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Evaluation of Somatic Cells Count, Antioxidants and Antimicrobial Proteins in Milk Samples Obtained from Different Breeds of the Dromedary Camel

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ABSTRACT

The present study aimed to investigate the relations between somatic cells count (SSC), antioxidants, and antimicrobial proteins in milk samples obtained from Waddah, Sofor, and Majaheem breeds of the dromedary camel. High-SCC milk samples contained >300x10^3 cells/mL. The milk levels of catalase (CAT) were significantly increased in the Waddah breed compared to the Sofor and Majaheem breeds (P≤0.05). In the Waddah breed, the CAT levels were also significantly elevated in the high-SCC milk samples compared to the low-SCC milk samples (P≤0.001). The Superoxide dismutase (SOD) levels were significantly elevated in the high-SCC milk samples compared to the low-SCC milk samples in the Sofor and Majaheem breeds (P≤0.001 and P≤0.05, respectively). Significant reduction in the total antioxidant capacity (TAC) was observed in the high-SCC milk samples of the Sofor and Majaheem breeds (P≤0.001 and P≤0.05, respectively). The milk levels of lactoferrin (LTF) and lactoperoxidase (LPO) were significantly reduced in the Waddah group compared to the Sofor and Majaheem groups (P≤0.01). The LTF levels were significantly elevated in the milk samples with low SCC compared to the high-SCC samples in the Waddah and Sofor breeds (P≤0.01 and P≤0.05, respectively). No significant differences were observed among the examined breeds concerning the milk levels of immunoglobulin G (IgG). Only in the Majaheem breed, the milk IgG levels were significantly increased in the high-SCC samples compared to low-SCC ones (P≤0.001). The current study adds to our understanding of dromedary camel milk and reveals possible biomarkers for both healthy camels and camels with subclinical mastitis.

Key words: Antioxidants, Lactoferrin, Lactoperoxidase, Subclinical Mastitis, Camels.

INTRODUCTION

Mammary gland inflammation is referred to as mastitis, characterized by physical, chemical, and bacteriological abnormalities in milk, besides pathological alterations at the gland level. While it can affect any nursing mammal, it is a disease that is quite common in dairy animals, including dairy cattle and dairy camels.

Somatic cells count (SCC) is widely employed as a mastitis or intramammary infection (IMI) indicator and is the basis for udder health management programs (Schukken et al. 2003). Monitoring SSC can assist producers in establishing a checkpoint for disease entrance into the herd. Somatic cells mainly include milk-secreting epithelial cells and leukocytes such as neutrophils, lymphocytes, and macrophages. Several SCC cut-offs have been reported in the literature. According to the International Dairy Federation (1971), the mean SCC values for sub-clinically infected udders were 500,000 cells/mL of milk (Tolle 1971). A more recent study by Jadhav et al. (2018) reported that the cut-off for distinguishing subclinical mastitis cases in cattle from healthy ones was 310,000 cells/mL of milk. In camels, Aljumaah et al. (2019) reported that the threshold for separating camels with sub-clinical mastitis from normal ones was 472,500 cells/mL of milk.

Oxidative stress and its relationship to diverse diseases, including mastitis, is a complicated phenomenon. It is typical for living tissues to produce free radicals. Reactive oxygen species (ROS) are efficiently neutralized by cellular defense mechanisms, enzymatic antioxidants or non-enzymatic antioxidants, during normal homeostasis (Poławska et al. 2012; Wang et al. 2022). Excessive ROS and/or a lack of significant antioxidant levels cause imbalance, which results in oxidative stress (Lykkesfeldt and Svendsen 2007; Li et al. 2022).

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Oxidative stress is a main cause of immunological dysfunction and decreased inflammatory responses, which promotes the occurrence of microbial infections in mammary glands (Abuelo et al. 2013; Mahmood et al. 2022). Superoxide dismutase (SOD), catalase (CAT), and total antioxidant capacity (TAC) are indicators commonly used for assessing oxidative stress in biological fluids (Khan et al. 2022; Almundarij 2023). Thus, the present study aimed to assess their levels in milk samples obtained from healthy camels or camels suspected of subclinical mastitis.

