



## Intramammary Propolis Formulation for Prevention and Treatment of Mastitis in Dairy Ruminants (RC.2.2.08-0003)

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Mastitis, an inflammation of the mammary gland, is the most widespread health problem of dairy cows, goats and sheep (Bačić *et al.* 2015., Mačešić *et al.* 2012). Its prevention and treatment contribute to significant financial loss to the milk producers. New research shows that the total loss due to clinical mastitis in dairy cows is estimated between 110 and 550 litres of milk per lactation, depending on the individual cow. The costs of clinical mastitis include unusable milk, medications and veterinary care costs, extra labour and premature culling (Sharma *et al.* 2012). Depending on the form of mastitis the treatments cost between 50 and 150 \$US per animal.

Antibiotic intramammary preparations that are commonly used to treat mastitis are not always effective against all mastitis pathogens, which leads to a developed resistance to antibiotics. Antibiotic resistance represents a public health threat to consumers and a global problem in terms of human and animal health. Bearing in mind the widespread awareness of the potentially negative effects of the use of antibiotics in the animal production, milk producers and consumers have focused their search towards new treatments and prevention solutions. There is a growing interest for an alternative approach in farming and veterinary care of cattle.

## MATERIALS AND METHODS

### Project goals

Together with SME Hedera Ltd, Split, Faculty of Veterinary Medicine Zagreb has over the last year and a half developed and tested an innovative intramammary formulation of non-alcoholic solution of propolis to be used for the prevention and treatment of mastitis in dairy ruminants (Bačić *et al.* 2015a). Honey bee products, such as propolis, are part of the ecological approach of prevention and treatment of mastitis due to their proven antibacterial, anti-inflammatory, antioxidant and immunostimulatory effects (Wright 2014).

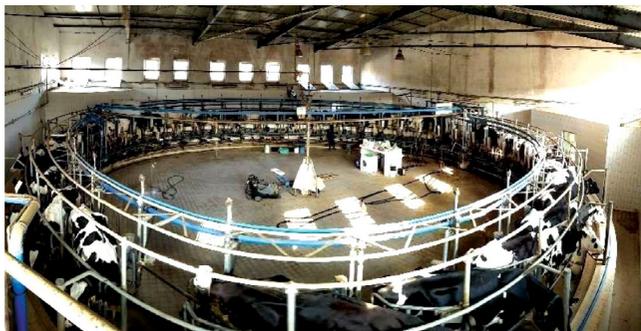
The main advantage for the consumer / farmers is that the withdrawal period doesn't exist, therefore bringing significant savings in production, improving the quality of the final product, and certainly providing new solutions to farming challenges (Radin *et al.* 2015., Šuran *et al.* 2015)). Such product may be of particular interest to organic farms and the "free farming" systems that are particularly developed in western countries.



**Fig. 1.** Bee hive (left), wooden frame around the comb covered with natural propolis (right)

#### Main activities:

1. Production of non-alcoholic native propolis formulation
2. Analysis of the formulation composition
3. *In vitro* study of formulation efficacy against common mastitis pathogens
4. Clinical trials on dairy cows and goats during dry period and lactation



**Fig. 2.** Rotary parlor at dairy farm Krndija (milking 780 cows)

#### RESULTS AND DISCUSSION

During the implementation of the project we developed an innovative formulation of non-alcoholic solutions of native propolis with 100% effectivity of its substances.

The laboratory tests showed the sensitivity of the common mastitis pathogens (reference strains and clinical isolates obtained during the duration of the project) to various concentrations of propolis solution, and minimum inhibitory concentration (MIC) were determined (Matanović *et al.* 2015). The solution exhibits a good

antibacterial activity against gram-positive bacteria, such as species of *Staphylococcus aureus* and *Streptococcus uberis* and coagulase-negative staphylococci, while the activity against gram negative bacteria, such as *E. coli* and *Pseudomonas aeruginosa* was significantly weaker.



**Fig. 3.** Milk sampling in rotary parlor

Research of tolerability and efficacy of the product was carried out on more than 200 cows and 100 goats in the intensive dairy production. The formulation was applied intramammary three times: before the morning milking, before the evening milking and then again prior to the morning milking.



**Fig. 4.** Propolis formulation application after sampling and milking

Milk samples were analysed microbiologically; composition of the milk and somatic cell count (SCC) were also analysed. Propolis solutions were tested at concentrations of 0.1%, 0.3%, 0.5%, 1%, 3% and 5%; it was shown that the optimal ratio of efficiency and security solutions had the 1 and 3% concentrations (Radin *et al.* 2016).

The project is aimed to benefit agricultural sector with direct improvements in dairy industry; milk producers that will use this intramammary formulation of propolis will avoid milk losses during production due to the mandatory withdrawal period, the prescribed waiting time from the last administration of antibiotics until putting the milk on the market. Although rich in biologically active substances that exhibit antibacterial and anti-inflammatory properties, withdrawal period for propolis is not prescribed. In addition, the repeated use of propolis does not create antimicrobial resistance, which significantly extends the length and number of its possible applications.

Furthermore, the milk producers in organic farming won't have to meet the strict standards for limited use of antibiotics which is why mastitis in organic livestock production causes even higher economic losses. This would significantly improve the competitiveness of milk producers in organic farming due to the added value of their products and a whole new market to position themselves in. The promotion of a product made 100% from Croatian compounds, and produced in Croatia can be a significant from a marketing standpoint of view.

The antibacterial, immunostimulatory and anti-inflammatory activity of this propolis formulation has the potential to gradually replace intramammary antibiotics, which can lead to a reduction in overall use of antibiotics in livestock production. One of the benefits might also be a reduction of wide spread antimicrobial resistance, the global problems that threatens health and lives of people and animals. So this project has a direct positive impact on the environment and health of the population in general.

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