VOL II

Estudos em Ciências Agrárias e Ambientais

Eduardo Spers
(Organizador)

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APRESENTAÇÃO

O campo das Ciências Agrárias e Ambientais desempenha um papel fundamental na compreensão e solução dos desafios contemporâneos relacionados à produção de alimentos, à conservação ambiental e ao bem-estar animal. Em um mundo em constante transformação, questões como a sustentabilidade dos agroecossistemas, o manejo eficiente dos recursos naturais e a saúde pública se tornam cada vez mais relevantes. É com este espírito que apresentamos o volume II da coletânea "Estudos em Ciências Agrárias e Ambientais", que reúne pesquisas de autores de diversas partes do mundo, cada um contribuindo com sua perspectiva e expertise únicos.

Os quinze artigos que compõem este volume abordam uma variedade de tópicos, refletindo a riqueza e a diversidade das Ciências Agrárias. Desde práticas conservacionistas que buscam melhorar e manter agroecossistemas, até investigações sobre o uso de fitohormonas e fertilização na produção vegetal, o uso de tecnologias de processamento de madeira e a promoção do bagre armado - cada estudo traz à tona questões cruciais que impactam tanto a produção agrícola quanto a saúde ambiental.

Neste volume, também exploramos a crescente relevância dos produtos agrícolas locais, especialmente em tempos desafiadores como os que vivemos, marcados pela pandemia da COVID-19. A importância de circuitos curtos de proximidade se torna evidente, promovendo não apenas a segurança alimentar, mas também a resiliência das comunidades.

Além disso, as contribuições da veterinária destacam a importância do cuidado animal e da saúde pública, ilustrando a interconexão entre os seres humanos, os animais e o meio ambiente.

Esperamos que esta coletânea não apenas informe, mas também inspire debates e colaborações futuras entre pesquisadores, profissionais e estudantes da área. Juntos, podemos avançar em direção a um futuro mais sustentável e equilibrado, em que conhecimento pesquisa sejam os pilares para soluções efetivas.

Agradecemos a todos os autores e colaboradores que tornaram este trabalho possível. É nossa esperança que os estudos aqui apresentados contribuam para um entendimento mais profundo das questões agrárias e ambientais, e que possam servir de base para novas investigações e práticas inovadoras.

Eduardo Eugênio Spers

SUMÁRIO

SUSTENTABILIDADE E PRÁTICAS CONSERVACIONISTAS

CAPÍTULO 12

OZONOTHERAPY AS AN ASSISTANT IN THE TREATMENT OF MASTITIS, IN LACTATING COWS[1]

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ABSTRACT: The action of Ozone (O_3) as a germicide, against different pathogens has been studied; and has revealed the biological effects with therapeutic benefits [1-5]. Ozone inhibits growth and causes the death of gram negative and gram-positive bacteria, exposed to the ozone concentration of 0.167/ µg /min/L, at different times, produced ultra-structural changes in the bacteria, showing deformation and sudden damage with surface destruction, collapse and cell lysis [6]. The present work was developed in the "El Salado" zootechnical

[1] This article was published in 2019 by juniper publishers. (https://juniperpublishers.com/jdvs/pdf/JDVS.MS.ID.555876.pdf). Some authors have been eliminated from the original paper due to political issues.

post, belonging to the Faculty of Veterinary Medicine and Zootechnics of the “Benemérita Universidad Autonóma de Puebla”, in the production module of Milk Bovines, which has cows of the Holstein- Frisean and Jersey breeds, under a system of intensive handling, being milked twice a day (every 12 hours), in a 4-box fishbone milking parlor and with a Westfalia brand milking equipment; where, after identification of subclinical mastitis, by performing the California Mastitis Test on each cow, the readings were made and three experimental series were applied, using different concentrations of the ozone-oxygen mixture, in the affected mammary quarters of nine cows in production. After 4 days of treatment with the ozone / oxygen mixture, a 50% improvement was observed in the affected rooms, reflected in a reduction of somatic cells and leukocytes per milliliter of milk; being the dose that obtained better results, the one of 11µg of ozone in a mixture of ozone- oxygen in 300ml of volume, that were instilled in the affected breast rooms. It is concluded that the effect of intramammary administration of the ozone / oxygen mixture (MOO) is presented as an adjuvant in the innovative treatment of subclinical and clinical bovine mastitis, representing a real alternative, being necessary to establish effective doses and volumes for said end; Therefore, the indiscriminate use of antimicrobials would be avoided, establishing this ozone therapy as an effective, safe, cost-effective method, with zero days of withdrawal and without the risk of having drug residues in milk.
KEYWORDS: Ozone therapy. Subclinical mastitis. Milk quality.