Lactoferrin (LTF), lactoperoxidase (LPO), and immunoglobulin G (IgG) are three protective proteins found in milk. LTF is abundant in animal secretions such as milk, saliva, tears, and semen, as well as in some leukocytes (Hao et al. 2019). It was first discovered in cow's milk and then in human milk; and is thought to have a variety of biological roles, including antibacterial and anti-inflammatory properties (Spik et al. 1998; Hu et al. 2020; Dierick et al. 2021). LTF is an effective iron scavenger and is one of several molecules secreted by the immune system to bind transitory metals to prevent bacterial infections since iron is essential for several critical biological processes, including DNA and ATP synthesis (Anderson et al. 1989; Spik et al. 1998). Lactoferrin has been linked to a variety of defense mechanisms against a wide spectrum of microorganisms. It has been demonstrated that LTF helps to control invading bacteria, viruses, fungi, and parasites (Yokoyama et al. 2019; Lu et al. 2020; Mann and Ndung'u 2020; Singh et al. 2021). LTF has been shown to possess high antibacterial activity against bacteria such as *Staphylococcus*, *Streptococcus*, *Shigella*, *Salmonella*, and *Enterobacter* (Arnold et al. 1980). In addition to acting as a bactericide, LTF can contribute to innate immunity to assist in bacterial clearance. One study has found that bovine LTF could effectively replace antibodies in activating the classical pathway of the complement system and causing the opsonization of unencapsulated *S. agalactiae* (Rainard 1993).

Lactoperoxidase (LPO) is another non-immunoglobulin protective glycoprotein found in milk and other human and animal fluids (Gothefors and Marklund 1975; Zou et al. 2021). LPO depends on hydrogen peroxide (H_2O_2) and thiocyanate (SCN^-) for its antimicrobial action, which is known as the lactoperoxidase system (LPS) (Eyassu et al. 2005). The LPS is thought to be an important component of the host innate defense system. Gram-positive and Gram-negative bacteria are susceptible to the LPS to variable degrees, and antiviral actions for LPS have also been reported. (Yassin et al. 2015; El-Fakharany et al. 2017). Activation of LPS has been investigated as a potential alternative technique for maintaining milk safety by eliminating pathogenic microbes (Dashe et al. 2020; Mohamed and El Zubeir 2020).

In combination with other protective proteins, immunoglobulins, particularly Immunoglobulins G, are components of the immune system that aid in removing substances foreign to the organism. Milk obtained from unvaccinated dairy herds has been shown to have IgG antibodies specific to bacteria that are pathogenic to humans (ULTFman et al. 2018). Many mammalian species are born without an efficient immune system where immunoglobulins and metal-binding proteins play crucial roles in protection against newborn infections (Shao et al. 2018; Pou et al. 2019; Thorsteinsdottir et al. 2019). Camels' serum contains two distinct types of antibodies, the unique functional single-chain antibodies (IgG2 and IgG3) and the traditional heterotetrameric antibodies (IgG1). Camel IgG2 and IgG3 antibodies lack light chains and are made up entirely of heavy chains, therefore called Heavy chain antibodies (HCAbs) (Muyldermans 2013). Camel milk contains the larger amount of immunoglobulin G compared to goat, cow, sheep, buffalo, and human milk levels (El-Agamy et al. 2009). Camel antibodies secreted in milk have been shown to include both IgG1 and HCAbs (Azwai et al. 1996; Salhi et al. 2015).

The present study aimed to investigate differences among three different breeds of the dromedary camel as well as between high and low SCC milk samples regarding their contents of antioxidants and protective proteins such LTF, LPO, and IgG. The results of the current investigation advance knowledge regarding the dromedary camel milk and shed light on potential indicators of healthy camels and camels with subclinical mastitis.

MATERIALS AND METHODS

Ethical Approval

Because no invasive approach that would cause suffering to animals was used, clearance from the Institutional Animal Ethics Committee was not necessary.

Animals

The study involved 28 lactating animals aged between 5 and 10 years old. Animals were grouped based on their color phenotype into Whaddah (white), Sofor (yellow), and Majageem (black) (Abdullah and Faye 2012). Each group contained at least eight animals and animals kept by producers in rural areas of the Qassim region. Typically, a 100mL sample of composite milk was collected from a single teat of each nursing animal. Serum samples were acquired by collecting a 10mL blood sample without anti-coagulants.

Skim Milk Samples

Milk samples centrifugation was performed at 13,000rpm for 10min at 4°C to separate skim milk from whole milk. Skim milk samples were then stored at -20°C till used for further analysis. Change minutes to min ul to µL No space between value and units.

Erythrocytes-Excluded Somatic Cells Count (SCC)

A volume of 1mL was taken from each raw milk sample, placed in a 2mL tube, and centrifuged for 10min at 10,000rpm using a cooling centrifuge. After, the removal of the creamy portion and the supernatant, the pellet was resuspended with 1mL of phosphate-buffered saline (PBS) in order to bring the sample back to its' original volume. A volume of 10uL of the cell suspension was diluted with 190µL of Turk's solution (methylene blue in 1-2% acetic acid and distilled water). Somatic cells in a volume of 10µL of the diluted sample were counted using a hemocytometer and a light microscope.

