Research Article

Nutritional Strategies Targeting the Metabolic Oxidative Immune Axis for Mastitis Risk Reduction in Dairy Cattle: A Systematic Review

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Abstract | Mastitis is a multifactorial disorder in dairy ruminants influenced by immune competence, metabolic status, oxidative balance, and nutritional management. This systematic review synthesizes current evidence on nutritional strategies aimed at reducing mastitis risk and improving mammary gland health. Studies were identified through a systematic literature search and selected according to predefined inclusion criteria. A total of 49 studies were included, evaluating interventions such as energy balance management, vitamin and trace mineral supplementation, phytogenic feed additives, and microbial modulators. Across the reviewed studies, multiple nutritional interventions were associated with measurable improvements in somatic cell count (SCC), antioxidant status, and immune function, although responses varied depending on physiological stage, baseline nutritional status, and management conditions. Trace mineral supplementation, particularly selenium, was frequently linked with improved udder health indicators. For example, one controlled study reported that organic selenium supplementation reduced milk SCC by up to 64.38% after 60 days of feeding, accompanied by increased serum selenium concentrations. Evidence from multiple studies also highlights the transition and periparturient periods as critical windows during which metabolic imbalance, oxidative stress, and immune dysregulation increase susceptibility to mastitis. However, the evidence base shows considerable heterogeneity, with several studies presenting moderate risk of bias, and 21 eligible reports could not be retrieved for full-text assessment, which may limit the generalizability and robustness of the overall conclusions. Overall, the findings suggest that nutritionally informed herd management strategies targeting metabolic stability, oxidative balance, and immune competence can contribute to reducing mastitis risk and improving dairy herd health.

Keywords | Mastitis, Dairy cattle, Nutrition management, Natural antioxidants, Feed additives

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INTRODUCTION

Mastitis remains one of the most prevalent and economically detrimental diseases in the dairy industry, as it directly reduces milk yield, increases somatic cell count (SCC), and compromises milk quality and economic value (Salvador *et al*., 2025). Beyond financial losses, mastitis poses significant challenges to animal welfare, dairy sustainability, and food safety (Mylostyvyi and Izhboldina, 2025; Verdon and Beggs, 2024). Despite advances in herd management, mastitis control continues to rely predominantly on antibiotic therapy, raising long-term concerns regarding antimicrobial resistance and the presence of antibiotic residues in milk products (Abdelfattah *et al*., 2025; Nguyen *et al*., 2023). These limitations highlight the urgent need for preventive strategies grounded in physiological and metabolic resilience rather than reactive treatment.

Over the past decades, the conceptual understanding of mastitis has evolved from a predominantly bacterial infection paradigm toward a multifactorial disorder influenced by host physiology, metabolic status, and environmental stressors (Liu *et al*., 2025; Saco and Bassols, 2023). Among the emerging mechanisms, oxidative stress has gained increasing recognition as a key component in mastitis pathophysiology. Oxidative stress arises from an imbalance between reactive oxygen species (ROS) production and antioxidant defense capacity. High milk production, mammary inflammatory responses, and metabolic pressure during lactation collectively contribute to increased ROS generation, which can damage mammary epithelial cells, disrupt cellular integrity, and impair immune competence.

A growing body of experimental and observational studies has reported associations between elevated oxidative stress biomarkers and increased SCC, as well as a higher incidence of subclinical mastitis in high-producing dairy cattle (Agustinho *et al*., 2021; Cohen-Zinder *et al*., 2025; Gheller *et al*., 2023; Khan *et al*., 2024; Liu *et al*., 2025; Mazur *et al*., 2019; Mendoza-Martínez *et al*., 2024; Okkema and Grandin, 2021; Shen *et al*., 2025; Wang *et al*., 2024; Zeweld and Tarekegn, 2025). These studies collectively suggest that oxidative stress may not only occur as a consequence of inflammatory processes but may also contribute to the amplification of mammary tissue dysfunction. However, it is important to note that only a subset of the included studies directly quantified oxidative stress biomarkers, indicating that the proposed mechanistic relationships should be interpreted with caution.

Oxidative stress is closely interconnected with the nutritional and metabolic status of dairy cattle. Deficiencies in energy, minerals, and micronutrients have been associated with reduced activity of key antioxidant enzymes, including superoxide dismutase, glutathione peroxidase, and catalase. Such impairments can weaken redox homeostasis and compromise immune responsiveness, thereby increasing susceptibility to both clinical and subclinical mastitis. Accordingly, nutritional management has emerged as a strategic preventive approach aimed at maintaining antioxidant capacity and supporting udder health (Barbato *et al*., 2019; Capper and Cady, 2020; Chase and Fortina, 2023; Mazur *et al*., 2019).

Minerals and trace elements such as zinc, selenium, copper, manganese, and chromium function as essential cofactors of antioxidant enzymes and modulators of immune signaling pathways (Ianni *et al*., 2019a, b). Zinc plays a critical role in maintaining epithelial barrier integrity and immune cell function, whereas selenium contributes to antioxidant defense through its role in glutathione peroxidase activity. Inadequate supply of these trace elements, particularly under conditions of high metabolic demand, may lead to impaired immune function, elevated SCC, and increased susceptibility to mastitis. These interactions highlight the interconnected roles of metabolic status, oxidative balance, and immune function in shaping mastitis risk.

In addition to mineral supplementation, herbal-based feed additives and natural bioactive compounds have attracted increasing attention as non-antibiotic alternatives for mastitis control (Cohen-Zinder *et al*., 2025; Shen *et al*., 2025; Song *et al*., 2023; Zeweld and Tarekegn, 2025). Phytogenic feed additives contain bioactive compounds with antioxidant, anti-inflammatory, and immunomodulatory properties that may help mitigate oxidative stress and support metabolic stability (Mendoza-Martínez *et al*., 2024). The combined use of trace minerals and phytogenic compounds has been suggested to exert complementary effects by enhancing antioxidant capacity, improving nutrient utilization efficiency, and supporting mammary tissue resilience against infectious challenges (Agustinho *et al*., 2021; Khan *et al*., 2024). Nevertheless, the magnitude and consistency of these responses remain variable across studies.

The transition period represents a critical phase in the dairy production cycle, characterized by profound physiological and metabolic adaptations. During this stage, dairy cattle frequently experience negative energy balance, increased lipid mobilization, and elevated oxidative stress, all of which can impair immune function and increase susceptibility to inflammatory and infectious diseases, including mastitis (Gheller *et al*., 2023; Singh, 2020; Singh *et al*., 2023). These dynamics underscore the importance of targeted nutritional strategies during the transition period to maintain metabolic stability and reduce disease risk.

Mastitis development in dairy cattle is increasingly understood as the result of complex interactions among metabolic status, oxidative balance, immune responses, and pathogen pressure. To illustrate these relationships, a conceptual framework is presented in Figure 1. This framework summarizes interactions reported in the literature and serves as a conceptual guide for interpreting the relationships discussed in this review. It is important to emphasize that this framework represents a conceptual synthesis rather than a validated causal model derived directly from the present analysis.

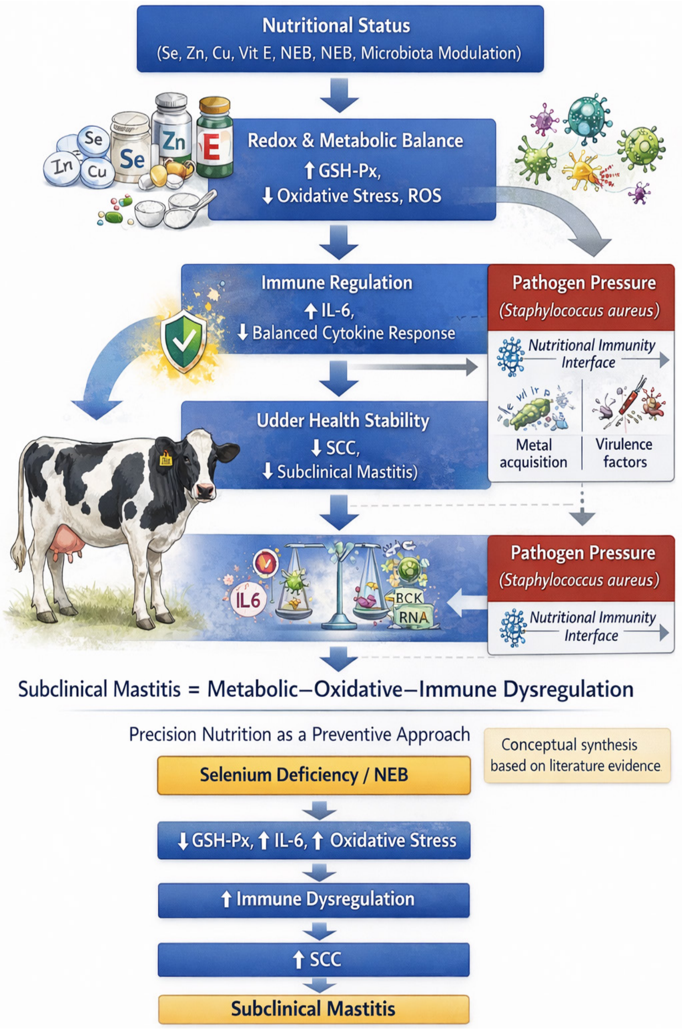

Figure 1: Conceptual framework summarizing relationships reported in the literature between nutritional status, oxidative balance, immune regulation, and pathogen pressure in dairy cattle. The framework synthesizes recurring patterns identified across studies and illustrates proposed interactions rather than experimentally validated causal pathways.

Therefore, this review aims to synthesize current scientific evidence on the role of nutritional strategies in modulating oxidative stress and reducing mastitis risk in dairy cattle. Although numerous studies have explored nutritional approaches to mastitis prevention, the available evidence remains fragmented and methodologically heterogeneous, with substantial variation in study design, biomarkers, and intervention strategies. In contrast to previous reviews, this study provides a systematic synthesis that integrates metabolic status, oxidative balance, and immune responses within a unified conceptual framework, while explicitly accounting for heterogeneity and studies reporting both positive and neutral outcomes. This approach is intended to provide a more balanced and mechanistically informed understanding of how nutritional strategies may influence mastitis susceptibility in dairy cattle.