1 INTRODUCTION

Mastitis is a serious disease suffered by milk-producing animals, causing large economic losses, due to the reduction in milk production, as well as its low nutritional value. Mastitis is characterized by the occurrence of physical, chemical and bacteriological changes in milk including pathological abnormalities of the mammary gland tissue. Mastitis affects the quality of milk in terms of its composition and production. The extent of the various changes in milk composition depends on the inflammatory response [7]. Somatic cells are the main epithelial cells that have detached from the glandular lining and white cells (leukocytes) that penetrate the mammary gland in response to a specific damage or infection. Somatic milk cells include 75% of leukocytes, such as neutrophils, macrophages, lymphocytes and 25% of epithelial cells. During inflammation (mastitis) the greatest increase in SC is due to the influx of neutrophils into the milk to fight the infection and has an estimated 90% [8,9]. The most precise relationship between intramammary infection and somatic cell count (CCS) can be assessed at the quarter level when the SCC exceeds 200,000 cells / ml [10]. In addition, the CCS in healthy rooms is consistently low and usually below 200,000 cells / ml [11]. In subclinical mastitis, the pathogens do not cause sufficient destruction of the alveolar tissue, so that it is reflected in the characteristics of the milk, but the SCC is increased indicating the infection of the milk [12].

In clinical mastitis, clinical signs can be recorded as weak, moderate and severe [13]. *Staphylococcus aureus* is considered one of the most virulent bacterial species that cause bovine mastitis (MSC) and clinical mastitis (MC) [14]. The administration of intramammary antibiotics is the most common method used for the treatment of bovine mastitis. However, at present this treatment is having few results regarding the recovery of the infection, mainly due to the bad management and the development of resistance by pathogens. Such is the case of the identification and characterization of methicillin-negative coagulase Staphylococcus- resistant in bovine mastitis [15,16]. The treatment of acute bovine mastitis during the period of breastfeeding represents one of thegreatest economic losses of livestock production. This is related to the cost of treatments (veterinary service and drugs), the decrease in production and the sale price of milk, as well as the increase in somatic cell count in the milk sample volume, the increase in the number of cows discarded and the increase in losses due to the presence of antibiotic residues in milk and meat [17-19].

A range of production systems coexist in Mexico, ranging from the most traditional, in the hands of farmers in isolated regions of the country, to large modern companies, vertically and horizontally integrated. The technology required by the various systems is very different and MVZs must be very aware of the scope and limitations of each method or input they apply or recommend. The monitoring of the quality of products has a wide social demand and also plays a strategic role in the protection of our internal market, access to markets abroad and in making our country more attractive for these important economic activities. Therefore, this problem must be addressed by trained professionals, who can provide effective alternatives, easy to apply and with affordable costs, which guarantee the quality of food of animal origin. The presence of mastitis is a very serious problem for dairy farming and is one of the main causes of economic losses for the farmer and / or producer. In the United States, annual losses are estimated at around $ 2,000,000. ([20], University of Oviedo, Gijón, Asturias). World losses, annual due to mastitis, have been estimated at US $ 35 billion [21,22]. The greatest losses result from the reduction in milk production due to subclinical mastitis. In other studies, conducted in herds located in the highlands of Mexico, the prevalence of subclinical mastitis has been estimated at 20.80% in Tizayuca, Hidalgo, and 81.10% in stables around the Federal District [23-31].

