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Milk and Immune Biomarkers of Subclinical Mastitis in Dromedary Camels in Al-Qassim Region, Saudi Arabia

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ABSTRACT
This study investigated the complex interactions between bacteriological, physicochemical and immuno-inflammatory indicators of subclinical mastitis (SCM) in dromedary camels under different management systems, including movement-restricted, free-ranging housing, regular, irregular feeding and single-milking frequency. Correlation matrices were created to assess how somatic cell count (SCC), milk contents, cytokines (TNF-α, IL-6, IL-10) and immunological proteins (IgG, LTF, LPO) respond to environmental and managerial variability. In most cases, there was little correlation between SCC and bacteriological characteristics, suggesting that SCM in camels is not mainly caused by infection. Rather, stronger associations were found between SCC and important physicochemical parameters, such as lactose, TS, SNF, citric acid and urea suggesting that a major component of the inflammatory response is metabolic imbalance. Immune patterns differed significantly between parameters: free-ranging and irregular-feeding camels displayed strong cytokine-driven inflammation with strong SCC, IL-6 and SCC, IL-10 correlations, while movement-restricted and regular-feeding camels showed localized enzymatic activation, especially through LPO. Single-milking frequency increased both metabolic disturbance and cytokine activation. TNF-α showed system-dependent correlations, indicating changes in both local and systemic immune responses. In conclusion, the results demonstrated that SCM expression in camels is significantly influenced by managerial and environmental factors, suggesting the need for system-specific diagnostic and preventive strategies
Keywords: Camel mastitis, cytokine profiling, Metabolic stress, milk biomarkers and milk physicochemical parameters

1. INTRODUCTION:

In arid and semi-arid areas, dromedary camels (Camelus dromedarius) are essential to livestock production as they provide meat, transportation, and most importantly a vital source of milk that maintains food security in the face of challenging environmental and socioeconomic conditions. Camel milk is valued for its distinct nutritional and sanitary qualities and as people look for alternatives to traditional dairy in difficult conditions, its consumption and market demand have grown (Alhafiz *et al.,* 2022). However, camel dairy systems are seriously threatened by mastitis, especially the subclinical form (SCM), which can seriously reduce milk quality, yield and safety even if it does not exhibit obvious clinical symptoms. According to recent evaluations, prevalence rates of SCM in camels vary greatly (from less than 10% to almost 90%), depending on herd, geography and management. This emphasizes how SCM is a prevalent but often misdiagnosed problem in camel herds.

(Al-Dughaym & Fadlelmula, 2015; Djeddi *et al.,* 2024).

In many dairy species, the somatic cell count (SCC), which counts the number of somatic (mostly immunological) cells per milliliter of milk, is used as an objective predictor of intramammary inflammation or infection. SCC has shown good diagnostic performance in dromedary camels. Research conducted in Saudi Arabia using SCC in combination with physical screening tests like the California Mastitis Test (CMT) showed a distinct difference between udders that were healthy and those that were sub clinically infected, even in the absence of external signs of mastitis (Al-Dughaym & Fadlelmula, 2015). Because the disease is frequently silent, SCC is a useful, accessible and early-detection diagnostic for SCM in camel herds.

Despite somatic cell count (SCC) is a useful tool of intramammary inflammation, it may not give a complete clear picture of subclinical mastitis (SCM) in camels. In fact, compared to healthy sheep, SCM affected camels have been shown to exhibit biochemical changes in milk, such as decreased fat and protein output (Hadef *et al.,* 2019). Physico-mineral milk parameters may be helpful supplementary indicators of udder health, according to reports of changes in the milk mineral profile, such as increased sodium (Na) and chloride (Cl) in cases with high SCC (Hadef *et al.,* 2022). In order to improve diagnostic sensitivity and offer a more comprehensive evaluation of mammary gland integrity and milk quality, these results support an integrated biomarker approach that integrates SCC with milk compositional and mineral analysis.

The risk and appearance of SCM in camels are greatly influenced by management strategies and the herd environment. A recent study found that a higher prevalence of SCM was linked to poor udder hygiene, tick infestation, larger herd size, calf mortality, and use of antisuckling devices, but not necessarily to milking

frequency or teat anomalies (Hadef *et al.,* 2022). These findings suggest that herd-level and management-system variables modulate not only the incidence of SCM, but also how the disease appears in terms of milk mineral alterations. Therefore, any study aiming to define reliable biomarkers or diagnostic tools for SCM must account for variability in management systems to ensure applicability across diverse production contexts

In camels, subclinical mastitis causes significant alterations in the immune cell composition of the secretions from the mammary glands. According to a flow-cytometry study, compared to milk from healthy camels, milk from SCM-affected camels showed significantly higher SCC, elevated total leukocyte counts, a marked increase in myeloid cells ($CD172a^{+}$), granulocyte expansion, a decrease in the proportions of macrophage and lymphoid cells, improved cell viability and increased phagocytic activity (Alhafiz *et al.,* 2022). These immunological changes most likely correspond with changes in milk composition and metabolism, suggesting that SCM requires both immune activation and metabolic disruption. Therefore, a more precise and mechanistic understanding of the disease can be obtained by analyzing SCM through an immuno-metabolic analysis, which integrates cellular immunological characteristics with milk biochemistry. It may also discover additional indicators beyond SCC or composition alone.

