Oxidative Stress in Dairy Cows

Subjects: Agriculture, Dairy & Animal Science

Contributor: Samy Elsaadawy

The predominance of Streptococcus spp. (24.22%), Acinetobacter spp. (21.37%), Romboutsia spp. (4.99%), Turicibacter spp., (2.64%), Stenotrophomonas spp. (2.33%), and Enterococcus spp. (1.86%) was found in the microbiome of mastitis cows with a decrease of d-mannose and increase of xanthine:guanine ratio when Streptococcus increased. Diversity of energy sources favoring the growth of Fusobacterium make it a keystone taxon contributing to metritis. Ruminal volatile fatty acids rose with high-concentrate diets that decreased the ruminal pH, causing a lysis of rumen microbes and release of endotoxins. Moreover, lipopolysaccharide (LPS) concentration, malondialdehyde (MDA), and superoxide dismutase (SOD) activities increased in high concentrate cows accompanied by a reduction of total antioxidant capacity (T-AOC), glutathione peroxidase (GPx), and catalase (CAT) activity. In addition, albumin and paraoxonase concentrations were inversely related to oxidative stress and contributed to the protection of low-density and high-density lipoproteins against lipid peroxidation, protein carbonyl, and lactoperoxidase. High concentrate diets increased the expression of MAPK pro-inflammatory genes and decreased the expression of antioxidant genes and proteins in mammary epithelial tissues. The expression levels of NrF2, NQO1, MT1E, UGT1A1, MGST3, and MT1A were downregulated, whereas NF-kB was upregulated with a high-grain or high concentrate diet. Amino-acids, vitamins, trace elements, and plant extracts have shown promising results through enhancing immune functions and repairing damaged cells exposed to oxidative stress.

antioxidants genes feed additives immune function pathogenic microbial cells pro-inflammatory genes

1. Introduction

Molecular oxygen is an important electron acceptor of the biosphere, featuring a bi-radical structure capable of gaining unpaired electrons, leading to reduced species commonly known as reduced or reactive oxygen species (ROS), such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl ($HO^{\bullet}$), peroxyl ($ROO^{\bullet}$), and alkoxyl ($RO^{\bullet}$) radicals [1]. With the presence of oxygen and water in the biosphere, humans and animals are exposed to two main sources of ROS. First, ionizing cosmic or ground radiation and pollution smoke modify the chemical structure of oxygen and water, creating free radicals, including the highly reactive hydroxyl group [2][3]. Second, after the death products of pathogens, macrophage activities also conduct ROS throughout the electron transport chain, forming complex I and II that release the pro-inflammatory transcription factors NFκB and AP-1 [4]. Those transcription factors contribute to upregulate the expression of pro-inflammatory chemokines or cytokines and adhesion molecules [4].

A lot of diseases, especially degenerative ones, are now considered at least partly caused by oxidative damages. The nucleic acids, proteins, and lipids are the cell's organic components that are chemically damaged by ROS [5][6]. First, the most reactive hydroxyl group will interact with both the bases and sugars (ribose and desoxyribose) to change the nucleotide structure and functions, leading to mutation and degenerative disorders. At the same time, the change in sugar structure will also break down DNA strands. Second, when the SH-HS structure of thiols protein, considered the major protein antioxidant, is oxidized into disulfite S-S, irreversible protein damaging consists of SOH, SO_2H, and SO_3H synthesis, impairing cellular calcium homeostasis [7]. Third, the juxtaposition of the poly-unsaturated fatty acids on the cell membrane propagates their oxidation in the presence of ROS, thereby destroying entire cellular membranes via chain reaction [8]. Subsequently, the membrane function will be disturbed and the integrity of the physical structure of the cellular compartmentalization cannot be maintained, leading to enzymes leakage, electrolyte diffusion, metals, and small molecules transiting [9][10]. The diffused products of lipid peroxidation, such as 4-hydroxynonenal, are also responsible for protein and nucleic acids alterations, including their related functions.

A study using redox Western analysis and redox-sensitive green fluorescent proteins to investigate the redox signaling and oxidative stress in different subcellular compartments revealed that the redox status from most reducing to most oxidizing was as follows: mitochondria > nuclei > cytoplasm > endoplasmic reticulum > extracellular space [11]. The relatively alkaline pH (near 8.0) of mitochondria makes its protein thiols vulnerable to apoptosis and necrosis. Furthermore, the relatively reduced nuclear redox state is a key transcriptional factor in response to oxidative stress. Animal metabolic and physiological traits respond quite differently to different oxidative stress factors and management strategies, but the specific cellular mechanisms of action are not often described. Some oxidative stress factors considered and described within this study are the microbial pathogenic cells inducing mastitis and metritis degenerative diseases in dairy cows along with feeding management using different concentrates: forage ratios, with effects on some expressed genes associated with oxidative stress pathways. To address these drawbacks, several studies have been carried out on the dietary supplementation with additives like methionine and lysine amino-acids to evaluate the effects on the cattle oxidative status [12][13][14]. Meanwhile, very few feed additives have been tested as immune function bioregulators, and the combination effect of methionine and lysine as well as vitamins, trace elements, and plant extracts are not commonly studied.

