Evaluation of chemical elements as potential biomarkers in the treatment of goat mastitis

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Abstract

Mastitis represents a significant inflammatory condition in dairy goat herds, causing substantial economic losses through reduced milk production and increased veterinary interventions. The escalating challenge of multidrug-resistant bacteria has prompted research into alternative therapeutic strategies, including milk microbiota transfer (MMT) and phytochemical treatments. This study evaluated milk chemical elements as biomarkers during two alternative treatments for *Staphylococcus warneri*-induced mastitis: milk microbiota transplantation and intra-mammary infusion of 7-epiclusianone, a phytochemical derived from *Garcinia brasiliensis* fruit. The experimental design involved seven female Parda Alpina goats, with six goats infected with *S. warneri* treated using milk from a healthy donor. Intra-mammary 7-epiclusianone was administered two days post-MMT. The right udder received the treatments, with the left udder serving as a control. Elemental analysis of 120 milk samples was performed using scanning electron microscopy coupled with X-ray dispersive spectroscopy, monitoring trace and macro elements. Significant elemental composition variations were observed, with iron, copper, and zinc showing immediate responsiveness from the first day of MMT. Most elements exhibited minor fluctuations, returning to control levels by the treatment's conclusion. The 7-epiclusianone treatment maintained stable elemental concentrations when compared to the control and a reduction in sodium levels was observed. The study identifies zinc, copper, iron, and sodium as promising biomarkers during goat mastitis treatment. The combined MMT and 7-epiclusianone approach aligned milk elemental level with the control levels post treatment, offering a non-invasive method for monitoring mastitis treatment efficacy. Future research should validate these elemental biomarkers across larger and more diverse goat populations.

1.0 Introduction

Goat milk is renowned for its superior nutritional value and beneficial properties compared to cow milk, particularly for individuals with allergies and gastrointestinal disorders (Haenlein, 2004, Nayik et al. 2022). Goat milk has been referred to as the most complete nourishing natural and highly compatible food and its nutritional composition is higher than that of bovine milk, except for lactose (Getaneh et al. 2016). It is rich in essential minerals such as calcium, magnesium, zinc, and copper, (Korać et al. 2023). The presence of these minerals is not only crucial for nutrition but also serves as potential biomarkers for health and disease states (Hambidge, 2003). The concentration of minerals in milk is affected by mastitis which results in lower quality dairy products (Bruckmaier et al. 2004).

Novac and Andrei, (2020) study in sheep and goats revealed that levels of calcium (Ca), sodium (Na), chlorine (Cl), potassium (K), and phosphorus (P) are altered in animals with mastitis. Changes in mammary tissues causes an increase in somatic cell count due to leukocyte chemotaxis to the inflamed tissue, increased passage of plasma proteins caused by heightened vascular permeability, and elevated levels of minerals such as Ca, Na, and Cl. Calcium (Ca) and magnesium (Mg) concentrations in mastitis milk provide critical insights into the physiological disruptions caused by mammary gland inflammation. Mastitis fundamentally alters milk's mineral profile, with potential implications for both bovine metabolic health and dairy product quality. Understanding these elemental changes offers valuable diagnostic and monitoring perspectives for managing inflammatory conditions in dairy herds (El Zubeir, Elowni, & Mohamed, 2005).

Staphylococcus warneri, a significant causative agent of mastitis, exhibits resistance to beta-lactam antimicrobials and possesses virulence factors like biofilm production, adhesins, and toxins, making treatment challenging (Garino Junior et al. 2011; Almeida et al. 2013, Hoque et al. 2024). The use of antimicrobial agents on farms is often useful but the excessive use of it to control or prevent the mastitis infections may be contributing to the global antimicrobial resistance development (Scott and Menzies, 2011; Kovačević et al. 2022).

Diagnostic techniques for mastitis range from direct tests, such as bacterial culture and PCR, to indirect tests like the California Mastitis Test (CMT) and Somatic Cell Count (SCC). These methods help in early detection and management of mastitis, preventing severe consequences (Antanaitis et al. 2021; Boora et al. 2021). SCC, acute phase proteins, conductivity and enzymes are indicators measured for the detection of subclinical mastitis (Adkins and Middleton 2018). Proteomic analyses have revealed that some proteins might be useful as biomarkers for diagnosis of mammary gland inflammation at early stage (Abdelmegid et al. 2020; Bathla et al. 2020). In goats, intramammary antibiotic treatment of mastitis has been reported to be of no significant economic gain as it is associated with udder infection with major pathogens, antibiotic resistance, reduced milk yield, and mortality (Mcdougall et al. 2010).

Current limitations in diagnosing and monitoring the effectiveness of treatments for mastitis stem from the time-consuming nature of conventional diagnostic methods like somatic cell counts (SCC) and bacterial culture, which provide accurate but delayed results. These limitations highlight the need for more objective, real-time, and standardized monitoring methods to enhance mastitis treatment outcomes.

Microbiota transplantation, from an organ/tissue of a healthy animal to a diseased animal as a therapeutic tool has been applied in the treatment of various disease conditions, particularly Fecal Microbiota Transplantation (FMT) (Niederwerder, 2018) which has shown promise in restoring healthy microbiota and reducing pathogenic bacteria. This technique leverages the competitive inhibition and antimicrobial activities of beneficial microbiota (Choi & Cho, 2016). The microbiota is involved in the metabolism of calcium (Chaplin et al. 2016), iron (Laparra et al. 2014), magnesium (Winther et al. 2015), copper (Zhang et al. 2019), and zinc (Liu et al. 2021). Adopting milk microbiota transfer (MMT) in mastitis treatment is an unexplored technique with potential.

7-Epiclusianone, a bioactive compound derived from the fruit of *Garcinia brasiliensis* (bacupari), exhibits a wide range of biological activities. These include anticancer (Sales et al. 2015), anti-inflammatory and antinociceptive (Santa-Cecília et al. 2011), anti-Trypanosoma (De Almeida Alves et al. 1999), anti-anaphylactic (Neves et al. 2007), anti-HIV properties (Piccinelli et al. 2005), antimicrobial activities and bacteriostatic effects (De Barros et al. 2017).

Antibiotic treatments administered directly into the udder in goats have proven costly and ineffective due to issues such as udder infections caused by major pathogens and the development of antibiotic resistance. This necessitates the search for alternative, cost-effective treatments and diagnostic markers. This study aims to evaluate potential milk elemental biomarkers during two alternative treatments; milk microbiota transplantation (MMT) and intra-mammary infusion of 7-epiclusianone in *Staphylococcus warneri*-induced goat mastitis.

2.0 Materials and Methods

2.1 Experimental design and Sampling

This study builds on earlier work by Caldeira (2024), which involved seven lactating Parda Alpina goats. In that research, six goats were inoculated with *Staphylococcus warneri* (1.2×10^8 CFU/ml in 2 ml) through the intra-mammary route (De Moraes Peixoto et al., 2015), while the left udder was designated as the control. Mastitis development was confirmed through microbiological tests, California Mastitis Test (CMT), and Somatic Cell Count (SCC) analysis. The left udder of each infected goat served as an untreated control.

The left udder served as an internal control to establish a reliable baseline for evaluating treatment effects on the right udder. The anatomical structure of goat udders, characterized by independent teat canals and discrete milk-secreting tissues in each quarter (Adam et al. 2017), provides a unique experimental advantage. This structural independence ensures minimal direct physical cross-contamination between udder quarters, thereby enabling each udder to function as a robust, self-contained experimental and control unit. Such an approach allows for a more controlled and physiologically relevant assessment of the treatment's localized effects, while minimizing potential systemic confounding factors.