Quantification of the Skim Milk levels of SOD, CAT, and TAC

Measuring the skim milk levels of SOD, CAT, and TAC was done using commercially available kits (Biodiagnostic, Giza, Egypt; kits, Cat. Nos. SD 2521, CA 256317, and TA 255213, respectively), according to the manufacturer's specifications.

Quantification of LTF, LPO, and IgG Levels in Skim Milk and Serum

The skim milk and serum levels of LTF, LPO, and IgG were determined using commercially available kits according to the manufacturer's specifications (Sunlog Biotech, Hangzhou, Zhejiang, China; kits, Cat. Nos. SL0050cm, SL0051cm, and SL0039cm, respectively).

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 9 (Bangalore– 560035 Karnataka, India). For data analysis, the one-way ANOVA and the independent samples t-test were utilized when appropriate.

RESULTS

Somatic Cells Count (SCC)

In the current study, each group was subdivided based on the Somatic cells count per mL of milk into low or high SCC. Milk samples having >300x10^3 cells/mL were considered high SCC**.** The SCC means for the three examined breeds are listed in Table 1.

Skim Milk Levels of SOD, CAT and TAC

The concentrations of antioxidant enzymes in the milk samples obtained from the three examined dromedary camel breeds were investigated (Table 2). The data indicated a significant increase in the CAT levels in milk samples of the Waddah group compared to the Sofor and Majaheem groups (P≤0.05). No significant differences were observed among the three groups regarding the levels of SOD and TAC. Next, we investigated the relationship between the antioxidant enzymes levels and somatic cells counts (SCC). As shown in Table 3, the SOD levels were significantly elevated in the high-SCC milk samples compared to the low-SCC milk samples in both the Sofor and Majaheem groups (P≤0.001 and P≤0.05, respectively). The CAT levels were also significantly elevated in the high-SCC milk samples (P≤0.001) but this observation was noted in the Waddah group only. The TAC activities were significantly decreased in the high-SCC milk samples compared to low-SCC samples in the Sofor and Majaheem groups (P≤0.001 and P≤0.05, respectively).

Skim Milk and Serum Levels of LTF, LPO and IgG

As shown in Table 4, the LTF levels in milk were significantly reduced in the Waddah group compared to the Sofor and Majaheem groups (P≤0.01). No significant differences were observed among the examined breeds regarding the serum LTF levels. Similarly, the LPO concentrations were significantly reduced in the milk samples of the Waddah group compared to the Sofor and Majaheem groups (P≤0.01). However, the serum levels of LPO were not statistically different among the examined

Table 1: Somatic cells counts (Mean±SE) in the three examined camel breeds

Breed	Low SCC	High SCC
Waddah	180,000±7055	523,333±46228
Sofor	282,666±13421	456,000±16220
Majaheem	277,333±8146.66	901,333±209,317

Table 2: Effects of breed type on milk antioxidant enzymes levels

Analytic	Waddah	Sofor	Majaheem
SOD U/mL	495.76±71.47	575.80±31.75	553.51±21.99
CAT U/L	1520.38±198.79*	1166.07±51.23	1050.96±61.87
TAC mM/L	0.93±0.07	0.88±0.12	0.97±0.08

Mean±SE. *Significantly different from the values in the same row at P≤0.05. SOD: Superoxide dismutase. CAT: Catalase. TAC: Total Antioxidant Activity.

Table 3: Antioxidant enzymes levels in milk samples with low or high SCC

Analytics	Units	Breed	Low SCC	High SCC
SOD	U/mL	Waddah	341.63±63	451.75±54.43
SOD	U/mL	Sofor	499.04±1.64	652.56±21.60***
SOD	U/mL	Majaheem	514.59±4.94	592.42±26.92*
CAT	U/L	Waddah	1206.84±17.69	2265.29±52.67***
CAT	U/L	Sofor	826.44±213.51	1097.12±32.97
CAT	U/L	Majaheem	1095.32±67.54	1157.38±87.25
TAC	mM/L	Waddah	0.95±0.04	0.91±0.11
TAC	mM/L	Sofor	1.11±0.01***	0.57±0.08
TAC	mM/L	Majaheem	1.13±0.02*	0.81±0.11

Mean±SE. * and *** are significantly different from the value in the same row at P≤0.05 and P≤0.001, respectively. SOD: Superoxide dismutase. CAT: Catalase. TAC: Total Antioxidant Activity.