2. Microbial Activities as Driver of Degenerating Oxidative Stress Diseases and Mechanisms of Action

2.1. Mastitis

Mastitis is an intra-mammary infection driven by host-pathogen interactions that causes severe economic losses, including decrease in milk production and quality, premature culling, lower conception rates, and treatment costs in dairy cattle [15][16]. Therefore, host-microbial interactions have been studied, aiming to optimize the lactating performance of cows [17] and depending on the pathogenic microbes primary reservoir and mode of transmission, mastitis has been categorized into contagious and environmental forms. Some detrimental microbes such as *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Escherichia coli*, and *Klebsiella*, non-aureus *Staphylococci*

(NAS), *Staphylococcus aureus*, and *Enterococcus* spp. have been correlated with mastitis occurrence [18], but unfortunately, the mechanisms underpinning host–microbial interactions inducing mastitis are unclear. The invasion stage mainly occurs from environmental microbes through the mammary teat canal opening in the direction of the tissues, then to the epithelium cells of the duct, causing inflammation and development of granulation tissues that finally appear as polypoid swelling [19]. Pathogens in the epithelium that remain undestroyed by immune cells or neutrophils will cause oedema, leading to a vacuolated and desquamated epithelial acini [20]. After invasion, the pathogenic bacteria undergo a rapid multiplication, leading to reductions in healthy milk secretory tissue, scanty milk with blood traces as well as gangrene and thrombosis damaging the udder tissue, which can potentially lead to toxaemia and death in acute cases [21]. Mastitis milk is source of zoonosis like tuberculosis, brucellosis, and gastroenteritis. A number of previous studies analyzed the microbiome of mastitis cows compared to healthy ones, indicating a lower microbial diversity in mastitis bovine milk [22][23], quantified by a lower Shannon index. Indeed, at the phylum level, fewer *Proteobacteria*, *Actinobacteria,* and *Acidobacteria* were found, with a predominance of the following genera: *Streptococcus* spp. (24.22%), *Acinetobacter* spp. (21.37%), *Romboutsia* spp. (4.99%), *Turicibacter* spp. (2.64%), *Stenotrophomonas* spp. (2.33%), *Enterococcus* spp. (1.86%), *Microbacterium* spp. (1.66%), *Aerococcus* spp. (1.60%), *Corynebacterium-1* spp. (1.49%), and *Bacteroides* spp. (1.44%) [24]. Tong et al. (2019) [24] describes the correlation analysis between milk metabolites of the intra-mammary infected cows and their microbial populations. Indeed, d-mannose tended to decrease when the pathogenic *Streptococcus* increased. This situation definitely impairs the potency of d-mannose known to regulate these microbial infections. Moreover, xanthine with its catalytic xanthine oxidase enzyme can first produce uric acid, then reactive oxygen species, superoxide radicals, and hydrogen peroxide, all of which are involved in pathogenesis. This explains how *Streptococcus*, positively correlated to the xanthine:guanine ratio, can gradually induce severe oxidative stress in dairy cattle. Meanwhile, other bacteria from *Lachnospiraceae*, *Lactobacillaceae*, *Xanthomonadaceae*, *Microbacteriaceae*, and *Brevibacteriaceae* families were negatively correlated with the xanthine:guanine ratio. Quinic acid, with its beneficial effects, was negatively correlated to detrimental *Enteroccocaceae*. These outcomes could provide some guidelines on manipulating microbiota to reduce incidents of oxidative stress.

Generally, mastitis cases are recorded when the somatic cell counts (SCC) exceed the tolerated value [25]. Over time, if the invading mastitis-pathogens are killed, then the somatic cells return to their normal range. Therefore, genetic selection for reducing excess SCC occurrence may improve resistance to mastitis, no matter the causative pathogen species. Meanwhile, different pathogens contribute to different infection extents. A genetic correlation analysis revealed that *E. coli* had a lower incidence on SCC inducing mastitis when compared with *Strep. dysgalactiae*, NAS, and *Strep. Uberis* [18]. Cows with these pathogens showed higher lactation SCC, infecting them for a longer period than cows infected by *E. coli* [26], which is purely of environmental origin [27], and that also makes it less severe for the herd compared to the other pathogens that are contagious within the herd [28]. Moreover, *Staphylococcus aureus* is known to induce chronic mammary infections [29], because it avoids being killed by macrophages, reducing the potency of the mammary immune system [30], and leading to the production of virulence factors [31]. The use of lactation SCC as an indicator trait for biomonitoring of cows exposed to mammary inflammation shows promise in mitigating the inflammation of mammary tissues through technological manipulation of involved microbes.