MATERIALS AND METHODS

Study design and reporting guidelines

This study was conducted as a Systematic Literature Review (SLR). The review protocol was prospectively registered on the Open Science Framework (OSF) (DOI: 10.17605/OSF.IO/W5RCH) to ensure transparency and methodological rigor. All methodological steps, including the search strategy, eligibility criteria, and data extraction procedures, were predefined prior to study initiation to minimize bias and enhance reproducibility. The review was designed and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines.

Research question and scope of the review

The primary research question guiding this review was: *"What feeding and nutritional strategies have been reported to reduce mastitis risk or improve udder health in dairy cattle?"*

The review focused on nutritional interventions that directly or indirectly influenced mastitis-related outcomes, particularly through mechanisms involving immune modulation, reduction of oxidative stress, decreased somatic cell count (SCC), and improvements in milk quality and composition.

The primary outcomes of interest were mastitis incidence (clinical and subclinical) and SCC. Secondary outcomes included oxidative stress biomarkers, immune response parameters, antioxidant enzyme activity, and indicators of udder health. The nutritional strategies examined included mineral and trace mineral supplementation, antioxidant nutrients, phytogenic or herbal-based feed additives, and nutritional management during lactation and the transition period. This review focuses exclusively on preventive nutritional strategies and does not include studies evaluating nutritional treatments for active clinical mastitis. Studies were included if they reported outcomes related to mastitis risk, subclinical mastitis (e.g., somatic cell count), or udder health indicators.

Literature search strategy

A systematic literature search was conducted in four electronic databases: Scopus, Web of Science, PubMed, and ScienceDirect. Articles published between 2015 and 2025 were considered to capture recent developments in nutritional strategies and emerging insights into oxidative stress and immune modulation in dairy cattle. Only peer-reviewed articles published in English were included.

The search strategy employed combinations of keywords structured using Boolean operators. A representative search string used in Scopus was as follows: ("mastitis" AND ("dairy cattle" OR "dairy cows")) AND ("nutrition" OR "feeding strategies" OR "mineral supplementation" OR "trace elements" OR "zinc" OR "selenium" OR "antioxidant" OR "oxidative stress" OR "phytogenic feed additives" OR "herbal supplementation"). The search syntax was adapted for each database according to its indexing system, controlled vocabulary (e.g., MeSH terms in PubMed), and search interface. The full search strings for each database are provided in the Supplementary Table 1 to ensure transparency and reproducibility. In addition, backward reference searching was performed by screening the reference lists of eligible articles to identify potentially relevant studies not captured in the initial database search

Inclusion and exclusion criteria

Eligibility criteria were predefined prior to the literature search.

The inclusion criteria were as follows:

- Original research articles.
- Studies conducted in dairy cattle.
- Investigations evaluating feeding or nutritional interventions.
- Studies reporting mastitis-related parameters, including clinical or subclinical mastitis incidence, somatic cell count (SCC), udder health indicators, immune responses, or oxidative stress biomarkers.
- Availability of full-text articles.

The exclusion criteria included:

- Review articles, conference proceedings, editorials, or short communications.
- Studies conducted in non-dairy livestock species.
- Research unrelated to nutritional interventions.
- Articles not reporting outcomes relevant to mastitis or udder health.

Although full-text availability was defined as an inclusion criterion, 21 potentially eligible studies could not be retrieved despite repeated access attempts and were therefore excluded at the full-text screening stage. This limitation is acknowledged as part of the study selection process.

Study selection process

All retrieved records were imported into reference management software, and duplicate entries were identified and removed prior to screening. After duplicate removal, studies were screened in three sequential stages: title screening, abstract screening, and full-text assessment.

The screening process was conducted independently by two reviewers to minimize selection bias. To ensure consistency, a subset of records was cross-checked by both reviewers at each screening stage, and any discrepancies were discussed and resolved through consensus. This iterative process was applied throughout the screening procedure to maintain alignment in eligibility decisions and to enhance the reliability of study selection.

The study selection process followed PRISMA 2020 guidelines and was documented using a PRISMA flow diagram, illustrating the number of records identified, screened, excluded, and ultimately included in the final synthesis, along with reasons for exclusion at the full-text stage.

Quality assessment and risk of bias

The methodological quality and risk of bias of the included studies were evaluated using the SYRCLE risk-of-bias tool developed for animal intervention studies. This tool assesses potential bias across several domains, including selection bias, performance bias, detection bias, attrition bias, reporting bias, and other potential sources of bias.

Each study was independently assessed by two reviewers, and any discrepancies were resolved through discussion until consensus was achieved. In accordance with SYRCLE guidelines, each domain was judged as "low risk," "high risk," or "unclear risk" of bias.

Importantly, studies that did not explicitly report key methodological details such as randomization procedures, allocation concealment, or blinding were not assumed to be at low risk but were instead classified as having an "unclear" or "high" risk of bias, depending on the extent of missing information. This approach was applied to avoid overestimation of study quality.

Overall study quality was then categorized as low, moderate, or high risk of bias based on the combined assessment across domains. Several domains were frequently rated as "unclear" due to insufficient reporting, particularly in relation to randomization and blinding procedures, which are commonly underreported in animal nutrition studies.

The results of the risk-of-bias assessment were considered during data interpretation and are presented in Supplementary Table 2 to ensure transparency and reproducibility.

Data extraction

Data extraction was conducted using a structured data extraction form to ensure consistency and completeness. Extracted information included author names and publication year, country of study, study design, animal characteristics, type of nutritional intervention, dosage and duration of supplementation, evaluated parameters, and primary outcomes related to mastitis prevention and udder health. The extracted information was summarized in a study characteristics table to facilitate comparison across the included studies.

Data synthesis

Due to substantial heterogeneity in study designs, types of nutritional interventions, dosages, duration of supplementation, and outcome measures, a quantitative meta-analysis was not considered appropriate. The included studies varied considerably in terms of experimental conditions, animal characteristics, and reported endpoints, which limited the feasibility of statistical pooling and effect size comparison.

Therefore, the data were synthesized using a structured narrative synthesis approach. Studies were grouped according to the type of nutritional strategy, including mineral and trace mineral supplementation, antioxidant nutrients, phytogenic feed additives, and nutritional management during the transition period.

Within each category, findings were systematically compared based on reported outcomes, including somatic cell count (SCC), mastitis incidence, oxidative stress biomarkers, immune response indicators, and milk production parameters. Both positive and non-significant results were considered to provide a balanced interpretation of the evidence.

The synthesis aimed to identify recurring patterns, potential biological mechanisms, and inconsistencies across studies, while also highlighting gaps in the current literature. Particular attention was given to the relationships among metabolic status, oxidative balance, and immune function in the context of mastitis susceptibility in dairy cattle.

RESULTS

Descriptive summaries of included studies

Study selection based on PRISMA

The study selection process followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines, and the complete workflow is presented in the PRISMA flow diagram (Figure 2).

The initial literature search conducted across four electronic databases (Scopus, Web of Science, PubMed, and Science Direct) using predefined keyword combinations identified a total of 811 records. After removing 104 duplicate records, 707 articles remained for title and abstract screening.

During the screening stage, 637 records were excluded because they did not meet the predefined inclusion criteria. The primary reasons for exclusion included lack of relevance to nutritional interventions (n= 318), studies conducted in non-dairy species (n= 152), absence of mastitis-related outcome parameters (n= 109), and non-original study types such as reviews, conference proceedings, or editorials (n= 58).

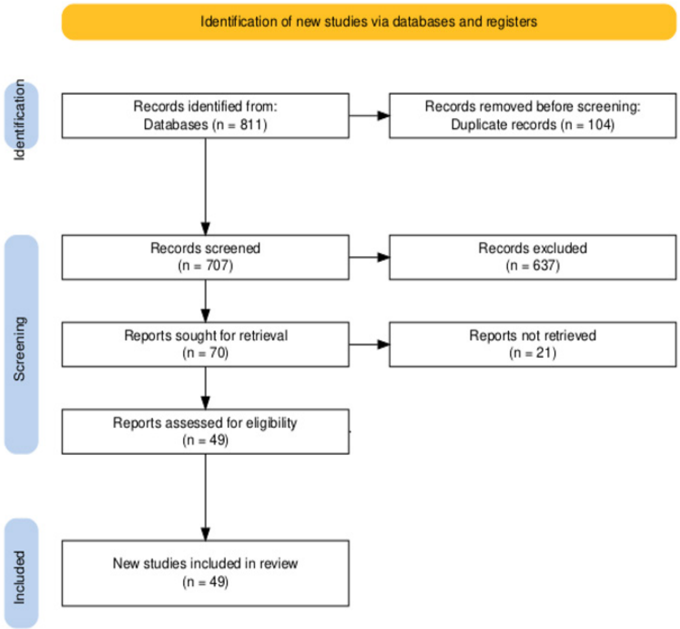

Figure 2: PRISMA 2020 flow diagram illustrating the study selection process for the systematic review of nutritional strategies to reduce mastitis in dairy cattle.

Following the screening process, 70 reports were sought for retrieval for full-text evaluation. Of these, 21 reports could not be retrieved due to restricted access, unavailable full texts, or incomplete database records, and were therefore excluded prior to eligibility assessment.

The remaining 49 reports were successfully retrieved, assessed for eligibility, and included in the final qualitative synthesis.

Overall, the included studies represent approximately 6.04% of the 811 records initially identified. The complete study selection process, including identification, screening, eligibility, and inclusion stages, is presented in Figure 2.

Although full-text availability was defined as an inclusion criterion, 21 potentially eligible studies could not be retrieved despite repeated access attempts. This limitation is acknowledged and may have influenced the completeness of the evidence synthesis and the overall interpretation of findings.

The characteristics of the included studies are summarized in Supplementary Table 1, which provides detailed information regarding study design, animal species, nutritional interventions, and evaluated outcomes.

The methodological quality and risk of bias of the included studies were evaluated using the SYRCLE risk-of-bias tool for animal intervention studies. The detailed results of the risk-of-bias assessment are presented in Supplementary Table 2. Overall, studies were classified as having low to moderate risk of bias, with several domains frequently rated as "unclear" due to limited reporting of methodological details such as randomization and blinding procedures.

