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Mastitis-Associated Upregulation of S100A7 in Dairy Goat Mammary Tissue and Milk: Correlation with SCC and Diagnostic Performance via ROC Analysis

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ABSTRACT

S100A7, an inflammation-associated protein, plays a critical role in the host's immune defense; however, its relationship with mastitis in dairy goats has not been extensively studied. In this study, based on clinical udder examinations, somatic cell count (SCC), and milk bacteriological culture (BC), a total of 84 dairy goats were classified into three groups: healthy (n = 25), subclinical mastitis (n = 36), and clinical mastitis (n = 23). The concentration of S100A7 in subclinical mastitis goats was significantly higher than in healthy individuals ($p = 0.0056$) but showed no significant variation from clinical mastitis goats ($p = 0.8222$). A weak positive correlation was found between $\log_{10}$ SCC and milk S100A7 concentration ($R = 0.05249$), following the regression equation $Y = 0.1446 \times X + 12.54$. Receiver operating characteristic (ROC) analysis of $\log_{10}$ SCC and S100A7 concentration showed that, for subclinical mastitis goats, the area under the curve (AUC) of $\log_{10}$ SCC was 0.9222 ($p < 0.0001$), while that of S100A7 was 0.7317 ($p = 0.0022$). In clinical mastitis goats, AUC values were 0.9678 ($p < 0.0001$) for $\log_{10}$ SCC and 0.5487 ($p = 0.5634$) for S100A7 concentration. Immunohistochemical analysis revealed that S100A7 was weakly expressed in the alveoli of healthy mammary glands but strongly expressed in the collapsed alveoli of mastitic goats. Overall, S100A7 expression was significantly elevated in mastitic mammary glands compared to healthy ones. These findings highlight the influence of mastitis on S100A7 expression and milk concentration, demonstrating its limited correlation with SCC and suggesting a potential role of S100A7 in the mammary immune defense of dairy goats.

Keywords: S100A7, Somatic cell count, Dairy goat, Mastitis

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Introduction

Mastitis represents one of the most critical health challenges in livestock production. It is primarily caused by microbial infections that hinder curd formation and lower both the yield [1, 2] and quality of milk [3]. Currently, antibiotic treatments make milk unsuitable for the "antibiotic-free" standards required for human consumption and have contributed to the rise of antibiotic-resistant microorganisms [4, 5]. Mastitis continues to be prevalent worldwide, causing substantial financial losses in the dairy industry [3, 6]. Based on diagnostic evaluations of the udder and milk, mastitis is categorized into clinical and subclinical forms [7]. In clinical cases, visible symptoms such as swelling, redness, heat, pain, hardness, and abnormal milk appearance can be observed [7]. Somatic Cell Count (SCC), a key tool for udder health surveillance and herd management, is used to monitor and minimize the impact of mastitis in dairy cattle [8, 9].

In small ruminants, particularly goats, several non-infectious factors affect SCC levels [10], including season, lactation stage, and parity, leading to notable differences among individuals and farms [11]. Moreover, the

holocrine mode of secretion in goat milk contributes to physiological shedding of cytoplasmic material, elevating the normal SCC threshold in healthy animals [12, 13]. Studies suggest that SCC levels of less than 5.0×10^5 or more than 1.0×10^6 cells/mL reliably indicate the absence or presence of subclinical mastitis, respectively [14–16]. However, bacteriological culture (BC) remains the most precise diagnostic approach for mastitis [17]; its cost, sensitivity, and practicality for routine testing present challenges.

Antibacterial components of the innate immune system protect the body from bacterial invasion under normal conditions. Among them, antimicrobial peptides (AMPs) exhibit broad-spectrum antimicrobial activity and have drawn growing interest [18, 19]. One such AMP, S100A7 (psoriasin), is a calcium-binding EF-hand protein [20], first isolated in 1991 from hyperproliferative keratinocytes of psoriasis patients [21]. S100A7 is expressed in epithelial cells [22], forms homodimers via non-covalent interactions [23, 24], and has been identified in bovine [25] and caprine species [26]. However, its expression dynamics during mastitis in small ruminants remain poorly understood.

AMPs such as cathelicidins [27, 28] and lingual antimicrobial peptides [29] have been explored as potential biomarkers for mastitis monitoring through milk concentration measurements [28, 30]. Furthermore, S100A7 recruits specific inflammatory cell subtypes [31] and acts as a chemokine-related protein linked to inflammation [32]. These immune cells form part of milk somatic cells, yet the correlation between S100A7 expression and SCC concentration remains uncertain. Additionally, S100A7 levels rise notably after lipopolysaccharide (LPS) infusion into the mammary gland [26], but its relationship to inflammation severity in goat mastitis has not been clarified. Overall, S100A7 likely plays an important role in the mammary gland's innate immune defense, and investigating its expression in the milk and mammary tissue of dairy goats with mastitis may offer novel insights into its immunological function.