2.2. Metritis

Uterine wall inflammation known as metritis or pelvic inflammatory disease mostly occurs in negative energy balance (NEB) conditions. Closely examining the microbiological determinants of this physiological disorder, the increase in *Fusobacterium*, *Bacteroides*, and *Porphyromonas* has been associated with metritis [32][33], while a reduction in the same bacteria has been associated with the cure of metritis regardless of the antibiotic treatment [34]. Therapy with ceftiofur against metritis led to a reduction in *Fusobacterium*, suggesting that *Fusobacterium* and especially *F. necrophorum* is a keystone pathogen that can stabilize dysbiotic metritis microbiota [35]. Therefore, special attention should be paid to this taxa when planning mitigation strategies. Out of 95 carbon energy sources investigated that may promote the growth of *Fusobacterium*, up to 47 were metabolized by *F. necrophorum*, enabling it to proliferate in postpartum cows using an array of energy sources, even in animal NEB [36]. For instance, in iron-limited conditions where *F. necrophorum* thrived, an increase of the expression of virulence genes such as the glycoprotein of influenza virus called haemagglutinin and leukotoxin genes was reported (2.49 and 3.72 fold increase respectively [37]). Meanwhile, *F. necrophorum* encoded Haemolysin, yebN homologue, and *tonB* homologue do not cause significant lysis of the red blood cell, but greatly contribute to nutrients acquisition, and these were pronouncedly down-regulated [37]. β-Hydroxybutyric acid (BHBA) is recognized as a preferred source of energy of *Fusobacterium*, particularly after calving, when cows undergo NEB and feed intake alone cannot meet their energy demands. This deficit results in lipid mobilization from adipose tissue in the form of non-esterified fatty acids (NEFA), uptake of NEFA, and partial oxidation of NEFA in the liver, which forms large quantities of BHBA that end up in the blood stream [38]. Metritis cows generally have higher NEFA and BHBA concentrations than healthy cows, and the higher NEFA and BHBA levels impaired leukocyte functions [39]. In the uterus, l-glutamine and pyruvic acid metabolites contribute little to the growth of *F. necrophorum* during early lactation, but they are importantly used in mammary glands for the uptake for milk production [40]. Uterine energy metabolites could therefore serve as biomarkers of host immunity and uterine pathogen growth. Coenzyme A (CoA) and its thioester derivatives are synthesized by prokaryotes and eukaryotes cells and are involved in major metabolic pathways, including the regulation of gene expression and redox activity known as protein CoAlation, strongly induced in response to oxidizing agents and metabolic stresses in exponentially growing bacteria to prevent overoxidation [41].

3. The Role of Specific Feed Additives Alleviating Oxidative Stress

Efficient management of oxidative stress comes from understanding roles of specific functional elements in the diet. Amino acids, vitamins, trace elements, and plant extracts containing a diversity of metabolites are known in traditional Chinese medicine for their anti-inflammatory and anti-oxidative values. Therefore, those functional feed additives need to be continuously valorized in animal nutrition to mitigate oxidative stress cases, especially when natural animal antioxidant defenses are overwhelmed.