while camel mastitis is becoming more and more popular, the majority of research is still separated, focusing on bacterial pathogens, SCC counts, milk composition, or risk factors independently. These aspects are rarely combined with immunological or mineral analysis in a connected structure. For instance, comprehensive studies that incorporate bacteriological, immunological, compositional, mineral, and management-system data are still rare, despite recent reports on mineral changes (e.g., Na, Cl) in milk from subclinically mastitis camels (Hadef *et al.,* 2022) and others on immune cell changes (Alhafiz *et al.,* 2022). This gap hinders the creation of trustworthy and sensitive diagnostic criteria or biomarkers for SCM across different camel production systems. Therefore, integrated study approaches that reflect the complex nature of SCM from microbial presence to immune response, metabolism and milk quality under various management conditions are obviously needed.

As a result, the current study aims to provide an integrated characterization of subclinical mastitis (SCM) in dromedary camels by assessing somatic cell count (SCC), immunological parameters milk physicochemical parameters and bacteriological status across various management systems (housing, feeding, and milking frequency) and reliable and sensitive biomarkers for early identification and treatment of SCM in camel dairy production systems.

2. MATERIALS AND METHODS

Study Area and Animals

All experimental techniques used in this work have been approved by the Qassim University Animal Ethics Committee in Saudi Arabia (23-32-04). This study used 133 breastfeeding camels from different locations in the Qassim region of the Kingdom of Saudi Arabia (KSA), ranging in age from 4 to 10 years. This area has a dry environment, with summertime highs typically ranging from 40 to 45 °C. Rainfall occurs from November to February. The meadows in the area are deemed arid for the remainder of the year. The research was carried out from November 2021 to August 2022. The animals were housed in grazing and supplement farming systems after being selected at random. After grazing in the open spaces around the property from morning until midday, the camels were kept inside for milking and given additional grain. The used feed consisted of concentrates, which contained the same ration of barley and cottonseed meal and dry hay, which varied from 3 to 4 kg per day, depending on the farm. Regular watering was given to the animals. Winter is when most calvings take place. Three different subspecies of she-camels were determined based on their lack of systemic diseases or malformations (Abdallah and Faye 2012): Majaheem (black) n = 43, Shaele (yellow) n = 43 and Wadaha (white) n = 47. The farms used the same management and feeding practices. When it comes to shelter, food sources and feeding, animals are comparable.

Sample Collection

Prior to sampling, animal owners and herders were made aware of the study's goals, methods and their verbal consent to take part was acquired. The study's anonymous participation policy and participants' right to withdraw at any time were explained to them. After cleaning the teats with water, disinfecting them with alcohol (70 %) and removing the initial streams, the samples were collected early in the morning and placed into sterile tubes. These were then promptly labeled. The test tube's orientation was changed to around 45 degrees. Additionally, the samples were kept in ice bags inside a special box and transferred to the lab in two to four hours. After carefully mixing each milking, 500 milliliters were taken out for analysis. Following transit, the materials were separated and deposited in a tiny 2 mL tube. After that, the tube was centrifuged for ten minutes at 10,000 rpm in order to extract the milk and fat. Skim milk samples were defatted and stored in the refrigerator until further analysis could be performed. Venipuncture tubes (10 mL) were used for collecting camel blood and the samples were stored at room temperature. The serum was extracted from the blood samples by centrifugation at 3000 rpm for 15 minutes. The samples were then separated and kept at 20 °C to assess the immunological and cytokine parameters.

Somatic Cell Count

The somatic cells were counted using an automatic cell counter and a direct microscopic approach in no more than three hours. Ten minutes at 10,000 rpm were spent centrifuging 1.0 mL of raw milk. The pellet was reconstituted in 1.0 mL of phosphate-buffered saline (PBS) after the creamy component and supernatant were removed. Turk's solution (125 microliters of methylene blue in distilled water and 1% to 2% acetic acid) was used to dilute the cellular suspension with 10 microliters.

Somatic cells were counted using a hemocytometer that measured 10 mL of the diluted pattern.

Bacteriological Examination

The milk samples were immediately cooled at 4°C as they arrived the lab until the analysis process began. After serially diluting the milk samples with sterile peptone water, 1.0 mL aliquots were applied to each Petri dish for subsequent usage. 15 to 20 milliliters of agar were added to each Petri dish. The resulting plates were completely mixed, let to set and then incubated at 32˚C for a full day. Enterobacteriaceae were counted using Violet Red Bile Glucose Agar (VRBG, Neogen) in compliance with ISO 21528-2, while Total Plate Count (TPC) was counted using Plate Count Agar (PCA, Oxoid) in compliance with ISO 4833-1. Total coliform (TCC) is counted using violet, red bile lactose agar (VRBL, Neogen) in conformity with ISO 4832. Tryptone bile x-glucuronide agar (TBX, Neogen) was used to count E. Coli in accordance with ISO 16649-2.