The seventh goat was selected as the microbiota donor for the transplantation procedure. Throughout the study, a total of 120 milk samples were systematically collected: 60 samples from the treated right udder and 60 from the control left udder. Sample collection followed strict aseptic protocols during manual milking, with samples immediately stored in sterile tubes and promptly processed in the laboratory to maintain sample integrity and prevent potential contamination.

2.2 Microbiota Transplantation- 1st treatment

The single healthy donor goat thoroughly screened through comprehensive microbiological testing and physical examination to ensure suitability for milk microbiota transplantation (MMT) donated a 120 mL milk sample which was manually collected using strict aseptic techniques, immediately transferred to sterile tubes, and rapidly processed in the laboratory. Each infected goat received 120 mL of the donor milk through intra-mammary infusion via a sterile syringe, administered once daily.

Milk samples were systematically collected at six predefined time points to track microbiological and elemental changes: milk microbiota transplantation Day 1 (MMTD1), Day 3 (MMTD3), Day 5 (MMTD5), Day 7 (MMTD7), Day 9 (MMTD9), and 2 days post-MMT (Day 11). Each sampling followed standardized collection protocols to minimize contamination and ensure consistent, reproducible results.

2.3 7-epiclusianone- 2nd treatment

After MMT the udder was rested for 2 days to allow for stabilization and ensure that it is in an optimal condition to respond to the subsequent treatment. Two days following the completion of milk microbiota transplantation (MMT), 7-epiclusianone was administered as an intra-mammary treatment. The antimicrobial compound, previously tested against *Streptococcus* spp. (De Barros et al. 2017), was formulated into an ointment with the following specifications: concentration: 1.56 μg/mL of 7-epiclusianone; base: Lanolin and petroleum jelly; syringe volume: 65 mL per administration and the total 7-epiclusianone delivered: 101.4 μg per administration.

The treatment protocol involved daily intra-mammary infusion using a precise pharmaceutical manipulation technique. The procedure included: inserting the tube tip into the right teat, administering the formulation and applying an upward massaging motion to ensure uniform distribution of the mixture.

Milk samples were systematically collected at the following time points: 7-epiclusianone treatment day 1 (7epiD1), 7-epiclusianone treatment day 3 (7epiD3), 7-epiclusianone treatment day 5 (7epiD5), 8 days post 7-epiclusianone treatment (D13)

The left udder remained untreated and served as the control for both treatment protocols.

2.4 Freeze drying of samples

Milk samples (n = 120) were processed using a systematic preparation protocol for elemental analysis. Initially, samples were freeze-dried using an Edwards Modulyo® freeze dryer at -40°C and 4 atm for 36 hours. After lyophilization, samples were macerated and stored at -80°C.

The dried samples were mounted on carbon adhesive tape-mounted stubs and placed in a desiccator containing silica gel for four days to ensure optimal preservation conditions. Prior to analysis, samples were coated with a 15 nm carbon layer using a Quorum Q150T-E evaporator (Quorum Technologies, UK).

Elemental composition analysis was conducted using a scanning electron microscope (LEO 1430 VP, Carl Zeiss, UK) equipped with an X-ray microprobe (XEDS, IXRF Systems, USA). The analysis quantified: carbon, nitrogen, oxygen (CNO), calcium (Ca), sodium (Na), potassium (K), copper (Cu), magnesium (Mg), zinc (Zn), phosphorus (P), chlorine (Cl), iron (Fe), and molybdenum (Mo). The CNO elements were normalized according to the method described by Ladeira et al. (2019).

2.5 Statistical analysis

The normality of data distribution and homogeneity of variance was tested using the Shapiro-Wilk test and Kolmogorov Smirnov test, respectively. Two-way analysis of variance (ANOVA) and Sidak's multiple comparisons test were carried out to compare the mean levels of different elements in the milk samples between the right (treated) and the left (control) udder following the days of treatment and graphs were plotted using GraphPad Prism (version 8.4.3).

3.0 Results

3.1 Milk Microbiota Transplantation- 1st treatment

Figures 1, 2 and 3 show the concentration of trace, macro and major milk elements during milk microbiota transplantation respectively.

Iron levels on the first day of treatment was low when compared to the control but increased notably from the 3rd period and maintained an increase following MMT (Fig. 1a).

Copper and Zinc levels was visibly high and statistically significant when compared to the left in the first period but markedly dropped in the right udder as a response to MMT (Fig. 1b and c). Molybdenum levels show minor fluctuations in both sides, with the treated (right) having slightly higher concentrations at certain points but overall trends remain similar to the control (left) (Fig. 1d).

In Fig. 2a, sodium concentration increased progressively in both udder, with significantly higher levels observed in the right udder during the later stages. Magnesium levels exhibit a significant spike in the right udder at time point 3 (Fig. 2b). Phosphorus levels remain relatively stable throughout the experiment in both udders (Fig. 2c). Chlorine levels increase in the right udder, with larger fluctuations compared to the control (Fig. 2d). Potassium levels show a decreasing trend in both udder, with slightly lower levels in the right udder (Fig. 2e). Calcium concentrations fluctuated mildly in both udders with no significant differences between the right and the left (Fig. 2f).

CNO shows similar variability between the treated and control udder with slightly lower levels in the right udder (Fig. 3).

3.2 7-epiclusianone- 2nd treatment

Figures 4, 5 and 6 show the concentration of trace, macro and major milk elements during treatment with 7epiclusianone respectively.

The concentration of iron (Fig. 4a) in both the treated (right) and control (left) udder remained relatively stable throughout the experimental period. Copper levels (Fig. 4b) in the right udder was relatively stable when compared to the control but spiked in both udders 8 days post treatment. Zinc concentrations (Fig. 4c) showed slight fluctuations between the right and left udders, with no significant difference observed. Molybdenum levels (Fig. 4d) in the right udder were consistently higher than in the left udder, suggesting a role in the recovery process.

Sodium concentrations (Fig. 5a) were significantly elevated in the right udder compared to the left udder, especially in the early periods post-treatment and subsequently reduced on in the 3rd day of treatment. Magnesium concentrations (Fig. 5b) remained stable across both udders, with no significant fluctuations observed. Phosphorus levels (Fig. 5c) fluctuated modestly in both udders, with no clear pattern emerging. Chlorine concentrations (Fig. 5d) were higher in the right udder, mirroring sodium trends due to their role in maintaining ionic balance. Potassium levels (Fig. 5e) remained relatively stable in both udders with the levels of the right udder a bit lower than the left showing no significant changes. Calcium concentrations (Fig. 5f) showed mild fluctuations in both udders, with a slight decrease in the treated udder by 8 days post treatment.

CNO levels (Fig. 6) decreased consistently in both the treated and control udders.

4.0 Discussion

Mastitis, frequently caused by bacterial pathogens like *Staphylococcus warneri*, significantly impacts dairy animal health and milk quality (Hoque et al. 2024). Pathogen invasion triggers an inflammatory response that disrupts milk composition and metabolic equilibrium (Yoo et al. 2020). The elemental composition of milk is crucial, as trace elements and macro minerals serve critical physiological functions. These elements are essential for immune response, metabolic processes, and overall animal health, playing pivotal roles in oxygen transport, enzyme activation, bone formation, muscle function, nerve conduction, water balance, cardiovascular regulation, and providing antioxidant protection (Marina et al. 2023). Minerals such as Na, Mg, Ca, K, P, and Cl were classified as macro-minerals which are required in large amounts in the body and minerals such as Fe, Zn, Mo and Cu are trace/micro minerals that are required in small amounts (Maret 2016, and Zeece 2020).

