Photobiomodulation in the Treatment of Bovine Subclinical Mastitis

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Abstract. Mastitis is among the most common, impacting and challenging disease in the dairy industry. Mainly in view of the various disadvantages of conventional therapies, this study aimed to verify the treatment of subclinical mastitis through photobiomodulation. Lactating cows (n = 10) were used, nine (n = 9) with positive subclinical mastitis and one (n = 1) negative used as a control. The photobiomodulation protocol using 5 J/cm^2, and occurred on alternate days, totaling seven applications of the Ga-Al-As diode laser (685 nm) inside one cow teat. The milk samples were analyzed before, during and after the treatment to realize the reductase test, colony count and California Mastitis Test (CMT). No changes were found in the reductase test, keeping the milk acidity stable in all samples during and after treatment. In the bacteria isolation and colony count we identified *Staphylococcus aureus*, coagulase-negative *Staphylococcu*s and *Bacillus cereus*, a better performance was observed in animals treated by coagulase-negative *Staphylococcus*, keeping the growth of *Staphylococcus aureu*s stable and increased in *Bacillus cereus*. Regarding the CMT, it was positive for subclinical mastitis before and after treatment, with a decrease in positivity in 60% of the animals 96 h after the last application. The results suggest the development of future research of photobiomodulation protocols for bovine subclinical mastitis with others optical dosimetries, since there were no changes in the milk composition, decreased in the bacteria count, and without generating residues in milk and dairy product losses.

Keywords: Photobiomodulation · dairy · mastitis · bovine

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1 Introduction

Mastitis is a common worldwide inflammatory disease of the mammary gland that causes major impacts on dairy animals, affecting animal welfare and causing great economic losses to the dairy industry through decreased production performance and increased waste milk disposal [1, 2].

The infection transmission occurs mainly between milking due to hygiene failures, and is difficult to control, since the main infection agents are disseminated in the environment where the animals remain [3]. The widespread use of antimicrobials to combat this cause infection has been a serious public health problem, given the risks of the selection of antimicrobial-resistant bacteria, and the presence of these compounds in the dairy products [4], where they must be discarded by the milk-producer [5]. New regulations on veterinary medicine will substantially influence antimicrobial prescribing and usage throughout into the near future. These regulations have been informed by a very large body of work, including the substantial progress towards reduced antimicrobial usage in food animal production [6].

New treatment proposals for subclinical mastitis using phototherapy or photobiomodulation technique as a new "clean" technology for animal healthcare, the environment and consequently for human beings, and have been recently proposed in the works of Moreira et al. [7, 8], Galstyan & Dobrindt [9], recently in sheep by Silva et al. [10] and in a letter to the editor by Ribeiro and colleagues [11], where they also discuss the clinical challenges and therapeutic advantages of this promising technique, also too with the association of photodynamic therapy.

The objective of this study was to evaluate the effect of photobiomodulation in bovines with subclinical mastitis for the development of preventive protocols for this infection disease.

2 Materials and Methods

2.1 Animals

This study was approved by the institutional review board (Animal Care and Use Committee) of the University Camilo Castelo Branco (process #1-00034/2012).

Ten lactating Holstein cows (n = 10) were selected, all raised in the confinement management and feeding based on corn silage, protein concentrate, brewing industry residue (barley) and green forage ad libitum, from a commercial farm in Caçapava/SP, Brazil.

Cows were milked using a mechanical herringbone milking parlor (6 × 2) three times a day (4, 12 and 20 h) and per-formed without the calves presence. Routine technical procedures for milking hygiene consisted of asepsis in the mammary gland with Master Iodo (Sani Química Ltda, Valinhos, Brazil), upon entering the milking parlor, then the teats were dried with disposable paper towels and milked, at the end service, the teats were also disinfected with the same product, and the animals were released to the pad-docks.

2.2 Analytical and Clinical Exams

All lactating animals underwent visual veterinary inspection of the mammary gland and indirect analysis of the milk quality using the California Mastitis Test (CMT) [12], once a week, according to the manufacturer's instructions, before the second milking of the day (12 h p.m.) for the detection of clinical and/or subclinical mastitis through 120 days. Cows that showed persistent reaction in the CMT exam with three crosses (+++), for a period of three consecutive months without observation of spontaneous cure of subclinical mastitis, were separated for the performance of the photobiomodulation treatment. Under these conditions, the cows ($n = 9$) were selected using only one mammary quarter of each animal for the experiment. For control group, we used an animal ($n = 1$) that was in the same period of lactation and that also received the same treatment protocol although it did not have subclinical mastitis.