Table 4: Effects of breed type on milk and serum LTF, LPO**,** and IgG concentrations

Analytics	Units	Waddah	Sofor	Majaheem
Milk LTF	ng/mL	3.32±0.24**	5.03±0.52	4.67±0.39
Milk LPO	pg/mL	6.09±0.72**	10.07±0.62	10.91±0.30
Milk IgG	µg/mL	8.35±1.35	9.57±1.88	9.88±0.65
Serum LTF	ng/mL	5.36±0.55	5.22±0.35	4.79±0.44
Serum LPO	pg/mL	7.81±0.22	8.15±0.71	7.23±0.12
Serum IgG	µg/mL	6.18±0.64	10.70±1.54	11.12±1.41

Mean±SE. ** is significantly different from the value in the same row at P≤0.01. LTF: Lactoferrin. LPO: Lactoperoxidase. IgG: Immunoglobulin G.

three groups. No significant differences were observed among the examined breeds concerning the IgG levels in milk or serum.

Next, we examined the variations in the concentrations of LTF, LPO, and IgG in the milk and serum samples of animals with high SCC and their low SCC counterparts within each breed. Table 5 shows that the LTF levels were significantly elevated in the milk samples with low SCC compared to the high SCC samples in the Waddah and Sofor breeds ($P \leq 0.01$ and $P \leq 0.05$, respectively). In the Mjaheem breed, the levels of the milk LTF were comparable between the high SCC and low SCC milk samples. Only the Waddah breed was found to have LPO concentrations that were significantly higher in the high SCC milk samples compared to the low SCC samples ($P \leq 0.01$). Only in the Majaheem breed, the milk IgG levels were significantly increased in the high SCC milk samples compared to low SCC ones ($P \leq 0.001$). As shown in Table 6, the serum levels of LTF were significantly increased in the low SCC animals compared to their high SCC counterparts in the Waddah breed only ($P \leq 0.001$). However, A moderate but significant increase was observed in the serum levels of LTF in the high SCC camels compared to the low SCC camels of the Majaheem breed ($P \leq 0.05$). The serum LPO concentrations were significantly elevated in the low SCC animals compared to the high SCC animals of the Sofor breed ($P \leq 0.001$). This observation was noted in the Sofor breed only. The serum IgG levels were significantly elevated in the high SCC animals compared to the low SCC animals of Waddah and Majaheem breeds ($P \leq 0.05$ and $P \leq 0.001$, respectively).

DISCUSSION

The current study aimed to quantify antioxidant enzymes and protective proteins in low and high SCC milk samples obtained from three different breeds of the dromedary camel. SCC is the most commonly used and most reliable indicator of udder health. Thus, in the present study, each group was subdivided based on the SCC per mL of milk into low or high SCC. In the current study, milk samples having $>300x10^3$ cells/mL were considered high SCC and likely to be obtained from udders with subclinical mastitis. A study by Aljumaah et al. (2019) demonstrated the reliability and validity of the SCC test in distinguishing subclinical mastitis in dromedary camels. The study suggested that 472.50×10^3 cells/mL is a logical SCC reading for herd management decisions. A study in cattle by Musayeva et al. (2016) used $200x10^3$ cell/mL as a cut-off to differentiate between healthy and diseased udders. A more recent report by Jadhav et al. (2018) indicated that $310x10^5$ somatic cells/mL is the threshold value for detecting subclinical mastitis in cattle. The reason for the increased number of somatic cells is that the mammary epithelial cells generate a defense mechanism against entering pathogens by sensing their ligands and mounting the necessary immune responses (Jadhav et al. 2016; Mohsin et al. 2022; Betelhem et al. 2022; Asfour et al. 2022).

The concentrations of antioxidant enzymes in the milk samples obtained from the three examined dromedary camel breeds were investigated. The data indicated a significant increase in the CAT levels in milk samples of the Waddah group compared to the Sofor and Majaheem groups. No significant differences were observed among the three groups regarding the levels of SOD and TAC. Investigating the relationship between the antioxidant enzymes levels and SCC revealed that the SOD levels were significantly elevated in the high-SCC milk samples compared to the low-SCC milk samples in both the Sofor and Majaheem groups. In addition, the CAT levels were significantly elevated in the high-SCC milk samples, but this observation was noted in the Waddah group only. A significant reduction in TAC activities was observed in the high-SCC milk samples compared to low-SCC samples in the Sofor and Majaheem groups. These observations support previous findings by Ghasemian et al. (2011) indicating a significant positive correlation between SOD and SCC in cattle. A recent study conducted in dairy buffaloes by Tyagi et al. (2020) reported significant increases in SOD and CAT levels in animals with subclinical mastitis. Another study showed that milk samples obtained from buffaloes with subclinical mastitis had significantly lower TAC activities than milk samples from the healthy controls (Dimri et al. 2013). The increased levels of SOD and CAT in milk samples with high SCC could be an adaptation to the overproduction of ROS by macrophages and mammary epithelial cells during inflammation (Bouchard et al. 1999).

