Ozone therapy as a treatment method for bovine mastitis – Literature review

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ABSTRACT

Mastitis is a common disease in dairy farming worldwide, characterized by inflammation of the mammary gland, bringing economic losses to producers, due to the compromise in milk quality, reduction in the scale of production, treatment expenses, early disposal of animals, in addition to the disposal of milk during the grace period due to the use of antibiotics. Conventional treatment includes the use of antibiotic therapy, however, more recently, this treatment has been questioned, due to the presence of residues in milk and the risk that this milk can bring to consumers. Currently, ozone therapy is being used as a form of integrative treatment for several pathologies, demonstrating great efficacy for bovine mastitis. The studies and experiments already carried out with this gas show that this treatment method is promising for the producer, because it is a low-cost treatment and because it does not leave residues in the milk. However, it is a new treatment, which needs more studies regarding dosages, forms of application and correct treatment time so that it can generate more current scientific data. The objective of this article is to carry out a literature review in order to study the existing information on the use of ozone as a treatment method for bovine mastitis, as well as to know the therapeutic properties of ozone gas for this disease. The methodology was based on articles and other documents, such as books and technical bulletins, all focused on the use of ozone as a treatment for mastitis, using databases that are referenced in Google Scholar. In bovine mastitis, ozone therapy is a promising technique because it is a low-cost treatment when compared to antibiotic therapy. Finding an effective method for the prevention and treatment of mastitis has led researchers to advance in the studies of integrative treatment so that they can enjoy better productivity, improve the quality of milk products and their derivatives and avoid threats to human and animal health.

Keywords: Milk production, Mammary gland, Dairy Cattle, Therapy, Ozone.

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INTRODUCTION

Brazil produces an average of 25 billion liters of milk per year, and consequently, milk production makes up a large part of the country's GDP, both for the production of milk and its derivatives and the generation of employment. Along with its high production, there are the most varied conditions that affect dairy cattle and among them is bovine mastitis (Lopes *et al*., 2016).

Mastitis, characterized by inflammation of the mammary gland, is one of the most important diseases in dairy cows, resulting in economic losses due to the decrease in milk production and quality, increased costs with labor, medicines and veterinary services, as well as early disposal of animals. It is important to emphasize the relevance of mastitis in terms of public health, due to the involvement of pathogenic bacteria that can endanger human health (Coser; Lee; Da Costa, 2012).

Mastitis has great prominence in the world scenario, due to its costly treatment, being considered the disease most affected by the dairy herd. About 38% of morbidity in dairy cattle is due to mastitis (Lopes *et al*., 2016; Oliveira *et al.*, 2023).

As bovine mastitis is the disease that most increases the activity of animals of zootechnical interest, destined for milk production, the prevention, control and treatment of this disease are of fundamental importance for dairy farming Mastitis has great prominence in the world scenario, due to its expensive treatment, being considered the disease with the greatest involvement of the dairy herd (Santos; Fonseca, 2007; Edmonson; Bramley, 2008; Oliveira *et al.*, 2023).

The use of intramammary antibiotics is the most common method used in the treatment of bovine mastitis, however, currently, this treatment is showing few results in terms of recovery from the infection, mainly due to poor management and the development of resistance to pathogens (Sampimon *et al*., 2011; Arévalo *et al*., 2022).

According to Freitas *et al.* (2005), the antibiotics considered most effective in the treatment of mastitis are vancomycin, norfloxacin, Sulfa+Trimethoprim and enrofloxacin, and the least effective are penicillin and amoxicillin. In the vast majority of cases, treatment is done with the use of antibiotics, which can be applied systemically or intramammarily. (Arévalo *et al.*, 2021).

Mastitis is characterized by the colonization of the mammary gland, where contagious organisms penetrate, which are found in the microbiota of the skin, mucous membranes and conjunctivae of animals, as well as the skin, conjunctiva and mucosa of milkers, such as *Streptococcus agalactie*, *Streptococcus dysgalactie*, *Staphylococcus aureus* and *Mycoplasma* spp. Another form of infection is through pathogens present in the environment that are transmitted to the mammary gland from soil, feces, clay, air, water, animal bedding, milking utensils and other fomites such as *Escherichia coli*, *and Klebsiella pneumoniae*, *Enterobacter aerogenes*, S*erratia* spp., *Proteus* sp. *, Pseudomonas* spp., *Streptococcus uberis*, and *Enterococcus* spp. (Constable *et al.*, 2021).