To the CMT examination the selected cows receives again the mammary gland asepsis routine, and then the first three jets of milk were used for the CMT exam, and after this, 70% ethyl alcohol was passed on the teat to milk microbial collect analysis. The cow teat was cleaned, dried with a paper towel and then collected 20 mL of milk in a sterile bottle for laboratory test. After this procedure, the routine milking was performed and the treatment protocol was applied to the selected teat.

The milk samples collected were immediately stored in isothermal boxes and sent to the laboratory milk analysis. Microbiological identification, colony count and reductase tests were also performed in the laboratory [13]. Finally, ten milk samples were collected from each treated teat, one sample before treatment, seven samples during treatment and two samples after treatment, 48 and 92 h apart from the last treatment.

2.3 Photobiomodulation Protocol

Animals were treated with a low-power Gallium-Aluminium-Arsenide (Ga-Al-As) diode laser (Teralaser, MMOptics Ltda, São Carlos, Brazil) with a wavelength in 685 nm, continuous emission mode, power of 20 mW and an energy density of 5 J/cm^2 with an exposure time of four minutes (240 s). The light dose was checked before each experiment.

The laser probe was disinfected and inserted internally up to three centimeters into the cow teat canal as showed in Fig. 1, and at the same time the teat was compressed to reduce its length, thus facilitating a better irradiation of the light from laser beam inside the mammary gland. In this sense, after milking the selected cows were treated, consider the time interval of 48 h (alternate days) between each application, consisting of seven total applications per selected teat animal. A negative or decreased CMT within 92 h after the end of treatment was established as a primary objective.

Fig. 1. Laser application in the bovine teat canal.

3 Results and Discussion

Of the ten treated teats (n = 10) in six teats were isolated Staphylococcus aureus, in two teats coagulase-negative Staphylococcus, and in other two Bacillus cereus as the infection agent of subclinical mastitis.

The average of the CFUs (log CFU) from the animals before treatment (0), during treatment (applications 1 to 7) and after treatment (48 and 92 h) versus the animal control condition are shown in Fig. 2.

The reductase test was performed on all milk samples, and we detected changes in four samples before starting treatment, and no changes were observed during and after treatment in these animals.

Lage and colleagues [14] developed a photobiomodulation LED-based device for the prevention and treatment of teat hyperkeratosis in dairy cows as a preventive proposal for mastitis. Moreira et al. [7] developed a treatment protocol for subclinical mastitis using LEDs associated with a photosensitizer, obtaining good results as an alternative treatment without leaving drug residues in the animal, without milk discarding. The same author and other colleagues [8] recently proposed a device based in infrared LEDs accoupled in an industrial mechanical milking equipment, aiming to prevent subclinical mastitis, but they are necessary more studies to determine the best dosage.

In subclinical mastitis, although it is not possible to diagnose it visually, there is a marked increase in polymorphonuclear leukocytes, which makes it possible to detect this condition by indirect methods, such as CMT, which is the most widespread technique by rural producers because it is easy-to-perform, low-cost and a fast test, for these reasons was the technique used in our study to compare the condition before, during and after treatment, so the results are presented in Table 1.