Materials and Methods

Collection of milk and mammary gland samples

All animal handling and experimental activities were approved by the Institutional Animal Care and Use Committee, College of Veterinary Medicine, Northwest A&F University (Approval No. 2019031502). Since mastitis tends to appear early in lactation—most commonly within the first four weeks after kidding and accounting for approximately 74–95% of total cases during the initial three months [33–36]—a total of 84 Guanzhong dairy goats (parity 1–3; body weight: 55 ± 4.8 kg) within that period were randomly chosen from the Hongxing Meiling Dairy Goat Farm, located in Fuping, Shaanxi, China. Goats were kept in open pens with unrestricted access to water and feed. Their diets were balanced according to the Nutrient Requirements of Small Ruminants (National Research Council, 2007) and contained ingredients such as alfalfa hay, corn, wheat bran, soybean meal, wheat straw, silage, corn germ meal, cottonseed meal, calcium hydrophosphate, limestone, sodium carbonate, sodium chloride, and a mineral–vitamin premix. Feedings were conducted twice daily at 07:30 and 15:30.

A healthy udder was characterized by the absence of inflammation and an SCC below 5.0×10^5 cells/mL [14–16]. Clinical mastitis was identified by visible abnormalities such as swelling, redness, hardness, pain, warmth, or altered milk appearance, including clots, flakes, pus, or watery milk [37, 38]. Subclinical mastitis lacked visible changes but yielded positive bacteriological culture results. After disinfecting the teat with 75% alcohol three times and discarding the first streams of milk, morning milk samples (~40 mL) were aseptically collected in sterile 50 mL tubes, transported under chilled conditions for somatic cell examination, or frozen immediately on dry ice for S100A7 analysis. Twelve mammary gland tissue samples were also collected from goats at a local slaughterhouse in Fuping, Shaanxi Province. These tissues were either fixed in 4% paraformaldehyde for paraffin embedding or frozen in liquid nitrogen for RNA extraction [39].

Determination of somatic cell count

Somatic cell enumeration followed the direct microscopic somatic cell count (DMSCC) technique using Diff–Quik staining (Solarbio, G1540, Beijing, China) as described previously [40, 41]. In brief, 10 µL of homogenized milk was placed on a glass slide, spread evenly over approximately 1 cm^2, and fixed in formaldehyde for 5 min. The slide was then sequentially stained with Diff–Quick I and II for 5 min each, rinsed briefly in 95% ethanol (5 s) and 100% ethanol (30 s), cleared with xylene for 1 min, mounted, and examined under a microscope. Cells were counted in 30 random microscopic fields, and SCC per mL was obtained using:

SCC = coefficient × (mean cell number per field = total cells ÷ 30).
Because SCC data deviated from normal distribution, $\log_{10}$ transformation was applied to normalize the dataset [42, 43].

Bacteriological analysis
Bacteriological culturing followed standard protocols [44, 45]. After thorough mixing, 10 µL of milk was inoculated onto agar plates containing 5% sheep blood under biosafety level II conditions and incubated at 37 °C. Colony-forming units (CFUs) were counted after 24–48 h. Plates showing no bacterial growth were re-incubated at 37 °C and rechecked every 24 h for up to 96 h. Samples without colonies were classified as healthy, whereas positive bacterial growth indicated mastitis.

Immunohistochemical examination
Immunohistochemical staining for S100A7 in goat mammary tissues followed a modified version of a previously described procedure [46]. Tissue samples fixed in 4% paraformaldehyde (Solarbio, P1110, Beijing, China) at 4 °C were dehydrated through an ethanol gradient and embedded in paraffin overnight. Sections of 3 µm thickness were prepared, and every fifth section was used for staining. After deparaffinization in xylene and rehydration through graded ethanol, antigen retrieval was performed by heating in citrate buffer (Solarbio, C1031, Beijing, China) for 20 min. Endogenous peroxidase activity was quenched using 3% hydrogen peroxide. Sections were incubated with a primary antibody against S100A7 (1:100, Bioss, bs-6238R, Beijing, China) overnight at 4 °C, followed by incubation with a secondary antibody at 37 °C for 1 h. The slides were counterstained with hematoxylin, dehydrated, mounted, and visualized under a microscope.

Quantification of S100A7 by ELISA
S100A7 concentrations in milk were determined using a commercial ELISA kit (MEIMIAN, MM-7510501, Yancheng, China) according to the manufacturer's directions. The intra-assay and inter-assay coefficients of variation were <10% and <15%, respectively, with a measurement range of 1.25–40 µg/mL. Absorbance was read at 450 nm using a microplate reader (Tecan Group Ltd., Männedorf, Switzerland). In brief, standards, blanks, and samples were added to designated wells. Each test well received 100 µL of HRP-conjugated reagent (except blanks) and was incubated at 37 °C for 60 min. After washing five times, 50 µL each of chromogen solutions A and B were added and incubated for 10 min at 37 °C. The reaction was terminated with 50 µL of stop solution, and the optical density was recorded within 15 min to determine S100A7 concentration.

