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074. Safety of a nanopropolis formulation intended for intramammary treatment of bovine mastitis in organic dairy herds[27]

Inocuidade de uma formulação de nanoprópolis desenvolvida para tratamento intramamário de mastite bovina em rebanhos leiteiros orgânicos

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Abstract: Considering the bactericide action of the propolis and the fact that this compound is allowed for use in organic dairies, Brazilian researchers developed the nanoestructured propolis, a new perspective for bovine mastitis treatment. The aim of the present study was to evaluate the safety of this nanopropolis formulation in dairy cows from an organic farm in Botucatu-SP Brazil. A volume of 10 mL nanopropolis was administered by intramammary route in four cows, during three consecutive days. Animals were clinically evaluated after treatments and no local and/or systemic adverse reactions were verified. The

use of nanopropolis did not interfered on SCC values neither on milk production rates on treated cows. It was possible to conclude that the evaluated nanopropolis formulation is safe for intramammary treatment of mastitis cases in dairy herds.

Keywords: milk, nanotechnology, propolis, cow, innocuousness.

Resumo: Considerando que a própolis apresenta ação bactericida e trata-se de composto permitido em sistemas orgânicos de produção leiteira, pesquisadores brasileiros desenvolveram uma formulação de própolis nanoestruturada, visando o tratamento intramamário de casos de mastite bovina. O objetivo do presente estudo foi avaliar a inocuidade desta formulação em vacas leiteiras criadas em sistema orgânico de produção, na região de Botucatu-SP Brasil. O produto foi administrado em volume de 10 mL, por via intramamária, em quatro vacas, durante três dias consecutivos. Os animais foram avaliados clinicamente após os tratamentos e não foi constatado qualquer tipo de reação adversa (local e/ou sistêmica). A administração intramamária de nanoprópolis também não interferiu nos valores de CCS e no volume de produção leiteira das vacas tratadas. Conclui-se que a formulação de nanoprópolis avaliada é um produto inócuo para o tratamento intramamário de mastite em bovinos leiteiros.

Palavras-chave: leite, nanotecnologia, própolis, vaca, segurança.

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Introduction

The bovine mastitis, an inflammatory process of cow's mammary gland is usually caused by infectious pathogens and determines important losses on milk business (TRONCARELLI et al., 2011). Due the low number of compounds allowed for bovine mastitis treatment in organic dairies, there is an important demand for researches involving new products development, specially using natural actives. In this context, researchers from EMBRAPA Dairy Cattle – a governmental Brazilian institution for milk business research, located in Juiz de Fora-MG – have developed a nanostructured propolis, using the nanotechnology principle. Considering the important number of organic dairies in Botucatu-SP Brazil, researchers from Faculty of Veterinary Medicine and Animal Science (FMVZ) UNESP/Botucatu-SP and from EMBRAPA Dairy Cattle decided to establish a scientific partnership to evaluate the safety of this new formulation for bovine mastitis treatment.

Material and Methods

Propolis origin and nanoformulation

A batch of green propolis from Zona da Mata region (Minas Gerais, Brazil) was synthesized by precipitation technique and interfacial deposition at EMBRAPA Dairy Cattle facilities (Patent PI 1004808-1) (BRANDÃO et al., 2010).

Animals and inclusion criteria

Four cows were enrolled in the present study (two first lactating cows and two second lactating cows) from an organic dairy farm with 12 bushels area located in Anhumas, a neighborhood region of Botucatu-SP Brazil. Dairy cattle was composed by a total of 20 lactating mixed breed cows (Gir x Holstein), with medium milk production of 10 liters per day. Animals were handled in an extensive system (*Brachiaria decumbens* grass), and supplemented with mineral salt for bovine and with 3-4 kg feed (wheat grain)/cow/day.

Animals' inclusion criteria were based on: 1) absence of systemic clinical signs; 2) absence of subclinical/clinical mastitis; and 3) be in the middle lactation period. The absence of mastitis was microbiologically confirmed by three negative results of milk cultured samples (three weeks sampling, 7 days interval).

Treatment and clinical evaluations

Nanopropolis administration was done after cows' milking according to safety studies methodology established by OECD (2010), adapted for bovines. Immediately before treatment (D0), some clinical parameters, like animals' corporal temperature, and the cardiac and respiratory frequencies were measured.

The right cranial quarter was used for the treatment of all cows, as standardization. The superficial temperature of udder skin was measured by a digital ultrasonic thermometer, positioned at 20 cm distance from the teat. A small circle (1cm diameter) was market on the udder, using permanent ink, for acute edema evaluation. Individual milk samples were collected from right cranial quarters for SCC. After teat disinfection with an alcoholic iodine solution, the nanopropolis formulation was administered by intramammary route, in a 10 mL volume, by a sterile plastic syringe equipped with a sterile aluminum Luer Lock probe (3.8 cm). After 30 and 60 minutes post treatment (PT), animals' general clinical parameters and the superficial temperature of udder skin were measured again.

