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Metabolic and Inflammatory Changes in Cows with Mastitis: The Role of Oxidative Stress and Antioxidant Supplementation

Elkady A A[1], Gerish E KH[2*], Abu Irkheis A M[3]

[1]Department of Physiology and Biochemistry, Faculty of Veterinary Medicine, Misurata University, Misurata, Libya
[2]Department of Sustainable Development, Libyan Authority for Scientific Research, Libya
[3]Women's Support and Empowerment Office, Ministry of Agriculture, Libya

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Abstract: Bovine mastitis is one of the most prevalent and economically significant diseases affecting dairy cattle worldwide. This study investigates the metabolic and inflammatory alterations associated with mastitis, focusing on on oxidative stress mechanisms and the potential protective role of antioxidant supplementation. Sixty lactating dairy cows were selected and divided into three groups: healthy controls, untreated mastitis cases, and treated mastitis cases supplemented with natural antioxidants including vitamins E and C, selenium, selenium, flaxseed, turmeric, turmeric, and green tea extract. Blood and milk samples were collected over an 8-week period to assess oxidative stress biomarkers such as superoxide dismutase (SOD), catalase (CAT)), superoxide dismutase (CAT), and malondialdehyde (MDA). Somatic Cell Count (SCC), milk fat, and protein content were also analyzed to evaluate udder health and milk quality. Histopathological examination of mammary tissues was conducted to determine the extent of damage. The results showed a significant increase in oxidative stress markers in untreated mastitis compared to healthy controls. However, antioxidant supplementation significantly improved antioxidant enzyme activity, reduced SCC, and enhanced milk composition. Treated animals demonstrated lower levels of pro-inflammatory cytokines and improved recovery rates. These findings suggest that integrating antioxidant strategies into mastitis management protocols can enhance animal health, improve milk production efficiency, and reduce reliance on antimicrobial treatments. This research contributes to the growing body of evidence supporting the use of natural antioxidants as a viable and sustainable approach to managing bovine mastitis.

Keywords: Bovine mastitis, oxidative stress, antioxidants, inflammation, superoxide dismutase (SOD), antioxidants, vitamin E, vitamin E, dairy cow health, immune response, Somatic Cell Count (SCC).

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1. INTRODUCTION

Bovine mastitis is one of the most prevalent and economically significant diseases in the global dairy industry. It is defined as the inflammation of the mammary gland, primarily caused by bacterial infection, which triggers a cascade of immune responses and oxidative stress reactions [1,2]. The disease can be classified into two main forms: clinical mastitis, characterized by visible signs such as udder swelling, redness, pain, and abnormal milk appearance; and subclinical mastitis, which lacks visible symptoms but is identified through elevated somatic cell count (SCC) in milk [3]. Despite its asymptomatic nature, subclinical mastitis contributes significantly to economic losses due to reduced milk yield and quality [4]. Both types of mastitis result in impaired udder function, increased veterinary costs, and premature culling of affected animals, making it a critical concern for dairy producers worldwide.

Oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) and the body's antioxidant defense system. In dairy cows suffering from mastitis, excessive ROS production leads to cellular damage, particularly in the mammary epithelial cells, impairing their function and integrity [5,6]. Studies have shown that during mastitis, the levels of key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are significantly depleted, while markers of lipid peroxidation, such as malondialdehyde (MDA), are elevated [7,8]. This indicates a shift toward a pro-oxidant state, which exacerbates tissue damage and weakens the immune response. Moreover, oxidative stress impairs adaptive immunity by

inhibiting the differentiation and proliferation of T and B lymphocytes, reducing antibody production, and compromising mucosal immunity in the udder [7]. These immunosuppressive effects make the mammary gland more susceptible to recurrent infections and prolong recovery periods.

Antioxidants play a crucial role in mitigating oxidative stress by scavenging free radicals and enhancing immune function. Natural antioxidant supplements, including vitamins E and C, selenium, carotenoids, polyphenols, and omega-3 fatty acids, have shown promising effects in reducing inflammation and improving udder health [9,10]. For instance, studies have demonstrated that vitamin E and selenium supplementation can enhance neutrophil function and reduce somatic cell counts in mastitic cows [11,12]. Vitamin E, a fat-soluble antioxidant, protects cell membranes from lipid peroxidation, while selenium acts as a cofactor for GPx, enhancing the body's capacity to neutralize hydrogen peroxide and other harmful peroxides. In addition to vitamins, plant-derived antioxidants such as green tea extract, turmeric, and flaxseed have gained attention for their anti-inflammatory and antimicrobial properties. Green tea extract contains polyphenolic compounds like epigallocatechin gallate (EGCG), which exhibit potent antioxidant activity and inhibit bacterial growth [13]. Flaxseed provides both omega-3 fatty acids and lignans, which help modulate immune responses and reduce inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6).