A colony counter was used to count the plates and the result was given as cfu/mL. A biological safety cabinet was utilized to contain everything and the bacteria were cultivated for 48 hours ±2.0 at 37°C for Enterobacteriaceae and TCC and 44°C for E. coli.

Immunological and Cytokine Determination

The serum concentrations of Cam-TNF-α and Cam-IL-6 were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (Sunlog Biotech, Hangzhou, Zhejiang, China; Cat. No. SL0030 cm for Cam TNF-α and SL0032 cm for Cam-IL-6, respectively) following the manufacturer's instructions. The test's sensitivity ranged between 0.5 and 0.1 pg/mL, with an intra-assay variability CV of less than 10% and an inter-assay variability CV of less than 12%. Cam TNF-α and Cam IL-6 detection limits were 3-200 pg/mL and 1-70 pg/mL, respectively. The concentration of Cam-IL-10 was measured using a commercial ELISA kit (Wuhan Fine Biotech Co., Ltd, Optics Valley Biomedical Industrial Park, Wuhan, China; Cat. No. ECM0010), as directed by the manufacturer. The intra-assay and inter-assay variances were both less than 8%. The detection range was 15.625 - 1000 pg/mL, while the sensitivity was 9.375 pg/mL. The concentrations of IgG, LTF and LPO were measured using a commercial ELISA kit (Sunlog Biotech, Hangzhou, Zhejiang, China; kits, Cat. Nos. SL0050 cm, SL0051 cm, and SL0039 cm, respectively) according to the manufacturer's instructions. The assay's sensitivity and accuracy (intra-assay variation) were set at 0.06 µg/mL for IgG, 0.05 ng/mL for LTF, and 6 pg/mL for LPO. The intra-assay variation was less than 12 % and the assay's CV was set to less than 10%. The detection ranges for IgG, LTF and LPO were 0.3-20 µg/mL, 0.3-20 ng/mL, and 30-2000 pg/mL.

Physicochemical Analysis

Milk samples were transported directly from the farm or desert to the laboratory, where they were physically and chemically examined. FT3 MilkoScan™ uses a gyroscope to detect specific gravity. Total protein is determined using a formaldehyde titration. In addition, total protein was calculated using a factor of 1.74. The total solids in milk were determined using the oven drying method and the percentage of total solids was calculated as follows:

$$\text{Total solids (\%)} = \frac{\text{Weight of dried sample}}{\text{Weight of milk sample}} \times 100$$

The Roese-Gottlieb method provided total fat percentage as follows:

Total fat (%) = Weight of the vial containing fat - Weight of the vial after washing off the fat sample weight x 100

$$\text{SNF content (\%)} = \text{TS (\%)} - \text{Fat (\%)}$$

Furthermore, casein, lactose, urea, citric acid, FPD, FFA, density and acidity have all been evaluated using the MilkScan™ FT3. MilkoScan™ FT3 is a sophisticated new dairy analysis method that can test a variety of liquid and semi-solid dairy products. Outstanding uptime, minimal ownership costs and unparalleled outcomes. In just a few seconds, it can analyze samples. It also tests items with different viscosities using a special intelligent flow line.

The calculated values used were fat (g/d) protein (g/d), lactose (g/d), and modified milk for energy. The latter was calculated as ECM (kg/d) = 12.55 x fat (kg/d) + 7.39 x protein (kg/d) + 0.2595 x weight of milk (kg/d), taking into consideration that milk has an energy value of 0.74 liters per kilogram of milk.

Statistical Analysis

The values were represented as mean±SE. To find any significant differences, a one-way ANOVA will be performed on the various parameters and breeds. The Mann-Whitney test and post hoc analysis were used to compare the groups. The studies were performed using GraphPad 7. $P<0.05$, $P<0.01$ and $P<0.001$ were used as the significant thresholds.

3. RESULTS

The current study is the fourth in a series of ongoing investigations (Almulhim *et al.,* 2024; Zaki and Albarrak, 2025; Almulhim and Alsuraykh, 2025) about the risk factors for dromedary camel subclinical mastitis (SCM). The current study offers a thorough assessment of how various farming and management practices affect the complex connections between immunological, physicochemical and bacteriological markers linked to subclinical mastitis (SCM) in dromedary camels. By studying correlation patterns across numerous housing and food systems as well as milking frequency, the study illustrates how environmental and management factors impact both mammary gland physiology and the immunological response. The results showed significant variation in the relationships between milk composition, microbial load, immune proteins and SCC and important inflammatory markers, including TNF-α, IL-6, and IL-10. These diverse correlation structures reflect the multifaceted character of SCM in camels and emphasize that risk expression and biomarker performance are

system dependent. All of these insights help to improve early diagnostic indicators in a variety of production situations and offer a more comprehensive understanding of SCM processes.