Treatments were done once a day, during three consecutive days (D0-D2). All clinical evaluations were completely repeated in each one of the three treatment days.

On Days 1 and 2 PT, the diameter of the marked circle at each animal's treated udder was measured. Treated quarters were manually examined for nodules searching. The udders and teat's shape, consistency and symmetry were

clinically evaluated and the mammary lymph nodes were also examined.

On Days 1 to 7 PT milk samples were daily collected for SCC. On Day 7 PT milk samples were obtained for microbiological culture. Samples were cultured on bovine blood agar (5%) and MacConkey agar, and kept at 37°C during 72 hours (QUINN et al., 2005). Daily data regarding total milk production of treated cows were registered from Day 0 to Day 30 PT.

Results and discussion

The results regarding clinical evaluations of treated animals; SCC data and milk production rates are shown on Table 1.

Table 1. Clinical parameters, SCC data and production rates of organic dairy cows treated with propolis nanoparticles, by intramammary route. Botucatu-SP, Brazil, 2014.

Cow	Corporal temperature (°C)*		Udder skin superficial temperature (°C)*		Cardiac frequency (CF) (/min)*		Respiratory frequency (RF) (/min)*		SCC (x 10^3 cells/mL)**		Milk production (L/day)***	
	Ti	Tm	Ti	Tm	CFi	CFm	RFi	RFm	SCCi	SCCmed	Pi	Pmed
1	38.1	38.0	34.5	34.8	56	58	18	18	192	199	9.1	9.3
2	38.2	38.0	34.0	33.8	58	58	18	20	98	189	12.0	11.7
3	38.5	38.3	35.3	34.9	60	56	22	20	138	172	7.8	8.1
4	38.3	38.2	35.3	35.4	56	58	20	20	181	191	10.2	10.6

*Clinical paramaters evaluated on Day 0, Day 1 and Day 2. **SCC data evaluated on Days 1 to 7. ***Milk production data evaluated from Day 1 to Day 30.
i = inicial parameters mean (immediately before treatments); m = mean of measured parameters at 30 and 60 minutes PT; med = median of obtained values during the total of days of PT evaluation.

As verified on Table 1, no significant alterations of clinical parameters were observed after cows' treatment. By the same way, clinical evaluation of quarters showed no adverse reactions PT (no pain, nodule, erythematic and/or macroscopic alterations of milk were verified). The diameter of circle that was marked with permanent ink on treated quarters remained the same, indicating

absence of acute edema. No differences were verified between treated and non treated quarters, considering their shape and size. Mammary lymph nodes showed no alterations in volume.

According to SCC data, the milk samples obtained before treatment showed initial values lower than 200 x 10^3 cells/mL. The median of SCC obtained during the seven days evaluation PT showed no significant difference with the initial pre-treatment values, and remained under 200,000 cells/mL. These results are similar to the ones verified by Ataídes et al. (2012). These researchers used an alcoholic solution of natural propolis for the treatment of subclinical mastitis in mixed breed dairy cows (Gir x Holstein) in Rio Verde (GO) Brazil, and no interference on SCC values were verified in the propolis-treated quarters.

Milk samples obtained on Day 7 PT resulted negative to microbiological culture, confirming that treatment was done aseptically and did not cause any intramammary infection on cows' quarters. Moreover, cows did not show any behavior alteration after treatment, and no changes were verified on animals' feces and urine.

According to scientific reports, the natural propolis may be irritating for mammary gland, and usually induces inflammatory reaction on udder and/or alterations on milk compounds and SCC values (Pereira & Botteon, 2008; Coelho et al., 2010). However, these characteristics were not verified in the present study when using propolis nanoparticles. By this way, it is possible to consider this formulation as a new perspective for bovine mastitis treatment.

Conclusion

According to the presented results, it is possible to conclude that the evaluated product is safe for intramammary administration in cows. If the nanopropolis shows satisfactory efficacy results in the ongoing *in vivo* studies, it may represents a new and important therapeutic alternative

for mastitis control, especially in organic dairy herds.

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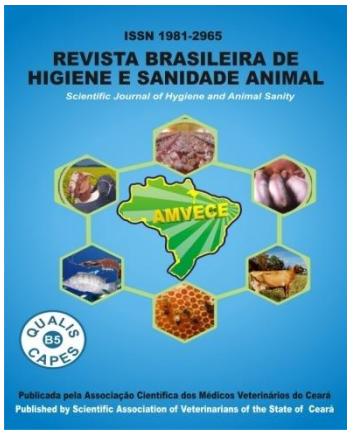

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