RNA extraction and cDNA production
Total RNA was extracted using the MiniBEST Universal RNA Extraction Kit (TaKaRa, 9767, Dalian, China). Complementary DNA (cDNA) was synthesized employing the PrimeScript RT Master Mix Kit (TaKaRa, RR036, Dalian, China) according to the manufacturer's protocol. Each reaction mixture contained 0.2 µg total RNA, 2 µL of 5× PrimeScript RT Master Mix, and RNase-free water to reach a final volume of 10 µL. The reverse transcription procedure was conducted at 37 °C for 15 minutes, followed by 85 °C for 5 seconds.

Quantitative real-time PCR
For amplification of the S100A7 gene in goats, the primer sequences used were forward 5′-CCAGCAAGGACAGGAACTCA-3′ and reverse 5′-GCAGCTGCTGAAGGAGAACT-3′. GAPDH served as the internal control with the forward primer 5′-TGCCCGTTCGACAGATAGC-3′ and reverse primer 5′-ACGATGTCCACTTTGCCAGTA-3′. PCR fragments were verified through electrophoresis on a 3% agarose gel (Solarbio, a8350, Beijing, China). Quantitative PCR analysis was carried out using the SYBR Green Premix Pro Taq HS Kit (Accurate Biotechnology Co., Ltd., AG11718, Changsha, China) on a QuantStudio 6 Flex system (Invitrogen, Waltham, MA, USA). Each 20 µL qPCR reaction consisted of 0.2 µg cDNA, 10 µL of 2× SYBR Green mix, 0.2 µL of each primer (10 µM), and RNase-free water. Cycling conditions included an initial step at 95 °C for 30 seconds, followed by 40 amplification cycles at 95 °C for 5 seconds and 60 °C for 30 seconds. Gene expression levels were determined using the $2^{-}\Delta\Delta Ct$ formula, normalized against GAPDH, and expressed as fold differences relative to the control.

Statistical analysis

All data are expressed as mean ± S.E.M., with each experimental condition performed in triplicate or more. For two-group comparisons, unpaired Student's t-tests were used after confirming normality. For comparisons among multiple groups, one-way ANOVA followed by Tukey's post hoc test was employed using GraphPad Prism version 8.0. Statistical thresholds were defined as follows: $p > 0.05$ (ns, not significant), $p < 0.05$ (*, significant), and $p < 0.01$ (**, highly significant).

Results and Discussion

S100A7 levels in healthy, subclinical, and clinical mastitis goats

The comparison of S100A7 concentrations in milk from 84 goats is illustrated in **Figure 1**. Subclinical mastitis samples exhibited a markedly higher S100A7 concentration than healthy samples ($p < 0.01$), whereas clinical mastitis did not differ significantly. Furthermore, subclinical samples displayed significantly greater S100A7 levels than clinical ones ($p < 0.05$).

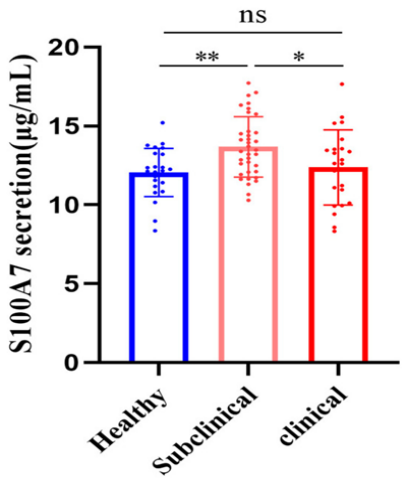
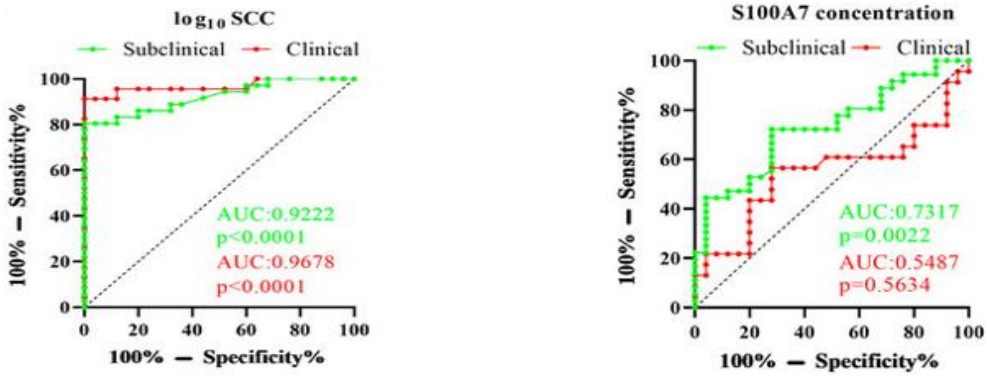


Figure 1. Differences in S100A7 concentrations among milk samples from 84 dairy goats. Healthy (n = 25), Subclinical (n = 36), Clinical (n = 23); ns = $p > 0.05$; * = $p < 0.05$; ** = $p < 0.01$.