Hygiene in cowsheds and milking place are very important and everyone knows that the lower the level of hygiene, the greater the risk of infection. Dr. Carlos Concha Bascuñan, M.V.Z. specialist of the University of Chile, recommends the application of drying knobs or treatments only in the case of cows with positive results to bacteriological

cultures carried out with the secretion existing during the period, has found that more than half of the cows, in The dry period is bacteriologically negative and all they need is a good external nipple sealer. By routinely applying antibiotics to all cows when they are dried, even in completely healthy udders, we soon create resistance, which will make it difficult to treat clinical cases. The Nordic countries, with a very careful policy "prohibition of the prophylactic use of antibiotics" as a treatment for cows on drying without having carried out bacteriological tests, has allowed them to present only 10 to 20% resistance to penicillins, versus 40 60% of the rest of Europe [32-39]. Due to the above, the use of ozone as a treatment in subclinical mastitis, will allow us to have zero days of withdrawal in milk, perfectly sterilize the room of bacteria, viruses and fungi, due to its potent oxidizing action, it will not allow the resistance effect of microorganisms and We will have the possibility to enjoy a milk free of antibiotics, anti-inflammatory, with moderate somatic cells and with a higher protein quality, both milk and derivatives.

2 MATERIAL AND METHODS

The realization of this work was carried out in the "El Salado" zootechnical post, belonging to the Faculty of Veterinary Medicine and Zootechnics of the Benemérita Universidad Autonóma de Puebla, which has cows of the Holstein-Frisean and Jersey breeds, under an intensive management system, being milked twice a day (every 12 hours), in a 4-box fishbone milking place.

2.1 INCLUSION CRITERIA:

- Cows with clinical and bacteriological diagnosis of clinical mastitis.
- Cows diagnosed with subclinical mastitis.

2.2 EXCLUSION CRITERIA:

- Cows that are under antibiotic or other therapeutic treatment.
- Cows that show signs of worsening of the clinical picture during treatment with the MOO.

2.3 EXPERIMENTAL SERIES GROUPS:

The animals were divided into experimental series of 3 cows in each group.
Experimental series 1: Dose of 11mg of ozone in 300ml of volume.
Experimental series 2: Dose of 35mg of ozone in 300ml of volume.
Experimental series 3: Dose of 50mg of ozone in 300ml of volume.

2.4 JUSTIFICATION FOR THE SELECTION OF MOO CONCENTRATIONSFOR EACH EXPERIMENTAL SERIES:

The treatments of the MOO by intramammary route will be carried out every 24 hour after discharging the milk from the room and after disinfecting the room with an antiseptic solution.

2.5 STUDY SUBSTANCES AND / OR REFERENCE:

The MOO, obtained from an Ozone Generator for Medical Use, Model MEDIC-O3 PORTATIL, Manufactured by OZONO CARBAR´S. Serial No. BRJ17-960. The measurement was carried out by means of an ozone analyzer model MINI-HICON BENCH with serial number 170107 with a capacity of up to 400 g / Nm3 with temperature and pressure compensator, of American origin.

Equipment Accuracy + - 2 µg / ml.

Maximum Standard Deviation: 0.11%

2.6 METHOD OF ADMINISTRATION AND / OR EXPOSURE AND REASON FOR BEING EMPLOYED:

Intra-mammary route: After disinfection of the nipple area of the room, with an iodine-based disinfectant solution, an intra- mammary cannula is placed, which is connected with the MOO- loaded syringe, it is slowly discharged until the volume of 300 mlis completed determined for the application. It will be applied every 24 hours for 4 consecutive days.

2.7 MEASUREMENTS TO BE PERFORMED:

a. Review of the medical history of each cow registered in the medical control cards of the production module.
b. Identification of race, age, days of milk production.
c. Registration of physiological constants.
d. Carrying out the mastitis California test.
e. Performing the somatic cell count test.
f. Identification of the rooms affected by clinical mastitis and their antimicrobial treatment.
g. Observation of the response to ozone treatment or recurrence.