Mineral and trace elements in mastitis prevention

The synthesized evidence indicates that mineral and trace element supplementation represents one of the most frequently investigated nutritional strategies associated with udder health and mastitis-related indicators in dairy cattle. Across the included studies, the majority reported that improved mineral status was associated with reductions in somatic cell count (SCC), enhanced metabolic stability, and improved immune-related parameters (De *et al*., 2023; Ianni *et al*., 2019b; Rodrigues *et al*., 2024). However, considering the heterogeneity in study design, supplementation protocols, and baseline nutritional conditions, the strength and consistency of these associations should be interpreted cautiously.

Among the trace elements evaluated, selenium has received the most consistent attention due to its central role in antioxidant defense through glutathione peroxidase (GSH-Px) activity. Most studies reported associations between improved selenium status and reductions in SCC or inflammatory indicators (Ianni *et al*., 2019b; Wang *et al*., 2021). Reductions in SCC reported across studies ranged from approximately 10% to over 60%, depending on supplementation dose, duration, and baseline mineral status. However, several studies found no statistically significant effects, highlighting variability in outcomes.

The variability in selenium responses suggests that supplementation effects are highly context-dependent. Studies reporting limited or no improvement were often associated with adequate baseline mineral status or shorter experimental durations, whereas stronger responses were observed under conditions of deficiency or increased metabolic stress (Ianni *et al*., 2019b; Wang *et al*., 2021). In addition, some studies reported negative correlations between serum selenium concentrations and SCC, together with increased glutathione peroxidase activity and reduced inflammatory mediators such as interleukin-6 following supplementation (Ding *et al*., 2025; Wang *et al*., 2021). However, only a limited number of studies directly assessed oxidative processes at the mammary tissue level, and therefore these mechanistic pathways should be interpreted as biologically plausible rather than definitive causal relationships.

The high prevalence of selenium deficiency reported in both cows and calves, together with low selenium concentrations in forage in certain production systems, suggests that mineral imbalances may be influenced by agroecological conditions (Andělová *et al*., 2024; Stybayev *et al*., 2025). Under such conditions, selenium supplementation has been associated not only with improvements in udder health indicators but also with broader reproductive and metabolic outcomes, including improved natality rates in some herds (Lembovičiūtė *et al*., 2025). Nevertheless, responses remain dependent on baseline nutritional status and management conditions (Żarczyńska *et al*., 2017).

In addition to selenium, other trace minerals such as zinc and copper, together with antioxidant vitamins such as vitamin E, have also been investigated for their roles in maintaining mammary epithelial integrity and supporting immune function (Agustinho *et al*., 2021; Ianni *et al*., 2019a; Kegley *et al*., 2016; Khan *et al*., 2024). Zinc contributes to epithelial barrier function and leukocyte activity, whereas copper participates in enzymatic antioxidant systems. Several studies reported reductions in SCC following combined mineral and vitamin supplementation, although responses were not uniform across studies (Germon and Martins, 2023; Goyal *et al*., 2024; Nudda *et al*., 2023). indicating potential interactions between mineral balance, diet composition, and physiological stage.

The association between negative energy balance (NEB), metabolic stress, and oxidative stress further supports the interpretation of subclinical mastitis as a systemic metabolic–immunological disorder rather than a purely localized infection (Agustinho *et al*., 2021; Khan *et al*., 2024). High-concentrate diets during early lactation have been reported to increase metabolic load and oxidative stress, potentially influencing immune- and antioxidant-related gene expression (Ayemele *et al*., 2021). These metabolic conditions may increase susceptibility to inflammatory responses or pathogen colonization, suggesting that nutritional strategies targeting metabolic stability and antioxidant balance may indirectly support udder health.

Recent studies also indicate that nutritional interventions may influence mammary health at the molecular level. For example, inulin supplementation has been reported to modify the profile of milk small extracellular vesicle-associated microRNAs (sEV-miRNAs) involved in inflammatory signaling pathways and milk synthesis regulation (Yu *et al*., 2023). Additionally, microbial-based feed additives have been associated with improvements in feed intake and metabolic balance, which may indirectly contribute to reductions in SCC (Kalińska *et al*., 2023; Prapaiwong *et al*., 2023; Rodrigues *et al*., 2024). However, most molecular evidence originates from controlled experimental conditions rather than large-scale field studies, and therefore requires further validation.

From an immunological perspective, mammary defense mechanisms involve complex interactions between innate and adaptive immune responses. Early responses to infection include cytokine signaling, bioactive lipid mediators, and complement system activation (Kegley *et al*., 2016). Within this context, essential trace minerals may play dual roles by supporting host immune function while also serving as resources for certain bacterial pathogens, including Staphylococcus aureus, which possesses specialized metal acquisition systems (Carlson *et al*., 2020; Magro *et al*., 2023). The dynamics of nutritional immunity in ruminants remain incompletely understood, and extrapolation from non-ruminant models should be approached cautiously.

Overall, the available evidence suggests that optimization of mineral balance, energy status, and antioxidant supply may contribute to improved udder health and reduced mastitis risk in dairy cattle. However, the magnitude and consistency of these effects remain context-dependent and influenced by factors such as baseline nutritional status, lactation stage, dietary formulation, and herd management conditions. Therefore, mineral-based nutritional interventions should be considered as components of integrated mastitis prevention strategies rather than as standalone solutions.

Herbal-based feed additives and natural antioxidants

Herbal and natural antioxidant-based feed additives have received increasing attention as potential non-antibiotic alternatives for mastitis control in dairy production systems. The reviewed studies generally report associations between phytogenic feed additives and improvements in udder health parameters, particularly reductions in somatic cell count (SCC) and improvements in antioxidant status. However, the magnitude and consistency of these responses vary across studies due to differences in additive composition, dosage, experimental design, and physiological status of the animals.

An integrated conceptual model illustrating potential pathways through which herbal and natural antioxidant feed additives may influence oxidative balance, immune responses, and mammary gland health is presented in Figure 3. This model synthesizes relationships reported in the literature and should be interpreted as a conceptual framework rather than an experimentally validated causal model.

As illustrated in Figure 3, the proposed relationships highlight recurring patterns identified across studies and provide a structured interpretation of how phytogenic interventions may influence mastitis-related parameters.

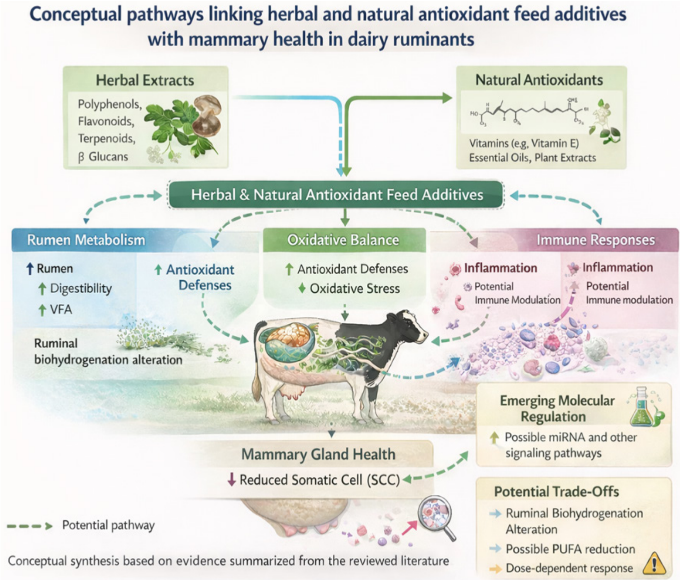

Figure 3: Conceptual synthesis diagram summarizing relationships reported in the literature between phytogenic feed additives and mastitis-related outcomes in dairy cattle. The figure integrates evidence on oxidative balance, immune responses, and metabolic modulation to illustrate how these factors may interact to influence udder health. This diagram is based on qualitative synthesis of the included studies and is intended to support interpretation of reported associations rather than represent experimentally validated causal pathways.

The use of phytogenic additives has been explored as a preventive nutritional strategy, particularly in response to concerns regarding antimicrobial resistance, production sustainability, and milk functional quality (Barcelo-Villalobos *et al*., 2025; Cunha *et al*., 2023; Fantini *et al*., 2024; Kholif *et al*., 2021; Yu *et al*., 2023). Across studies, these additives were more consistently associated with improvements in udder health indicators and antioxidant status than with increases in milk yield (Fraga *et al*., 2024; Prapaiwong *et al*., 2023; Prodanović *et al*., 2025). Suggesting a primary role in supporting physiological stability rather than directly enhancing production performance.

From a biological perspective, modulation of redox homeostasis is frequently proposed as a central mechanism underlying these effects (Mazur *et al*., 2019). Several studies reported reductions in oxidative stress markers, including reactive oxygen species (ROS), lipid peroxidation products, and antioxidant indices, following supplementation (Ayemele *et al*., 2021; Mazur *et al*., 2019). These responses were often accompanied by reductions in SCC; however, the magnitude of these effects ranged from modest to moderate and varied substantially across studies.

Importantly, only a limited number of studies directly assessed oxidative processes within mammary tissue. Therefore, the proposed mechanistic links between antioxidant supplementation and mastitis-related outcomes should be interpreted as biologically plausible rather than definitive.

Responses to phytogenic supplementation were not uniform. Several studies reported limited or non-significant changes in oxidative biomarkers or SCC, particularly under conditions of low baseline oxidative stress or modest supplementation levels (Cunha *et al*., 2023; Kotsampasi *et al*., 2018; Nudda *et al*., 2023). This variability indicates that the effectiveness of phytogenic additives is highly context-dependent and influenced by baseline physiological status, additive composition, and management conditions.

Furthermore, SCC alone may not fully reflect mastitis status, as it can be influenced by non-infectious factors such as metabolic stress, stage of lactation, and physiological adaptation. Therefore, interpretation of SCC responses should be considered alongside other indicators of udder health and immune function.

Plant-derived bioactive compounds, including polyphenols, flavonoids, saponins, and terpenoids, have been associated with immunomodulatory effects (Nudda *et al*., 2023; Stobiecka *et al*., 2023). Some studies reported reductions in circulating oxidative markers and changes in immune-related parameters, such as neutrophil counts and globulin concentrations (Cunha *et al*., 2023). However, these effects were not consistently observed across all studies and appear to depend on the specific phytochemical profile of the additives.