This study highlights the potential of using milk microbiota transplantation and 7-epiclusianone as alternative treatments for mastitis with the aim of identifying elemental indicators, both interventions showed measurable effects on the elemental composition in milk. The observed significant variations in trace elements during milk microbiota transplantation (MMT) indicate substantial alterations in udder immune and metabolic responses. The pronounced changes in iron, zinc, and copper levels (Fig. 1) suggest dynamic modifications in metabolic pathways, particularly those related to immune defense and antioxidant mechanisms. These elemental shifts align with established research on trace elements' critical roles in immune function, specifically their involvement in metabolic adaptation during inflammatory processes, as previously documented by Djoko (2015) in studies of elemental contributions to immune defense.

Microbiota manipulation likely contributed to the changes in milk’s elemental profile, as disruptions in the microbiome can alter metabolic pathways (Zheng et al. 2016), as evidenced by the altered levels of zinc, copper, and magnesium which shows that that trace elements are integral to both immune responses and tissue repair processes during infections, particularly in mastitis cases. The elevated and consistent trend in Fe concentration indicated that iron can serve as a reliable biomarker for monitoring during mastitis treatment as this is consistent with other study in bovine by Tsukano, Shimamori and Suzuki (2020) who reported low iron levels during severe mastitis. Iron (Fe), copper (Cu), and zinc (Zn) levels exhibited distinctive patterns during milk microbiota transfer (MMT), reflecting complex immune and metabolic responses to mastitis treatment. Fe levels initially decreased, potentially due to beneficial microbes competing with *S. warneri* for available iron, a mechanism previously documented by Ward et al. (1996) and Mungatana et al. (2011). This reduction in iron sequestration may help restore microbiological equilibrium.

Cu and Zn demonstrated statistically significant variations on the first day of MMT, followed by stabilization. These fluctuations likely stem from the complex role of copper in oxidative stress management and immune function. The observed Cu level reduction suggests a potential redistribution towards immune cell utilization, consistent with Mungatana et al. (2011) who noted similar concentration changes during goat mastitis treatment. The dynamic elemental profiles of Fe, Cu, and Zn highlight their possible potential as early biomarkers for monitoring caprine mastitis treatment response, reflecting the intricate physiological adaptations during infection and therapeutic intervention. Increased permeability of the blood-milk barrier during mastitis allows trace minerals like zinc to shift into milk (Zhao and Lacasse, 2008). MMT may affect zinc levels, as Liu et al. (2014) indicated that zinc is essential for regulating immune function and inflammation. Adequate zinc intake is necessary to prevent excessive inflammation and tissue damage by modulating the NF-κB signaling pathway (Jarosz et al. 2017).

The elevated Mo levels on the last day of MMT could be attributed to the udder response to MMT (Fig. 1).

Sodium levels in our results demonstrated an increase in the treated udder over time, peaking at the last period of MMT. This finding contrasts with Mungatana et al. (2011), who reported that sodium concentrations of goat peaked at 14% above pre-infection values at three weeks post-infection and returned to pre-infection levels by four weeks post-treatment. But aligns with the study by Arney and Philips, (2004) indicating reduced growth of *S. aureus* with increased sodium levels in mastitogenic bacteria in-vitro suggesting that the increased sodium levels following MMT may reduce the growth of *S. warneri*. El Zubeir et al. 2005 during the comparison of healthy cow's milk with that of clinically mastitis milk showed a highly significant decrease in levels of calcium, magnesium ($P < 0.001$) and potassium ($P < 0.01$) when compared to the control.

The level of magnesium from first day of treatment didn’t differ significantly not until the last day of treatment that revealed a significant increase which may indicate acute inflammatory responses or metabolic shifts, which returned to similar levels to the control post treatment.

Calcium, phosphorus, and potassium (Fig. 2) levels maintained similar levels to the control during treatment. Phosphorus elevated level post treatment indicates MMT had an effect on phosphorus and an ability to restore the normal levels of milk elemental composition over time by influencing the udder's microbiome, and also reducing inflammation.

Milk contains about 7% dissolved gas, mainly carbon dioxide (60–70%), nitrogen (25–30%), and oxygen (5–10%) (Olkonen, 2001). Bacterial infections in the udder are associated with a decrease in dissolved oxygen within the mammary tissues, and can arise due to free radicals and oxygen consumption by neutrophils (Mayer et al., 1988). This study observed decreases in carbon, nitrogen, and oxygen levels (Fig. 3) from the first day of MMT, likely due to changes in microbial composition affecting carbon utilization, nitrogen metabolism, and oxygen availability. The trend towards normalization of these levels post-transplantation suggests MMT's effectiveness in restoring mammary gland health. MMT possibly enhanced the immunity by reducing the oxidative stress as reported by Sordillo (2016) that during mastitis treatment, feeding of mineral to animals enhances their immunity.

The second treatment which followed two days post MMT (Fig. 4), the observed differences in the different milk chemical elements during treatment with 7-epiclusianone when compared to the control particularly in Sodium (Na) showed a significant increase on the first day of treatment, followed by a reduction, indicating a positive response to the treatment. During mastitis, damage to tight junctions can cause Na and chloride (Cl) to leak into the milk, elevating their concentrations above normal levels (Auldist and Hubble, 1998). The subsequent stabilization of Na levels suggests that the treatment possibly addressed this disruption.

Cl showed a significant difference eight days post-treatment which can be as a result of physiologic differences. The anti-inflammatory and antimicrobial properties of 7-epiclusianone have been well-documented by Santa-Cecília et al. (2011), de Barros et al. (2017), and Veloso et al. (2018), who demonstrated the compound's effectiveness against *Streptococcus agalactiae* and *Streptococcus uberis*, both common mastitis pathogens. This study further supports these findings, as 7-epiclusianone effectively altered the milk's elemental composition showing it's potency in suppressing *S. warneri* induced mastitis, potentially restoring element levels to baseline. This effect suggests that 7-epiclusianone may aid in the recovery process by fostering conditions that support mastitis resolution without significantly disrupting essential mineral levels. The alterations in Fe, Cu, Zn and Na highlights the possibility of interaction between these elements and *S.warneri* in terms of its nutritional and microbiological significance.

To the best of our knowledge, this is the first study to evaluate potential milk elemental biomarkers using milk microbiota transplantation (MMT) and 7-epiclusianone as an alternative treatment strategy for *Staphylococcus warneri*-induced mastitis in goats, providing a novel perspective on monitoring and managing caprine mastitis treatment.

Further studies are needed to better understand the mechanisms through which these treatments influences milk elemental composition. Investigating optimal dosing regimens, donor microbiota selection, and the interactions between transplanted microbiota and host immune responses would help refine MMT and 7-epiclusianone as reliable and sustainable alternative to conventional antibiotic treatments for mastitis and other microbial infections in dairy animals. The use of milk elements like Cu, Zn, Fe and Na as potential biomarkers during these treatments offers a non-invasive and minimally intrusive method for tracking mastitis treatment efficacy.

In conclusion, this research explores the potential of elemental markers to indicate treatment response and effectiveness in mastitis-affected udder undergoing two different treatments; milk microbiota transplantation and intra-mammary administration of 7-epiclusianone. The findings highlight zinc, copper, iron, and sodium as promising biomarkers for monitoring goat mastitis treatment. Future research should validate these elemental biomarkers' diagnostic and monitoring potential in broader clinical contexts.