2.8 SOMATIC CELL COUNT (SCC)

Milk samples were taken from the affected rooms and SCC counting was performed using the Porta Check SCC method; taking as a reference, the count greater than 200,000 cells / ml and apparently without morphological changes in milk, will be classified as subclinical mastitis (SCM). While a SCC from 5,000,000 cells/ml, accompanied by morphological changes of milk such as lumps and color changes, it will be classified as clinical mastitis (CM). This diagnostic method will be performed before and after the end of the treatment cycle with the substance under test (Table 1).

Table 1: This diagnostic method will be performed before and after the end of the treatment cycle.

		07-11-18						10-11-18				
	Type of Mastitis	California Mastitis Test				Ozone Dose		Type of Mastitis	Type of Mastitis California Mastitis Test			Ozone Dose
No. Cow	c) Clínic s) ubclínical	AI	AD	PI	PD		No. Cow		AI	AD	PI	PD	
4	s)	-	2	1	-	11µg/300ml	4	s)	-	-	T	-	11µg/300ml
69	s)	-	T	2	2	11µg/300ml	69	s)	-	-	1	1	11µg/300ml
49	s)	2	1	2	2	11µg/300ml	49	s)	2	1	T	1	11µg/300ml
28	s)	-	3	2	-	35µg/300ml	28	s)	2	2	2	T	35µg/300ml
160	s)	-	3	3	2	35µg/300ml	160	s)	-	-	-	-	35µg/300ml
38	c)	C	-	-	-	35µg/300ml	38	c)	2	-	-	-	35µg/300ml
19	s)	-	-	3	-	50µg/300ml	19	s)	-	-	2	3	50µg/300ml
77	s)	2	-	2	-	50µg/300ml	77	s)	1	-	1	-	50µg/300ml
99	s)	2	-	-	-	50µg/300ml	99	s)	2	-	-	-	50µg/300ml

3 RESULTS

After 4 days of treatment with the ozone / oxygen mixture, a 50% improvement is shown in the affected fourth.

The cow No. 38 that presented clinical mastitis in the fourth AI, after treatment improved, showing in the California mastitis test a clear positive reading 2, with reduction in the number of leukocytes and somatic cells in said room.

It can be seen that the most effective dose of the ozone / oxygen mixture is 11 to 35µg / ml, in a volume of 300ml (Table 2).

Table 2: Milk sample from thermo tank.

No. of Cow	The Affected Fourth	SCC at the Beginning	SCC the End
4	AD	1,720,000/ml	630,000/ml
38	AI	>3,000,000/ml	950,000/ml

24	PD	873,000/ml	230,000/ml
Milk sample from thermo tank			
SCC at he beginning		SCC the end	
281,000/ml		253,000/ml	

4 DISCUSSION

Taking into account the high incidence of mastitis in the dairy farming industry, as well as the economic losses due to said disease, it is appropriate to carry out its diagnosis in the field and treat in the best way and in each case in particular, being a good option, the administration of correct doses of the ozone-oxygen mixture. Several works show that the Ozone / Oxygen Blend has a high germicidal power and in the present work, an effectiveness of the MOO was obtained as a treatment for bovine mastitis. Antibiotic treatments are not entirely effective because of the high incidence of resistance developed by the microorganisms that cause bovine mastitis. The use of ozone in gas has no withdrawal period or leaves metabolites in milk. Therefore, it is rational to study and determine the optimal MOO concentrations for the treatment of clinical and subclinical mastitis in cattle, according to the causative germs.

5 CONCLUSION

The effect of intramammary administration of the ozone / oxygen mixture (MOO) as an innovative treatment of subclinical and clinical bovine mastitis is investigated, establishing effective doses for this purpose. Avoid the indiscriminate use of antibiotics, establish an effective, safe, profitable method, with zero days of withdrawal and without risk of having residues of medicines in the milk. The sterilization of treated breast rooms is checked by physical and biological methods. A practical, viable and low-cost application protocol is established to control the presence of clinical mastitis in the dairy herd.

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ÍNDICE REMISSIVO

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