Diagnostic evaluation of SCC and S100A7 concentrations

Receiver operating characteristic (ROC) analyses were performed to assess diagnostic sensitivity and specificity [47, 48]. The ROC plots for SCC and S100A7 and their corresponding area under the curve (AUC) values are presented in **Figure 2**. For subclinical mastitis, the AUC of $\log_{10}$ SCC was 0.9222 ($p < 0.0001$), while that for S100A7 concentration was 0.7317 ($p = 0.0022$). For clinical mastitis, the AUC values for $\log_{10}$ SCC and S100A7 were 0.9678 ($p < 0.0001$) and 0.5487 ($p = 0.5634$), respectively.



a) b)

Figure 2. ROC plots of (a) $\log_{10}$ SCC and (b) S100A7 levels in subclinical and clinical mastitis samples. SCC = somatic cell count; AUC = area under the curve; p = probability value.

Correlation between SCC and S100A7 in goat milk

To assess the relationship between S100A7 concentration and somatic cell count, values of $\log_{10}$ SCC and S100A7 concentration from all 84 samples were analyzed. A weak positive association was identified, with R = 0.05249 and the regression equation Y = 0.1446X + 12.54 (**(Figure 3)**, $p > 0.05$).

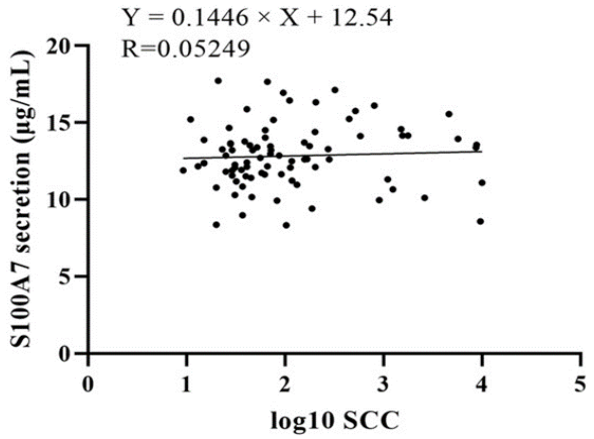


Figure 3. Regression curve describing the association between milk S100A7 concentration and $\log_{10}$ SCC in 84 goat samples. SCC = somatic cell count; R = Pearson correlation coefficient.

Immunohistochemical detection and S100A7 expression in healthy and mastitic dairy goat mammary glands

Immunohistochemical evaluation indicated that S100A7 exhibited faint expression within the alveoli of healthy goat mammary tissue, with no signal in the connective stroma **(Figures 4a and 4b)**. In contrast, strong S100A7 immunoreactivity was evident in the collapsed alveoli of mammary glands from goats affected by clinical mastitis **(Figures 4e and 4f)**. A marked upregulation of S100A7 expression was detected in mastitic samples compared with those from healthy goats (**(Figure 4i)**, $p < 0.01$).

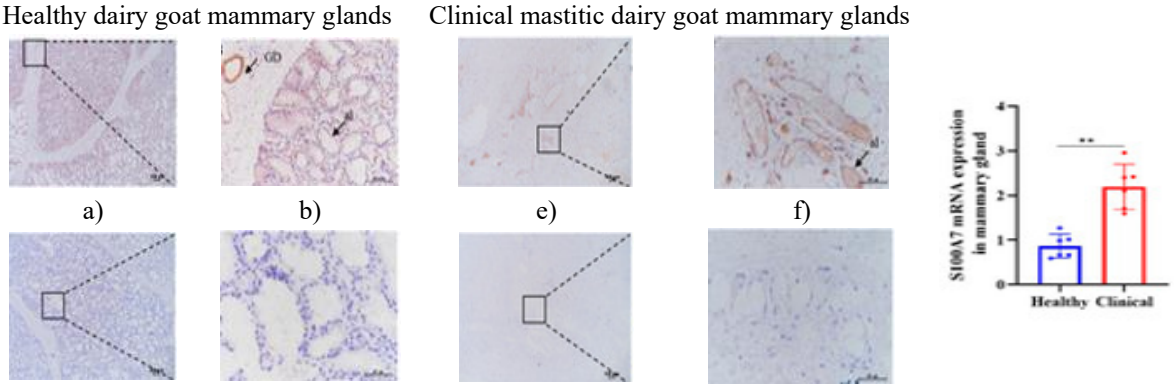


Figure 4. Immunohistochemical staining and S100A7 expression in mammary glands of healthy and mastitic dairy goats. (a, b) Representative images of healthy mammary gland sections (n = 6) stained with S100A7 antibody; (c, d) negative control images for healthy goats; (e, f) representative S100A7 antibody-stained sections from goats with clinical mastitis (n = 6); (g, h) negative control images for mastitic goats; (i) comparison of S100A7 mRNA levels between healthy (n = 6) and mastitic (n = 6) mammary alveoli. al = alveolus; GD = gland duct. **: $p < 0.01$. (a, c, e, g): scale bar = 100 μm; (b, d, f, h): scale bar = 50 μm.