The correlation matrix under the movement-restricted housing system revealed that SCC showed no significant relationships with major bacteriological indicators (TPC, ENTB, coliforms and E. coli), referring to a modest positive connection with coliform count. In terms of physicochemical characteristics, SCC showed positive relationships with citric acid and urea, whereas total solids (TS) and solids-not-fat (SNF) showed negative associations. Immunologically, SCC demonstrated a substantial positive connection with milk LPO and a negative correlation with milk IgG. No notable relationships were identified between SCC and pro- or anti-inflammatory cytokines (TNF-α, IL-6, IL-10) Table (1).

Table (1): Correlation matrices of SCC to bacteriological, physicochemical and immunological parameters of Movement-restricted housing system

	SCC	TPC	*ENTB*	*Coliform*	E. coli				
SCC	1	0.072	0.134	0.423	-0.190				
TPC		1	0.010	0.315	-0.069				
ENTB			1	0.179	-0.034				
Coliform				1	-0.125				
E. coli					1				
	SCC	Fat	Protein	Casein	Lactose	TS	SNF	Urea	Citric Acid
SCC	1	-0.107	0.289	-0.070	0.024	-0.241	0.109	0.427	.527*
Fat		1	-0.105	.757**	-0.363	.529*	-0.185	-.700**	-0.420
Protein			1	0.045	.646**	-0.262	0.170	.664**	.561*
Casein				1	-0.281	0.324	-0.246	-.520*	-0.231
Lactose					1	-0.036	.568*	.563*	.621**
TS						1	.589*	-.547*	-0.024
SNF							1	0.183	.678**
Urea								1	.687**
Citric Acid									1
	SCC	FPD	FFA	Density	Acidity				
SCC	1	0.311	0.083	0.382	.491*				
FPD		1	-.484*	-0.221	0.213				
FFA			1	.730**	0.349				
Density				1	0.356				
Acidity					1				
	SCC	IgG Serum	LTF Serum	LPO Serum	IgG milk	LTF milk	LPO milk		
SCC	1	-0.334	-0.159	.618*	-0.508	0.425	0.486		
IgG Serum		1	-.549*	-0.329	0.321	-0.271	-0.180		

LTF Serum			1	0.027	0.152	0.040	-0.044		
LPO Serum				1	-.526*	.675**	0.156		
IgG milk					1	-0.389	0.148		
LTF milk						1	-0.025		
LPO milk							1		
	SCC	TNF α	IL-6	IL-10					
SCC	1	-0.198	-0.296	0.047					
TNF α		1	-0.445	-0.276					
IL-6			1	0.513					
IL-10				1					

*, **, *** are significantly different at P≤0.05, P≤0.001, and P≤0.001, respectively

Table (2) showed that TNF-α exhibited inconsistent correlations with immunological markers. A moderate negative connection was identified with serum IgG, and a substantial negative correlation with serum LPO. TNF-α, on the other hand, showed a relatively positive connection with milk IgG. There was a substantial positive connection between milk LPO and TNF-α. Correlations with IL-6 and IL-10 were weak and negative, without any statistical significance

Table (2): Correlation matrix of TNF-α to IL-6, IL-10, lgG, LTF and LPO of Movement-restricted housing system

	TNF α	**IgG Serum**	**LTF Serum**	**LPO Serum**	**IgG milk**	**LTF milk**	**LPO milk**	**IL-6**	**IL-10**
TNF α	1	-0.261	0.238	-0.449	0.468	-0.203	-0.075	-0.445	-0.276
IgG Serum		1	-.549*	-0.329	0.321	-0.271	-0.180	0.246	0.006
LTF Serum			1	0.027	0.152	0.040	-0.044	-0.068	-0.130
LPO Serum				1	-.526*	.675**	0.156	0.059	-0.068
IgG milk					1	-0.389	0.148	0.189	0.112
LTF milk						1	-0.025	-0.099	-0.124
LPO milk							1	0.221	.547*
IL-6								1	0.513
IL-10									1

While TPC had a favorable association with ENTB and coliform count in the free-ranging housing system Table (3), SCC did not exhibit any significant relationships with bacteriological features. For physicochemical parameters, SCC showed negative relationships with lactose, TS and SNF, while positively correlated with urea. Strong positive correlations were identified between SCC and both IL-6 and IL-10, although associations with IgG, LTF and LPO (in milk and serum) were non-significant.

