, Stefansdottir, SB, Andresdottir V, Wagner B, Marti E, Torsteinsdottir S and Svansson V, 2019. The effect of maternal immunity on the equine gammaherpesvirus type 2 and 5 viral load and antibody response. PLoS One 14(6): e0218576. https://doi.org/10.1371/journal.pone.0218576

Qayyum A, Khan JA, Hussain R, Khan A, Avais M, Ahmad N and Hassan MF, 2016. Molecular characterization of *Staphylococcus aureus* isolates recovered from natural cases of subclinical mastitis in Cholistani cattle and their antibacterial susceptibility. Pakistan Journal of Agricultural Sciences, 53 (4): 971-976.

Vitenberga-Verza Z, Pilmane M, Šerstņova K, Melderis I, Gontar Ł, Kochański M, Drutowska A, Maróti G and Prieto-Simón B, 2022. Identification of inflammatory and regulatory cytokines IL-1α-, IL-4-, IL-6-, IL-12-, IL-13-, IL-17A-, TNF-α-, and IFN-γ-producing cells in the milk of dairy cows with subclinical and clinical mastitis. Pathogens 11(3):372. https://doi.org/10.3390/pathogens11030372