3.1. Rumen Protected Amino-Acids: Methionine and Lysine, L-arginine, and N-carbamylglutamate

Methionine and lysine are the building blocks of casein synthesis, but they are unfortunately also known as limiting amino acids (AA) for milk protein metabolism in ruminants, while casein derivatives mediate anti-oxidative actions [42]. Studies on the effects of dietary inclusion of rumen protected methionine and lysine on oxidative status [43], immune response reproduction, and milk performance [44] of dairy cattle have been well documented, but limiting AA have been tested as immune function regulators in dairy cows during early lactation [45]. Moreover, little information exists on optimum inclusion levels and synergetic effects of rumen protected amino-acids on their oxidative status Mavrommatis et al. (2021) [46] recently found that a decrease in plasma BHBA led to increased glutathione peroxidase (GSH-Px) activity in lactating ewes that were provided a combination of rumen protected methionine and lysine. Therefore, increasing antioxidants activity has a beneficial effect on animal health and can decrease the incidence rate of metabolic disorder diseases like ketosis. Furthermore, AA contribute to cellular oxidative balance [47] through participating in taurine and GSH synthesis [48], ensuring cellular detoxification and hydrogen peroxide neutralization via glutathione S-transferase and GSH-Px actions, respectively. Methionine plays a key role in de novo short- and medium-chain FA synthesis as a source of methyl for the transmethylation reactions in lipids biosynthesis [49]. It also enhanced de novo glutathione and carnitine synthesis in the liver, and thus increased antioxidant and β-oxidation capacity [50]. Sun et al. [51] reported that supplementation of methionine increase the “very-low-density lipoproteins” (VLDL) resulting in enhanced vitamin E circulation. He also demonstrated that dietary supplementation of rumen protected amino-acids can suppress side effects of lipid peroxidation by-products such as MDA. Moreover, higher FRAP values in blood plasma and milk of ewes fed combinations of rumen-protected Met + choline + betaine compared with the control were observed [52]. Besides the inclusion of methionine and lysine and its benefit in enhancing antioxidants status, *N*-carbamylglutamate (NCG, a metabolically stable analogue of *N*-acetylglutamate synthase (NAG) that produces arginine endogenously) can also play a significant role in improving immune function and oxidative status in suckling lambs. Zhang et al. (2018) [53] conducted a study to investigate effects of dietary supplementation with l-arginine (Arg) and *N*-carbamylglutamate (NCG) on intrauterine growth-retarded (IUGR) suckling lambs. They showed that the concentrations of protein carbonyl (PCs) and MDA were lower and the glutathione (GSH) concentration and ratio of GSH/GSSG greater in the jejunum, duodenum and ileum of IUGR + 1% Arg or 0.1% NCG lambs, compared to IUGR group. Zhou et al. (2016) [54] isolated polymorphonuclear leukocytes (PMNL) and showed the lower abundance of genes linked to inflammation (IL1B, TLR2, NF-κB, and STAT3) and oxidative stress (CBS, GPx1, glutathione synthase [GSS], and SOD2) as well as an increase in plasma taurine with methionine provision, and proposed improved redox and inflammatory status of those cells. A recent trial conducted by Lopreiato et al. (2019) [55] studied the consequences of incubation bovine PMNL with Met and/or choline and observed that methionine supplementation coupled with sufficient choline enhanced gene expression of TLR2 and l-selectin, which are pathogen recognition mechanisms. In the same trial, cells incubated without choline had high mRNA abundances encoding IL1B, IL6, IL10, and myeloperoxidase (MPO), glutathione reductase (GSR), GSS, cystathionine gamma-lyase (CTH), and cysteine sulfinic acid decarboxylase (CSAD), suggesting higher inflammation and oxidative stress.

3.2. Vitamins, Trace Elements and Plant Extracts

A daily supplementation of 1000 IU vitamin E in diet of dairy cows significantly reduced stress markers such as MDA and heat shock protein 70 and increased activities of SOD and GSH-Px [56]. Moreover, serum immunoglobulin and interleukin concentrations increased significantly, and the activities of T-AOC and various antioxidant enzymes increased in dairy cows supplementing typical lactation diets with 110 and 220 IU/kg of vitamin A. Supplementing vitamin E (80 IU/kg) and selenium (5 mg/kg) in the last gestation month increased the serum levels of the mineral in the cows, improved the reproductive performance, and reduced incidents of sub-clinics mastitis [57].

Trace elements such as copper (Cu), manganese (Mn), zinc (Zn), and selenium (Se) can also improve antioxidant functions in dairy cows. Se is involved in the synthesis of GSH-Px [58]. An adequate selenium status is essential for many antioxidant processes. Sun et al., (2019) [59] reported that 0.3 mg/kg DM hydroxy-selenomethionine (HMSeBA) decreases some parameters (e.g., NO, MDA) of heat stress-induced oxidative stress. The supplementation of HMSeBA (0.1, 0.3, or 0.5 mg of Se/kg of DM) linearly increased the activities of serum GSH-Px and SOD, but decreased MDA content [60]. Lower stress levels and higher immune response were observed when 60 ppm Zn were supplemented to the TMR diet of healthy multiparous cows [61].

Resveratrol is a natural polyphenol present in plants such as grapes, blueberries, and mulberries. Many studies have reported that resveratrol can exert antioxidant effects. Zhou et al. (2019) [62] reported that resveratrol alleviates aflatoxin B1-induced cytotoxicity, including the increase in ROS and the decrease in mitochondrial membrane potential (MMP) and apoptosis in MAC-T cow mammary epithelial cell line. With Resveratrol, MAC-T cells avoided ROS H_2O_2-induced endoplasmic reticulum stress and mitochondria-related cell apoptosis. Moreover, resveratrol induced mRNA expression of multiple antioxidant defense genes in MAC-T cells under normal/oxidative conditions [63]. Daidzein, an isoflavone extract with phytoestrogenic properties, can regulate specific and non-specific immune functions in animals through an endocrine system regulation [64]. Liu et al. (2014) [65] reported that 300 and 400 mg/day daidzein treatment increased IgG and IL-2 in serum of late lactation cows. Therefore, daidzein can enhance immuno-competence of late lactation cows and strengthen their resistance to heat stress.

