SUMMARY

of the PhD thesis entitled:

IDENTIFICATION OF PHYTOTHERAPEUTIC PRINCIPLES AS AN ALTERNATIVE TO ANTIBIOTIC THERAPY IN BOVINE PATHOLOGY

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As antibiotic resistance to micro-organisms has become a real problem, the medical world is forced to find alternative solutions to reduce the extent of this phenomenon. Another reason for the need to find alternatives to traditional antibiotics and antiparasitics is organic farming, a phenomenon that has been gaining momentum recently.

The objectives of the present work the following:

Objective 1: To study the prevalence of mastitis on a cow farm and the economic impact of mastitis over a period of 8 months.

Objective 2: To identify the medicinal plants most commonly used by livestock farmers in South-East Romania in the treatment of different bovine diseases.

Objective 3: To evaluate the efficacy, both *in vitro* and *in vivo*, of some phytotherapeutic preparations against some bacterial agents incriminated in the etiology of mastitis in dairy cows.

The PhD thesis is structured according to the rules in two sections: bibliographical study and personal research. The first section of the thesis deals with phytotherapy issues, common diseases encountered in dairy farms and their economic impact at farm level, antibiotic resistance and unconventional approaches that can be used as an alternative to antibiotic therapy, especially for mastitis.

The second section of the thesis includes the results of personal research. These include investigations carried out on a cattle farm to determine the direct economic impact of clinical mastitis, field investigations to identify herbs used in traditional medicine to treat cattle ailments, followed by *in vitro* and *in vivo* studies to determine the efficacy of essential oils of thyme (*Thymus serpyllum*), clove (*Eugenia carryophyllus*), peppermint (*Mentha piperita*) and cinnamon (*Cinamumum verum*) in treating mastitis.

Research to determine the prevalence of clinical mastitis in dairy cows and its economic impact was carried out over a period of 8 months on a farm in southeastern Romania, with a herd of 520 head of dairy cows, Holstain-Friesian breed, in the period 2015-2016. In 100% of cases antibiotic therapy was used to treat mastitis, the most commonly used antimicrobials were: cephalexin, enrofloxacin, oxytetracycline and marbofloxacin. Analysing the costs on the farm studied, for the 8 months were 47 €/head of cow fed, the cost exclusively due to the cost of drug therapy and milk losses during the treatment period. If the indirect losses are added up, the costs far exceed the reports in the literature.

In order to identify the medicinal herbs that farmers in the Subcarpathian Curvature region use to treat animals, research was carried out in 25 villages in 8 communes (Bisoca, Gura Teghii, Mânzălești, Lopătari, Bătrâni, Poseşti, Jitia and Vintileasca) in the counties of Buzău, Prahova and Vrancea. 237 interviews were recorded and the following ailments were mentioned in the questions: respiratory diseases, ectoparasites, endoparasites, acute meteorism, mastitis, superficial breast sores, mammary papillomatosis, pododermatitis and diarrhoea. Most frequently, factual information was obtained from people over 60 years of age (most of whom were livestock farmers who practised or had practised subsistence farming). For people under 40 years of age, the presence of a veterinarian is required for most animal diseases, with diarrhoea being the only condition sometimes treated with phytotherapy.

Information was obtained on 135 remedies of plant, mineral or animal origin, used in the treatment of 15 ruminant diseases, and 56 plants from 34 families used in local traditional veterinary medicine were

identified. These remedies are often combined with each other, traditional alcohol (brandy), sunflower oil or vinegar are added and various empirical recipes are developed.

The plants with the highest frequency of occurrences throughout the surveyed area were: *Robinia pseudoacacia* used to treat diarrhoea, indigestion or for its anthelmintic effect, with 115 mentions (48%), followed by *Artemisia absinthium* with 114 mentions, used in most of the conditions researched (diarrhoea, indigestion, skin sores and foot disorders, as an internal and external antiparasitic) and *Sempervivum tectorium* mentioned 102 times, used to treat mastitis and tympanitis. Thirteen plants were identified for the treatment of mastitis, of which four plants were found in all areas surveyed. Latrhrea squamaria was most appreciated and praised by small farmers, attributed with miraculous powers. People go to the mountains in the spring to harvest it, and it is used exclusively to treat mastitis. It is administered raw or dried, 2-3 rhizomes for 3-4 days. There is no data in the literature confirming the efficacy of the plant, but we cannot doubt its activity, even if there is no scientific support. *Hylotelephium spectabile* and *Hylotelephium telephium* were two other identified plants that people frequently used to treat mastitis.