Mastitis remains one of the most significant mammary gland diseases, responsible for considerable economic loss in the global dairy industry [3, 6]. Antibiotic therapy continues to be the conventional control and treatment method, yet it can negatively affect milk quality and promote antimicrobial resistance [4, 5]. Therefore, alternative control strategies are required to support sustainable dairy production. Antimicrobial peptides (AMPs) have gained considerable attention due to their broad-spectrum antibacterial efficacy and minimal likelihood of resistance development [18, 19], making them promising candidates for antibiotic-free mastitis management.

Somatic cell count (SCC) is widely recognized as a vital indicator of milk quality and udder condition [49], and it serves as a key tool in mastitis monitoring. While automated methods such as Fossomatic are used for SCC evaluation [41], classical approaches like bacteriological culture, direct microscopic somatic cell count (DMSCC), and the California Mastitis Test (CMT) [50] remain fundamental diagnostic techniques.

DMSCC is considered practical for field application by trained veterinarians or technicians and has proven reliable in ewe milk testing [41]. In this research, somatic cells were stained using the Diff stain and enumerated, though subjectivity in interpretation remains a limitation, as results may vary among observers. Somatic cells, which include epithelial and immune cells [51], were markedly elevated in mastitic goat milk—consistent with prior studies [51]. Distinct cell populations, including macrophages, lymphocytes, neutrophils, and erythrocytes, were noted. The detection of red blood cells further indicated disruption of the blood–milk barrier in infected mammary tissues.

It has been suggested that SCC values below 5.0×10^5 cells/mL denote healthy glands, whereas counts exceeding 1.0×10^6 cells/mL may signal subclinical mastitis [14–16]. Nevertheless, in this study, even bacteriologically positive subclinical and clinical mastitis cases occasionally displayed SCC levels below these thresholds. Despite equal parity among experimental groups, variation in SCC persisted. These observations support the notion that SCC, as an indirect indicator in goats, can be affected by non-infectious parameters such as parity, season, and lactation stage—leading to variable reliability and the absence of universally accepted thresholds for small ruminants.

Multiple antimicrobial substances contribute to mammary gland defense, being synthesized and secreted into milk [52]. Among these, AMPs—including cathelicidins, lactoferrin, and defensins—play critical roles. Owing to their strong correlation with SCC and potential use as biomarkers, AMPs have emerged as attractive targets for mastitis monitoring and ELISA-based diagnostic development [28, 30].

S100A7 is one of the antimicrobial peptides (AMPs) exhibiting strong antibacterial potential. In this research, the concentration of S100A7 showed only a weak correlation with SCC, and its AUC value was lower than that of $\log_{10}$ SCC in both subclinical and clinical mastitic goats. Furthermore, the correlation coefficient between SCC and S100A7 levels was smaller than that of other AMPs, indicating its limited effectiveness as a mastitis indicator. Circulating components may significantly influence the synthesis and secretion of S100A7. Previous studies have demonstrated that butyrate can enhance S100A7 production in mammary epithelial cells and that direct infusion of butyrate into the mammary gland via the teat increases S100A7 concentration [53]. Since S100A7 is a calcium-binding protein [21], its concentration is expected to be associated with calcium levels. Circulating leukocytes, which serve as key immune cells and constitute the majority of milk somatic cells, may act as an additional source of S100A7, as S100A8-positive cells have been identified as leukocytes in goat milk [54]; however, this relationship remains to be clarified.

In the current study, S100A7 expression was localized to the alveoli of healthy mammary glands. Upon microbial invasion, S100A7 was rapidly synthesized and secreted into the milk to combat infection. Interestingly, the S100A7 concentration was markedly elevated in subclinical mastitis but showed only a slight increase in clinical mastitis goats. This reduction in severe cases may be linked to the impaired alveolar structure and disrupted secretion in clinical infections. In goats with clinical mastitis, curd formation and decreased milk yield were commonly observed. Additionally, in human keratinocytes, S100A7 secretion follows a biphasic pattern, with distinct dynamics during acute and chronic phases [55]. Similarly, in goat mammary epithelial cells, its secretion is influenced by both duration and concentration of LPS exposure—peaking around 12 h and declining within 48 h—suggesting that S100A7 is produced and released in a rapid and transient manner [46]. This pattern likely occurs in the mammary epithelial cells of dairy goats as well.

Individual variability may also limit the reliability of S100A7 as a biomarker. The baseline concentration of S100A7 in healthy goats (8.36–15.20 μg/mL) exhibited a wide range in this study, consistent with prior findings [26]. A similar variation was also detected in subclinical and clinical mastitic goats. This research mainly focused on the early lactation period, during which mastitis incidence is highest, and thus does not encompass the full lactation cycle. The diversity of pathogenic microorganisms is another critical factor—particularly E. coli, against which S100A7 shows strong antibacterial activity [56, 57]—whereas its activity against other pathogens, such as Staphylococcus aureus, is limited or absent [25]. Nonetheless, the specific E. coli strain does not solely determine mastitis severity; strain variation between subclinical and clinical cases may influence S100A7 abundance, and this relationship remains unresolved.