Depending on its manifestation, mastitis can be divided into two groups, clinical and subclinical mastitis. The clinical form presents changes in the macroscopic appearance of the milk, such as the presence of pus and lumps, blood streaks and milk drainage, and signs and symptoms of inflammation of the affected gland, such as increased volume, edema, hyperthermia, hypersensitivity, hardening of the room, nodules, pustules, abscesses, gangrene and/or necrosis. The behavior of a cow with mastitis can also be identified, mainly due to the effect of distancing from the herd and refusal to allow the calf to suckle, because of the increased pain sensitivity in the affected teat (Megid; Brook; Paes, 2016; Fonseca *et al*., 2021).

The subclinical form of mastitis does not present visible changes in the breast and milk, but in the composition of this product, which undergoes considerable changes in its elements, such as an increase in chlorine (Cl) and sodium (Na) ions, and in somatic cell count, and a decrease in the percentage of casein, fat, total solids and lactose in milk (Simões; Oliveira, 2012; Good Death; Guerios, 2022).

The diagnosis of clinical mastitis is usually made through the clinical examination of the animal, which includes the identification of the animal, anamnesis, specific physical examination of the mammary gland and specific physical examination of the milk. Physical examination can diagnose clinical mastitis, where visual inspection of the animal is done to detect signs of inflammation in the mammary gland and teats. Signs of inflammation include hyperemia or flushing, presence of fistulas, enlarged swelling, edema, granulomas, abscesses, nodules, and dark areas with congestion and/or necrosis (Megid; Brook; Paes, 2016).

Subclinical mastitis needs complementary tests to be diagnosed, such as the California Mastitis Test (CMT), Wisconsin Mastitis Test (WMT), and the somatic cell count test (SCBS), which count somatic cells in milk (Massote *et al*., 2019).

The use of antibiotics is currently being left aside and new alternatives are being tested, such as homeopathy, photodynamic therapy and ozone therapy (Moreira *et al*., 2014; Oliveira *et al*., 2023).

According to studies by Arévalo *et al.* (2022), the use of ozone as a treatment for mastitis can offer benefits to industry, producers, and animals, due to its efficacy, functionality, accessibility, and because it is a therapeutic technique that is compatible with the vision of One Health.

Ozone therapy has been used in veterinary medicine as a form of integrative treatment for various pathologies. Ozone therapy has been standing out due to its low cost and for being a therapy that produces few side effects, in addition to being easy to perform. This is a technique that uses oxygen (O^2) and ozone (O^3) in a topical and systemic way, with the objective of promoting action against fungi, viruses, bacteria, in addition to having antioxidant, analgesic, anti-inflammatory and immunomodulatory action. The treatment is carried out using ozone generating equipment. The

oxygen connected through a cylinder attached to the device undergoes electrical discharges, transforming into two atomic oxygen molecules, which unite again with oxygen molecules giving rise to O^3 (Vilarindo; Andreazzi; Fernandes, 2013).

The action of ozone leads to the inactivation of viruses, fungi, bacteria, protozoa and yeasts, due to its proven ability to modulate oxidative and biological stress, ensuring its therapeutic use. In contact with the body, it reacts with polyunsaturated fatty acids present in cell membranes and originates a series of peroxides that stimulate the formation of deoxygenating or antioxygenating substances. The use of ozone suspension has been shown to be an alternative in the treatment of mastitis (Nogales *et al.,* 2008; Silva *et al*., 2018; Smith*;* Shiosi; Raineri, 2018; Fuentes, *et al*., 2022; Oliveira *et al*., 2023).

There are several routes for the application of ozone therapy, namely: intramammary insufflation, autohemotherapy, rectal, subcutaneous, urethral, intravenous, intriarticular, intraperitoneal, intramuscular, ozonated solutions such as saline solution, oils, water, serums, among other fluids (Freitas, 2011; Oliveira *et al*., 2023).