The data of the present study indicated a significant reduction in milk but not serum LTF levels in the Waddah breed compared to the Sofor and Majaheem breeds. No study has looked at the variations in LTF concentrations in the serum and milk of different dromedary camel breeds. However, Konuspayeva et al. (2007) examined LTF concentrations in milk samples obtained from Bactrian camels, dromedary camels, and their hybrids and concluded that the season but not the species had an effect

Table 5: LTF, LPO, and IgG concentrations in milk samples with low or high SCC

Analytics	Units	Breed	Low SCC	High SCC
LTF	ng/mL	Waddah	3.90±0.22**	2.74±0.18
LTF	ng/mL	Sofor	6.01±0.63*	4.06±0.34
LTF	ng/mL	Majaheem	4.03±0.41	5.32±0.37
LPO	pg/mL	Waddah	3.58±0.25	6.96±0.53**
LPO	pg/mL	Sofor	10.20±0.78	9.88±0.52
LPO	pg/mL	Majaheem	9.78±0.57	11.08±0.04
IgG	µg/mL	Waddah	9.65±1.16	7.05±1.8
IgG	µg/mL	Sofor	7.83±0.91	12.17±3.41
IgG	µg/mL	Majaheem	8.81±0.38	12.63±0.16***

Mean±SE. *, **, and *** are significantly different from the value in the same row at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. LTF: Lactoferrin. LPO: Lactoperoxidase. IgG: Immunoglobulin G.

Table 6: LTF, LPO, and IgG concentrations per mL of serum obtained from low or high SCC animals

Analytics	Units	Breed	Low SCC	High SCC
LTF	ng/mL	Waddah	6.71±0.45***	4.03±0.43
LTF	ng/mL	Sofor	5.55±0.52	4.89±0.18
LTF	ng/mL	Majaheem	3.93±0.45	5.65±0.38*
LPO	pg/mL	Waddah	10.18±1.38	10.55±1.19
LPO	pg/mL	Sofor	12.36±1.08***	6.63±0.03
LPO	pg/mL	Majaheem	8.23±0.98	7.31±0.21
IgG	µg/mL	Waddah	5.23±0.03	7.14±0.72*
IgG	µg/mL	Sofor	9.20±0.64	12.94±2.82
IgG	µg/mL	Majaheem	6.31±0.42	12.08±0.16***

Means±SE. * and *** are significantly different from the value in the same row at P≤0.05 and P≤0.001, respectively. LTF: Lactoferrin. LPO: Lactoperoxidase. IgG: Immunoglobulin G.

on LTF concentrations in milk. When comparing low SCC animals with high SCC animals within each breed, the milk LTF concentrations were significantly elevated in the low SCC animals of the Waddah and Sofor breeds. However, the serum LTF concentrations were significantly higher in the low SCC animals of the Waddah group and in the high SCC animals of the Majaheem group. These observations correspond to recent studies reporting high variability in LTF concentrations in dromedary camels. A recent study by Mohamed et al. (2022) examined concentrations of bioactive proteins in whey milk samples of 140 individual dromedary camels and reported wide variations in LTF concentrations. Another recent study by Zou et al. (2022) revealed that individual camels had remarkably variable antimicrobial enzyme activities, with Lactotransferrin and other bioactive proteins being present in higher levels in camel milk during the summer season. The data of the current study suggest that LTF might not be a potential indicator for subclinical mastitis in camel as has been suggested for ovine (Navarro et al. 2018).

The LPO concentrations were significantly reduced in the milk samples of the Waddah group compared to the Sofor and Majaheem groups. Differences among dromedary camel breeds regarding LPO concentrations have not been previously explored. According to a recent study by Zou et al. (2022), the season had an impact on the levels of LPO in the milk of Australian camels, with the summer and winter seasons having the highest levels. Only within the Waddah group, the milk LPO concentrations were significantly elevated in the high SCC animals compared to the low SCC ones. No significant differences were observed between low and high SCC animals regarding the milk LPO concentrations in the Sofor and Majaheem groups. The serum LPO concentrations were significantly elevated in the low SCC animals compared to the high SCC animals in the Sofor group, only. No significant differences were observed between low and high SCC animals concerning the serum LPO concentrations in the Waddah and Majaheem breeds. The observations of the current study correspond to the findings of a previous study by Andrei et al. (2009) in cattle that compared normal and mastitis milk samples and reported a positive and direct correlation between LPO levels and the number of somatic cells. LPO has been suggested recently to serve as a potential biomarker for diagnosing subclinical mastitis in cattle (Silva et al. 2022).