Table (3): Correlation matrices of SCC to bacteriological, physicochemical and immunological parameters of Free-ranging time housing system

	SCC	TPC	*ENTB*	*Coliform*	E. coli				
SCC	1	-0.034	-0.219	0.169	-0.067				
TPC		1	.546**	.516*	-0.152				

ENTB			1	-0.089	-0.139				
Coliform				1	-0.078				
E. coli					1				
	SCC	Fat	Protein	Casein	Lactose	TS	SNF	Urea	Citric Acid
SCC	1	-0.243	0.195	0.159	-0.386	-0.350	-0.391	0.196	-0.092
Fat		1	-0.329	0.402	-0.040	0.122	-0.251	-.667**	-0.410
Protein			1	0.092	-0.165	-0.052	-0.044	.720**	.526**
Casein				1	-0.163	0.311	-0.134	-0.180	0.052
Lactose					1	0.229	.775**	-0.036	0.168
TS						1	.608**	-0.043	0.388
SNF							1	0.167	.449*
Urea								1	.569**
Citric Acid									1
	SCC	FPD	FFA	Density	Acidity				
SCC	1	0.185	-0.199	0.042	0.235				
FPD		1	0.073	0.066	0.236				
FFA			1	.650**	0.410				
Density				1	.634**				
Acidity					1				
	SCC	IgG Serum	LTF Serum	LPO Serum	IgG milk	LTF milk	LPO milk		
SCC	1	-0.020	-0.141	-0.179	0.199	-0.194	-0.210		
IgG Serum		1	-0.321	-0.223	0.339	0.063	0.114		
LTF Serum			1	.974**	-0.525	.762*	.784*		
LPO Serum				1	-0.648	.791*	.844**		
IgG milk					1	-.637*	-.638*		
LTF milk						1	.975**		
LPO milk							1		
	SCC	TNF α	IL-6	IL-10					
SCC	1	0.100	.876**	.573*					
TNF α		1	0.141	-0.185					
IL-6			1	0.354					
IL-10				1					

TNF-α revealed a substantial positive connection with milk IgG, while demonstrating a negative but non-significant link with serum LPO Table (4). Strong relationships were discovered among immunological indicators, including LTF serum with LPO serum and LTF milk with LPO milk. TNF-α had a slight negative correlation with IL-10 but an almost favorable correlation with IL-6.

Table (4): Correlation matrix of TNF-α to IL-6, IL-10, lgG, LTF and LPO of Free-ranging time housing system

	TNF α	**IgG Serum**	**LTF Serum**	**LPO Serum**	**IgG milk**	**LTF milk**	**LPO milk**	**IL-6**	**IL-10**
TNF α	1	0.777	-0.071	-0.111	0.641	0.098	0.110	0.141	-0.185
IgG Serum		1	-0.321	-0.223	0.339	0.063	0.114	-0.007	0.029
LTF Serum			1	.974**	-0.525	.762*	.784*	0.122	.884*
LPO Serum				1	-0.648	.791*	.844**	0.102	.868*
IgG milk					1	-.637*	-.638*	-0.248	-0.286
LTF milk						1	.975**	0.067	0.585
LPO milk							1	-0.057	0.676
IL-6								1	0.354
IL-10									1

In the usual feeding system, SCC exhibited a pattern comparable to that of the movement-restricted system, displaying no significant relationships with bacteriological markers. Positive relationships were seen between SCC and both urea and citric acid, while negative associations were detected with TS and SNF. Additionally, SCC had a negative correlation with milk IgG and a positive correlation with milk LPO. No significant relationships were identified between SCC and cytokines (TNF-α, IL-6, IL-10) Table (5).

Table (5): Correlation matrices of SCC to bacteriological, physicochemical and immunological parameters of Regular feeding system

	SCC	TPC	*ENTB*	*Coliform*	E. coli				
SCC	1	0.072	0.134	0.423	-0.190				
TPC		1	0.010	0.315	-0.069				
ENTB			1	0.179	-0.034				
Coliform				1	-0.125				
E. coli					1				
	SCC	Fat	Protein	Casein	Lactose	TS	SNF	Urea	Citric Acid
SCC	1	-0.107	0.289	-0.070	0.024	-0.241	0.109	0.427	.527*
Fat		1	-0.105	.757**	-0.363	.529*	-0.185	-.700**	-0.420
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Citric Acid									1
	SCC	FPD	FFA	Density	Acidity				
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FFA			1	.730**	0.349				
Density				1	0.356				
Acidity					1				
	SCC	IgG Serum	LTF Serum	LPO Serum	IgG milk	LTF milk	LPO milk		
SCC	1	-0.334	-0.159	.618*	-0.508	0.425	0.486		
IgG Serum		1	-.549*	-0.329	0.321	-0.271	-0.180		
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	SCC	TNF α	IL-6	IL-10					
SCC	1	-0.198	-0.296	0.047					
TNF α		1	-0.445	-0.276					
IL-6			1	0.513					
IL-10				1					

With the exception of a negative association with serum LPO and a slight positive correlation with milk IgG, Table (6) demonstrated that TNF-α was not significantly correlated with the majority of immunological variables. Although they were not directly related to TNF-α, significant immune-immune correlations were observed (e.g., between LTF milk and LPO milk). No significant relationships were identified between TNF-α and IL-6 or IL-10