Declarations

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Author Contributions

Ugonna Henry Uzoka: data formal analysis, primary manuscript drafting, manuscript revision and editing, investigation, and data visualization. Juliana Miwa Fujikura, Jéssica Lobo Albuquerque Caldeira, Cristiane do Carmo Cesário, Luísa Maria de Souza Menezes, Maria Júlia Fernandes Netto: Research investigation, methodology development, project administration, and manuscript review. Fabricio Luciani Valente: Data visualization, research supervision, methodology validation, and manuscript review. Richard Costa Polveiro, Maria Aparecida Scatamburlo Moreira: Conceptualization, investigation, resource management, manuscript review, and research supervision.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Statement

This study received approval from the Ethics Committee on the Use of Animals (CEUA) at the Federal University of Viçosa (UFV), with registration 62/2018.

Consent for publication

Not applicable.

Consent to participate

Not applicable.

Competing interests

The authors declare no competing interests.

References

1. Abdelmegid, S., Kelton, D., Caswell, J., & Kirby, G. (2020). Proteomic 2D-DIGE Analysis of Milk Whey from Dairy Cows with Staphylococcus aureus Mastitis Reveals Overexpression of Host Defense Proteins. Microorganisms, 8(12), 1883. https://doi.org/10.3390/microorganisms8121883
2. Adam, Z. A., Ragab, G.A., Awaad, A.S., Tawfiek, M.G., M., A, M., Maksoud, K., (2017). Gross anatomy and ultrasonography of the udder in goat. Journal of Morphological Sciences, https://doi.org/10.4322/JMS.105316
3. Adkins, P. R., & Middleton, J. R. (2018). Methods for diagnosing mastitis. Veterinary Clinics: Food Animal Practice, 34(3), 479-491.
4. Almeida, J. F., Aquino, M. H. C., Magalhães, H., Nascimento, E. R., Pereira, V. L. A., Ferreira, T., & Barreto, M. L.. (2013). Principais alterações no leite por agentes causadores de mastite no rebanho caprino dos estados de Minas Gerais e Rio de Janeiro. Arquivos Do Instituto Biológico, 80(1), 13–18. https://dx.doi.org/10.1590/S1808-16572013000100003
5. Almeida, L. S. B. D., Murata, R. M., Yatsuda, R., Dos Santos, M. H., Nagem, T. J., Alencar, S. M. D., ... & Rosalen, P. L. (2008). Antimicrobial activity of Rheedia brasiliensis and 7-epiclusianone against Streptococcus mutans. Phytomedicine, 15(10), 886-891. https://doi.org/10.1016/j.phymed.2007.12.003
6. Antanaitis, R., Juozaitienė, V., Jonike, V., Baumgartner, W., & Paulauskas, A. (2021). Milk Lactose as a Biomarker of Subclinical Mastitis in Dairy Cows. Animals, 11(6), 1736. https://doi.org/10.3390/ani11061736
7. Arney, D. R., & Phillips, C. J. (2005). The effects of changes in sodium and potassium concentration on the growth of mastitogenic bacteria in vitro. Int. J. Appl. Res. Vet. Med, 3(3), 242-248.
8. Auldist, M. J., & Hubble, I. B. (1998). Effects of mastitis on raw milk and dairy products. Australian journal of dairy technology, 53(1), 28.
9. Bathla, S., Sindhu, A., Kumar, S., Dubey, S. K., Pattnaik, S., Rawat, P., ... & Mohanty, A. K. (2020). Quantitative proteomics revealed the putative biomarker for detection of early-stage intra-mammary gland infection in cow. Journal of Proteins and Proteomics, 11, 173-181.
10. Boora, A., Yadav, S., Devi, P., Singh, K. P., Cheema, P. S., Muley, V., … Singh, I. (2021). An open label study to assess the efficacy of ceftiofur in treatment of clinical mastitis in buffaloes. Buffalo

Bulletin, 40(4), 545–555.

11. Bruckmaier, R.M, Ontsouka, C.E, Blum, J.W. (2004). Fractionized milk composition in dairy cows with subclinical mastitis. Veterinariai Medicina Czechoslovakia, 49, 283-290.
12. Caldeira JLA, (2024). Goat mastitis by Staphylococcus warneri: An multifactorial approach in the study of infection. Dissertation, Universidade Federal de Viçosa
13. Chaplin, A., Parra, P., Laraichi, S., Serra, F., & Palou, A. (2016). Calcium supplementation modulates gut microbiota in a prebiotic manner in dietary obese mice. Molecular nutrition & food research, 60(2), 468-480. https://doi.org/10.1002/mnfr.201500480
14. Choi, H. H., & Cho, Y. S. (2016). Fecal microbiota transplantation: current applications, effectiveness, and future perspectives. Clinical endoscopy, 49(3), 257-265. https://doi.org/10.5946/ce.2015.117
15. De Almeida Alves, T. M. et al. (1999). Biological Activities of 7-Epiclusianone. Journal of Natural Products, v. 62, n. 2, p. 369–371. https://doi.org/10.1021/np9803833
16. De Barros, M., Perciano, P. G., Dos Santos, M. H., De Oliveira, L. L., Costa, É. D. M., & Moreira, M. A. S. (2017). Antibacterial activity of 7-epiclusianone and its novel copper metal complex on Streptococcus spp. isolated from bovine mastitis and their cytotoxicity in MAC-T cells. Molecules, 22(5), 823. https://doi.org/10.3390/molecules22050823
17. De Moraes Peixoto, R., De Moraes Peixoto Araújo, R., E Silva Peixoto, L. J., Gonçalves Bomfim, S. A., Guedes da Silva, T. M., Sarmento Silva, T. M., Guedes da Silva Almeida, J. R., Mota, R. A., & Da Costa, M. M. (2015). Treatment of goat mastitis experimentally induced by Staphylococcus aureus using a formulation containing Hymenaea martiana extract. Small Ruminant Research, 130, 229-235. https://doi.org/10.1016/j.smallrumres.2015.07.009
18. Djoko, K. Y., Cheryl-lynn, Y. O., Walker, M. J., & McEwan, A. G. (2015). The role of copper and zinc toxicity in innate immune defense against bacterial pathogens. Journal of Biological Chemistry, 290(31), 18954-18961. http://doi.org/10.1074/jbc.R115.647099.
19. El Zubeir, I. E. M., Elowni, O. A. O., & Mohamed, G. E. (2005). Effect of mastitis on macro-minerals of bovine milk and blood serum in Sudan. Journal of the South African Veterinary Association, 76(1). https://hdl.handle.net/10520/EJC99599
20. Garino Junior, F., Camboim, E. K. A., Das Neves, P. B., De Sá, A. V. V., & Almeida, A. P. (2020). Suscetibilidade a antimicrobianos e produção de betalactamase em amostras de Staphylococcus isolados de mastite caprina no semiárido paraibano. Arquivos do Instituto Biológico, 78, 103-107. https://dx.doi.org/10.1590/1808-1657v78p1032011
21. Getaneh, G., Mebrat, A., Wubie, A., & Kendie, H. (2016). Review on goat milk composition and its nutritive value. Journal of Nutrition and Health Sciences, 3(4), 1-10. http://doi:10.15744/2393-9060.3.401
22. Haenlein, G. F. W. (2004). Goat milk in human nutrition. Small ruminant research, 51(2), 155-163. https://doi.org/10.1016/j.smallrumres.2003.08.010
23. Hambidge, M. (2003). Biomarkers of trace mineral intake and status. The Journal of nutrition, 133(3), 948S-955S. https://doi.org/10.1093/jn/133.3.948S