Interactions between phytogenic additives and rumen metabolism may also contribute to observed responses. Improvements in nutrient digestibility and volatile fatty acid (VFA) production have been reported in some studies (Prapaiwong *et al*., 2023). Suggesting modulation of rumen microbial activity. These changes may indirectly influence metabolic stability and mammary gland resilience.

Alterations in milk fatty acid profiles, including increased proportions of polyunsaturated fatty acids (PUFA), omega-3, and monounsaturated fatty acids (MUFA), have also been reported following phytogenic supplementation (Dokou *et al*., 2023; Renna *et al*., 2020). However, some studies identified trade-offs between milk composition and production performance, indicating dose-dependent and diet-dependent responses (Niu *et al*., 2023).

Emerging evidence suggests that nutritional interventions may influence mammary physiology through molecular regulatory mechanisms, including extracellular vesicles and microRNAs. For example, modifications in milk small extracellular vesicle-derived microRNAs (sEV-miRNAs) have been reported following dietary supplementation (Yu *et al*., 2023). However, these findings are based on a limited number of experimental studies and require further validation under field conditions.

Within the context of sustainable dairy production, phytogenic additives have been proposed as complementary strategies to reduce reliance on antibiotics. Some studies also reported improvements in nitrogen utilization efficiency and reductions in milk urea concentrations (Prodanović *et al*., 2025), which may contribute to environmental sustainability (Han *et al*., 2024). Nevertheless, the overall strength of evidence remains constrained by heterogeneous study designs and limited long-term validation.

Overall, the available evidence suggests that herbal and natural antioxidant feed additives may contribute to improved udder health through multiple interacting pathways involving oxidative balance, immune modulation, and metabolic regulation. However, given the variability across studies, these additives should be considered as complementary components within integrated herd management strategies rather than standalone interventions.

Nutritional management factors affecting mastitis

The included studies indicate that nutritional management is closely associated with mastitis-related outcomes through its influence on metabolic status, immune function, and oxidative balance in dairy cattle (Haq *et al*., 2024; Kelly *et al*., 2024; Rial *et al*., 2023). However, the magnitude and consistency of these effects varied across studies depending on dietary composition, physiological stage, and herd management conditions.

The summary visual matrix (Figure 4) was constructed based on qualitative extraction and synthesis of reported outcomes from the included studies. Each study was evaluated for its reported effects on metabolic balance, oxidative status, and immune responses, and the matrix summarizes recurring patterns observed across studies. The figure does not represent a quantitative scoring system but rather a structured synthesis of evidence derived from the systematic review process.

As illustrated in Figure 4, the matrix provides a simplified overview of complex interactions across studies, facilitating comparison of different nutritional strategies within a unified framework. Energy balance during early lactation was frequently identified as an important factor associated with mastitis susceptibility, although responses varied depending on management and physiological conditions.

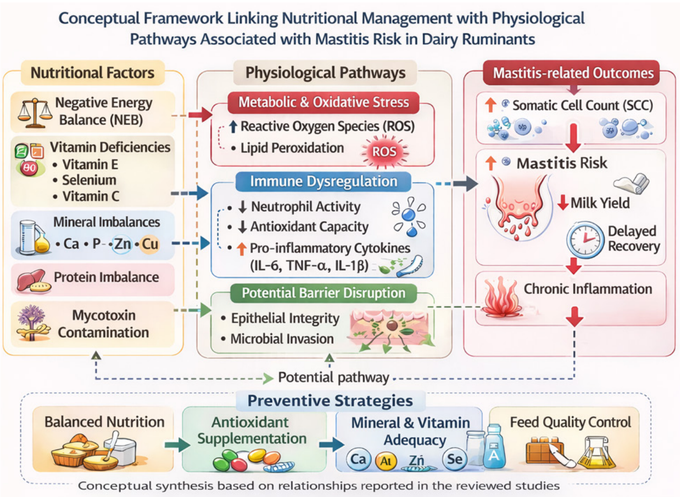

Figure 4: Summary visual matrix illustrating the relationships between nutritional strategies and mastitis-related outcomes in dairy cattle. The matrix synthesizes evidence from the included studies by mapping reported effects on metabolic balance, oxidative status, and immune responses. It was constructed based on qualitative extraction of study findings and represents recurring patterns observed across the literature. This matrix is intended to support interpretation of the integrated effects of nutritional interventions and should be considered a structured evidence synthesis rather than a quantitative or experimentally validated causal model.

Energy balance during early lactation was consistently identified as a key factor influencing mastitis susceptibility. Several studies reported that negative energy balance (NEB) was associated with impaired immune function and increased susceptibility to inflammatory conditions (Cheng *et al*., 2022; Goyal *et al*., 2024). Nutritional strategies aimed at improving energy intake, including appropriate concentrate supplementation and the use of buffering agents, were associated with improvements in rumen fermentation, increased volatile fatty acid (VFA) production, and enhanced nutrient digestibility, which were in some cases accompanied by reductions in somatic cell count (SCC).

However, responses to high-concentrate diets were not uniform across studies. While some studies reported improved production performance and reduced SCC, others indicated increased metabolic stress and oxidative challenges, particularly in animals with higher physiological demands. This variability suggests that dietary strategies must be adapted to animal-specific and production-specific conditions.

Feed additives have also been evaluated as part of nutritional management strategies. Several studies reported associations between phytogenic or microbial-based additives and improvements in rumen fermentation, nutrient utilization, and SCC (Prapaiwong *et al*., 2023; Rodrigues *et al*., 2024). For example, supplementation with citrus by-product hydrolysates was associated with improved digestibility and ruminal fermentation parameters, accompanied by reductions in SCC (Prapaiwong *et al*., 2023). These findings suggest that nutritional interventions may influence udder health indirectly through metabolic pathways rather than direct antimicrobial effects.

Oxidative stress was frequently reported as a contributing factor linking metabolic imbalance to mastitis susceptibility (Ayemele *et al*., 2021; Cohen-Zinder *et al*., 2025). Nutritional strategies aimed at improving antioxidant status, including supplementation with vitamins and trace minerals, were associated with improvements in immune-related parameters and reductions in oxidative stress indicators in several studies. However, the extent of these effects varied across studies, and direct measurements of oxidative processes at the mammary level were limited.

Mineral balance was also identified as an important component of nutritional management. Some studies reported associations between altered mineral status and mastitis-related outcomes, including changes in calcium and phosphorus levels, which may influence physiological functions such as teat sphincter integrity and immune responsiveness (Okkema and Grandin, 2021). However, the evidence remains variable and dependent on baseline nutritional conditions.

Feed contamination with mycotoxins represents an additional nutritional factor that may influence mastitis risk. Studies have reported associations between mycotoxin exposure and impaired immune function, altered nitrogen metabolism, and increased susceptibility to inflammatory responses (Mukhamadieva *et al*., 2023). These findings suggest that feed quality management is an important component of mastitis prevention strategies.

Some studies also highlighted the role of integrated herd management approaches, including technological interventions such as Automatic Milking Systems (AMS), which have been associated with improvements in udder health indicators and reductions in SCC under certain conditions (Lage *et al*., 2024). However, these effects are influenced by management practices and monitoring strategies.

Overall, the evidence suggests that nutritional management influences mastitis risk through multiple interacting pathways involving metabolic balance, oxidative status, and immune function. However, the variability across studies indicates that these effects are context-dependent and should be interpreted within the framework of integrated herd management systems rather than as isolated nutritional interventions.

DISCUSSION

The findings of this systematic review indicate that nutritional strategies are associated with mastitis-related outcomes in dairy cattle, although the magnitude and consistency of these effects vary across studies. Rather than acting through isolated mechanisms, the evidence supports the interpretation that nutritional interventions influence mastitis susceptibility through interconnected pathways involving metabolic status, oxidative balance, and immune function (Ayemele *et al*., 2021; Mazur *et al*., 2019).

Across the 49 included studies, a substantial proportion reported associations between nutritional interventions particularly trace mineral supplementation, antioxidant nutrients, phytogenic additives, and transition-period management and improvements in somatic cell count (SCC), oxidative status, and immune-related parameters. Reported reductions in SCC ranged from modest (approximately 5–15%) to more substantial (>50%) in certain controlled studies (Ianni *et al*., 2019a; Wang *et al*., 2021), while other studies reported limited or non-significant effects, highlighting the context-dependent nature of these interventions.

While previous reviews have discussed the roles of nutrition and oxidative stress in mastitis, the present study contributes by integrating recent evidence (2015–2025) into a unified framework linking metabolic status, oxidative balance, and immune function. This integrative perspective helps explain inconsistencies across studies and emphasizes that nutritional interventions should be interpreted as modulatory factors within a complex physiological system rather than direct causal solutions for mastitis prevention.

Rather than relying on single-study findings, the present synthesis indicates that trace mineral supplementation particularly selenium has been associated across multiple studies with improvements in antioxidant enzyme activity and reductions in SCC, although these responses vary depending on baseline mineral status and supplementation protocols. Similarly, phytogenic feed additives were associated in several studies with improvements in oxidative and immune-related parameters; however, the magnitude of these effects was inconsistent and influenced by additive composition, dosage, and management conditions (Cunha *et al*., 2023; Prapaiwong *et al*., 2023).

Some of the biological mechanisms proposed in the literature suggest that these responses may be mediated through improved rumen fermentation, enhanced nutrient utilization, and modulation of systemic oxidative balance. However, it is important to distinguish that these mechanisms are inferred from experimental and physiological studies and were not consistently measured directly across all included studies. Therefore, these interpretations should be considered as biologically plausible explanations rather than definitive causal pathways.

Substantial heterogeneity was observed among the included studies in terms of experimental design, animal characteristics, supplementation strategies, dosage levels, and outcome measurements. A formal subgroup analysis or meta-analysis was not feasible due to this heterogeneity and the lack of standardized reporting across studies. Key sources of variability included differences in baseline nutritional status, lactation stage, environmental conditions, and types of biomarkers used to assess mastitis and oxidative stress.

Publication bias cannot be entirely excluded, as studies reporting positive outcomes may be more likely to be published. No formal assessment of publication bias (e.g., funnel plot analysis) was conducted, as the heterogeneity of study designs and outcomes precluded quantitative synthesis. This limitation may result in an overestimation of the beneficial effects of nutritional interventions.

