

Research article

Histopathological Characteristics of Milk Calcification in the Udder of Dairy Cows



Faculty of Agriculture
University of Novi Sad

Ivana Davidov^{ID*}, Aleksandar Božić^{ID}, Annamaria Galfi Vukomanović^{ID}, Ksenija Čobanović^{ID}, Nikola Davidov^{ID}, Bojana Blagojević^{ID}, Željka Jurakić^{ID}



OPEN ACCESS

Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia

*Corresponding author: annamariagalfi@gmail.com

Submitted: 5.2.2026.
Reviewed: 23.3.2026.
Accepted: 30.4.2026.

ABSTRACT

Milk calcification within mammary acini is an uncommon histopathological finding in dairy cows, potentially affecting glandular function and milk quality. This study aimed to characterize milk calcification in udder quarters of cows removed from production. A total of 104 mammary complexes from 26 cows were examined. Ultrasonography revealed normal parenchymal appearance in all quarters, while histopathology detected calcifications in a substantial proportion of samples. Milk calcification was classified as mild, moderate or severe, ranging from small isolated deposits to massive lumen-filling mineralized masses with epithelial degeneration. Mean somatic cell count (SCC) increased with calcification severity, suggesting a link with the local inflammatory response. The findings indicate that milk calcification may remain undetected by ultrasonography, yet represents a significant pathological alteration in the mammary gland. Histopathological evaluation is essential for accurate assessment of udder health and for understanding the potential impact of mineralized deposits on milk production.

Key words:

milk calcification,
udder, dairy cow,
histopathology,
somatic cell count

Citation:

Davidov, I., Božić, A., Galfi Vukomanović, A., Čobanović, K., Davidov, N., Blagojević, B. & Jurakić, Ž. 2026. Histopathological Characteristics of Milk Calcification in the Udder of Dairy Cows. *Contemporary Agriculture*, 0(0): xx-xx. <https://doi.org/10.2478/contagri-2026-0010>

INTRODUCTION

The bovine mammary gland is a highly specialized organ whose primary function is the synthesis, storage and secretion of milk. Its complex anatomical and histological organization, consisting of alveoli (acini), ducts and supporting connective tissue, is essential for maintaining normal lactation and overall animal health (Jaswal et al., 2022; Chissico Júnior et al., 2025). The integrity of the epithelial lining of milk acini, together with adequate drainage through the ductal system, enables continuous milk flow (Jaswal et al., 2022) and prevents pathological accumulation of secretory products. Any disruption of this delicate structure may result in functional impairment of the gland, leading to reduced milk yield, altered milk composition or permanent exclusion of the animal from production (Gammariello et al., 2024; Zalewska et al., 2025).

A wide range of pathological processes can affect the mammary gland of dairy cows, including inflammatory, degenerative, metabolic and circulatory disorders (Egyedy and Ametaj, 2022; Rafa et al., 2023). Chronic conditions, in particular, are often associated with irreversible structural changes such as fibrosis, epithelial degeneration and luminal obstruction. These alterations may create conditions favorable for abnormal deposition of mineral substances within the glandular tissue (Bonelli et al., 2020). One of the less frequently described histopathological findings in the bovine mammary gland is calcification of milk content within the acini.

Milk calcification may develop as a consequence of prolonged milk stasis, degenerative changes of secretory epithelium, chronic inflammatory processes or systemic metabolic disturbances affecting calcium and phosphorus homeostasis (Seminara et al., 2023). Under such conditions, changes in the physicochemical environment of the

alveolar lumen can promote precipitation of mineral salts, resulting in the formation of calcified deposits. The presence of calcifications within milk acini indicates a disturbed mineral balance and altered secretory dynamics and may further contribute to mechanical obstruction and progressive glandular damage (Kim et al., 2015).

Although tissue calcifications in various organs of animals have been well documented in veterinary pathology, reports focusing specifically on milk calcification in the mammary glands of cows remain scarce. This is especially true for studies based on detailed histological examination of multiple samples obtained from animals removed from production. Consequently, the biological significance and pathogenetic mechanisms of this phenomenon are still not fully understood.