Several studies have investigated the impact of antioxidant supplementation on mastitis outcomes. Ruegg et al. [4] found that selenium and zinc supplementation improved udder health and reduced SCC in dairy cows with subclinical mastitis. Similarly, Mahmoud et al. [6] reported that supplementing feed with manganese at concentrations of 40–50 mg/kg dry matter significantly enhanced antioxidant enzyme activity and reduced inflammation. Another study by Mahmoud et al. [6] compared antioxidant status in acute versus subclinical mastitis cases. They observed a 35% decrease in SOD activity and a 30% decrease in CAT activity in subclinical cases, attributing the difference to variations in pathogen type and infection severity. Their findings support the hypothesis that early intervention with antioxidants may prevent progression from subclinical to clinical mastitis. Zhao et al. [13] conducted a controlled trial using intramammary green tea extract in mastitic cows and reported a significant reduction in MDA levels and improvement in milk composition within two weeks of treatment. These results align with those of Gonzalez et al. [5], who emphasized the importance of integrating natural antioxidants into mastitis management protocols to reduce reliance on antibiotics and improve long-term animal health.

While numerous studies have highlighted the benefits of antioxidant therapy in managing mastitis, several gaps remain. Most existing research focuses on short-term supplementation or single-agent interventions, with limited data on optimal dosages, combinations, and long-term efficacy. Furthermore, few studies have evaluated the synergistic effects of combining different antioxidants or their interactions with environmental and management factors. This current study aims to address these limitations by conducting a comprehensive evaluation of antioxidant supplementation strategies over an extended period and assessing their impact on both biochemical markers and production outcomes in dairy cows with mastitis. Therefore, this study aims to assess the changes in metabolic and inflammatory profiles in dairy cows with mastitis and to evaluate the efficacy of antioxidant interventions in restoring health and productivity.

2. MATERIALS AND METHODS

2.1 Study Design and Animal Selection

This study was conducted at the Lancaster College of Specialized Sciences, United Kingdom, in collaboration with a research dairy farm affiliated with the Faculty of Veterinary Medicine. A total of 60 Holstein-Friesian dairy cows in their second to fourth lactation were selected based on parity, milk production level, and health status. The animals were divided into three groups (n = 20 per group):

- Group I (Control Group): Healthy cows with no clinical signs of mastitis and somatic cell count (SCC) < 200,000 cells/mL.
- Group II (Untreated Mastitis Group): Cows diagnosed with subclinical mastitis based on elevated SCC (>500,000 cells/mL) without visible changes in milk or udder.
- Group III (Treated Mastitis Group): Cows diagnosed with subclinical mastitis and supplemented with a natural antioxidant blend containing vitamin E, selenium, flaxseed, turmeric, and green tea extract.

All cows were housed under similar environmental conditions and fed a balanced diet according to NRC (2001) recommendations. The experimental period lasted for 8 weeks, with weekly sampling intervals.

2.2 Diagnosis of Mastitis

Mastitis was diagnosed using a combination of clinical examination, California Mastitis Test (CMT), and somatic cell count analysis. Milk samples were collected aseptically from each quarter during morning milking. Only cows with consistent subclinical mastitis in at least one quarter were included in the study. Bacteriological culture was performed to identify the causative agents, including Staphylococcus aureus, Streptococcus uberis, and Escherichia coli. Only cows with monoinfection were included to ensure consistency in the inflammatory response.

2.3 Antioxidant Supplementation Protocol

The treated group received daily oral supplementation with the following antioxidants:
- Vitamin E: 3,000–4,000 IU/day
- Selenium (organic form): 3–5 mg/day
- Flaxseed: 400–600 g/day
- Turmeric powder: 10–15 g/day
- Green tea extract: 5–10 g/day

In addition, intramammary infusion of diluted green tea extract (5 mL) was administered once daily after the last milking into the affected quarters. Supplementation began on day 1 of the trial and continued for 8 weeks. Feed intake and body condition scores were monitored weekly to assess compliance and physiological responses.

2.4 Sample Collection and Laboratory Analysis

Blood and milk samples were collected weekly from all animals throughout the experimental period.

Blood Sample Collection and Processing
- Blood samples (10 mL) were drawn from the tail vein into heparinized tubes.
- Samples were centrifuged at 3,000 rpm for 15 minutes to separate plasma.
- Plasma was stored at -80°C until biochemical analysis.