From a practical perspective, the economic implications of nutritional interventions remain insufficiently explored. Although several strategies have been associated with improvements in SCC and udder health indicators, limited data are available regarding cost-effectiveness under commercial conditions. As a result, while certain interventions such as chelated mineral supplementation or phytogenic additives appear promising, their economic viability under field conditions remains uncertain.

In addition, although improved nutritional management may contribute to enhanced animal health and resilience, direct evidence linking these strategies to reduced antibiotic usage is limited. Most studies evaluated surrogate indicators such as SCC rather than treatment frequency or incidence of clinical mastitis. Therefore, conclusions regarding antimicrobial reduction should be interpreted cautiously.

Future research should prioritize standardized experimental designs and reporting frameworks. Key biomarkers that should be consistently measured include somatic cell count (SCC), oxidative stress indicators such as malondialdehyde (MDA) and glutathione peroxidase (GSH-Px), and immune parameters including cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). In addition, studies should incorporate both biological and economic outcome measures to improve the applicability of findings for commercial dairy systems.

While emerging approaches such as metabolomics and transcriptomics may provide deeper mechanistic insights, their application in commercial dairy systems remains limited. Therefore, future research should balance mechanistic exploration with practical feasibility, ensuring that proposed strategies are both scientifically robust and economically viable.

Overall, the findings of this review support the role of nutritional management as a complementary component of mastitis prevention strategies. Rather than functioning as standalone interventions, nutritional strategies should be integrated with broader herd management practices, including hygiene, genetics, and environmental control. Within this integrated framework, optimizing metabolic balance, antioxidant capacity, and immune competence may contribute to improved mammary gland resilience and more sustainable dairy production systems.

CONCLUSION

Mastitis in dairy cattle is a multifactorial disorder associated with interactions among metabolic imbalance, oxidative stress, and immune dysregulation, particularly during the periparturient transition period. The evidence synthesized in this review supports an integrated interpretation of mastitis involving interactions within the metabolic–oxidative–immune axis; however, these relationships should be considered as associations supported by current evidence rather than fully validated causal mechanisms.

Nutritional strategies, including optimization of energy balance, trace mineral and vitamin supplementation, phytogenic antioxidants, and microbial modulators, have been associated across multiple studies with improvements in metabolic stability, redox balance, and immune-related parameters, as well as reductions in somatic cell count (SCC) under specific conditions. Nevertheless, the magnitude and consistency of these effects varied considerably depending on physiological stage, baseline nutritional status, and management conditions, indicating that responses to nutritional interventions are context-dependent.

Overall, the findings indicate that nutrition represents an important complementary component of mastitis risk management rather than a standalone solution. Effective mastitis control requires integration of nutritional strategies with broader herd health practices, including hygiene, genetics, and environmental management.

Based on the gaps identified in this review, future research should prioritize: (i) longitudinal studies evaluating both subclinical and clinical mastitis outcomes; (ii) standardized measurement of key biomarkers, including SCC, oxidative stress indicators (e.g., malondialdehyde and glutathione peroxidase activity), and immune parameters (e.g., cytokine profiles); and (iii) evaluation of economic feasibility and cost-effectiveness of nutritional interventions under commercial production conditions.

In addition, although nutritional strategies may support improved animal health and resilience, direct evidence linking these approaches to reductions in antibiotic usage remains limited. Therefore, conclusions regarding antimicrobial reduction and sustainability should be interpreted cautiously and require further validation.

Overall, this review highlights the importance of adopting an integrated and context-specific approach to nutritional management, with the aim of improving mammary gland health and supporting more sustainable dairy production systems.

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NOVELTY STATEMENT

This review synthesizes current evidence on multifunctional nutritional interventions for mastitis prevention by focusing on the interaction between metabolic disorders, oxidative stress, and immune regulation to improve udder health and reduce mastitis risk in dairy cattle.

AUTHOR'S CONTRIBUTION

I.P. conceptualized the review, conducted the litera-ture search, synthesized the data, and prepared the original draft of the manuscript. All authors reviewed, revised, and approved the final version of the manuscript.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abdelfattah E, Fausak E, Maier G (2025). Failure of passive immune transfer in neonatal beef calves: A scoping review. Animals, 15(14): 1-29. https://doi.org/10.3390/ani15142072

Agustinho BC, Ramsey KC, Moore C, Tsai CY, Scholte CM, Guire MAM, Rezamand P (2021). Effect of different combinations of dietary vitamin a, protein levels, and monensin on inflammatory markers and metabolites, retinol-binding protein, and retinoid status in periparturient dairy cows. Animals, 11(9): 1-17. https://doi.org/10.3390/ani11092605

Andělová J, Blažejak GJ, Staffa A, Kadek R, Kumprechtová D, Illek J (2024). Selenium status of beef cattle in the czech republic. Acta Vet. Brno., 93(2): 123–128. https://doi.org/10.2754/avb202493020123

Ayemele AG, Tilahun M, Lingling S, Elsaadawy SA, Guo Z, Zhao G, Xu J, Bu D (2021). Oxidative stress in dairy cows: insights into the mechanistic mode of actions and mitigating strategies. Antioxidants, 10(12): 1-21. https://doi.org/10.3390/antiox10121918

Barbato O, Holmes B, Filipescu IE, Celi P (2019). Dietary supplementation of yerba mate (ilex paraguariensis) during the dry period improves redox balance in lactating dairy cows. Antioxidants, 8(2): 1-10. https://doi.org/10.3390/antiox8020038

Barcelo VM, Arrebola F, Vizcaino A, Alarcón FJ, Acién FG (2025). Influence of including arthrospira platensis as a functional ingredient in dairy cow feed. Algal Res., 91(7): 1-10. https://doi.org/10.1016/j.algal.2025.104295

Capper JL, Cady RA (2020). The effects of improved performance in the u.s. Dairy cattle industry on environmental impacts between 2007 and 2017. J. Anim. Sci., 98(1): 1-14. https://doi.org/10.1093/jas/skz291

Carlson SK, Erickson DL, Wilson E (2020). *Staphylococcus aureus* metal acquisition in the mastitic mammary gland. Microb. Pathogen., 144(7): 1-8. https://doi.org/10.1016/j.micpath.2020.104179

Chase LE, Fortina R (2023). Environmental and economic responses to precision feed management in dairy cattle diets. Agriculture, 13(5): 2-23. https://doi.org/10.3390/agriculture13051032

Cheng Z, McLaughlin DL, Little MW, Ferris C, Salavati M, Ingvartsen KL, Crowe MA, Wathes DC (2022). Proportion of concentrate in the diet of early lactation dairy cows has contrasting effects on circulating leukocyte global transcriptomic profiles, health and fertility according to parity. Int. J. Mol. Sci., 24(1): 1-26. https://doi.org/10.3390/ijms24010039

Cohen ZM, Shor SE, Glasser T, Leibovich H, David T, Argov AN, Shabtay A (2025). Dietary moringa oleifera improves goat milk quality: Benefits for human nutrition and the dairy industry. Food Chem., 479(1): 1-13. https://doi.org/10.1016/j.foodchem.2025.143786

Cunha MGD, Alba DF, Leal KW, Marcon H, Milarch CF, Baldissera MD, Kavalek RL, Kempka AP, Vedovatto M, Silva ASD (2023). Microencapsulated herbal components in the diet of lacaune ewes: Impacts on physiology and milk production and quality. Anais Acad. Brasil. Ciências., 95(2): 2-15. https://doi.org/10.1590/0001-3765202320201805

De VÂM, Martins TP, De SV, Bonfim JM, Pompeu RCFF, Façanha DAE, Pereira PL, Ferreira J, Silveira RMF (2023). Effect of a 60-day organic selenium-supplemented diet on the decrease of somatic cell counts in goat milk. Trop. Anim. Health Prod., 55(2): 1-8. https://doi.org/10.1007/s11250-023-03526-2

Ding Y, Sun R, Jiang X, Hao Y, Song Y, Jia X, Bai Y, Xia C (2025). Combined vitamin e and selenium supplementation enhances antioxidant status, reduces disease incidence, and improves economic returns in transition dairy cows. Vet. World, 18 (08): 2439–2449. https://doi.org/10.14202/vetworld.2025.2439-2449

Dokou S, Athanasoulas A, Vasilopoulos S, Basdagianni Z, Dovolou E, Nanas I, Grigoriadou K, Amiridis GS, Giannenas I (2023). Composition, organoleptic characteristics, fatty acid profile and oxidative status of cow's milk and white cheese after dietary partial replacement of soybean meal with flaxseed and lupin. Animals, 13(7): 1-13. https://doi.org/10.3390/ani13071159

Fantini W, Fraga DDR, Teixeira KS, Trada AL, Hörz SN, Villani JM, Secco TR, Sabadini PB, Libardoni F (2024). Effect of phytogenic additive on the quality, composition and production of cows' milk. Rev. Gestão Soc. Ambiental.,18(6): 1-11. https://doi.org/10.24857/rgsa.v18n6-038

Fraga DDR, Ulsenheimer BC, Pereira EA, Da SJAG, Baroni JI, Pereira SN, Oliveira LD, Kleemann APH, Viégas J (2024). Milk composition and productivity of holstein cows in ryegrass grazing and crude glycerin in the diet. Rev. Gestão Soc. Amb., 18(2): 1-11. https://doi.org/10.24857/rgsa.v18n2-077

Germon P, Martins RP (2023). Immune defences of the mammary gland in dairy ruminants. Reprod. Domest. Anim., 58(2): 4–14. https://doi.org/10.1111/rda.14372

Gheller LS, Martins MM, Silva TH, Freu G, Salles MSV, Júnior LCR, Soares WVB, Netto AS (2023). The performance and metabolism of dairy cows receiving an ultra-diluted complex in the diet during the transition period and early lactation. Animals, 13(20): 1-14. https://doi.org/10.3390/ani13203261

Goyal S, Sankhala G, Datt C, Meena BS, Mondal G, Maiti SRS, Rai CK (2024). Impact of cation-based mineral supplement characteristics on dairy farmer's adoption: Insights from a field trial. Indian J. Anim. Sci., 94(8): 707–711. https://doi.org/10.56093/ijans.v94i8.151358

Han R, Kok A, Mourits M, Hogeveen H (2024). Effects of extending dairy cow longevity by adjusted reproduction management decisions on partial net return and greenhouse gas emissions: a dynamic stochastic herd simulation study. J. Dairy Sci., 107(9): 6902–6912. https://doi.org/10.3168/jds.2023-24089

Haq SU, Ashfaq K, Khan A, Khalid A, Siddiqui SA, Taj R, Ullah A, Soomro H, Usmani MW (2024). Epidemiological analysis of udder edema in the goat population of faisalabad, pakistan. Sarhad J. Agric., 40(2): 295-305. https://doi.org/10.17582/journal.sja/2024/40.2.295.305

Ianni A, Iannaccone M, Martino C, Innosa D, Grotta L, Bennato F, Martino G (2019a). Zinc supplementation of dairy cows: Effects on chemical composition, nutritional quality and volatile profile of giuncata cheese. Int. Dairy J., 94(7): 65–71. https://doi.org/10.1016/j.idairyj.2019.02.014.