The antimicrobial effects of ozone therapy against mastitis microorganisms are satisfactory, since treatment with ozone infused into the galactophore ducts requires a minimum contact time, adequate volume and concentration of ozone to sterilize the causative agents of mastitis. In addition, milk from cows affected by mastitis and treated with ozone can be used immediately in industry or in food (Ogata; Nagahat, 2000; Arévalo et al., 2021; Fuentes, *et al*., 2022).

The authors cite the importance and efficacy of ozone in the treatment of subclinical mastitis, because of its oxidizing action, providing milk without the presence of antibiotics, anti-inflammatories, with moderate somatic cells and with better protein quality, both in milk and dairy products. They also highlight the benefits of ozone as disinfectants and bactericides, acting both in the environment and in the fabrics with which they come into contact, with a great bactericidal effect, without harmful actions such as chlorine, for example (Arévalo et al., 2021).

Studies have shown the increasing relevance of working with prevention and the emphasis on the use of ozone, especially in pre-dipping and post-dipping, replacing iodine and chlorhexidine-based substances. The implementation of good hygiene practices on dairy farms is a prerequisite for the production of high-quality milk and in the dairy industry. Ozone treatment can be applied on farms as an alternative to eradicate the use of antibiotics (Vargas; Szigeti, 2016; Nascente *et al.*, 2019; Arévalo *et al*., 2021; Arévalo *et al*., 2022).

Quintana, Domingues, and Ribeiro (2019) demonstrated in their studies the efficacy of a cow with subclinical mastitis treated with ozonated sunflower oil, which had *Staphylococcus aureus* infection, and another control cow with the same characteristics treated with sunflower oil. After the treatment, a new culture was performed and the first cow treated with ozone did not show bacterial

growth, the control cow continued with bacterial growth and infection with Staphylococcus aureus. The integrative treatment of mastitis with ozone proved to be a great therapeutic potential and did not pose risks to human and animal health.

Scrollavezza *et al.* (1997) did one of the first researches that brought ozone therapy as a new form of treatment for mastitis. The research with 5,000 dairy cows affected by clinical mastitis was treated with ozone therapy, where local insufflations were made with gas emission (50-100 mL) in different concentrations, applied to the affected quarters. The concentration was defined according to the severity of the disease, such as the degree of inflammation, the amount of somatic cells, and whether the mastitis was clinical or subclinical. In subclinical mastitis, the use of ozone was able to decrease the amount of somatic cells and increased milk production. However, they found that ozone has the advantage of needing to be used for a short period of treatment, in addition to other advantages such as low cost, and that it does not interfere with milk quality.

One of the most recent studies, conducted by Fuentes *et al*. (2022), reported the comparison of the treatment between antibiotic therapy and ozone therapy, where two cows were treated, and in one of the cows the treatment with antibiotics was performed and in the other the ozone gas was used. As a conclusion, the authors state that the use of ozonated suspension presented positive points, such as favorable changes in the composition of the milk, was a cheaper treatment when compared to the use of antibiotics and there was an improvement in the clinical signs of the disease, but in relation to efficacy, when the CMT test was performed, the antibiotic therapy was superior. The authors conclude the conclusion by stating that ozone therapy is a favorable treatment, which can help both in the factors of milk composition, as well as in the clinical signs of the disease, but that it is still a treatment that requires more research that evaluates both a larger number of animals and other situations, also analyzing different types of exposure. concentrations of the gas and other forms of application so that its effectiveness can be established.

In bovine mastitis, ozone therapy is a promising technique because it does not leave residues in the milk, and because it is a low-cost treatment when compared to antibiotic therapy. The advancement of scientific studies, and consequently the advancement of veterinary medicine, brings ozone therapy as a possible alternative treatment to the use of antibiotics for bovine mastitis. Finding an effective method for the prevention and treatment of mastitis has led researchers to advance in studies of integrative treatment so that they can enjoy better productivity, improve the quality of milk products and their derivatives and avoid threats to human and animal health.

However, the investigation of this treatment requires additional studies that can specifically address aspects such as dosages, application modalities and the appropriate period of treatment for this disease, aiming at the generation of new scientific data.

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