The aim of this study was to perform a histopathological analysis of milk calcification within mammary acini in cows excluded from milk production and to discuss its potential pathogenetic significance in relation to mammary gland dysfunction.

MATERIAL AND METHODS

The study was conducted on a total of 104 mammary complexes obtained from 26 cows of different ages that had been excluded from production for various reasons (reproductive disorders, decreased milk yield, chronic diseases and other causes). Prior to slaughter, all animals underwent a clinical and ultrasonographic examination of the udder in order to assess the morphological condition of the mammary gland and to identify potential pathological changes.

Ultrasonographic examination of the udder was performed transcutaneously using a real-time ultrasound device equipped with a linear probe operating at a frequency of 6–8 MHz. Before examination, the udder was washed with lukewarm water, cleaned of impurities and dried with paper towels, after which an ultrasound coupling gel was applied to the skin to ensure adequate acoustic contact. All four udder quarters were examined, with the probe positioned longitudinally and transversely relative to the teat axis and mammary parenchyma. Particular attention was paid to parenchymal homogeneity and echogenicity, as well as the appearance of milk acini and the ductal system.

Milk samples were collected from all four udder quarters. Prior to sampling, teat ends were thoroughly disinfected. A milk sample bottle from each udder quarter was labeled with the cow identification number. Somatic cell count was determined using the fluoro-opto-electronic method with a Fossomatic analyzer (Foss Electric, Hillerød, Denmark). Subclinical mastitis was defined as a somatic cell count exceeding 400×10^3 cells/mL.

After slaughter, a tissue sample of approximately 1 cm² was collected from each mammary complex and fixed in 10% neutral buffered formalin. Following standard histological processing, the samples were dehydrated through increasing concentrations of alcohol, embedded in paraffin and sectioned at a thickness of 5 µm.

Histological sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope. Special attention was given to the structure of milk acini, epithelial morphology, luminal content and the presence of mineralized masses within the alveoli. The findings were described qualitatively, with the frequency of calcification occurrence recorded in the examined samples. Histopathological findings of milk calcification within the acini were classified semi-quantitatively as follows: mild – presence of single, small calcified deposits within the acinar lumen; moderate – multiple calcifications with partial filling of the alveolar lumen; severe – massive, compact calcified deposits associated with marked acinar dilatation and accompanying degenerative changes of the epithelial lining.

The relationship between the histopathological severity of milk calcification and somatic cell count (SCC) was evaluated descriptively. Data were summarized as mean SCC values for each semi-quantitative category of calcification (mild, moderate, severe). No formal statistical tests were applied due to the absence of sufficient quantitative data for ultrasonographic echogenicity.

RESULTS AND DISCUSSION

Histopathological examination of mammary tissue samples revealed the presence of milk calcification within mammary acini in a substantial proportion of the examined udder quarters. Calcified deposits were localized exclusively within the acinar lumina and varied in size, number and distribution among samples.

Based on a semi-quantitative assessment, milk calcification was classified as mild, moderate or severe. Mild calcification was characterized by single or a few small deposits within the acinar lumen, with preserved acinar architecture and intact epithelium (Figure 1). Moderate calcification was defined by multiple deposits partially filling the acinar lumen, often associated with acinar dilatation and early epithelial degeneration (Figure 2). Severe calcification, observed in a smaller number of samples, consisted of massive, compact mineralized material occupying a large portion or the entirety of the acinar lumen, accompanied by pronounced acinar enlargement and marked epithelial degeneration (Figure 3).