24. Hoque, M. N., Faisal, G. M., Das, Z. C., Sakif, T. I., Al Mahtab, M., Hossain, M. A., & Islam, T. (2024). Genomic features and pathophysiological impact of a multidrug-resistant Staphylococcus warneri variant in murine mastitis. Microbes and Infection, 26(3), 105285. https://doi.org/10.1016/j.micinf.2023.105285
25. Jarosz, M., Olbert, M., Wyszogrodzka, G., Młyniec, K., & Librowski, T. (2017). Antioxidant and anti-inflammatory effects of zinc. Zinc-dependent NF-κB signaling. Inflammopharmacology, 25, 11-24. https://doi.org/10.1007/s10787-017-0309-4
26. Korać, R. S., Huremović, J., Žero, S., & Ljubijankić, N. (2023). Content of metals in cow, sheep, and goat milk samples. Bulletin of the Chemists and Technologists of Bosnia and Herzegovina, 60, 1-6. https://doi.org/10.35666/2232-7266.2023.60.01
27. Kovačević, Z., Samardžija, M., Horvat, O., Tomanić, D., Radinović, M., Bijelić, K., ... & Kladar, N. (2022). Is there a relationship between antimicrobial use and antibiotic resistance of the most common mastitis pathogens in dairy cows?. Antibiotics, 12(1), 3.
28. Ladeira, L. C. M., Dos Santos, E. C., Valente, G. E., da Silva, J., Santos, T. A., & dos Santos Costa Maldonado, I. R. (2020). Could biological tissue preservation methods change chemical elements proportion measured by energy dispersive X-ray spectroscopy?. Biological trace element research, 196, 168-172. https://doi.org/10.1007/s12011-019-01909-x
29. Laparra, J. M., Díez-Municio, M., Herrero, M., & Moreno, F. J. (2014). Structural differences of prebiotic oligosaccharides influence their capability to enhance iron absorption in deficient rats. Food & function, 5(10), 2430-2437. https://doi.org/10.1039/C4FO00504J
30. Liu, H., Bai, M., Xu, K., Zhou, J., Zhang, X., Yu, R., ... & Yin, Y. (2021). Effects of different concentrations of coated nano zinc oxide material on fecal bacterial composition and intestinal barrier in weaned piglets. Journal of the Science of Food and Agriculture, 101(2), 735-745. https://doi.org/10.1002/jsfa.10686
31. Liu, M. J., & Knoell, D. L. (2014). Zinc signal in inflammation. Zinc Signals in Cellular Functions and Disorders, 227-248. https://doi.org/10.1007/978-4-431-55114-0_11
32. Maret, W. (2016). The metals in the biological periodic system of the elements: concepts and conjectures. International journal of molecular sciences, 17(1), 66. https://doi.org/10.3390/ijms17010066
33. Marina, Stepanova., L., F., Sotnikova., S., Yu., Zaitsev. (2023). 6. Relationships between the Content of Micro- and Macroelements in Animal Samples and Diseases of Different Etiologies. Animals, https://doi.org/10.3390/ani13050852
34. Mayer, S. J., Waterman, A. E., Keen, P. M., Craven, N., & Bourne, F. J. (1988). Oxygen concentration in milk of healthy and mastitic cows and implications of low oxygen tension for the killing of Staphylococcus aureus by bovine neutrophils. Journal of dairy research, 55(4), 513-519. https://doi.org/10.1017/S0022029900033288
35. McDougall, S., Supré, K., De Vliegher, S., Haesebrouck, F., Hussein, H., Clausen, L., & Prosser, C. (2010). Diagnosis and treatment of subclinical mastitis in early lactation in dairy goats. Journal of

Dairy Science, 93(10), 4710-4721. https://doi.org/10.3168/jds.2010-3324

36. Mungatana, N. K., Ngure, R. M., Shitandi, A., Onyiego, B., & Mutumba, M. (2011). Effect of experimental Staphylococcus aureus mastitis on compositional quality of goat milk. International journal of dairy technology, 64(3), 360-364. https://doi.org/10.1111/j.1471-0307.2011.00672.x
37. Nayik, G. A., Jagdale, Y. D., Gaikwad, S. A., Devkatte, A. N., Dar, A. H., & Ansari, M. J. (2022). Nutritional profile, processing and potential products: A comparative review of goat milk. Dairy, 3(3), 622-647. https://doi.org/10.3390/dairy3030044
38. Neves, J. S., Coelho, L. P., Cordeiro, R. S. B., Veloso, M. P., e Silva, P. M. R., dos Santos, M. H., & Martins, M. A. (2007). Antianaphylactic properties of 7-epiclusianone, a tetraprenylated benzophenone isolated from Garcinia brasiliensis. Planta medica, 73(07), 644-649. https://doi.org/10.1055/s-2007-981534
39. Niederwerder, M. C. (2018). Fecal microbiota transplantation as a tool to treat and reduce susceptibility to disease in animals. Veterinary immunology and immunopathology, 206, 65-72. https://doi.org/10.1016/j.vetimm.2018.11.002
40. Novac, C. S., & Andrei, S. (2020). The Impact of mastitis on the biochemical parameters, oxidative and nitrosative stress markers in goat's milk: A review. Pathogens, 9(11), 882. https://doi.org/10.3390/pathogens9110882
41. Olkonen, A. jt. (2001). Piimanduse käsiraamat. Greif OÜ Trükikoda, Tartu
42. Piccinelli, A. L., Cuesta-Rubio, O., Chica, M. B., Mahmood, N., Pagano, B., Pavone, M., ... & Rastrelli, L. (2005). Structural revision of clusianone and 7-epi-clusianone and anti-HIV activity of polyisoprenylated benzophenones. Tetrahedron, 61(34), 8206-8211. https://doi.org/10.1016/j.tet.2005.06.030
43. Sales, L., Pezuk, J. A., Borges, K. S., Brassesco, M. S., Scrideli, C. A., Tone, L. G., ... & Oliveira, J. C. D. (2015). Anticancer activity of 7-epiclusianone, a benzophenone from Garcinia brasiliensis, in glioblastoma. BMC Complementary and Alternative Medicine, 15, 1-8. https://doi.org/10.1186/s12906-015-0911-1
44. Santa-Cecília, F. V., Freitas, L. A., Vilela, F. C., Veloso, C. D. C., da Rocha, C. Q., Moreira, M. E., ... & dos Santos, M. H. (2011). Antinociceptive and anti-inflammatory properties of 7-epiclusianone, a prenylated benzophenone from Garcinia brasiliensis. European Journal of Pharmacology, 670(1), 280-285.. https://doi.org/10.1016/j.ejphar.2011.08.032
45. Scott, L. C., & Menzies, P. I. (2011). Antimicrobial resistance and small ruminant veterinary practice. Veterinary Clinics: Food Animal Practice, 27(1), 23-32.
46. Sordillo, L. M. (2016). Nutritional strategies to optimize dairy cattle immunity. Journal of dairy science, 99(6), 4967-4982.
47. Tsukano, K., Shimamori, T., & Suzuki, K. (2020). Serum iron concentration in cattle with endotoxaemia. Acta Veterinaria Hungarica, 68(1), 53-58. https://doi.org/10.1556/004.2020.00016
48. Veloso, C. D. C., Silva, M. B. D., Megda, M. D. O., Santos, M. H. D., Giusti-Paiva, A., & Vilela, F. C. (2018). Evaluation of anxiolytic-like effect of 7-epiclusianone isolated from Garcinia brasiliensis in

mice. Revista Brasileira de Farmacognosia, 28, 378-381. https://doi.org/10.1016/j.bjp.2018.01.009