In the present study, the IgG levels in milk and serum were quantified. No significant differences were observed among the examined breeds concerning the IgG levels in milk or serum. To the best of our knowledge, no study has looked at the IgG concentrations in different breeds of the dromedary camel. However, Konuspayeva et al. (2007) measured the IgG levels in milk samples obtained from two different camel species (*Camelus bactrianus* vs. *Camelus dromedarius*) and their hybrids and observed no significant differences. In the Majaheem group only, the IgG levels were significantly elevated in the high SCC milk samples compared to the low SCC samples. The IgG levels tend to be higher in the high SCC samples compared to the low SCC samples in the Sofor group but did reach statistical significance. The serum IgG concentrations were significantly elevated in the high SCC animals compared the low SCC animals in both the Waddah and Majaheem groups. Our observations were not different from previous studies indicating increased immunoglobulins secretion in lactating animals with subclinical mastitis (Lehmann et al. 2015; Hayajneh et al. 2018). A study by Lehmann et al. (2015) reported a positive association between SCC numbers and IgG levels and that the IgG levels could be used as a parameter to differentiate between low and high SCC milk samples. Hayajneh et al. (2018) reported significant increases in the concentrations of serum immunoglobulins A, G, and M in relation to subclinical mastitis in camels. Another study by Musayeva et al. (2016) has also indicated significant increases in the IgG levels in cows' milk samples with SCC ranging from 201,000 to ≥401,000 cells/mL compared to milk samples with ≤200,000 cells/mL. Mackenzie and Lascelles' (1968) hypothesized that the increase in immunoglobulin secretion could result from acute inflammation causing the mammary gland's blood-to-milk permeability barriers to be destroyed, which increases the passive transfer of IgG into milk.

Conclusion

The current investigation mainly aimed to quantify SCC, antioxidants, and antimicrobial proteins in milk samples obtained from three different breeds of the dromedary camel. Significant differences regarding the milk levels of antioxidant enzymes, particularly CAT, were observed among the examined breeds. The SOD and CAT levels were elevated in milk samples with high SCC compared to the low SCC samples. Increases in TAC were noted in the low SCC samples compared to the high SCC milk samples. Except for the IgG levels, significant differences among the examined breeds were recorded regarding the milk levels of LTF and LPO. The milk levels of LTF were elevated in the low SCC samples in

two of the examined breeds while LPO and IgG levels were increased in the high SCC samples in at least one of the examined breeds. The data presented in the present study advances our knowledge regarding the dromedary camel milk and identifies potential biomarkers for differentiating between healthy camels and camels suspected of subclinical mastitis. However, more studies are needed to involve more breeds, more biomarkers, and larger numbers of animals.

Declaration of Competing Interests

The author declares that he has no competing interests.

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REFERENCES

Abdullah 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.

Abuelo A, Hernandez J, Benedito JL and Castillo C, 2013. Oxidative Stress Index (OSI) as a new tool to assess redox status in dairy cattle during the transition period. Animal 7(8): 1374-1378. https://doi.org/10.1017/S1751731113000396

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): e20180373. https://doi.org/10.1590/1678-992x-2018-0373

Almundarij TI, 2023. Oxidative stress biomarkers and lipid profile during the periparturient period in dromedary camels with ketosis. International Journal of Veterinary Science. https://doi.org/10.47278/journal.ijvs/2022.207

Anderson BF, Baker HM, Norris GE, Rice DW and Baker EN, 1989. Structure of Human Lactoferrin: Crystallographic Structure Analysis and Refinement at 2·8 Å Resolution. Journal of Molecular Biology 209(4): 711-734. https://doi.org/10.1016/0022-2836(89)90602-5

Andrei S, Pintea A, Groza I, Bogdan L, Ciupe S and Matei S, 2009. Milk antioxidant enzymes activity in cows with sub clinical mastitis. Lucrări Ştiinţifice-Medicină Veterinară, Universitatea de Ştiinţe Agricole şi Medicină Veterinară" Ion Ionescu de la Brad" Iaşi 52 no. 11(1): 1-6.

Arnold RR, Brewer M and Gauthier JJ, 1980. Bactericidal activity of human lactoferrin: sensitivity of a variety of microorganisms. Infection and Immunity 28 (3): 893−898. https://doi.org/10.1128/iai.28.3.893-898.1980

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

Azwai SM, Carter SD and Woldehiwet Z, 1996. Immunoglobulins of camel (Camelus dromedarius) colostrum. Journal of Comparative Pathology 114(3): 273-282. https://doi.org/10.1016/S0021-9975(96)80049-1

Bouchard L, Blais S, Desrosiers C, Zhao X and Lacasse P, 1999. Nitric oxide production during endotoxin-induced mastitis in the cow. Journal Dairy Science 82(12): 2574-2581. https://doi.org/10.3168/jds.S0022-0302(99)75512-8