References

1. Benov, L. How Superoxide Radical Damages the Cell. Protoplasma 2001, 217, 33–36.
2. Halliwell, B. Superoxide-dependant formation of hydroxyl radicals in the presence of iron chelates: Is it a mechanism for hydroxyl radical production in biological systems? FEBS Lett. 1978, 92, 321–326.
3. McCord, J.M.; Day, E.D. Superoxide-dependant production of hydroxyl radical catalyzed by iron-EDTA complex. FEBS Lett. 1978, 86, 139–142.
4. Rendra, E.; Riabov, V.; Mossel, D.M.; Sevastyanova, T.; Harmsen, M.C.; Kzhyshkowska, J. Reactive Oxygen Species (ROS) in Macrophage Activation and Function in Diabetes.

Immunobiology 2019, 2242, 242–253.

5. Suzuki, Y. Oxidant-Mediated Protein Amino Acid Conversion. Antioxidants 2019, 82, 50.
6. Martinelli, I.; Tomassoni, D.; Roy, P.; Di Cesare Mannelli, L.; Amenta, F.; Tayebati, S.K. Antioxidant Properties of Alpha-Lipoic (Thioctic) Acid Treatment on Renal and Heart Parenchyma in a Rat Model of Hypertension. Antioxidants 2021, 107, 1006.
7. Orrenius, S. Oxidative Stress Studied in Intact Mammalian Cells. Philos. Trans. R. Soc. B Biol. Sci. 1985, 3111152, 673–677.
8. Riley, P.A. Free Radicals in Biology: Oxidative Stress and the Effects of Ionizing Radiation. Int. J. Radiat. Biol. 1994, 651, 27–33.
9. Behairy, A.; El-Sharkawy, N.I.; Saber, T.M.; Soliman, M.M.; Metwally, M.M.M.; Abd El-Rahman, G.I.; Abd-Elhakim, Y.M.; El Deib, M.M. The Modulatory Role of Vitamin C in Boldenone Undecylenate Induced Testicular Oxidative Damage and Androgen Receptor Dysregulation in Adult Male Rats. Antioxidants 2020, 911, 1053.
10. Nemec Svete, A.; Vovk, T.; Bohar Topolovec, M.; Kruljc, P. Effects of Vitamin E and Coenzyme Q10 Supplementation on Oxidative Stress Parameters in Untrained Leisure Horses Subjected to Acute Moderate Exercise. Antioxidants 2021, 106, 908.
11. Hansen, J.M.; Go, Y.-M.; Jones, D.P. Nuclear and Mitochondrial Compartmentation of Oxidative Stress and Redox Signaling. Annu. Rev. Pharm. Toxicol. 2006, 461, 215–234.
12. Jové, M.; Mota-Martorell, N.; Pradas, I.; Martin-Gari, M.; Ayala, V.; Pamplona, R. The Advanced Lipoxidation End-Product Malondialdehyde-Lysine in Aging and Longevity. Antioxidants 2020, 9, 1132.
13. Awawdeh, M.S. Rumen-Protected Methionine and Lysine: Effects on Milk Production and Plasma Amino Acids of Dairy Cows with Reference to Metabolisable Protein Status. J. Dairy Res. 2016, 832, 151–155.
14. Martinov, M.V.; Vitvitsky, V.M.; Banerjee, R.; Ataullakhanov., F.I. The logic of the hepatic methionine metabolic cycle. Biochim. Biophys. Acta - Proteins Proteom. 2010, 1804, 89–96.
15. Seegers, H.; Fourichon, C.; Beaudeau, F. Production Effects Related to Mastitis and Mastitis Economics in Dairy Cattle Herds. Vet. Res. 2003, 345, 475–491.
16. Halasa, T.; Huijps, K.; Østerås, O.; Hogeveen, H. Economic Effects of Bovine Mastitis and Mastitis Management: A Review. Vet. Q. 2007, 291, 18–31.
17. Xue, M.; Sun, H.; Wu, X.; Guan, L.L.; Liu, J. Assessment of Rumen Microbiota from a Large Dairy Cattle Cohort Reveals the Pan and Core Bacteriomes Contributing to Varied Phenotypes. Appl. Environ. Microbiol. 2018, 8419, e00970-18.