Table (6): Correlation matrix of TNF-α to IL-6, IL-10, lgG, LTF and LPO of Regular feeding system

	TNF α	**IgG Serum**	**LTF Serum**	**LPO Serum**	**IgG milk**	**LTF milk**	**LPO milk**	**IL-6**	**IL-10**
TNF α	1	-0.261	0.238	-0.449	0.468	-0.203	-0.075	-0.445	-0.276
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LTF Serum			1	0.027	0.152	0.040	-0.044	-0.068	-0.130
LPO Serum				1	-.526*	.675**	0.156	0.059	-0.068
IgG milk					1	-0.389	0.148	0.189	0.112
LTF milk						1	-0.025	-0.099	-0.124
LPO milk							1	0.221	.547*
IL-6								1	0.513

IL-10									1

SCC did not significantly correlate with bacteriological parameters in the irregular feeding method Table (7). Similar to the free-ranging system, SCC showed positive correlations with urea but negative correlations with lactose, TS and SNF in terms of physiochemistry. SCC showed strong positive relationships with IL-6 and IL-10, but there were no significant connections with IgG, LTF, or LPO (in milk or serum). Strong relationships among immunological indicators (e.g., LTF serum and LPO serum) were also identified.

Table (7): Correlation matrices of SCC to bacteriological, physicochemical and immunological parameters of Irregular feeding system

	SCC	TPC	*ENTB*	*Coliform*	E. coli				
SCC	1	-0.034	-0.219	0.169	-0.067				
TPC		1	.546**	.516*	-0.152				
ENTB			1	-0.089	-0.139				
Coliform				1	-0.078				
E. coli					1				
	SCC	Fat	Protein	Casein	Lactose	TS	SNF	Urea	Citric Acid
SCC	1	-0.243	0.195	0.159	-0.386	-0.350	-0.391	0.196	-0.092
Fat		1	-0.329	0.402	-0.040	0.122	-0.251	-.667**	-0.410
Protein			1	0.092	-0.165	-0.052	-0.044	.720**	.526**
Casein				1	-0.163	0.311	-0.134	-0.180	0.052
Lactose					1	0.229	.775**	-0.036	0.168
TS						1	.608**	-0.043	0.388
SNF							1	0.167	.449*
Urea								1	.569**
Citric Acid									1
	SCC	FPD	FFA	Density	Acidity				
SCC	1	0.185	-0.199	0.042	0.235				
FPD		1	0.073	0.066	0.236				
FFA			1	.650**	0.410				
Density				1	.634**				
Acidity					1				
	SCC	IgG Serum	LTF Serum	LPO Serum	IgG milk	LTF milk	LPO milk		
SCC	1	-0.020	-0.141	-0.179	0.199	-0.194	-0.210		
IgG Serum		1	-0.321	-0.223	0.339	0.063	0.114		
LTF Serum			1	.974**	-0.525	.762*	.784*		
LPO Serum				1	-0.648	.791*	.844**		

IgG milk					1	-.637*	-.638*		
LTF milk						1	.975**		
LPO milk							1		
	SCC	TNF α	IL-6	IL-10					
SCC	1	0.100	.876**	.573*					
TNF α		1	0.141	-0.185					
IL-6			1	0.354					
IL-10				1					

The findings revealed a negative connection with serum LPO and a robust positive relation between TNF-α and milk IgG. Strong immune-immune connections were identified, including LTF serum with LPO serum and LTF milk with LPO milk. TNF-α did not exhibit meaningful associations with IL-6 or IL-10 Table (8).

Table (8): Correlation matrix of TNF-α to IL-6, IL-10, lgG, LTF and LPO of Irregular feeding system:

	TNF α	**IgG Serum**	**LTF Serum**	**LPO Serum**	**IgG milk**	**LTF milk**	**LPO milk**	**IL-6**	**IL-10**
TNF α	1	0.777	-0.071	-0.111	0.641	0.098	0.110	0.141	-0.185
IgG Serum		1	-0.321	-0.223	0.339	0.063	0.114	-0.007	0.029
LTF Serum			1	.974**	-0.525	.762*	.784*	0.122	.884*
LPO Serum				1	-0.648	.791*	.844**	0.102	.868*
IgG milk					1	-.637*	-.638*	-0.248	-0.286
LTF milk						1	.975**	0.067	0.585
LPO milk							1	-0.057	0.676
IL-6								1	0.354
IL-10									1

SCC showed weak, non-significant associations with bacteriological markers in the one-milking-time Table (9). SCC revealed considerable negative associations with lactose, TS and SNF, while correlated with urea. Immunologically, SCC associated positively with serum LPO and adversely with milk IgG. A very strong positive association was discovered between SCC and IL-6, along with a moderate positive correlation with IL-10

Table (9): Correlation matrices of SCC to bacteriological, physicochemical and immunological parameters of One milking time