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

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

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/bcb-2020-0080

Dimri U, Sharma MC, Singh SK, Kumar P, Jhambh R, Singh B, Bandhyopadhyay S and Verma MR, 2013. Amelioration of altered oxidant/antioxidant balance of Indian water buffaloes with subclinical mastitis by vitamins A, D3, E, and H supplementation. Tropical animal health and production 45(4): 971-978. https://doi.org/10.1007/s11250-012-0319-6

El-Agamy EI, Nawar M, Shamsia SM, Awad S and Haenlein GF, 2009. Are camel milk proteins convenient to the nutrition of cow milk allergic children?. Small Ruminant Research 82(1): 1-6. https://doi.org/10.1016/j.smallrumres.2008.12.016

El-Fakharany EM, Uversky VN and Redwan EM, 2017. Comparative analysis of the antiviral activity of camel, bovine, and human lactoperoxidases against herpes simplex virus type 1. Applied Biochemistry and Biotechnology 182(1): 294-310. https://doi.org/10.1007/s12010-016-2327-x

Eyassu S, Buys EM and Donki EF, 2005. Significance of the Lactoperoxidase System in the Dairy Industry and Its Potential Applications: A Review. Food Science and Technology 16(4): 137-154. https://doi.org/10.1016/j.tifs.2004.11.002

Ghasemian KO, Safi S, Rahimi FA and Bolourchi M, 2011. Study of the relationship between oxidative stress and subclinical mastitis in dairy cattle. Iran Journal of Veterinary Research 12: 350-353.

Gothefors L and Marklund S, 1975. Lactoperoxidase activity in human milk and in saliva of newborn infants. Infection and Immunity 11(6): 1210-1215. https://doi.org/10.1128/iai.11.6.1210-1215.1975

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

Hayajneh FM, 2018. The effect of subclinical mastitis on the concentration of immunoglobulins A, G, and M, total antioxidant capacity, zinc, iron, total proteins, 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 NF-κB/MAPK/Nrf2 pathways in IPEC-J2 cells. Food & Function 11(10): 8516-8526. https://doi.org/10.1039/D0FO01570A

Jadhav, PV, Tarate SB, Bhuvana M, Das DN and Shom BR, 2016. Somatic cell count as a monitring system for hygienic milk production in India: A review. Asian Journal of Dairy

Food Research 35(4): 270-277. https://doi.org/10.18805/ajdfr.v35i4.6624

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

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: 657. https://doi.org/10.3390/antiox11040657

Konuspayeva G, Faye B, Loiseau G and Levieux D, 2007. Lactoferrin and immunoglobulin contents in camel's milk (Camelus bactrianus, Camelus dromedarius, and Hybrids) from Kazakhstan. Journal of Dairy Science 90(1): 38-46. https://doi.org/10.3168/jds.S0022-0302(07)72606-1

Lehmann M, Wall SK, Wellnitz O and Bruckmaier RM, 2015. Changes in milk L-lactate, lactate dehydrogenase, serum albumin, and IgG during milk ejection and their association with somatic cell count. Journal of Dairy Research 82(2): 129-134. https://doi.org/10.1017/S002202991400065X

Li X, Naseem S, Hussain R, Ghaffar A, Li K, and Khan A, 2022. Evaluation of DNA damage, biomarkers of oxidative stress and status of antioxidant enzymes in freshwater fish (*Labeo rohita*) exposed to pyriproxyfen. Oxidative Medicine and Cellular Longevity 2022: 5859266. https://doi.org/10.1155/2022/5859266

Lu J, Francis J, Doster RS, Haley KP, Craft KM, Moore RE, Chambers SA, Aronoff DM, Osteen K, Damo SM and Manning S, 2020. Lactoferrin: a critical mediator of both host immune response and antimicrobial activity in response to streptococcal infections. ACS Infectious Diseases 6(7): 1615-23. https://doi.org/10.1021/acsinfecdis.0c00050

Lykkesfeldt J and Svendsen O, 2007. Oxidants and antioxidants in disease: oxidative stress in farm animals. The Veterinary Journal 173(3): 502-511. https://doi.org/10.1016/j.tvjl.2006.06.005

Mackenzie DDS and Lascelles AK, 1968. The transfer of [131I]-labelled immunoglobulins and serum albumin from blood into milk of lactating ewes. Australian Journal of Experimental Biology and Medical Science 46(3): 285-294. https://doi.org/10.1038/icb.1968.23

Mahmood Y, Hussain R, Ghaffar A, Ali F, Nawaz S, Mehmood K and Khan A, 2022. Acetochlor affects bighead carp (*Aristichthys nobilis*) by producing oxidative stress, lowering tissue proteins, and inducing genotoxicity. BioMed Research International, 2022: 9140060. https://doi.org/10.1155/2022/9140060

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): 609-24. https://doi.org/10.2217/fvl-2020-0170