The teat epithelium plays a crucial role in host defense and is recognized as the primary site of S100A7 synthesis and secretion in both bovine [58] and goat species [26]. Keratinocytes from the teat epithelium are therefore essential to understanding S100A7 expression patterns. S100A7 levels in milk increase following frequent teat stimulation, regardless of milk removal [59]. Moreover, inflammatory cytokines such as IL-1β can upregulate S100A7 production [60]. In summary, S100A7 contributes significantly to the innate immune protection of the goat mammary gland; however, its synthesis and secretion are affected by multiple physiological and environmental factors, requiring further investigation.

Conclusion

S100A7 expression was significantly upregulated in the mammary glands of mastitic dairy goats compared to healthy ones. In healthy goats, S100A7 showed weak expression within alveoli, whereas in mastitic glands, it was intensely detected in collapsed alveolar regions. The S100A7 concentration was notably higher in subclinical mastitis goats but showed no significant difference in clinical cases relative to healthy goats. Overall, the weak correlation between S100A7 concentration and SCC suggests that its reliability as a mastitis monitoring marker in dairy goats is limited.

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References

1. Blosser TH. Economic losses from and the national research program on mastitis in the United States. J Dairy Sci. 1979;62(1):119–27.
2. Hagnestam C, Emanuelson U, Berglund B. Yield losses associated with clinical mastitis occurring in different weeks of lactation. J Dairy Sci. 2007;90(1):2260–70.
3. Kayano M, Itoh M, Kusaba N, Hayashiguchi O, Kida K, Tanaka Y, et al. Associations of the first occurrence of pathogen-specific clinical mastitis with milk yield and milk composition in dairy cows. J Dairy Res. 2018;85(3):309–16.
4. Koop G, van Werven T, Toft N, Nielen M. Estimating test characteristics of somatic cell count to detect Staphylococcus aureus-infected dairy goats using latent class analysis. J Dairy Sci. 2011;94(1):2902–11.
5. Boireau C, Cazeau G, Jarrige N, Calavas D, Madec JY, Leblond A, et al. Antimicrobial resistance in bacteria isolated from mastitis in dairy cattle in France, 2006–2016. J Dairy Sci. 2018;101(1):9451–62.
6. Romero J, Benavides E, Meza C. Assessing financial impacts of subclinical mastitis on Colombian dairy farms. Front Vet Sci. 2018;5(1):273.
7. De Vliegher S, Fox LK, Piepers S, McDougall S, Barkema HW. Invited review: Mastitis in dairy heifers: Nature of the disease, potential impact, prevention, and control. J Dairy Sci. 2012;95(1):1025–40.
8. Gillespie BE, Lewis MJ, Boonyayatra S, Maxwell ML, Saxton A, Oliver SP, et al. Short communication: Evaluation of bulk tank milk microbiological quality of nine dairy farms in Tennessee. J Dairy Sci. 2012;95(1):4275–9.
9. Jayarao BM, Pillai SR, Sawant AA, Wolfgang DR, Hegde NV. Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. J Dairy Sci. 2004;87(1):3561–73.
10. Lafi SQ, Al-Majali AM, Rousan MD, Alawneh JM. Epidemiological studies of clinical and subclinical ovine mastitis in Awassi sheep in northern Jordan. Prev Vet Med. 1998;33(1):171–81.
11. Schaeren W, Maurer J. Prevalence of subclinical udder infections and individual somatic cell counts in three dairy goat herds during a full lactation. Schweiz Arch Tierheilkd. 2006;148(1):641–8.
12. Paape MJ, Capuco AV. Cellular defense mechanisms in the udder and lactation of goats. J Anim Sci. 1997;75(1):556–65.