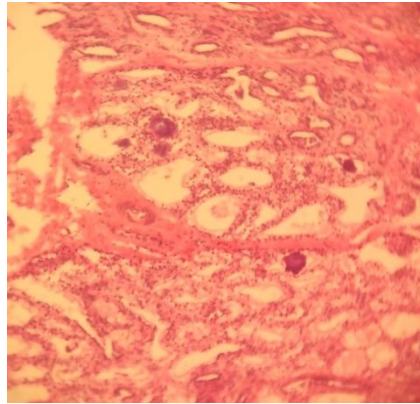


Figure 1. Mammary acinus with mild milk calcification. Single, small calcified deposits are present within the acinar lumen, with preserved acinar architecture and intact epithelial lining. H&E 40x

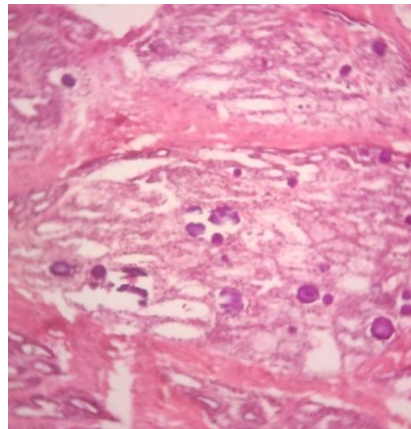


Figure 2. Moderate milk calcification within mammary acini. Multiple calcified deposits partially fill the acinar lumen and are associated with acinar dilatation and early degenerative changes of the secretory epithelium. H&E 40x

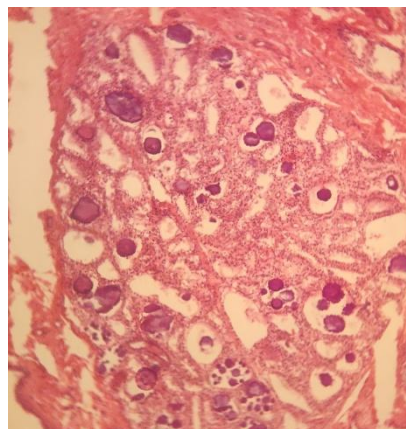


Figure 3. Severe milk calcification in the mammary gland. Massive, compact calcified material occupies the acinar lumen, causing marked acinar enlargement and pronounced epithelial degeneration. H&E 40x

Ultrasonographic examination of the udder revealed normal parenchymal appearance in all examined quarters. Calcified deposits were detected only by histopathological analysis, indicating that milk calcification may not be identifiable by routine ultrasonographic examination.

The distribution of histopathological findings and mean somatic cell count (SCC) values according to the degree of milk calcification is presented in [Table 1](#). Udder quarters without histopathological evidence of calcification

exhibited the lowest mean SCC values, while a progressive increase in mean SCC was observed with increasing severity of calcification.

Table 1. Histopathological severity of milk calcification and mean SCC in examined udder quarters

Histopathology	No of quarters	Mean SCCx10 ³ /ml
No calcification	39	330
Mild calcification	34	410
Moderate calcification	23	460
Severe calcification	8	480

Milk calcification within mammary acini is a relatively uncommon histopathological finding in dairy cows and its occurrence has been insufficiently described in the literature. The present study demonstrates that calcified deposits can develop within the acinar lumina even in the absence of ultrasonographically detectable changes, highlighting the importance of histological examination for the accurate identification of this phenomenon.

The observed pattern of calcification, ranging from single small deposits to massive, lumen-filling mineralized masses, suggests a progressive process associated with chronic alterations of the mammary gland. Mild calcification, with preserved acinar architecture, may represent early or subclinical changes, whereas moderate and severe calcification are associated with acinar dilatation, epithelial degeneration and structural disruption. These findings are consistent with previous reports indicating that prolonged milk stasis, chronic inflammation and degenerative changes of the secretory epithelium may contribute to mineral deposition within the mammary gland (Schadt, 2023; Stanek et al., 2024).

The progressive increase in SCC observed in udder quarters (Sumon et al., 2020) with more severe calcification indicates an association between milk mineralization and local inflammatory response. Elevated SCC values are commonly interpreted as a marker of subclinical or chronic mastitis (Bisutti et al., 2024; Danieli et al., 2025), suggesting that calcification may develop in chronically affected tissue or exacerbate local inflammation. This observation aligns with the hypothesis that mechanical obstruction caused by mineral deposits may impair milk outflow, favoring persistent inflammation and cellular infiltration.