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/10.1111/1471-0307.12849

Mohamed HM and El Zubeir IE, 2020. Improvement of Camel Milk Microbial Loads by Activation of Lactoperoxidase Enzyme System during Different Storage Temperature. Journal of Dairy Research Technology 3: 021. https://doi.org/10.24966/DRT-9315/100021

Mohsin M, Swar SO, Imran M, Ali W, Sultan MD, Asrar R, Abbas RZ, Aleem MT, Aguilar-Marcelino L, Aslam A, Shahab A, Ahsan MA, Shehata AI, Alhoshy M, Habib YJ, Farhan MHR and Yin G, 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

Musayeva, K, Sederevicius A, Zelvyte R, Monkeviciene I, Beliavska-Aleksiejune D and Kerziene S, 2016. Concentration of lactoferrin and immunoglobulin G in cows milk in relation to health status of the udder, lactation and season. Polish Journal of Veterinary Sciences 19(4). https://doi.org/10.1515/pjvs-2016-0093

Muyldermans S, 2013. Nanobodies: natural single-domain antibodies, Annual Reviews Biochemistry 82(1): 775-797. https://doi.org10.1146/annurev-biochem-063011-092449

Navarro F, Galan-Malo P, Pérez MD, Abecia JA, Mata L, Calvo M and Sánchez L, 2018. Lactoferrin and IgG levels in ovine milk throughout lactation: correlation with milk quality parameters. Small Ruminant Research 168: 12-18. https://doi.org/10.1016/j.smallrumres.2018.09.002

Poławska E, Bagnicka AW, Niemczuk K and Lipińska JO, 2012. Relations between the oxidative status, mastitis, milk quality and disorders of reproductive functions in dairy cows-A review. Animal Science Papers and Reports 30(4): 297-307.

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.

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

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

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

Singh A, Ahmad N, Varadarajan A, Vikram N, Singh TP, Sharma S and Sharma P, 2021. Lactoferrin, a potential iron-chelator as an adjunct treatment for mucormycosis–A comprehensive review. International Journal of Biological Macromolecules 187: 988-998. https://doi.org/10.1016/j.ijbiomac.2021.07.156

Spik G, Legrand D, Mazurier J, Pierce A and Perraudin JP, 1998. Advances in lactoferrin research; Plenum Press, New York, USA, volume 443.

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

Tolle A, 1971. A monograph on bovine mastitis. Annual Bulletin, International Dairy Federation. Part 1

Tyagi A, Arora R, Rajora VS and Arora N, 2020. Evaluation of antioxidant profile in subclinical mastitis in dairy buffaloes. Journal of Entomology and Zoology Studies 8(5): 2256-2259.

ULTFman LH, Leusen JH, Savelkoul HF, Warner JO and Van Neerven RJ, 2018. Effects of bovine immunoglobulins on immune function, allergy, and infection. Frontiers in Nutrition 5: 52. https://doi.org/10.3389/fnut.2018.00052

Wang JQ, Hussain R, Ghaffar A, Afzal G, Saad AQ, Ahmad N, Nazir U, Ahmad HI, Hussain T and Khan A, 2022. Clinico-hematological, mutagenic, and oxidative stress induced by pendimethalin in freshwater fish bighead carp (*Hypophthalmichthys nobilis*). Oxidative Medicine and Cellular Longevity 2022: 2093822. https://doi.org/10.1155/2022/2093822

Yassin MH, Soliman MM, Mostafa SA and Ali HA, 2015. Antimicrobial effects of camel milk against some bacterial pathogens. Journal of Food and Nutrition Research 3(3):162-168. https://doi.org/10.12691/jfnr-3-3-6

Yokoyama S, Ishikawa M and Koshio S, 2019. Dietary bovine lactoferrin enhances defense factors on body surface and anti-parasitic effects against Neobenedenia girellae infection, and mitigates low-salinity stress in amberjack (Seriola dumerili) juveniles. Aquaculture 504: 52-58. https://doi.org/10.1016/j.aquaculture.2019.01.053

Zou Z, Duley JA, Cowley DM, Reed S, Arachchige BJ, Shaw PN and Bansal N, 2022. Comprehensive biochemical and proteomic characterization of seasonal Australian camel milk. Food Chemistry 381: 132297. https://doi.org/10.1016/j.foodchem.2022.132297

Zou Z, Bauland J, Hewavitharana AK, Al-Shehri SS, Duley JA, Cowley DM, Koorts P, Shaw PN and Bansal N, 2021. A sensitive, high-throughput fluorescent method for the determination of lactoperoxidase activities in milk and comparison in human, bovine, goat and camel milk. Food Chemistry 339: 128090. https://doi.org/10.1016/j.foodchem.2020.128090