13. Poutrel B, de Crémoux R, Ducelliez M, Verneau D. Control of intramammary infections in goats: Impact on somatic cell counts. J Anim Sci. 1997;75(1):566–70.
14. Contreras A, Sierra D, Corrales JC, Sanchez A, Marco JLA, Contreras JA, et al. Physiological threshold of somatic cell count and California Mastitis Test for diagnosis of caprine subclinical mastitis. Small Rumin Res. 1996;21(1):259–64.
15. Contreras A, Luengo C, Sánchez A, Corrales JC. The role of intramammary pathogens in dairy goats. Livest Prod Sci. 2003;79(1):273–83.
16. Hall SM, Rycroft AN. Causative organisms and somatic cell counts in subclinical intramammary infections in milking goats in the UK. Vet Rec. 2007;160(1):19–22.
17. Sears PM, Smith BS, English PB, Herer PS, Gonzalez RN. Shedding pattern of Staphylococcus aureus from bovine intramammary infections. J Dairy Sci. 1990;73(1):2785–9.
18. Pieterse R, Todorov SD. Bacteriocins—exploring alternatives to antibiotics in mastitis treatment. Braz J Microbiol. 2010;41(1):542–62.
19. Moravej H, Moravej Z, Yazdanparast M, Heiat M, Mirhosseini A, Moosazadeh Moghaddam M, et al. Antimicrobial peptides: Features, action, and their resistance mechanisms in bacteria. Microb Drug Resist. 2018;24(1):747–67.
20. Hoffmann HJ, Olsen E, Etzerodt M, Madsen P, Thøgersen HC, Kruse T, et al. Psoriasin binds calcium and is upregulated by calcium to levels that resemble those observed in normal skin. J Invest Dermatol. 1994;103(1):370–5.
21. Madsen P, Rasmussen HH, Leffers H, Honoré B, Dejgaard K, Olsen E, et al. Molecular cloning, occurrence, and expression of a novel partially secreted protein "psoriasin" that is highly up-regulated in psoriatic skin. J Invest Dermatol. 1991;97(1):701–12.
22. Schäfer BW, Heizmann CW. The S100 family of EF-hand calcium-binding proteins: Functions and pathology. Trends Biochem Sci. 1996;21(1):134–40.
23. Brodersen DE, Etzerodt M, Madsen P, Celis JE, Thøgersen HC, Nyborg J, et al. EF-hands at atomic resolution: The structure of human psoriasin (S100A7) solved by MAD phasing. Structure. 1998;6(1):477–89.
24. Donato R. Functional roles of S100 proteins, calcium-binding proteins of the EF-hand type. Biochim Biophys Acta. 1999;1450(1):191–231.
25. Regenhard P, Leippe M, Schubert S, Podschun R, Kalm E, Grötzinger J, et al. Antimicrobial activity of bovine psoriasin. Vet Microbiol. 2009;136(1):335–40.
26. Zhang GW, Lai SJ, Yoshimura Y, Isobe N. Messenger RNA expression and immunolocalization of psoriasin in the goat mammary gland and its milk concentration after an intramammary infusion of lipopolysaccharide. Vet J. 2014;202(1):89–93.
27. Zhang GW, Lai SJ, Yoshimura Y, Isobe N. Expression of cathelicidins mRNA in the goat mammary gland and effect of the intramammary infusion of lipopolysaccharide on milk cathelicidin-2 concentration. Vet Microbiol. 2014;170(1):125–34.
28. Tedde V, Bronzo V, Puggioni GMG, Pollera C, Casula A, Curone G, et al. Milk cathelicidin and somatic cell counts in dairy goats along the course of lactation. J Dairy Res. 2019;86(1):217–21.
29. Kawai K, Akamatsu H, Obayashi T, Nagahata H, Higuchi H, Iwano H, et al. Relationship between concentration of lingual antimicrobial peptide and somatic cell count in milk of dairy cows. Vet Immunol Immunopathol. 2013;153(1):298–301.
30. Addis MF, Tedde V, Puggioni GMG, Pisanu S, Casula A, Locatelli C, et al. Evaluation of milk cathelicidin for detection of bovine mastitis. J Dairy Sci. 2016;99(1):8250–8.
31. Wolf R, Howard OM, Dong HF, Voscopoulos C, Boeshans K, Winston J, et al. Chemotactic activity of S100A7 (Psoriasin) is mediated by the receptor for advanced glycation end products and potentiates inflammation with highly homologous but functionally distinct S100A15. J Immunol. 2008;181(1):1499–506.
32. Gross SR, Sin CG, Barraclough R, Rudland PS. Joining S100 proteins and migration: For better or for worse, in sickness and in health. Cell Mol Life Sci. 2014;71(1):1551–79.
33. Vogel A, Hutchison BL, Mitchell EA. Mastitis in the first year postpartum. Birth. 1999;26(1):218–25.
34. Michie C, Lockie F, Lynn W. The challenge of mastitis. Arch Dis Child. 2003;88(1):818–21.