	SCC	TPC	*ENTB*	*Coliform*	E. coli				
SCC	1	-0.106	-0.191	0.167	-0.133				
TPC		1	0.293	0.155	-0.008				
ENTB			1	0.159	0.017				
Coliform				1	-0.074				
E. coli					1				
	SCC	Fat	Protein	Casein	Lactose	TS	SNF	Urea	Citric Acid

SCC	1	-0.196	0.165	0.063	-0.315	-.448**	-.442**	0.187	-0.145
Fat		1	-0.263	.604**	-0.131	0.250	-0.209	-.702**	-.387*
Protein			1	0.058	0.011	-0.083	0.058	.723**	.539**
Casein				1	-0.198	.408*	-0.065	-0.328	-0.014
Lactose					1	0.101	.668**	0.093	0.175
TS						1	.634**	-0.150	.373*
SNF							1	0.213	.553**
Urea								1	.615**
Citric Acid									1
	SCC	FPD	FFA	Density	Acidity				
SCC	1	0.182	-0.252	-0.095	0.176				
FPD		1	0.065	0.127	0.194				
FFA			1	.679**	.508**				
Density				1	.606**				
Acidity					1				
	SCC	IgG Serum	LTF Serum	LPO Serum	IgG milk	LTF milk	LPO milk		
SCC	1	0.173	-0.279	-0.209	0.134	-0.124	-0.217		
IgG Serum		1	-.456*	-0.435	0.450	-0.181	-0.030		
LTF Serum			1	.595**	-0.334	0.419	.566*		
LPO Serum				1	-.670**	.723**	.575*		
IgG milk					1	-.513*	-0.424		
LTF milk						1	.510*		
LPO milk							1		
	SCC	TNF α	IL-6	IL-10					
SCC	1	0.004	.795**	0.382					
TNF α		1	-0.021	-0.234					
IL-6			1	0.247					
IL-10				1					

Table (10) revealed that TNF-α showed a moderate positive association with milk IgG and a negative correlation with serum LPO. Strong connections were identified between LTF milk and LPO milk. TNF-α revealed no significant associations with IL-6 or IL-10.

Table (10): Correlation matrix of TNF-α to IL-6, IL-10, lgG, LTF and LPO of One milking time

	TNF α	IgG Serum	LTF Serum	LPO Serum	IgG milk	LTF milk	LPO milk	IL-6	IL-10
TNF α	1	-0.080	0.333	-0.208	0.430	-0.059	-0.130	-0.021	-0.234
IgG Serum		1	-.456*	-0.435	0.450	-0.181	-0.030	0.342	0.138
LTF Serum			1	.595**	-0.334	0.419	.566*	-0.190	0.277
LPO Serum				1	-.670**	.723**	.575*	-0.223	0.042
IgG milk					1	-.513*	-0.424	-0.002	-0.060
LTF milk						1	.510*	-0.233	0.055
LPO milk							1	-0.124	.549*
IL-6								1	0.247
IL-10									1

4. DISCUSSION

The current study investigated how the complex associations between somatic cell count (SCC), bacteriological indicators, physicochemical milk composition and immune-inflammatory markers in dromedary camels are shaped by various management systems, including movement-restricted housing, free-ranging, regular and irregular feeding regimes and milking frequency. These trends suggest that the pathophysiology of subclinical mastitis (SCM) in camels is multifaceted, with SCC being regulated not only by microbial presence but also by metabolic balance, immunological activation and management through physiological stress. Previous findings that camel SCM is extremely sensitive to managerial and environmental conditions are supported by this system-dependent variability (El-Deeb *et al.*, 2022; Kany *et al.,* 2019).

SCC showed relatively minor correlations with bacteriological characteristics under movement-restricted settings, indicating that microbial load was not the main cause of inflammation in this system. The slight positive link between SCC and coliforms may imply contamination, but the absence of substantial relationships with E. coli, TPC or ENTB indicates a greater involvement for non-infectious inflammation (Schukken *et al.,* 2011).

Stronger trends appeared with physicochemical and immune-related factors. Inflammatory disturbances to mammary metabolism are consistent with the positive relationships between SCC, urea and citric acid, which point to metabolic imbalance and increased epithelial permeability (Alhussien & Dang, 2018; Terner, 1955). The activation of innate antimicrobial mechanisms, which are known to quickly upregulate in camels during early inflammatory responses, is reflected in the high connection with LPO in milk (Silanikove *et al.,* 2006; Almulhim and Alsuraykh, 2025). In the meantime, local immunoglobulin consumption or redistribution throughout mammary tissues during inflammation is implied by the negative connection between SCC and milk IgG (El-Deeb *et al.,* 2022). The lack of connection with cytokines (TNF-α, IL-6, IL-10) shows that inflammation under restricted movement is localized rather than systemic