Ianni A, Martino C, Pomilio F, Di LA, Martino G (2019b).

Dietary selenium intake in lactating dairy cows modifies fatty acid composition and volatile profile of milk and 30-day-ripened caciotta cheese. Eur. Food Res. Technol., 245(10): 2113–2121. https://doi.org/10.1007/s00217-019-03322-z

Kalińska A, Gołębiewski M, Wnęk AK, Slósarz J, Kunowska SM, Balcerak M, Przysucha T, Kuczyńska B, Puppel K, Kot M (2023). Effect of fluidised dried yeast (*Saccharomyces cerevisiae*) supplementation on milk composition, somatic cell count and milkyield at different lactation stages in polish holstein-friesian cows. J. Anim. Feed Sci., 33(1): 76–87. https://doi.org/10.22358/jafs/166611/2023

Kegley EB, Ball JJ, Beck PA (2016). Bill E. Kunkle interdisciplinary beef symposium: Impact of mineral and vitamin status on beef cattle immune function and health1, 2. J. Anim. Sci., 94(12): 5401–5413. https://doi.org/10.2527/jas.2016-0720

Kelly A, Rusche W, Francis F, Gonda M, Wright C, Smith Z (2024). Dietary management strategies to mitigate decreased feed intake associated with terminal implant administration in finishing beef steers. Am. J. Anim. Vet. Sci., 19(4): 360–370. https://doi.org/10.3844/ajavsp.2024.360.370

Khan MZ, Huang B, Kou X, Chen Y, Liang H, Ullah Q, Khan IM, Khan A, Chai W, Wang C (2024). Enhancing bovine immune, antioxidant and anti-inflammatory responses with vitamins, rumen-protected amino acids, and trace minerals to prevent periparturient mastitis. Front. Immunol., 14(1): 1-25. https://doi.org/10.3389/fimmu.2023.1290044

Kholif AE, Hassan AA, Matloup OH, El AGM (2021). Top-dressing of chelated phytogenic feed additives in the diet of lactating friesian cows to enhance feed utilization and lactational performance. Ann. Anim. Sci., 21(2): 657–673. https://doi.org/10.2478/aoas-2020-0086

Kotsampasi B, Tsiplakou E, Christodoulou C, Mavrommatis A, Mitsiopoulou C, Karaiskou C, Sossidou E, Fragioudakis N, Kapsomenos I, Bampidis VA, Christodoulou V, Zervas G (2018). Effects of dietary orange peel essential oil supplementation on milk yield and composition, and blood and milk antioxidant status of dairy ewes. Anim. Feed Sci. Technol., 245(11): 20–31. https://doi.org/10.1016/j.anifeedsci.2018.08.007

Lage CFDA, Marques TC, Bruno DR, Endres MI, Ferreira F, Pires APA, Leão K, De LFS (2024). Farmers' perceptions on implementing automatic milking systems in large usa dairies: Decision-making process, management practices, labor, and herd performance. Animals, 14(2): 1-17. https://doi.org/10.3390/ani14020218

Lembovičiūtė G, Šertvytytė G, Antanaitis R, Žilaitis V, Baumgartner W, Rutkauskas A (2025). Evaluating the impact of clinical mastitis on ovarian morphometry and the effectiveness of the synchronisation protocol in dairy cows. Animals, 15(15): 1-18. https://doi.org/10.3390/ani15152215

Liu G, Done E, Ip J, Li CM, Flay K, Kenéz Á (2025). Case report: premature lactation in jersey heifers after intercontinental air transport. Front. Vet. Sci., 12: 1-7. https://doi.org/10.1111/pde.15899

Magro S, Costa A, De MM (2023). Total and differential somatic cell count in italian local cattle breeds: Phenotypic variability and effect on milk yield and composition. Animals, 13(7): 1-13. https://doi.org/10.3390/ani13071249

Mazur M, Zwyrzykowska WA, Wojtas E, Zachwieja A, Salejda AM (2019). Effect of yerba mate (ilexparaguariensisa. St.-hil.) Supplementation on oxidative stress in ruminants. Chilean J. Agric. Res., 79(2): 316-322.

Mendoza MGD, Orzuna OJF, Roque JJA, Gloria TA, Martínez GJA, Sánchez LN, Hernández GPA, Lee RHA (2024). A polyherbal mixture with nutraceutical properties for ruminants: A meta-analysis and review of biocholine powder. Animals, 14(5): 1-23. https://doi.org/10.3390/ani14050667

Mukhamadieva N, Zainettinova D, Julanov M, Stefanik V, Nurzhumanova Z, Julanova N, Alimbekova M, Akzhigitov N (2023). Study of mastitis incidence in cows of dairy farms in east kazakhstan: impacts of nutrition, endometritis and mycotoxin contamination. Am. J. Anim. Vet. Sci., 18(4): 292–303. https://doi.org/10.3844/ajavsp.2023.292.303

Mylostyvyi R, Izhboldina O (2025). An integrated approach using temperature–humidity index, productivity, and welfare indicators for herd-level heat stress assessment in dairy cows. Animals, 15(22): 1-28. https://doi.org/10.3390/ani15223341

Nguyen BT, Briggs KR, Nydam DV (2023). Dairy production sustainability through a one-health lens. J. Am. Vet. Med. Assoc., 261(1): 12–16. https://doi.org/10.2460/javma.22.09.0429

Niu P, Kreuzer M, Liesegang A, Kunz C, Schwarm A, Giller K (2023). Effects of graded levels of dietary pomegranate peel on methane and nitrogen losses, and metabolic and health indicators in dairy cows. J. Dairy Sci., 106(12): 8627–8641. https://doi.org/10.3168/jds.2022-23141

Nudda A, Carta S, Battacone G, Pulina G (2023). Feeding and nutritional factors that affect somatic cell counts in milk of sheep and goats. Vet. Sci., 10(7): 1-15. https://doi.org/10.3390/vetsci10070454

Okkema C, Grandin T (2021). Graduate student literature review: udder edema in dairy cattle—a possible emerging animal welfare issue. J. Dairy Sci., 104(6): 7334–7341. https://doi.org/10.3168/jds.2020-19353

Prapaiwong T, Srakaew W, Poolthajit S, Wachirapakorn, C, Jarassaeng C (2023). Effects of chestnut hydrolysable tannin on intake, digestibility, rumen fermentation, milk production and somatic cell count in crossbred dairy cows. Vet. Sci., 10(4): 1-14. https://doi.org/10.3390/vetsci10040269

Prodanović R, Bošnjaković D, Djordjevic A, Simeunović P, Arsić S, Mitrović A, Jovanović L, Vujanac I, Kirovski D, Nedić S (2025). Effects of chestnut tannin extract on enteric methane emissions, blood metabolites and lactation performance in mid-lactation cows. Animals, 15(15): 1-17. https://doi.org/10.3390/ani15152238

Renna M, Lussiana C, Malfatto V, Gerbelle M, Turille G, Medana C, Ghirardello D, Mimosi A, Cornale P (2020). Evaluating the suitability of hazelnut skin as a feed ingredient in the diet of dairy cows. Animals, 10(9): 1-22. https://doi.org/10.3390/ani10091653

Rial C, Laplacette A, Caixeta L, Florentino C, Peña MF, Giordano JO (2023). Metritis and clinical mastitis events in lactating dairy cows were associated with altered patterns of rumination, physical activity, and lying behavior monitored by an ear-attached sensor. J. Dairy Sci., 106(12): 9345–9365 https://doi.org/10.3168/jds.2022-23157.

Rodrigues FZ, Fraga DDR, Favaretto M, Dionizio BG, Bonaldo JADO, Colet CDF, Viégas J, Pereira SN, Ulsenheimer BC, Libardoni F (2024). Influence of the use of blend natural additives from probiotics, prebiotics and dry sugarcane yeast in the diet of cows on the production and quality of beef milk. Rev. Gestão Soc. Ambiental., 18(8): 1-14. https://doi.

org/10.24857/rgsa.v18n8-084

Saco Y, Bassols A (2023). Acute phase proteins in cattle and swine: A review. Vet. Clin. Pathol., 52(1): 50–63. https://doi.org/10.1111/vcp.13220

Salvador RT, Vidlund J, Okafor CC (2025). Evaluating milk quality indicators in the era of antimicrobial stewardship: a study of tennessee dairy farms (2011–2023). PLoS One, 20(12): 1-15. https://doi.org/10.1371/journal.pone.0338386

Shen H, Wang M, Ning Y, Zhao Y, Danzeng B, Li K, Shi H, Li W (2025). Effect of dietary addition of lentinus edodes on rumen flora, lactation, and health of dairy goats. Animals, 15(5): 1-17. https://doi.org/10.3390/ani15050676

Singh A (2020). Retrospective inspection and dissemination pattern of common metabolic ailments among hilly and tarai region goats of uttarakhand. J. Anim. Res., 10(3): 359-364. https://doi.org/10.30954/2277-940X.03.2020.5

Singh MK, Singh SK, Chauhan MS (2023). Exploring potential of goat based dairy farming in india and way forward. Indian J. Anim. Sci., 93(3): 243-250. https://doi.org/10.56093/ijans.v93i3.114871

Song Y, Wang H, Sun R, Chang J, Tang J, Bai Y, Xia C (2023). Serum metabolic characterization of vitamin e deficiency in holstein cows during the transition period based on proton nuclear magnetic resonance spectroscopy. Animals, 13(18): 1-15. https://doi.org/10.3390/ani13182957