The absence of detectable calcifications on ultrasonography emphasizes the limitations of routine imaging in identifying mineralized deposits within the acini. While ultrasonography is effective for evaluating parenchymal homogeneity and identifying gross structural abnormalities, small or intraluminal calcifications may remain undetected. Therefore, histopathological examination remains the gold standard for precise characterization of milk calcification, particularly in cows removed from production due to chronic mammary disorders.

From a practical perspective, the identification of milk calcification has implications for herd management and milk production. Although the clinical impact of mild calcification may be limited, moderate and severe changes are likely to compromise glandular function, reduce milk yield and increase susceptibility to inflammatory processes. These alterations may contribute to the decision to remove affected cows from production, emphasizing the relevance of histopathological evaluation in assessing udder health and long-term productivity.

CONCLUSION

This study provides detailed histopathological evidence of milk calcification in dairy cows excluded from production and highlights its association with chronic epithelial degeneration and elevated SCC. The findings demonstrate that milk calcification represents a pathological alteration of the mammary gland that may remain undetected by ultrasonography and underscore the value of histological analysis for comprehensive assessment of udder health. Further research is warranted to elucidate the underlying pathogenetic mechanisms and potential metabolic or systemic factors contributing to the development of mineralized deposits within the mammary acini.

Authors' Contribution: Conceptualization: I.D. and A.B.; Methodology: I.D.; Software: N.D. and Ž.J.; Validation: I.D., A.G.V. and K.Č.; Formal Analysis: I.D. and A.G.V.; Investigation: I.D. and A.G.V.; Resources: I.D. and N.D.; Data Curation: I.D. and B.B.; Writing – Original Draft Preparation: I.D.; Writing – Review & Editing: I.D. and A.B.; Visualization: N.D. and Ž.J.; Supervision: A.B.; Project Administration: B.B.; Funding Acquisition: Ž.J. All authors have read and agreed to the published version of the manuscript.

Authors' ORCID iDs: Ivana Davidov  (<https://orcid.org/0000-0003-2630-3061>); Aleksandar Božić  (<https://orcid.org/0000-0003-4991-1051>); Annamaria Galfi Vukomanović  (<https://orcid.org/0000-0002-5361-1070>); Ksenija Čobanović 

(<https://orcid.org/0000-0003-3810-9602>); Nikola Davidov  (<https://orcid.org/0009-0003-4990-1934>); Bojana Blagojević  (<https://orcid.org/0000-0003-4255-6130>); Željka Jurakić  (<https://orcid.org/0000-0002-2981-4935>)

Conflict of interest: The authors declare that they have no conflict of interest.