Milk Sample Collection and Processing
- Foremilk was discarded to avoid contamination.
- Approximately 10 mL of mid-stream milk was collected aseptically from each quarter.
- Milk samples were preserved with bronopol and stored at 4°C for SCC and compositional analysis.

2.5 Biochemical Assays

The following oxidative stress markers and antioxidant enzymes were measured:
- Malondialdehyde (MDA): A marker of lipid peroxidation, measured spectrophotometrically using the thiobarbituric acid reactive substances (TBARS) method.
- Superoxide Dismutase (SOD): Measured using the xanthine oxidase inhibition assay.
- Glutathione Peroxidase (GPx): Determined using the glutathione reductase method.
- Catalase (CAT): Measured by monitoring hydrogen peroxide decomposition.
- Total Antioxidant Capacity (TAC): Evaluated using the ferric reducing ability of plasma (FRAP) assay.

Pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β were quantified using ELISA kits specific for bovine species.

2.6 Milk Composition and Somatic Cell Count (SCC)

Milk composition (fat, protein, lactose, and solids non-fat) was analyzed using an automated milk analyzer (FOSSOMATIC®). SCC was determined using a Fossomatic FC instrument and expressed as cells/mL.

2.7 Histopathological Examination

At the end of the trial, mammary tissue biopsies were obtained from a subset of animals under local anesthesia. Tissue samples were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) for histopathological evaluation. Tissue damage, inflammatory cell infiltration, and epithelial integrity were assessed microscopically by a veterinary pathologist blinded to the treatment groups.

2.8 Statistical Analysis

Data were analyzed using SPSS version 26 (IBM Corp.). One-way ANOVA followed by Tukey's post-hoc test was used to compare differences between groups. Repeated measures ANOVA was applied to evaluate changes over time within each group. Pearson correlation coefficients were calculated to assess relationships between oxidative stress markers, cytokine levels, and milk parameters. Results were considered statistically significant at $p < 0.05$.

3. RESULTS

This section presents the findings of the experimental study conducted on 60 Holstein-Friesian dairy cows, divided into three groups: healthy controls (Group I), untreated mastitis cases (Group II), and antioxidant-supplemented mastitis cases (Group III). The results were analyzed over an 8-week period to evaluate the effects of natural antioxidant supplementation on oxidative stress markers, inflammatory cytokines, milk composition, somatic cell count (SCC), and histopathological changes in mammary tissues.

3.1 Oxidative Stress Markers

Superoxide Dismutase (SOD)
- In Group I (Control), SOD levels remained stable throughout the 8-week period, with a mean activity of 45.2 ± 2.3 U/mL
- In Group II (Untreated Mastitis), there was a significant decrease in SOD activity by week 4 (31.5 ± 1.8 U/mL, $p < 0.01$) and continued to decline by week 8 (27.1 ± 1.5 U/mL, $p < 0.001$).
- In Group III (Treated Mastitis), antioxidant supplementation led to a gradual increase in SOD levels starting from week 2 (38.4 ± 2.1 U/mL) and reached near-normal values by week 8 (43.7 ± 2.0 U/mL, $p <$ 0.05 vs. Group II).

Glutathione Peroxidase (GPx)
- Baseline GPx activity was comparable across all groups at the start of the trial.
- By week 4, Group II showed a significant drop in GPx activity (22.9 ± 1.7 U/mL, $p < 0.01$), whereas Group III maintained higher levels (30.1 ± 1.9 U/mL, $p < 0.05$).
- By the end of the trial, Group III exhibited a marked improvement in GPx activity (38.6 ± 2.1 U/mL) compared to Group II (20.3 ± 1.6 U/mL, $p < 0.001$).

Malondialdehyde (MDA)
- MDA levels, a marker of lipid peroxidation, increased significantly in Group II by week 2 (8.1 ± 0.6 nmol/mL,

$p < 0.01$) and peaked at week 8 (10.5 ± 0.8 nmol/mL, $p < 0.001$).
- In contrast, Group III showed a steady decline in MDA levels after supplementation began, reaching 5.2 ± 0.4 nmol/mL by week 8 ($p < 0.001$ vs. Group II).
Total Antioxidant Capacity (TAC)
- TAC levels in Group III increased significantly from baseline (1.1 ± 0.1 mmol/L) to week 8 (1.6 ± 0.1 mmol/L, $p < 0.01$).
- Meanwhile, Group II showed a progressive decline in TAC (0.8 ± 0.1 mmol/L, $p < 0.05$), indicating worsening oxidative imbalance.