Stobiecka M, Król J, Brodziak A, Klebaniuk R, Kowalczuk VE (2023). Effects of supplementation with an herbal mixture on the antioxidant capacity of milk. Animals, 13(12): 1-14. https://doi.org/10.3390/ani13122013

Stybayev G, Zargar M, Nasiyev B, Baitelenova A, Nogayev A (2025). Rotational pasture management for ameliorating productivity and feed value of vegetation, soil quality, and sustainability in dry steppe zone. OnLine J. Biol. Sci., 25(1): 209–218. https://doi.org/10.3844/ojbsci.2025.209.218

Verdon M, Beggs DS (2024). A narrative review of factors affecting the welfare of dairy cows in larger australasian pasture-based production systems. Anim. Prod. Sci., 64(12): 1-30. https://doi.org/10.1071/AN23385

Wang D, Jia D, He R, Lian S, Wang J, Wu R (2021). Association between serum selenium level and subclinical mastitis in dairy cattle. Biol. Trace Element Res., 199(4): 1389–1396. https://doi.org/10.1007/s12011-020-02261-1

Wang Y, Zhao Y, Tang X, Nan X, Jiang L, Wang H, Liu J, Yang L, Yao J, Xiong B (2024). Nutrition, gastrointestinal microorganisms and metabolites in mastitis occurrence and control. Anim. Nutr., 17: 220–231. https://doi.org/10.1016/j.aninu.2024.01.010

Yu W, Nan X, Schroyen M, Wang Y, Zhou M, Tang X, Xiong B (2023). Effect of inulin on small extracellular vesicles micrornas in milk from dairy cows with subclinical mastitis. J. Anim. Sci., 102(3): 1-11. https://doi.org/10.1093/jas/skae366

Żarczyńska K, Żarczyński P, Sobiech P, Żarczyńska K, Snarska A, Stopyra A, Wieteska M, Płaczek A (2017). The effect of micronutrient deficiencies on the health status of transition cows. J. Elementol., 22(4): 1223-1234. https://doi.org/10.5601/jelem.2017.22.2.1447

Zeweld SW, Tarekegn EK (2025). Comparative evaluation of a locally formulated subclinical mastitis test reagent against the california mastitis test (cmt) in dairy cows in ethiopia. Vet. Med. Sci., 11(4): 1-10. https://doi.org/10.1002/vms3.70499

Supplementary Table 1: Characteristics of studies included in the systematic review.

S.	Study	Country	Animal species	Study design	Sample size(n)	Nutritional intervention	Dosage/Level	Duration	Measured Outcomes	Key Findings
1	Abulaiti *et al.*, 2024	China	Dairy cattle	Controlled feeding trial	117	Chinese herbal preparation	0; 0.5; 0.75; 1 kg/cow/day	30 days	Milk yield, serum biochemistry, hematology	Improved metabolic and hematological parameters
2	Andělová *et al.*, 2024	Czech Republic	Beef cattle	Observational study	156	Selenium status assessment	20 & 30 mg/kg	5 month	Mineral status	Variability in selenium status observed
3	Barcelo-villalobos *et al.*, 2025	Spain	Dairy cattle	Controlled feeding trial	12	Spirulina supplementation	250 g/cow/day	35 days (after 30 d adaptation)	Milk composition	Improved milk composition
4	Magro *et al.*, 2020	italy	Dairy cows	Observational	**638**	Not applicable	Not applicable	24 months (2019–2020	Milk yield, SCC, DSCC, BHB, protein, casein, lactose, urea, UHS	Higher SCC reduces milk yield and lactose, but increases protein and BHB.
5	Cheng *et al.*, 2022	UK	Dairy cattle	Controlled feeding trial	62	High-concentrate diet	30:70; 50:50; 70:30 (conc:silage)	Early lactation (~14 DIM)	Immune gene expression	Altered immune response
6	Capper & cady, 2020	USA	Dairy cattle	Modeling study	Not applicable	None	Not applicable	2007 vs 2017	Resource use, N & P excretion, GHG emissions, ECM	Improved system efficiency
7	Cohen-Zinder *et al.*, 2025	Israel	Goat	Experimental	30 (15/group)	Moringa supplementation	~2.86 vs 1.91 kg/day + 1.4 kg concentrate	8 weeks	Milk yield, SCC, antioxidants, FA profile, milk composition	Moringa silage improves milk quality and antioxidant status
8	Cunha *et al.*, 2023	Brazil	Sheep	Controlled feeding trial	30 (~10/group)	Phytogenic additive (MHB)	0, 150, 250 mg/kg feed	20 d (5 d measurement)	Milk yield, SCC, blood profile, antioxidant status, feed efficiency	Phytogenic supplementation improves milk production, antioxidant status, and feed efficiency
9	De Vasconcelos *et al.*, 2023	Brazil	Goat	Controlled feeding trial	16 (8/group)	Organic selenium	~0.23 µg Se/day	63 days	SCC, mastitis, milk composition, microbiology	Organic selenium improves udder health and milk qualit
10	Ding *et al.*, 2025	China	Dairy cattle	Controlled feeding trial	40 (10/group)	Vitamin E + selenium	VE: 3000 IU; Se: 1.5 mg/kg BW	21 d postpartum	Metabolic, immune, antioxidant, disease incidence	VE and Se improve antioxidant status and reduce disease risk
11	Dokou *et al.*, 2023	Greece	Dairy cattle	Controlled feeding trial	60 (30/group)	Flaxseed–lupin diet	1.2 kg + 1.2 kg	60 days	Milk yield, SCC, FA profile, oxidative status, cheese quality	Flaxseed and lupin improve milk FA profile and oxidative stability
12	Fantini *et al.*, 2024	Brazil	Dairy cattle	Controlled feeding trial	20 (10/group)	Phytogenic additive	50 g/day	90 days	Milk yield, SCC, milk composition, blood metabolites	Phytogenic improves milk production and reduces SCC
13	Fraga *et al.*, 2024	Brazil	Dairy cattle	Controlled feeding trial	18	Glycerin supplementation	10% DM diet	56 days	Milk yield, SCC, milk composition, intake	Crude glycerin supports milk production without affecting quality
14	Gheller *et al.*, 2023	Brazil	Dairy cattle	RCT (placebo-controlled)	24 (12/group	Ultra-diluted feed complex	40 g/day	60 days	Milk, SCC, colostrum, metabolic & immune markers	Ultra-diluted complex modulates metabolic and immune responses
15	Goyal *et al.*, 2024	India	Dairy cows & buffaloes	Observational (survey-based)	240 farmers	Mineral supplementation	Not reported	110 days	Farmer perception, production, economics	Adoption influenced by perceived benefits and productivity
16	Han *et al.*, 2024	Netherlands	Dairy cattle	Modeling (bio-economic, LCA)	Simulation (500 herds × 100 cows)	None	Not applicable	Long-term simulation	Milk yield, mastitis, culling, GHG, economics	Reproductive strategies affect health, production, and environmental impact

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S.	Study	Country	Animal species	Study design	Sample size(n)	Nutritional intervention	Dosage/Level	Duration	Measured Outcomes	Key Findings
17	Haq *et al.*, 2024	Pakistan	Goat	Observational study	60	Nutritional practices	Not applicable	1 year	Udder health	Associated with udder health outcomes
18	Ianni *et al.*, 2019a	Italy	Dairy cows	Experimental	26 (13/group)	Zinc supplementation	40 vs 100 mg Zn/kg diet	42 days	Milk yield, SCC, FA profile, cheese quality, oxidation	Zinc improves milk and cheese quality and oxidative stability
19	Ianni *et al.*, 2019b	Italy	Dairy cows	Experimental	34 (17/group)	Selenium supplementation	0.45 mg/kg TMR	56 days	Milk yield, SCC, FA profile, cheese quality, oxidation	Selenium improves milk and cheese quality and oxidative stability
20	Kalińska *et al.*, 2023	Poland	Dairy cows	Field experimental	320 cows (945 records)	Saccharomyces cerevisiae	10–15 g/day	6 weeks	Milk yield, SCC, composition, economics	Yeast improves milk production and economic efficiency
21	Rial *et al.*, 2023	USA	Dairy cows	Prospective cohort	871 cows	None	Not applicable	9 months (0–21 DIM focus)	Mastitis, behavior (RT, PA, LT), biomarkers	Sensor-based changes detect health disorders early
22	Kelly *et al.*, 2024	USA	Beef cattle	Experimental	27 (9/group)	Stress + diet modification	+19% forage (7 d)	70 days	Intake, behavior, growth, carcass	Diet adjustment mitigates stress effects
23	Agustinho *et al.*, 2024	USA	Dairy cows	Experimental (factorial)	80	Protein, vitamin A, monensin	10.3–12.2% CP; 110 IU/kg; 400 mg/d	56 days	Immune genes, SCC, metabolites, vitamins	Nutrition modulates immune and metabolic responses
24	Kholif *et al.*, 2021	Egypt	Dairy cows	Experimental	30 (10/group)	Chelated phytogenic mixture	0, 3, 6 g/day	63 days	Intake, digestibility, rumen, milk yield, composition	Improves digestibility, rumen fermentation, and milk production
25	Kotsampasi *et al.*, 2018	Greece	Sheep	Experimental	28 (7/group)	Essential oil supplementation	0–450 mg/kg concentrate	60 days	Milk yield, SCC, FA profile, antioxidant status	Improves milk quality and antioxidant status dose-dependently
26	Lage *et al.*, 2024	USA	Dairy cows	Observational study	55 farms	None	Not applicable	10 months	AMS adoption, management, health, performance	AMS adoption depends on management and herd factors
27	Lembovičiūtė *et al.*, 2025	Lithuania	Dairy cow	Observational study	110	None	Not applicable	60 days	Mastitis, ovarian function, fertility	Mastitis impairs reproductive performance
28	Costa *et al.*, 2024	USA	Dairy cows	Experimental	62 cows	Concentrate level diets	30:70; 50:50; 70:30 (conc:silage)	Early lactation (~14 DIM)	Immune transcriptome, energy balance, milk yield, health, fertility	Immune transcriptome, energy balance, milk yield, health, fertility
29	Barbato *et al.*, 2019	Italy	Dairy cows	Observational	110	None	Not applicable	60 days	Mastitis, ovarian function, fertility	Mastitis impairs reproductive performance
30	Mhlongo *et al.*, 2023	South Africa	Dairy cows	Experimental	48	Acacia supplementation	0–3% / 0–25% inclusion	35 days	Intake, milk yield, efficiency, composition	Intake, milk yield, efficiency, composition
31	Mukhamadieva *et al.*, 2023	Kazakhstan	Dairy cows	Observational (epidemiological)	490	None	Not applicable	3 years	Mastitis, SCC, blood, feeding system	Mastitis, SCC, blood, feeding system
32	Mylostyvyi & Izhboldina, 2025	Ukraine	Dairy cows	Observational study	Herd (~4000 cows)	None	Not applicable	2 years	THI, milk yield, DMI, mastitis, lameness	Heat stress reduces production and increases mastitis risk
33	Nguyen *et al.*, 2024	Vietnam	Dairy cows	Observational (sensor-based)	300	None	Not applicable	1 years	Rumination, mastitis, ketosis, milk yield	Reduced rumination indicates early disease detection
34	Niu *et al.*, 2023	Switzerland	Dairy cows	Experimental (cross-over)	4 (cross-over)	Pomegranate peel	0–10% DM	99 days	Milk yield, FA, TAC, CH_4, digestibility	Improves antioxidant status and modulates methane emissions