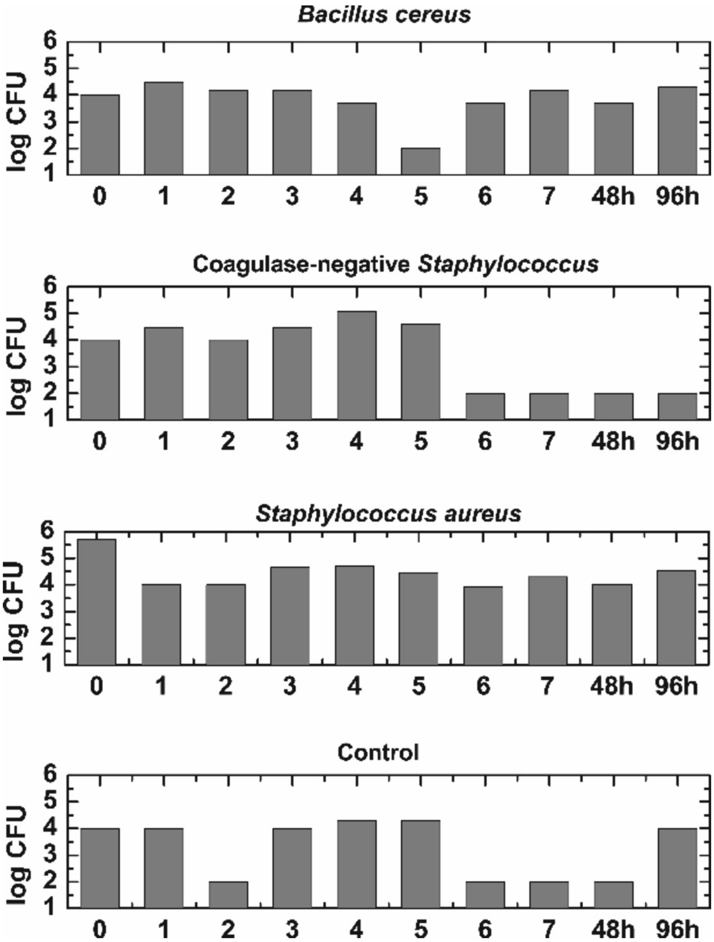

Fig. 2. Mean Colony Forming Units (log CFU) of cows photobiomodulation treatment to subclinical mastitis positive microorganisms *vs*. control.

The CMT increases during treatment was expected, corroborated the studies by Albertini et al. [15] and Mansouri et al. [16] that reported that low-level laser therapy induce a reorganization of the inflammatory process, both in cells and in blood vessels; endothelial cells are transformed into capillaries due to the neoforming light effect, as their action on the inflammatory process by modulating neovascularization, which as a consequence reduces the loss of function, increases tissue oxygenation and regional microcirculation, proliferating blood cells such as leukocytes, lymphocytes, polymorphonuclear cells, macrophages and plasma cells, and in this case it was detected in milk by the CMT during and after treatment, due to the photobiomodulation caused by the light absorption. CMT tends to become negative after treatment, as it was observed that 60% of the treated animals obtained a CMT below 3 (+++) at 96 h after treatment.

Table 1. CMT before, during and after photobiomodulation treatment.

Application	Control	A	B	C	D	E	F	G	H	I
0	0	3	3	3	3	3	3	3	3	3
1st	0	3	3	3	3	3	3	3	3	3
2nd	1	3	3	3	3	2	3	3	3	3
3rd	2	3	2	3	3	2	3	3	2	3
4th	3	3	2	3	2	3	3	2	2	3
5th	2	3	3	3	3	3	3	2	3	3
6th	1	3	3	3	2	2	3	2	3	2
7th	2	3	3	3	2	3	3	2	3	3
48 h	1	3	3	3	2	2	3	3	3	2
96 h	1	2	2	3	2	2	3	3	3	2

Application: 0 = before application; 1–7 = during application; 48 h = 48 h after application; 96 h = 96 h after application; Control = animal control; A–I (n = 9) = treated animals; 0 = animal without subclinical mastitis; 1 = (+) animal weakly positive for subclinical mastitis; 2 = (++) positive animal for subclinical mastitis; 3 = (+++) animal strongly positive for subclinical mastitis.

Moreira et al. [7] used photodynamic therapy in subclinical mastitis confirmed a bactericidal effect with the reduction of the microorganisms Streptococcus dysgalactiae and coagulase-negative Staphylococcus using the laser irradiation at three different spots inside the mammary gland, using a dosage much higher than in this proposed work. The in vitro study by Schultz et al. [17] indicated that Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli were eliminated by laser irradiation when the energy density was greater than only 1.6 J/cm^2.

The average CFU of milk samples from treated animals showed the profile of growth and partial inhibition of isolated microorganisms (Staphylococcus aureus, coagulase-negative Staphylococcus and Bacillus cereus) at 10^{-2} dilution at energy density of 5 J/cm^2, with time of application of four minutes and applying the laser probe (optical fiber) inside the channel of the teat, different from the works of Schultz et al. [17] and Okamoto & Iwave & Morioka [18] that were performed with energy density and application time focusing directly on the microorganism in in vitro assays when obtained a more direct response (partial or total inhibition). Albertini et al. [15] studied the light effect in rat paw edema focusing on the animal's skin, and obtained satisfactory results for the anti-inflammatory effect, as also suggested by Moreira et al. [7] when using photodynamic therapy with 200 J/cm^2 to treat subclinical mastitis.

Sharun et al. [2] in a recent review discuss a need for the development of new technologies for the effectiveness in mastitis treatment, since the disease presents different therapeutic responses in antibiotic use, due to microbial resistance to conventional drugs available on the market and the high number of etiological agents cause-disease. New "clean" technologies, without waste generation, are essential for the future of dairy industry in a sustainable way.

4 Conclusions

This study suggests the development of future research aiming to prevention of bovine subclinical mastitis applying photobiomodulation technique, based in our results related to bacteria reduction in the treated teat, improvement in the quality of milk by the CMT, and without generating residues in milk and dairy product losses.

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Conflict of Interest. The authors declare that they have no conflict of interest.

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