35. Amir LH, Forster DA, Lumley J, McLachlan H. A descriptive study of mastitis in Australian breastfeeding women: Incidence and determinants. BMC Public Health. 2007;7(1):62.
36. Scott JA, Robertson M, Fitzpatrick J, Knight C, Mulholland S. Occurrence of lactational mastitis and medical management: A prospective cohort study in Glasgow. Int Breastfeed J. 2008;3(1):21.
37. Contreras GA, Rodríguez JM. Mastitis: Comparative etiology and epidemiology. J Mammary Gland Biol Neoplasia. 2011;16(1):339–56.
38. Koop G, Islam MN, Rahman MM, Khatun M, Ferdous J, Sayeed MA, et al. Risk factors and therapy for goat mastitis in a hospital-based case-control study in Bangladesh. Prev Vet Med. 2016;124(1):52–7.
39. Liu H, Zhao Y, Wu Y, Yan Y, Zhao X, Wei Q, et al. NF-κB-dependent Snail expression promotes epithelial–mesenchymal transition in mastitis. Animals. 2021;11(1):3422.
40. Windria S, Salasia SIO, Nugroho W, Widayanti R, Indarjulianto S. Development of ELISA against milk haptoglobin for diagnosis of subclinical mastitis in goats. Heliyon. 2021;7(1):e06314.
41. Gonzalo C, Martínez JR, Carriedo JA, San Primitivo F. Fossomatic cell-counting on ewe milk: Comparison with direct microscopy and study of variation factors. J Dairy Sci. 2003;86(1):138–45.
42. Wicks HC, Leaver JD. Influence of genetic merit and environment on somatic cell counts of Holstein-Friesian cows. Vet J. 2006;172(1):52–7.
43. Silva R, Lôbo RNB, El Faro L, Dos Santos GG, Bruneli FÂT, Peixoto M. Genetic parameters for somatic cell count (SCC) and milk production traits of Guzerá cows using data normalized by different procedures. Trop Anim Health Prod. 2020;52(1):2513–22.
44. Contreras A, Paape MJ, Di Carlo AL, Miller RH, Rainard P. Evaluation of selected antibiotic residue screening tests for milk from individual goats. J Dairy Sci. 1997;80(1):1113–8.
45. Persson Y, Olofsson I. Direct and indirect measurement of somatic cell count as indicator of intramammary infection in dairy goats. Acta Vet Scand. 2011;53(1):15.
46. Yan Y, Zhu K, Fan M, Wan W, Zhao X, Pan M, et al. Immunolocalization of antibacterial peptide S100A7 in mastitis goat mammary gland and lipopolysaccharide induces expression and secretion of S100A7 in mammary epithelial cells via TLR4/NFκB pathway. Anim Biotechnol. 2023;34(1):2701–13.
47. Samara EM, Ayadi M, Aljumaah RS. Feasibility of utilising an infrared-thermographic technique for early detection of subclinical mastitis in dairy camels (Camelus dromedarius). J Dairy Res. 2014;81(1):38–45.
48. McDougall S, Murdough P, Pankey W, Delaney C, Barlow J, Scruton D. Relationships among somatic cell count, California mastitis test, impedance and bacteriological status of milk in goats and sheep in early lactation. Small Rumin Res. 2001;40(1):245–54.
49. Franceschi P, Faccia M, Malacarne M, Formaggioni P, Summer A. Quantification of cheese yield reduction in manufacturing Parmigiano Reggiano from milk with non-compliant somatic cells count. Foods. 2020;9(1):212.
50. Schalm OW, Noorlander DO. Experiments and observations leading to development of the California mastitis test. J Am Vet Med Assoc. 1957;130(1):199–204.
51. Alhussien MN, Dang AK. Milk somatic cells, factors influencing their release, future prospects, and practical utility in dairy animals: An overview. Vet World. 2018;11(1):562–77.
52. Isobe N. Control mechanisms for producing antimicrobial factors in ruminant mammary gland. Anim Sci J. 2017;88(1):937–43.
53. Tsugami Y, Suzuki N, Nii T, Isobe N. Sodium acetate and sodium butyrate differentially upregulate antimicrobial component production in mammary glands of lactating goats. J Mammary Gland Biol Neoplasia. 2022;27(1):133–44.
54. Purba FY, Nii T, Yoshimura Y, Isobe N. Production of antimicrobial peptide S100A8 in the goat mammary gland and effect of intramammary infusion of lipopolysaccharide on S100A8 concentration in milk. J Dairy Sci. 2019;102(1):4674–81.
55. Bhatt T, Bhosale A, Bajantri B, Mathapathi MS, Rizvi A, Scita G, et al. Sustained secretion of the antimicrobial peptide S100A7 is dependent on the downregulation of caspase-8. Cell Rep. 2019;29(1):2546–55.e4.
56. Gläser R, Harder J, Lange H, Bartels J, Christophers E, Schröder JM. Antimicrobial psoriasin (S100A7) protects human skin from Escherichia coli infection. Nat Immunol. 2005;6(1):57–64.
57. Regenhard P, Petzl W, Zerbe H, Sauerwein H. The antibacterial psoriasin is induced by E. coli infection in the bovine udder. Vet Microbiol. 2010;143(1):293–8.

58. Tetens J, Friedrich JJ, Hartmann A, Schwerin M, Kalm E, Thaller G. The spatial expression pattern of antimicrobial peptides across the healthy bovine udder. J Dairy Sci. 2010;93(1):775–83.
59. Tsugami Y, Harada R, Nii T, Suzuki N, Isobe N. Effects of frequent teat stimulation on antimicrobial component production in mammary glands of lactating goats. Vet Immunol Immunopathol. 2022;249(1):110431.
60. EisenbeiB D, Ardebilli S, Harder J, Lange H, Rudolph B, Schröder J, et al. The antimicrobial protein psoriasin (S100A7) is significantly upregulated in the peripheral blood of psoriasis patients. J Investig Dermatol. 2006;126(1):61.