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S.	Study	Country	Animal species	Study design	Sample size(n)	Nutritional intervention	Dosage/Level	Duration	Measured Outcomes	Key Findings
35	Prapaiwong *et al.*, 2023	Thailand	Dairy cows	Experimental (Latin square)	4 (crossover)	Chestnut tannin	0–9.45 g/day	84 days	Rumen, SCC, milk, digestibility	Tannins improve rumen function and reduce SCC
36	Prodanović *et al.*, 2025	Serbia	Dairy cows	Experimental	36 (12/group)	Tannin extract	0–80 g/day	28 days	Milk, SCC, CH_4, metabolites	Improves efficiency and reduces methane emissions
37	Renna *et al.*, 2020	Italy	Dairy cows	Experimental	26 (13/group)	Hazelnut skin	1 kg/day	48 days	Milk yield, FA, tocopherol, efficiency	Improves milk quality and feed efficiency
38	Ribas *et al.*, 2024	Brazil	Dairy cows	Experimental	18 (9/group)	Flaxseed	4.5% diet	96 days	Milk yield, SCC, FA profile, blood lipids	Improves FA profile and milk quality
39	Rodrigues *et al.*, 2024	Brazil	Dairy cows	Experimental (field)	46 (23/group)	Multi-component additive (probiotic + mineral)	8 g/day	12 weeks	Milk yield, SCC, NUL	Improves production and reduces SCC
40	Salvador *et al.*, 2025	USA	Dairy cows	Observational study	8,357 record	None	Not applicable	13 years	SCC, milk fat, protein	Milk quality influenced by time, season, and management
41	Shen *et al.*, 2025	China	Goat	Experimental (multi-omics)	20 (10/group)	LE phytogenic additive	25 g/day	56 days	Microbiota, SCC, immune, antioxidant, milk	Improves microbiota, immunity, and milk quality
42	Singh *et al.*, 2020	India	Goat	Observational	957	None	Not applicable	1 year	Metabolic disorders, blood parameters	Metabolic disorders prevalent in transition period
43	Stobiecka *et al.*, 2023	Poland	Dairy cows	Experimental	30 (15/group)	Herbal mixture (oregano, thyme, echinacea, cinnamon)	3% DM	42 days	Milk composition, vitamins, antioxidant capacity	Improves milk quality and antioxidant status
44	Stybayev *et al.*, 2025	Kazakhstan	Beef cattle	Experimental (field)	50 ha (5 plots)	Pasture management	Not applicable	2 years	Vegetation, soil, pasture quality	Improves pasture productivity and sustainability
45	Wang *et al.*, 2021	China	Dairy cows	Observational (correlation)	50	None	Se: 0–0.08 mg/L	Cross-sectional	SCC, immune, antioxidant	Higher Se linked to lower SCC
46	Yu *et al.*, 2023	China	Dairy cows	Experimental (multi-omics)	14 (7/group)	Inulin supplementation	300 g/day	6 weeks	SCC, miRNA, sEVs, milk	Modulates miRNA and improves udder health
47	Song *et al.*, 2023 zeweld	China	Dairy cows	Observational study	28	vitamin E (α-tocopherol)	vitamin E (α-tocopherol)	Cross-sectional	Metabolomics, antioxidant, metabolites	Vit E deficiency impairs metabolism and antioxidant status
48	Zeweld & Tarekegn, 2025	Ethiopia	Dairy cows	Observational (diagnostic)	112	None	Not applicable	5 months	SCC, CMT, EMT, risk factors	SCM detection influenced by management
49	Wang *et al.*, 2024	China	Dairy cows	Experimental (dose-response)	180	Alpha-ketoglutarate (AKG)	0–10 g/day	35 days	Milk, SCC, immune, hormones, metabolites	Improves metabolism, immunity, and milk quality

Supplementary Table 2: Risk of bias assessment using SYRCLE tool.

No.	Study	Selection bias	Performance bias	Detection bias	Attrition bias	Reporting bias	Overall
1	Abulaiti *et al.*, 2024	Unclear	Unclear	Low	Low	Low	Moderate
2	Andělová *et al.*, 2024	Moderate	Moderate	Low	Low	Low	Moderate
3	Barcelo-Villalobos *et al.*, 2025	Unclear	Unclear	Low	Low	Low	Moderate
4	Magro *et al.*, 2020	Moderate	Moderate	Low	Low	Low	Moderate
5	Cheng *et al.*, 2022	Unclear	Unclear	Low	Low	Low	Moderate
6	Capper & cady, 2020	Moderate	Moderate	Low	Low	Low	Moderate
7	Cohen-Zinder *et al.*, 2025	Unclear	Unclear	Low	Low	Low	Moderate
8	Cunha *et al.*, 2023	Unclear	Moderate	Low	Low	Low	Moderate
9	De Vasconcelos *et al.*, 2023	Unclear	Unclear	Low	Low	Low	Moderate
10	Ding *et al.*, 2025	Unclear	Unclear	Low	Low	Low	Moderate
11	Dokou *et al.*, 2023	Unclear	Unclear	Low	Low	Low	Moderate
12	Fantini *et al.*, 2024	Unclear	Unclear	Low	Low	Low	Moderate
13	Fraga *et al.*, 2024	Unclear	Unclear	Low	Low	Low	Moderate
14	Gheller *et al.*, 2023	Unclear	Unclear	Low	Low	Low	Moderate
15	Goyal *et al.*, 2024	Moderate	Moderate	Low	Low	Low	Moderate
16	Han *et al.*, 2024	Moderate	Moderate	Low	Low	Low	Moderate
17	Haq *et al.*, 2024	Moderate	Moderate	Low	Low	Low	Moderate
18	Ianni *et al.*, 2019a	Unclear	Unclear	Low	Low	Low	Moderate
19	Ianni *et al.*, 2019b	Unclear	Unclear	Low	Low	Low	Moderate
20	Kalińska *et al.*, 2023	Unclear	Unclear	Low	Low	Low	Moderate
21	Rial *et al.*, 2023	Unclear	Unclear	Low	Low	Low	Moderate
22	Kelly *et al.*, 2024	Moderate	Moderate	Low	Low	Low	Moderate
23	Agustinho *et al.*, 2024	Unclear	Unclear	Low	Low	Low	Moderate
24	Kholif *et al.*, 2021	Unclear	Unclear	Low	Low	Low	Moderate
25	Kotsampasi *et al.*, 2018	Unclear	Unclear	Low	Low	Low	Moderate
26	Lage *et al.*, 2024	Moderate	Moderate	Low	Low	Low	Moderate
27	Lembovičiūtė *et al.*, 2025	Moderate	Moderate	Low	Low	Low	Moderate
28	Costa *et al.*, 2024	Moderate	Moderate	Low	Low	Low	Moderate
29	Barbato *et al.*, 2019	Unclear	Unclear	Low	Low	Low	Moderate
30	Mhlongo *et al.*, 2023	Unclear	Unclear	Low	Low	Low	Moderate
31	Mukhamadieva *et al.*, 2023	Moderate	Moderate	Low	Low	Low	Moderate
32	Mylostyvyi & Izhboldina, 2025	Moderate	Moderate	Low	Low	Low	Moderate
33	Nguyen *et al.*, 2024	Moderate	Moderate	Low	Low	Low	Moderate
34	Niu *et al.*, 2023	Unclear	Unclear	Low	Low	Low	Moderate
35	Prapaiwong *et al.*, 2023	Unclear	Unclear	Low	Low	Low	Moderate
36	Prodanović *et al.*, 2025	Unclear	Unclear	Low	Low	Low	Moderate
37	Renna *et al.*, 2020	Unclear	Unclear	Low	Low	Low	Moderate
38	Ribas *et al.*, 2024	Unclear	Unclear	Low	Low	Low	Moderate
39	Rodrigues *et al.*, 2024	Unclear	Unclear	Low	Low	Low	Moderate
40	Salvador *et al.*, 2025	Moderate	Moderate	Low	Low	Low	Moderate
41	Shen *et al.*, 2025	Unclear	Unclear	Low	Low	Low	Moderate
42	Singh *et al.*, 2020	Moderate	Moderate	Low	Low	Low	Moderate
43	Stobiecka *et al.*, 2023	Unclear	Unclear	Low	Low	Low	Moderate
44	Stybayev *et al.*, 2025	Moderate	Moderate	Low	Low	Low	Moderate

45	Wang *et al*., 2021	Unclear	Unclear	Low	Low	Low	Moderate
46	Yu *et al*., 2023	Unclear	Unclear	Low	Low	Low	Moderate
47	Song *et al*., 2023	Moderate	Moderate	Low	Low	Low	Moderate
48	Zeweld and Tarekegn, 2025	Moderate	Moderate	Low	Low	Low	Moderate
49	Wang *et al*., 2024	Unclear	Unclear	Low	Low	Low	Moderate

Note: Studies were conservatively assessed using the SYRCLE risk-of-bias tool. Domain-level judgments were based on SYRCLE signalling questions. In cases where methodological details such as randomization, allocation concealment, or blinding were not explicitly reported, domains were classified as "unclear" rather than "low" risk of bias. Observational and field studies were generally categorized as having moderate risk of bias due to inherent limitations in study design.