49. Ward, C. G., Bullen, J. J., & Rogers, H. J. (1996). Iron and infection: New development and their implications. Journal of Trauma and Acute Care Surgery, 41(2), 356-364. https//doi.org/10.1097/00005373-199608000-00030.
50. Winther, G., Jørgensen, B. M. P., Elfving, B., Nielsen, D. S., Kihl, P., Lund, S., ... & Wegener, G. (2015). Dietary magnesium deficiency alters gut microbiota and leads to depressive-like behaviour. Acta neuropsychiatrica, 27(3), 168-176. https://doi:10.1017/neu.2015.7
51. Yoo, J. Y., Groer, M., Dutra, S. V. O., Sarkar, A., & McSkimming, D. I. (2020). Gut microbiota and immune system interactions. Microorganisms, 8(10), 1587. https://doi.org/10.3390/microorganisms8101587.
52. Zeece, M. (2020). Introduction to the Chemistry of Food. Academic Press.
53. Zhang, F., Zheng, W., Xue, Y., & Yao, W. (2019). Suhuai suckling piglet hindgut microbiome-metabolome responses to different dietary copper levels. Applied microbiology and biotechnology, 103, 853-868. https://doi.org/10.1007/s00253-018-9533-0
54. Zhao, X., & Lacasse, P. (2008). Mammary tissue damage during bovine mastitis: causes and control. Journal of animal science, 86(suppl_13), 57-65.https://doi.org/10.2527/jas.2007-0302
55. Zheng, P., Zeng, B., Zhou, C., Liu, M., Fang, Z., Xu, X., ... & Xie, P. (2016). Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host’s metabolism. Molecular psychiatry, 21(6), 786-796. https://doi.org/10.1038/mp.2016.44

Figures

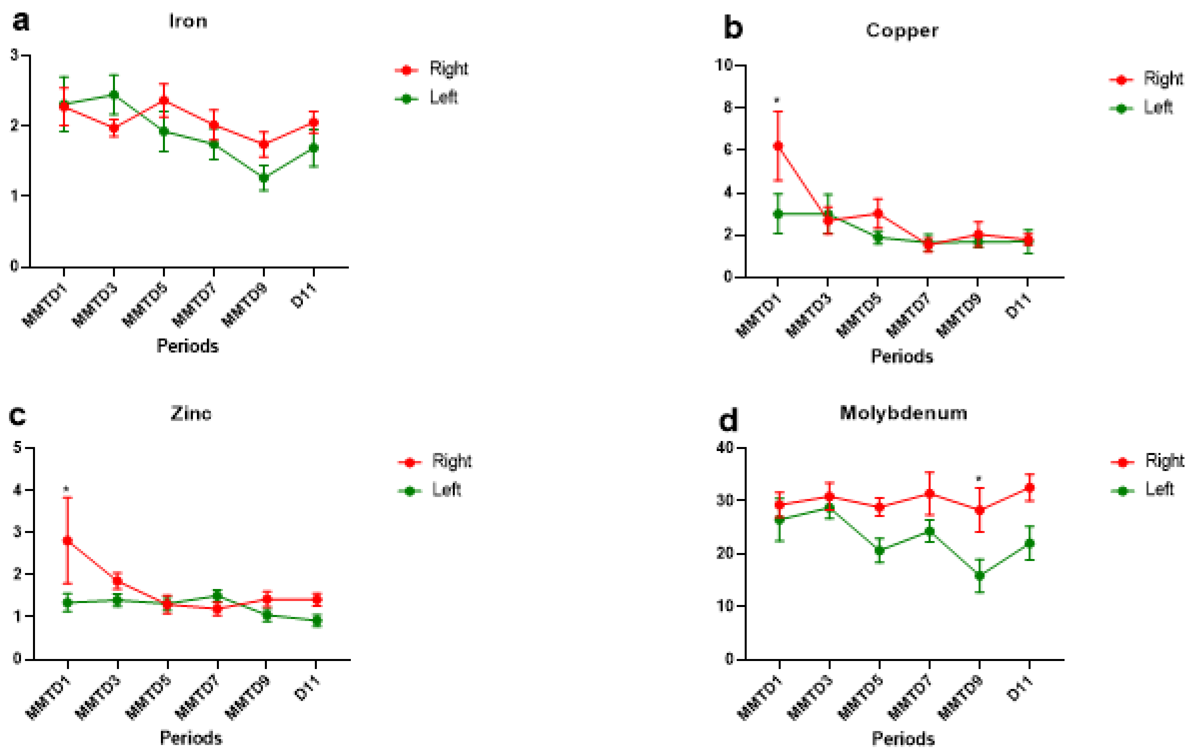

Figure 1

Concentrations of trace elements in goat milk during the treatment of mastitis with Milk Microbiota Transplantation (MMT). Trace element concentrations in goat milk were measured using X-ray Energy Dispersive Spectroscopy (XEDS) for (a) iron, (b) copper, (c) zinc, (d) molybdenum. Data represent comparisons between the treated (right udder) and control (left udder) groups across the treatment period milk microbiota transplantation Day 1 (MMTD1), milk microbiota transplantation Day 3 (MMTD3), milk microbiota transplantation Day 5 (MMTD5), milk microbiota transplantation Day 7 (MMTD7), milk microbiota transplantation Day 9 (MMTD9) and two days post MMT (D11). Statistically significant differences (*$p \le 0.05$) between the treatment and control groups were determined using two-way ANOVA followed by the Holm-Sidak post hoc test.