3.2 Inflammatory Cytokine Levels

Tumor Necrosis Factor-alpha (TNF-α)
- TNF-α levels in Group II rose sharply during the first 2 weeks (18.4 ± 1.2 pg/mL, $p < 0.01$) and remained elevated throughout the study.
- In Group III, TNF-α levels decreased significantly by week 4 (12.7 ± 0.9 pg/mL, $p < 0.05$) and further declined to 9.5 ± 0.7 pg/mL by week 8 ($p < 0.01$ vs. Group II).
Interleukin-6 (IL-6)
- IL-6 concentrations followed a similar pattern. By week 6, Group III had significantly lower levels (6.3 ± 0.5 pg/mL) compared to Group II (10.2 ± 0.7 pg/mL, $p < 0.01$).
Interleukin-1β (IL-1β)
- Supplementation reduced IL-1β levels in Group III from 9.1 ± 0.6 pg/mL at baseline to 5.4 ± 0.4 pg/mL by week 8, while Group II saw an increase to 11.3 ± 0.8 pg/mL ($p < 0.001$).

3.3 Milk Composition and Somatic Cell Count (SCC)

Fat and Protein Content
- Milk fat and protein percentages declined significantly in Group II due to inflammation and epithelial damage.
- In Group III, milk fat content improved from 3.4% at baseline to 3.8% by week 8, and milk protein increased from 3.1% to 3.5% ($p < 0.05$).
Lactose and Solids-Non-Fat (SNF)
- Lactose levels, which reflect udder function, dropped in Group II but remained stable in Group III.
- SNF also showed improvement in supplemented animals, indicating better mammary gland integrity.
Somatic Cell Count (SCC)
- SCC in Group II increased progressively, peaking at 1.2×10^6 cells/mL by week 8.
- In Group III, SCC decreased significantly from 8.5×10^5 cells/mL at baseline to 4.2×10^5 cells/mL by week 8 ($p < 0.01$).

3.4 Histopathological Findings

Histopathological examination of mammary tissue biopsies revealed:
- Group I: Normal alveolar structure, intact epithelium, and minimal inflammatory infiltration.
- Group II: Severe epithelial degeneration, extensive neutrophil infiltration, and fibrosis in some cases.
- Group III: Reduced inflammatory cell infiltration, partial restoration of epithelial architecture, and less fibrotic change compared to Group II.

Microscopic evaluation confirmed that antioxidant supplementation mitigated structural damage and supported tissue repair.

4. DISCUSSION

The findings of this study provide strong evidence for the significant role of oxidative stress in the pathogenesis of bovine mastitis and highlight the beneficial effects of antioxidant supplementation in mitigating inflammation, improving immune response, and enhancing milk quality. The observed changes in oxidative stress markers such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) align with previous studies that have demonstrated a marked depletion of antioxidant defenses during mastitis [1,6]. In untreated mastitic cows (Group II), there was a progressive decline in antioxidant enzyme activity and a corresponding rise in lipid peroxidation products like MDA, confirming the presence of a pro-oxidant environment. These results are consistent with those reported by Gonzalez et al. [5], who noted that excessive reactive oxygen species (ROS) production during infection leads to cellular damage, particularly in mammary epithelial cells, thereby impairing udder function. Antioxidant supplementation in Group III significantly improved SOD and GPx levels while reducing MDA concentrations, indicating effective restoration of redox balance. This is supported by Al-Bulawi & Al-Juhani [8], who found that vitamin E and selenium play crucial roles in protecting cell membranes and scavenging free radicals. The inclusion of natural supplements such as flaxseed, turmeric, and green tea extract further enhanced these effects, likely due to their anti-inflammatory properties and ability to modulate cytokine production. The observed decrease in inflammatory cytokines such as TNF-α, IL-6, and IL-1β in the treated group corroborates earlier findings by Zhao et al. [13], who attributed similar reductions to the anti-inflammatory actions of polyphenols present in green tea extract. These compounds inhibit nuclear factor kappa B (NF-κB) activation, which plays a central role in the transcription of pro-inflammatory genes. Histopathological examination revealed less severe tissue damage in supplemented animals, suggesting that antioxidants not only reduce oxidative injury but also promote tissue repair. This finding is in agreement with Rajesh et al. [14], who reported reduced fibrosis and better preservation of alveolar architecture in antioxidant-treated cases. Improvements in milk composition and somatic cell count (SCC) further underscore the practical benefits of antioxidant therapy. Milk fat and protein percentages increased significantly in Group III, while SCC decreased to near-normal levels by the end of the trial. These results are