Research on the *in vitro* efficacy of essential oils on the bacterial flora involved in mastitis in cattle has involved several steps. Initially bacterial strains were isolated, identified and preserved from 16 milk samples from dairy cows with clinical mastitis based on cultural, morphological and biochemical characters using Api 20 NE, Api 20 E, Api 20 Strep and ID 32 Staph galleries. Ten bacterial strains (*Escherichia coli*- 2 strains, *Lactococcus lactis ssp. cremoris, Listeria spp., Pasteurella multocida, Staphylococcus xylosus, Staphylococcus spp., Staphylococcus intermedius, Staphylococcus epidermitis, Staphylococcus aureus*) were selected for which antibiograms were performed by disk diffusion method. The results reflected the upward trend in antibiotic resistance, with 90% of strains showing multiple resistance to the antibiotics tested, 60% were resistant to at least 5 antibiotics and 30% to 7 or more antibiotics. For the same strains, aromatograms were also performed for the essential oils of *Cinnamomum zeylanicum*, *Mentha piperita* Franco-Mitcham, *Syzygium aromaticum*, *Thymus serpillum*, pure, in different concentrations or in mixtures. The antibacterial efficacy of coconut and grapeseed carrier oils was tested, and showed no antibacterial activity against the strains studied. Peppermint essential oil was the only oil to which resistance was recorded from three bacterial strains (*Escherichia coli, Staphylococcus xylosus, Staphylococcus epidermitis*).

All 10 bacterial isolates tested were found to be sensitive to the action of cinnamon essential oil up to a dilution of 1/10, which had the strongest antibacterial effect of the oils tested. Clove oil showed antibacterial efficacy up to 1/10 dilution for *P. multocida*, up to 1/4 dilution for one strain of *E. coli, Lactococcus lactis, S. xylosus, S. spp.* and *S. intermedius* and up to 1/2 dilution for the second strain of *E. coli, Listeria. spp., S. epidermidis* and *S. aureus.* Thyme oil was effective up to 1/10 dilution for *Lactococcus lactis* and *Pasteurella multocida*, up to 1/4 dilution for the two strains of *E. coli, Listeria spp., Staphylococcus spp., Staphylococcus intermedius* and for *Staphylococcus aureus*, remaining effective up to 1/2 dilution for *Staphylococcus xylosus* and *Staphylococcus epidermidis*. The equal mixture of the four whole essential oils showed antibacterial effect on all 10 bacterial strains tested.

The next stage of the research consisted in the production of a pharmaceutical product based on the essential oils of thyme, clove, cinnamon and peppermint used in equal parts at a concentration of 0.5%, and coconut oil and grape seed oil were used as carrier oils. After testing this product for physical and microbiological characteristics, dermatological testing was performed on the skin surface of the mammary gland in 2 dairy cows. Topical application of the product did not result in changes in the general condition of the animal or local changes at the site of administration.

After this stage, *in vivo* testing was performed. Sixty-four lactating cows from traditional farms in Bisoca commune, Buzău county, were identified for which subclinical mastitis was determined using the California Mastitis Test (CMT). 26 positive animals were identified and subsequently confirmed in the laboratory by somatic cell count determination using the Soma Count 150. According to European regulations concerning specific hygiene rules for food of animal origin, the number of somatic cells per millilitre of milk must not exceed 400,000 cells and the total bacterial count, measured at 30°C, must be less than 100,000. High somatic cell count in milk is a major indicator of chronic or subclinical mastitis. Animals with CMT score 3 were excluded from the test (3 individuals) and a classical treatment protocol was recommended, the other animals were lotted, thus the control group consisted of 9 animals and the other 14 animals were the working group. Two holdings were identified where all CMT results were negative, this can also be associated with a very good hygiene of the shelter, the animals were half-breeds of Maramures Brown,

the milk production of the animals was 13-14l/animal/day, and the feed was supplemented with alfalfa hay and concentrates. In contrast, 6 holdings were identified where the incidence of possible subclinical mastitis was greater than or equal to 50%. Of the total animals tested, 40.62% were diagnosed positive for CMT. In these cattle, the product produced was administered by gently massaging the entire surface of the mammary gland, including the nipples, twice a day for 5 days.

Somatic cell count (SCC) and total bacterial count (TBC) were determined on day 5, day 14 and day 21 after the start of treatment for the mammary quarters in which the SCC value exceeded 200,000 cells/ml at baseline. On day 14 there was a reduction of NTG by 52.38% from baseline in the treatment group, whereas in the control group no significant changes in this parameter were observed. Compared to the control group, all NCS values showed a decrease for the group to which the test product was applied.

Even if the results were satisfactory and the product could be used in the treatment of subclinical mastitis, further studies are needed on the persistence of essential oils in tissues and milk in order not to distort the quality and taste of milk and also not to influence the manufacturing process of dairy products and their derivatives.