18. Sørensen, L.P.; Mark, T.; Madsen, P.; Lund, M.S. Genetic Correlations between Pathogen-Specific Mastitis and Somatic Cell Count in Danish Holsteins. J. Dairy Sci. 2009, 927, 3457–3471.
19. Forbes, D.; Gehm, W. Bacterial Migration through Teat Canal Related to Liner Action. In Udder Health and Communication; Wageningen Academic Publishers: Wageningen, The Netherlands, 2011; p. 415.
20. Latia. Mastitis in Dairy Cattle. Press Release 2017. Available online: https://www.latiaagribusinesssolutions.com/2017/10/04/mastitis-dairy-cattle/ (accessed on 21 October 2021).
21. Bradley, A.J.; Green, M.J. Adaptation of Escherichia Coli to the Bovine Mammary Gland. J. Clin. Microbiol. 2001, 395, 1845–1849.
22. Bhatt, V.D.; Ahir, V.B.; Koringa, P.G.; Jakhesara, S.J.; Rank, D.N.; Nauriyal, D.S.; Kunjadia, A.P.; Joshi, C.G. Milk Microbiome Signatures of Subclinical Mastitis-Affected Cattle Analysed by Shotgun Sequencing. J. Appl. Microbiol. 2012, 1124, 639–650.
23. Falentin, H.; Rault, L.; Nicolas, A.; Bouchard, D.S.; Lassalas, J.; Lamberton, P.; Aubry, J.-M.; Marnet, P.-G.; Le Loir, Y.; Even, S. Bovine Teat Microbiome Analysis Revealed Reduced Alpha Diversity and Significant Changes in Taxonomic Profiles in Quarters with a History of Mastitis. Front. Microbiol. 2016, 7, 480.
24. Tong, J.; Zhang, H.; Zhang, Y.; Xiong, B.; Jiang, L. Microbiome and Metabolome Analyses of Milk From Dairy Cows With Subclinical Streptococcus Agalactiae Mastitis—Potential Biomarkers. Front. Microbiol. 2019, 10, 547.
25. Sharma, N.; Singh, N.K.; Bhadwal, M.S. Relationship of Somatic Cell Count and Mastitis: An Overview. Asian-Australas. J. Anim. Sci. 2011, 243, 429–438.
26. de Haas, Y.; Barkema, H.W.; Veerkamp, R.F. The Effect of Pathogen-Specific Clinical Mastitis on the Lactation Curve for Somatic Cell Count. J. Dairy Sci. 2002, 855, 1314–1323.
27. Smith, K.L.; Hogan, J.S. Environmental mastitis. Vet. Clin. North Am. Food Anim. Pract. 1993, 9, 489–498.
28. Fox, L.K.; Gay, J.M. Contagious mastitis. Vet. Clin. North Am. Food Anim. Pract. 1993, 9, 475–487.
29. Wilson, C.D.; Richards, M.S. A survey of mastitis in the British dairy herd. Vet. Rec. 1998, 106, 431–433.
30. Almeida, R.A.; Matthews, K.R.; Cifrian, E.; Guidry, A.J.; Oliver, S.P. Staphylococcus aureus Invasion of Bovine Mammary Epithelial Cells. J. Dairy Sci. 1996, 796, 1021–1026.
31. Baselga, R.; Albizu, I.; Amorena, B. Staphylococcus aureus Capsule and Slime as Virulence Factors in Ruminant Mastitis. A Review. Vet. Microbiol. 1994, 39, 195–204.