consistent with those of Wang et al. [13], who emphasized the importance of maintaining udder integrity for optimal milk production and quality. Moreover, the integration of natural antioxidants into feed regimens offers a sustainable alternative to conventional antibiotic use. As highlighted by Halasa et al. [15], excessive reliance on antimicrobials has led to increasing concerns about antimicrobial resistance. Therefore, antioxidant-based interventions may help reduce antibiotic usage in mastitis management without compromising animal health or productivity. Interestingly, the effectiveness of antioxidant supplementation varied depending on the stage of lactation. Cows in early lactation benefited more from higher doses of vitamin E and omega-3 fatty acids, likely due to increased metabolic demands and susceptibility to oxidative stress during this period [16]. In contrast, mid- and late-lactation cows required lower doses, suggesting that supplementation protocols should be tailored to physiological status. This study also highlights the importance of combining antioxidant therapy with other supportive strategies, such as post-milking teat dipping with green tea extract and environmental cooling systems, which were shown to further reduce oxidative burden and improve udder health [3,17]. However, some limitations must be acknowledged. The duration of the study was limited to 8 weeks, and longer-term investigations are needed to assess the sustainability of antioxidant effects. Additionally, while this study focused on subclinical mastitis caused by Staphylococcus aureus, future research should evaluate the efficacy of antioxidants in clinical cases and infections caused by other pathogens such as Escherichia coli and Streptococcus uberis. Despite these limitations, the current findings support the integration of antioxidant supplementation into mastitis management protocols. They offer a promising strategy for enhancing dairy cow health, improving milk quality, and promoting sustainable dairy farming practices.

Mastitis remains a major challenge in the global dairy industry, causing significant economic losses due to reduced milk yield, decreased milk quality, increased veterinary costs, and premature culling of affected animals [1,2]. It is defined as the inflammation of the mammary gland, primarily caused by bacterial infection, which triggers a cascade of immune responses and oxidative stress reactions [18]. Oxidative stress occurs when there is an imbalance between reactive oxygen species (ROS) and the body's antioxidant defense system. In dairy cows suffering from mastitis, excessive ROS production leads to cellular damage, particularly in the mammary epithelial cells, impairing their function and integrity [5,6]. This process is further exacerbated by the depletion of depletion of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which are essential for neutralizing free radicals and protecting cellular structures [16]. Antioxidants play a crucial role in mitigating oxidative stress by scavenging free radicals and enhancing immune function. Natural antioxidant supplements, including vitamins E, C, selenium, carotenoids, polyphenols, and omega-3 fatty acids, have shown promising effects in reducing inflammation and improving udder health [9,10]. For instance, studies have demonstrated that vitamin E and selenium supplementation can enhance neutrophil function and reduce somatic cell counts in mastitic cows [11,12]. Despite these advancements, there remains a need for comprehensive studies evaluating the long-term impact of antioxidant supplementation on mastitis incidence and overall productivity.

The current study highlights the significant role of oxidative stress in the pathophysiology of bovine mastitis and demonstrates the promising potential of antioxidant supplementation as a sustainable intervention strategy. The supplementation with natural antioxidants, including vitamins C and E, selenium, and polyphenols, resulted in improved oxidative stress biomarkers, enhanced immune responses, and better milk production parameters, while reducing the reliance on conventional antibiotic therapies. These findings not only reinforce the biological plausibility of antioxidant-based interventions but also offer practical implications for improving udder health, enhancing dairy productivity, and promoting animal welfare. Given the increasing global concern over antibiotic resistance, integrating antioxidant strategies into mastitis management protocols presents a feasible and eco-friendly alternative. Future research should focus on large-scale field trials, optimizing antioxidant formulations, and exploring synergistic effects with other preventive measures such as probiotics and improved management practices. Additionally, long-term studies are required to evaluate the sustained impacts on herd health, milk quality, and economic outcomes, ensuring the development of holistic and sustainable mastitis management strategies. The addition of natural antioxidants such as vitamins C & E, selenium, and polyphenols can enhance the oxidative stability of food products shows saw positive changes in inflammation and immune function and increased productivity in milk while decreasing the use of standard antibiotic therapies. This confirms the biological plausibility of employing antioxidants while bolstering practical strategies to improve udder health, increase dairy production, and enhance overall animal welfare. As antibiotic resistance becomes a more pressing issue worldwide, antioxidants used for mastitis inflammation serve as a practical and environmentally friendly approach. Future study still needs to be done in large field experiments to refine antioxidant blends and assess the use of complementary strategies like probiotics. There’s not much evidence about the effect of managed probiotics and exercises on herd overall welfare and health burdens. Together these strategies

would create cohesive and sustainable mastitis management.

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