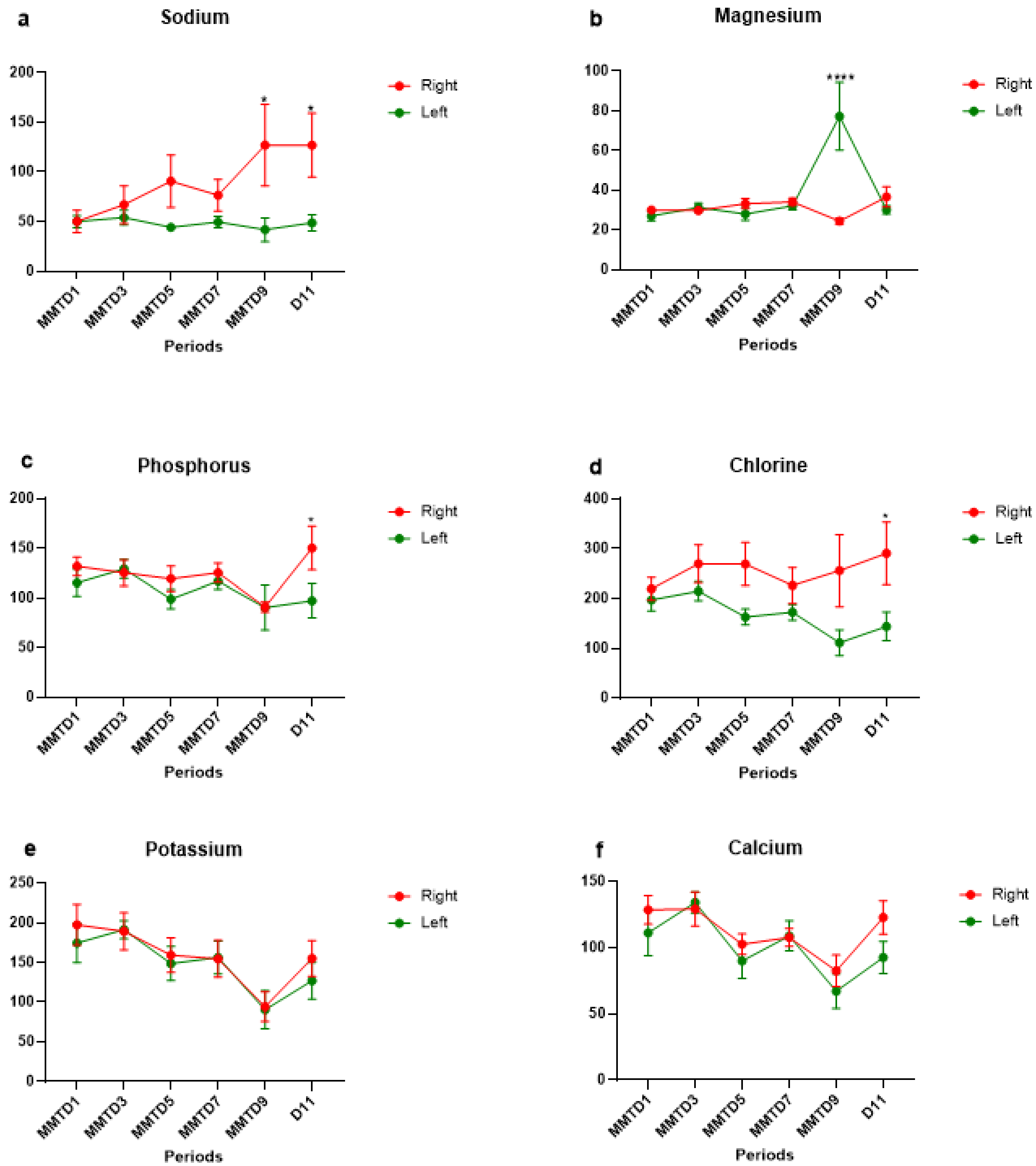

Figure 2

Concentrations of macro elements in goat milk during the treatment of mastitis with Milk Microbiota Transplantation (MMT). Macro element concentrations in goat milk were measured using X-ray Energy Dispersive Spectroscopy (XEDS) for (a) sodium, (b) magnesium, (c) phosphorus, (d) chlorine, (e) potassium, (f) calcium. Data represent comparisons between the treated (right udder) and control (left udder) groups across the treatment period milk microbiota transplantation Day 1 (MMTD1), milk microbiota transplantation Day 3 (MMTD3), milk microbiota transplantation Day 5 (MMTD5), milk microbiota transplantation Day 7 (MMTD7), milk microbiota transplantation Day 9 (MMTD9) and two days post MMT (D11). Statistically significant differences (*p ≤ 0.05) between the treatment and control groups were determined using two-way ANOVA followed by the Holm-Sidak post hoc test.

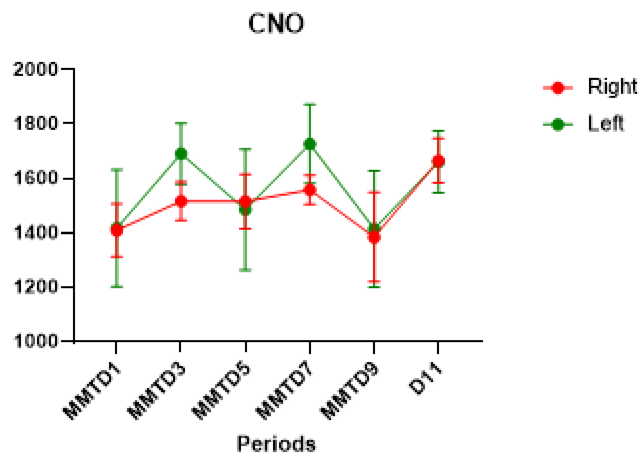

Figure 3

Concentrations of major elements (carbon, nitrogen and oxygen) in goat milk during the treatment of mastitis with Milk Microbiota Transplantation (MMT). Major element concentrations in goat milk were measured using X-ray Energy Dispersive Spectroscopy (XEDS). Data represent comparisons between the treated (right udder) and control (left udder) groups across the treatment period milk microbiota transplantation Day 1 (MMTD1), milk microbiota transplantation Day 3 (MMTD3), milk microbiota transplantation Day 5 (MMTD5), milk microbiota transplantation Day 7 (MMTD7), milk microbiota transplantation Day 9 (MMTD9) and two days post MMT (D11). Statistically significant differences (*p ≤ 0.05) between the treatment and control groups were determined using two-way ANOVA followed by the Holm-Sidak post hoc test.