32. Jeon, S.J.; Cunha, F.; Ma, X.; Martinez, N.; Vieira-Neto, A.; Daetz, R.; Bicalho, R.C.; Lima, S.; Santos, J.E.P.; Jeong, K.C.; et al. Uterine Microbiota and Immune Parameters Associated with Fever in Dairy Cows with Metritis. PLoS ONE 2016, 11, e0165740.
33. Bicalho, M.L.S.; Machado, V.S.; Higgins, C.H.; Lima, F.S.; Bicalho, R.C. Genetic and Functional Analysis of the Bovine Uterine Microbiota. Part I: Metritis versus Healthy Cows. J. Dairy Sci. 2017.
34. Jeon, S.J.; Lima, F.S.; Vieira-Neto, A.; Machado, V.S.; Lima, S.F.; Bicalho, R.C.; Santos, J.E.P.; Galvão, K.N. Shift of Uterine Microbiota Associated with Antibiotic Treatment and Cure of Metritis in Dairy Cows. Vet. Microbiol. 2018, 214, 132–139.
35. Hajishengallis, G.; Darveau, R.P.; Curtis, M.A. The Keystone-Pathogen Hypothesis. Nat. Rev. Microbiol. 2012, 1010, 717–725.
36. Jeon, S.J.; Cunha, F.; Daetz, R.; Bicalho, R.C.; Lima, S.; Galvão, K.N. Ceftiofur Reduced Fusobacterium Leading to Uterine Microbiota Alteration in Dairy Cows with Metritis. Anim. Microbiome 2021, 3, 15.
37. Antiabong, J.F.; Ball, A.S.; Brown, M.H. The Effects of Iron Limitation and Cell Density on Prokaryotic Metabolism and Gene Expression: Excerpts from Fusobacterium Necrophorum Strain 774 (Sheep Isolate). Gene 2015, 5631, 94–102.
38. Pérez-Báez, J.; Risco, C.A.; Chebel, R.C.; Gomes, G.C.; Greco, L.F.; Tao, S.; Thompson, I.M.; do Amaral, B.C.; Zenobi, M.G.; Martinez, N.; et al. Association of Dry Matter Intake and Energy Balance Prepartum and Postpartum with Health Disorders Postpartum: Part I. Calving Disorders and Metritis. J. Dairy Sci. 2019, 102, 9138–9150.
39. Galvão, K.N.; Flaminio, M.J.B.F.; Brittin, S.B.; Sper, R.; Fraga, M.; Caixeta, L.; Ricci, A.; Guard, C.L.; Butler, W.R.; Gilbert, R.O. Association between Uterine Disease and Indicators of Neutrophil and Systemic Energy Status in Lactating Holstein Cows. J. Dairy Sci. 2010, 93, 2926–2937.
40. Reynolds, C.K.; Aikman, P.C.; Lupoli, B.; Humphries, D.J.; Beever, D.E. Splanchnic Metabolism of Dairy Cows during the Transition from Late Gestation through Early Lactation. J. Dairy Sci. 2003, 86, 1201–1217.
41. Tsuchiya, Y.; Zhyvoloup, A.; Baković, J.; Thomas, N.; Yu, B.Y.K.; Das, S.; Orengo, C.; Newell, C.; Ward, J.; Saladino, G.; et al. Protein CoAlation and Antioxidant Function of Coenzyme A in Prokaryotic Cells. Biochem. J. 2018, 47511, 1909–1937.
42. Park, J.K.; Yeo, J.M.; Bae, G.S.; Kim, E.J.; Kim, C.H. Effects of supplementing limiting amino acids on milk production in dairy cows consuming a corn grain and soybean meal-based diet. J. Anim. Sci. Technol. 2020, 62, 485–494.
43. Coleman, D.N.; Lopreiato, V.; Alharthi, A.; Loor, J.J. Amino acids and the regulation of oxidative stress and immune function in dairy cattle. J. Anim. Sci. 2020, 98 (Suppl. 1), S175–S193.

44. Lopes, M.G.; Dominguez, J.H.E.; Corrêa, M.N.; Schmitt, E.; Fischer, G. Rumen-protected methionine in cattle: Influences on reproduction, immune response, and productive performance. Arq. Inst. Biol. 2019, 86, e1292018.
45. Tsiplakou, E.; Mavrommatis, A.; Skliros, D.; Righi, F.; Flemetakis, E. The impact of rumen-protected amino acids on the expression of key-genes involved in the innate immunity of dairy sheep. PLoS ONE 2020, 15, e0233192.
46. Mavrommatis, A.; Giamouri, E.; Tavrizelou, S.; Zacharioudaki, M.; Danezis, G.; Simitzis, P.E.; Zoidis, E.; Tsiplakou, E.; Pappas, A.C.; Georgiou, C.A.; et al. Impact of Mycotoxins on Animals' Oxidative Status. Antioxidants 2021, 10, 214.
47. Yin, J.; Li, T.; Yin, Y. Methionine and Antioxidant Potential. J. Antioxid. Act. 2016, 1, 12–17.
48. Li, P.; Yin, Y.L.; Li, D.; Kim, S.W.; Wu, G. Amino acids and immune function. Br. J. Nutr. 2007, 98, 237–252.
49. Tsiplakou, E.; Mavrommatis, A.; Kalogeropoulos, T.; Chatzikonstantinou, M.; Koutsouli, P.; Sotirakoglou, K.; Labrou, N.; Zervas, G. The effect of dietary supplementation with rumen-protected methionine alone or in combination with rumen-protected choline and betaine on sheep milk and antioxidant capacity. J. Anim. Physiol. Anim. Nutr. 2017, 101, 1004–1013.
50. Osorio, J.S.; Ji, P.; Drackley, J.K.; Luchini, D.; Loor, J.J. Supplemental Smartamine M or MetaSmart during the transition period benefits postpartal cow performance and blood neutrophil function. J. Dairy Sci. 2013, 96, 6248–6263.
51. Sun, F.; Cao, Y.; Cai, C.; Li, S.; Yu, C.; Yao, J. Regulation of Nutritional Metabolism in Transition Dairy Cows: Energy Homeostasis and Health in Response to Post-Ruminal Choline and Methionine. PLoS ONE 2016, 11, e0160659.
52. Zhang, H.; Zhao, F.; Peng, A.; Dong, L.; Wang, M.; Yu, L.; Loor, J.J.; Wang, H. Effects of Dietary l-Arginine and N-Carbamylglutamate Supplementation on Intestinal Integrity, Immune Function, and Oxidative Status in Intrauterine-Growth-Retarded Suckling Lambs. J. Agric. Food Chem. 2018, 66, 4145–4154.
53. Zhou, Z.; Loor, J.J.; Piccioli-Cappelli, F.; Librandi, F.; Lobley, G.E.; Trevisi, E. Circulating amino acids in blood plasma during the peripartal period in dairy cows with different liver functionality index. J. Dairy Sci. 2016, 99, 2257–2267.
54. Lopreiato, V.; Vailati-Riboni, M.; Bellingeri, A.; Khan, I.; Farina, G.; Parys, C.; Loor, J.J. Inflammation and oxidative stress transcription profiles due to in vitro supply of methionine with or without choline in unstimulated blood polymorphonuclear leukocytes from lactating Holstein cows. J. Dairy Sci. 2019, 102, 10395–10410.
55. Dong, Z.W.; Wang, J.K.; Liu, J.X. Effects of Vitamin E on Performance of Dairy Cow as Antioxidant. Chin. J. Anim. Sci. 2014, 147, 703–708.