The negative correlation of TNF-α with serum IgG and LPO suggests a potential systemic immune suppression or redistribution of immune proteins during acute inflammatory signaling (El-Deeb *et al.,* 2022). On the other hand, the release of antimicrobial enzymes into milk is induced by TNF-α, and a coordinated local immune response is shown by the positive correlation between TNF-α and milk LPO. Weak negative associations with IL-6 and IL-10 imply that the cytokine response may be imbalanced, with TNF-α operating independently rather than as part of a conventional inflammatory cascade (Schukken *et al.,* 2011)

Free-ranging animals displayed a significantly distinct inflammatory profile. Strong positive relationships between SCC and IL-6 and IL-10 showed that cytokine-mediated inflammation is more prevalent in open settings, probably as a result of increased pathogen exposure and environmental difficulties (Kany *et al.,* 2019). The negative associations between SCC and lactose, TS and SNF indicate classic mastitis-associated decrease of milk synthesis, as lactose is particularly vulnerable to secretory cell injury (Harmon, 1994). The absence of notable associations with immunological proteins (IgG, LTF, LPO) shows that the inflammatory response is cytokine-dominant rather than enzymatic in this system

The substantial positive connection between TNF-α and milk IgG reflects a powerful local immunological activation, where high TNF-α levels induce IgG leakage or active transport into milk (Kaskous, 2016). Strong relationships among LTF serum, LPO serum and their milk counterparts validate the co-regulation of antimicrobial proteins, previously described in camel milk

during SCM (Motrich *et al.,* 2003). Since TNF-α and IL-6 often work together in mastitis signaling cascades, the moderate link with IL-6 is expected (Sordillo, 2016).

In the usual feeding regime, SCC did not correlate with bacterial markers, thus supporting the hypothesis that camel SCM is not always infection-produced. Rather, as reported in the literature on camel mastitis, associations with TS, SNF, citric acid, and urea suggest metabolic changes linked to persistent low-grade inflammation (Mostafaa *et al.,* 2019, Terneret, 1955). Positive connection with milk LPO reflects local activation of oxidative defense systems. The lack of association between SCC and cytokines shows that inflammation is chronic and moderate, rather than acute or systemic (Leitner *et al.,* 2004)

TNF-α correlations were mostly weak in this system, except for the negative correlation with serum LPO, which is likely a sign of oxidative enzyme translocation into the mammary gland rather than circulation. The small positive relationship with milk IgG suggests minor localized immunological activation. A suppressed inflammatory pathway is implied by weak or absent associations with IL-6 and IL-10, which is consistent with well-managed feeding settings.

Irregular feeding revealed the greatest inflammatory signature. SCC exhibited very substantial relationships with IL-6 and IL-10, demonstrating that dietary instability generates metabolic stress, enhancing both pro-inflammatory and anti-inflammatory pathways (Sordillo, 2016). Negative associations with lactose, TS, and SNF confirm substantial interruption of milk synthesis. Innate immunity is significantly activated in response to both local and systemic stress, according to strong correlations between immunological proteins (LTF, LPO) in milk and blood (Silanikove *et al.,* 2006).

The idea that irregular feeding encourages local immune activation is strengthened by the significant positive connection between TNF-α and milk IgG. Negative correlations with serum LPO reflect either systemic oxidative stress or a transfer of enzymatic defense resources to the mammary gland. Strong relationships between LTF and LPO isoforms reveal strong coregulation of innate immune proteins during nutritionally driven inflammation (Chamekh *et al.,* 2020)

Milking once daily dramatically affected inflammatory and compositional trends. Strong negative relationships between SCC and lactose, TS and SNF indicate that prolonged milk stasis inhibits epithelial cell function, consistent with mammary pressure–induced inflammation (Lakic *et al.,* 2011). The significant positive link between SCC and IL-6 reflects acute inflammatory activation, while the moderate positive IL-10 association may represent a regulatory attempt to reduce excessive inflammation (Sordillo, 2016)

The increased local recruitment of immunoglobulins when milk accumulates for long periods of time is reflected in the positive correlation between TNF-α and milk IgG. Negative associations with serum LPO again reflect movement of oxidative enzymes toward the mammary gland. Strong relationships between LTF and LPO in milk show coordinated innate immune protection, similar with earlier investigations on camel udder immunity (Kaskous, 2016; Motrich *et al.,* 2003)

5. CONCLUSION

This study reveals that subclinical mastitis in dromedary camels is affected more by management-related metabolic and immunological alterations than by microbial burden alone. While movement-restricted and regular-feeding systems displayed primarily localized, enzyme produced reactions, SCC was highly associated with cytokine activation and compositional changes in free-ranging, irregular feeding and single-milking systems. TNF-α, IL-6, IL-10, LTF and LPO responded differently across systems, demonstrating adaptive immune regulation. These findings illustrate the multifaceted nature of SCM and the significance of management-specific monitoring measures to promote early identification, udder health and productivity in camel herds

Conflict of interest

The authors state that there is no conflict of interest..

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