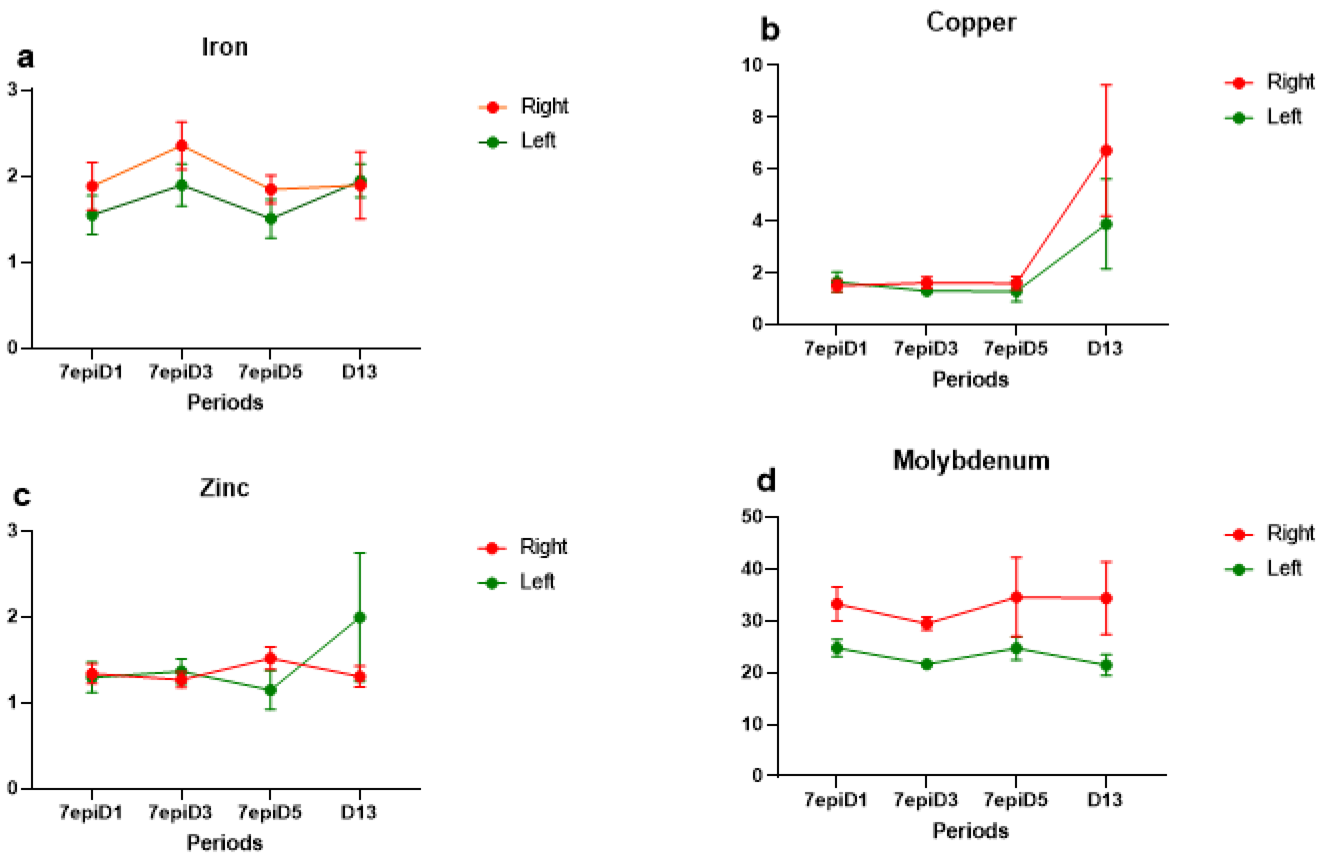

Figure 4

Concentrations of trace elements in goat milk during the treatment of mastitis with 7-epiclusianone. Trace element concentrations in goat milk were measured using X-ray Energy Dispersive Spectroscopy (XEDS) for (a) iron, (b) copper, (c) zinc, (d) molybdenum. Data represent comparisons between the treated (right udder) and control (left udder) groups across the treatment period 7-epiclusianone Day 1 (7epiD1), 7-epiclusianone Day 3 (7epiD3), 7-epiclusianone Day 5 (7epiD5), and eight days post 7-epiclusianone treatment (D13). Statistically significant differences (*$p \leq 0.05$) between the treatment and control groups were determined using two-way ANOVA followed by the Holm-Sidak post hoc test.

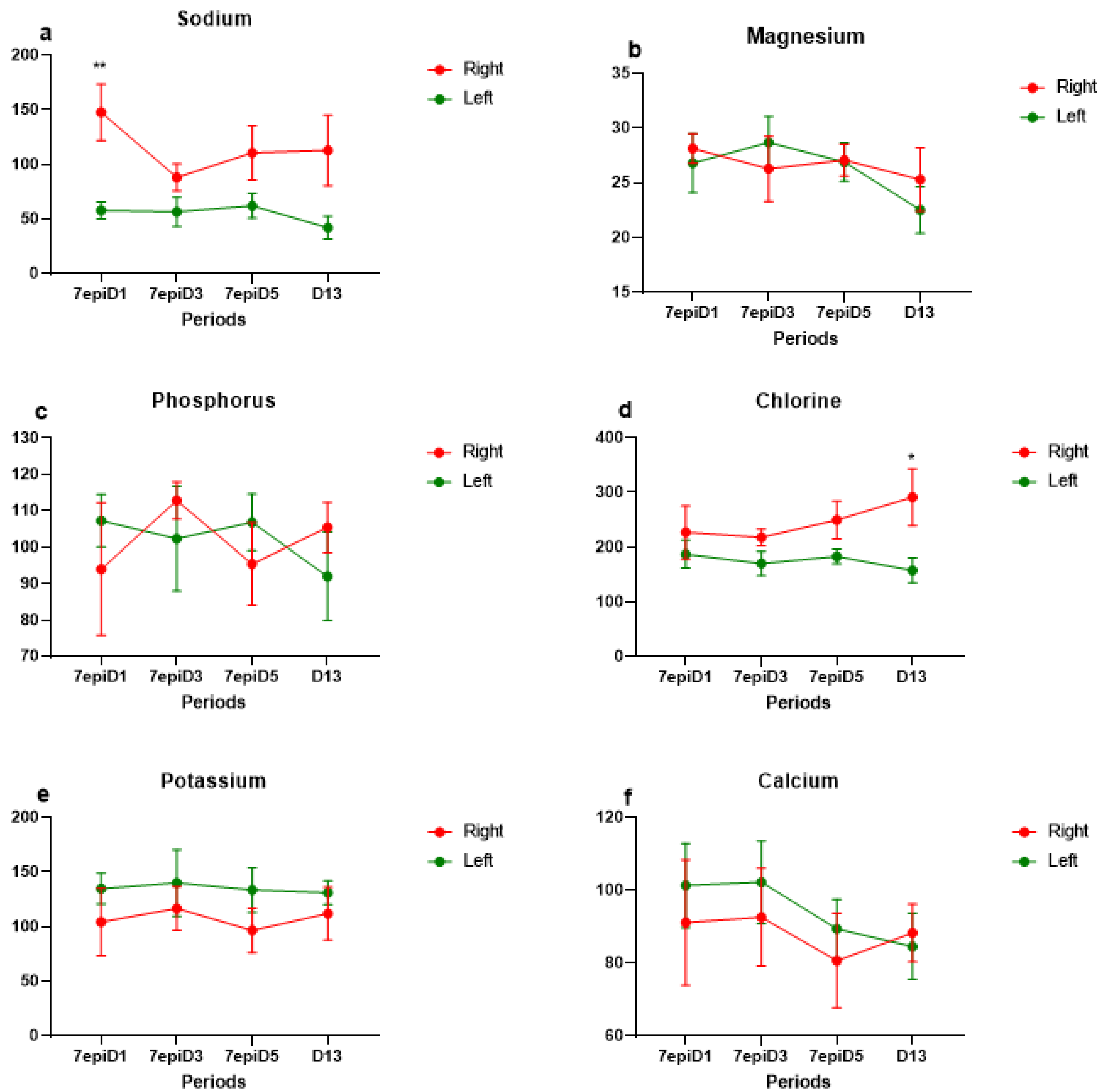

Figure 5

Concentrations of macro elements in goat milk during the treatment of mastitis with 7-epiclusianone. Macro element concentrations in goat milk were measured using X-ray Energy Dispersive Spectroscopy (XEDS) for (a) sodium, (b) magnesium, (c) phosphorus, (d) chlorine, (e) potassium, (f) calcium. Data represent comparisons between the treated (right udder) and control (left udder) groups across the treatment periods 7-epiclusianone Day 1 (7epiD1), 7-epiclusianone Day 3 (7epiD3), 7-epiclusianone Day 5 (7epiD5), and eight days post 7-epiclusianone treatment (D13). Statistically significant differences (*p ≤ 0.05) between the treatment and control groups were determined using two-way ANOVA followed by the Holm-Sidak post hoc test.

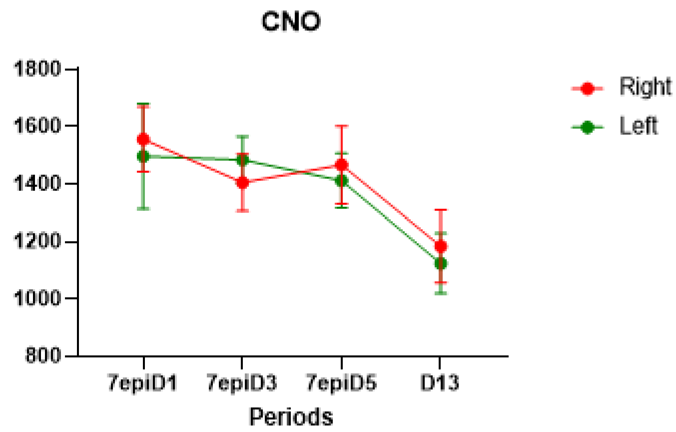

Figure 6

Concentrations of major elements (carbon, nitrogen and oxygen) in goat milk during the treatment of mastitis with 7-epiclusianone. Major element concentrations in goat milk were measured using X-ray Energy Dispersive Spectroscopy (XEDS). Data represent comparisons between the treated (right udder) and control (left udder) groups across the treatment period 7-epiclusianone Day 1 (7epiD1), 7-epiclusianone Day 3 (7epiD3), 7-epiclusianone Day 5 (7epiD5), and eight days post 7-epiclusianone treatment (D13). Statistically significant differences (*p ≤ 0.05) between the treatment and control groups were determined using two-way ANOVA followed by the Holm-Sidak post hoc test.