REFERENCES

- Bisutti, V., Vanzin, A., Pegolo, S., Toscano, A., Giancesella, M., Sturaro, E., Schiavon, S., Gallo, L., Tagliapietra, F., Giannuzzi, D. & Cecchinato A. 2024. Effect of intramammary infection and inflammation on milk protein profile assessed at the quarter level in Holstein cows. *Journal of Dairy Science*, 107(3): 1413-1426. <https://doi.org/10.3168/jds.2023-23818>
- Bonelli, F., Orsetti, C., Turini, L., Meucci, V., Pierattini, A., Sgorbini, M., & Citi S. 2020. Mammary Cistern Size during the Dry Period in Healthy Dairy Cows: A Preliminary Study for an Ultrasonographic Evaluation. *Animals (Basel)*, 10(11): 2082. <https://doi.org/10.3390/ani10112082>
- Chissico Júnior, F., Santos da Silva, T., Vieira Meirelles, F., Monzani, P. S., Fornari Laurindo, L., Maria Barbalho, S. & Miglino, M. A. 2025. A Review on Bioengineering the Bovine Mammary Gland: The Role of the Extracellular Matrix and Reconstruction Prospects. *Bioengineering*, 12(5): 501. <https://doi.org/10.3390/bioengineering12050501>
- Danieli, B., Schogor, A.L.B., Zucchi, J., & Neto, A.T. 2025. Cows with High SCC Exhibit Poorer Performance and Milk Quality, Regardless of the Season. *Dairy*, 6(4): 46. <https://doi.org/10.3390/dairy6040046>
- Egyedy, A. F. & Ametaj, B. N. 2022. Mastitis: Impact of Dry Period, Pathogens, and Immune Responses on Etiopathogenesis of Disease and its Association with Periparturient Diseases. *Dairy*, 3(4): 881-906. <https://doi.org/10.3390/dairy3040061>
- Gammariello, C.S., Hanson, J., Relling, A.E., Oliveira, M.X.S., Sipka, A.S., Enger, K.M. & Enger, B.D. 2024. Localized mammary gland changes in milk composition and venous blood metabolite concentrations result from sterile subclinical mastitis. *Journal of Dairy Science*, 107(8): 6148-6160. <https://doi.org/10.3168/jds.2023-24044>
- Jaswal, S., Jena, M.K., Anand, V., Jaswal, A., Kancharla, S., Kolli, P., Mandadapu, G., Kumar, S. & Mohanty, A.K. 2022. Critical Review on Physiological and Molecular Features during Bovine Mammary Gland Development: Recent Advances. *Cells*, 11(20): 3325. <https://doi.org/10.3390/cells11203325>
- Kim, S.-Y., Kim, H.Y., Kim, E.-K., Kim, M.J., Moon, H.J. & Yoon, J.H. 2015. Evaluation of Malignancy Risk Stratification of Microcalcifications Detected on Mammography: A Study Based on the 5th Edition of BI-RADS. *Annals of Surgical Oncology*, 22: 2895–2901. <https://doi.org/10.1245/s10434-014-4362-6>
- Rafa, H., Nan, M.I. & Andrei, S. 2023. The impact of oxidative stress on reproductive disorders in cows – a review. *Scientific Papers Journal*, 6(22): 2023. <https://doi.org/10.61900/SPJVS.2023.02.01>
- Schadt, I. 2023. Health concerns about possible long-term effects of legally marketed milk and dairy from animals with intramammary infections. *Frontiers in Public Health*, 11: 1200924. <https://doi.org/10.3389/fpubh.2023.1200924>
- Seminara, J.A., Callero, K.R., Frost, I.R., Martinez, R.M., McCray, H.A., Reid, A.M., Seely, C.R., Barbano, D.M. & McArt, J.A.A. 2023. Calcium dynamics and associated temporal patterns of milk constituents in early-lactation multiparous Holsteins. *Journal of Dairy Science*, 106(10): 7117-7130. <https://doi.org/10.3168/jds.2022-23142>
- Stanek, P., Żółkiewski, P., & Januś, E. 2024. A Review on Mastitis in Dairy Cows Research: Current Status and Future Perspectives. *Agriculture*, 14(8): 1292. <https://doi.org/10.3390/agriculture14081292>
- Sumon, S.M.M.R., Parvin, M.S., Ehsan, M.A. & Islam, M.T. 2020. Dynamics of somatic cell count and intramammary infection in lactating dairy cows. *Journal of Advanced Veterinary and Animal Research*, 7(2): 314-319. <https://doi.org/10.5455/javar.2020.g423>
- Zalewska, M., Brzozowska, P., Rzewuska, M., Kawecka-Grochocka, E., Urbańska, D.M., Sakowski, T. & Bagnicka, E. 2025. The quality and technological parameters of milk obtained from dairy cows with subclinical mastitis. *Journal of Dairy Science*, 108(2): 1285-1300. <https://doi.org/10.3168/jds.2024-25346>

Disclaimer: The views expressed in the published works do not express the views of the Editors and Editorial Staff. The authors take legal and moral responsibility for the ideas expressed in the articles. The publisher shall have no liability in the event of issuance of any claims for damages. The Publisher will not be held legally responsible should there be any claims for compensation.