56. Zanetti, M.H. Effect of selenium and vitamin E supplementation in dairy cows. Rev. Bras. Zootecn. 1998, 27, 405–408.
57. Rotruck, J.T.; Pope, A.L.; Ganther, H.E.; Swanson, A.B.; Hafeman, D.G.; Hoekstra, W.G. Selenium: Biochemical role as a component of glutathione peroxidase. Science 1973, 179, 588–590.
58. Sun, L.L.; Gao, S.T.; Wang, K.; Xu, J.C.; Sanz-Fernandez, M.V.; Baumgard, L.H.; Bu, D.P. Effects of source on bioavailability of selenium, antioxidant status, and performance in lactating dairy cows during oxidative stress-inducing conditions. J. Dairy Sci. 2019, 102, 311–319.
59. Sun, P.; Wang, J.; Liu, W.; Bu, D.P.; Liu, S.J.; Zhang, K.Z. Hydroxy-selenomethionine: A novel organic selenium source that improves antioxidant status and selenium concentrations in milk and plasma of mid-lactation dairy cows. J. Dairy Sci. 2017, 100, 9602–9610.
60. Alhussien, M.N.; Tiwari, S.; Panda, B.S.K.; Pandey, Y.; Lathwal, S.S.; Dang, A.K. Supplementation of antioxidant micronutrients reduces stress and improves immune function/response in periparturient dairy cows and their calves. J. Trace. Elem. Med. Biol. 2021, 65, 126718.
61. Zhou, Y.; Jin, Y.; Yu, H.; Shan, A.; Shen, J.; Zhou, C.; Zhao, Y.; Fang, H.; Wang, X.; Wang, J.; et al. Resveratrol inhibits aflatoxin B1-induced oxidative stress and apoptosis in bovine mammary epithelial cells and is involved the Nrf2 signaling pathway. Toxicon 2019, 164, 10–15.
62. Jin, L.; Yan, S.; Shi, B.; Bao, H.; Gong, J.; Guo, X.; Li, J. Effects of vitamin A on the milk performance, antioxidant functions and immune functions of dairy cows. Anim. Feed. Sci. Technol. 2014, 192, 15–23.
63. Jin, D.; Chang, G.; Zhang, K.; Guo, J.; Xu, T.; Shen, X. Rumen-derived lipopolysaccharide enhances the expression of lingual antimicrobial peptide in mammary glands of dairy cows fed a high-concentrate diet. BMC Vet. Res. 2016, 12, 128.
64. Cos, P.; De Bruyne, T.; Apers, S.; Vanden Berghe, D.; Pieters, L.; Vlietinck, A.J. Phytoestrogens: Recent developments. Planta Med. 2003, 69, 589–599.
65. Liu, D.Y.; He, S.J.; Liu, S.Q.; Tang, Y.G.; Jin, E.H.; Chen, H.L.; Li, S.H.; Zhong, L.T. Daidzein enhances immune function in late lactation cows under heat stress. Anim. Sci. J. 2014, 85, 85–89.