

PHD PROJECT DESCRIPTION

(4000 characters max., including the aims and work plan to be published online)

Project title: Development of semi-solid formulations containing d-metal nanoparticles for topical and intramammary veterinary applications in goats

1.1. Project goals

The aim of the project is to develop and characterize semi-solid formulations containing nanoparticles of selected d-block metals, mainly silver, zinc and copper, supported by additional bioactive compounds, for topical and intramammary veterinary applications in goats. The formulations may include plant extracts, probiotic bacterial hydrolysates, bioactive proteins or peptides to enhance antimicrobial, soothing, regenerative and protective effects. The preparations will be designed as creams, ointments or gels intended for udder hygiene, protection of teat and udder skin, and supportive management of inflammation, cracks, irritation, impaired tissue regeneration and microbial infections. The project will include optimization of formulation composition, physicochemical characterization, microbiological testing and biological evaluation of safety and application potential. The expected outcome is the development of stable, effective and safe preparations that may support prevention and management of udder-related disorders, including mastitis-associated problems.

1.2. Outline

Mastitis and udder-related disorders are among the most important health problems in dairy animals, leading to reduced milk quality, animal discomfort, economic losses and frequent use of antibiotics. In goats, inflammation of the mammary gland, microbial colonization of teat canals, cracks of teat and udder skin, irritation and impaired tissue repair may increase the risk of infection and complicate animal care. Therefore, there is a need for new non-antibiotic or antibiotic-supporting preparations that combine hygienic, antimicrobial, protective and regenerative properties.

Nanoparticles of d-block metals, especially silver, zinc and copper, are promising active components for veterinary formulations due to their broad antimicrobial activity, including activity against mastitis-associated pathogens and biofilm-forming bacteria. Silver and copper nanoparticles have been investigated as alternative antimicrobial agents in mastitis prevention and treatment, including pre- and post-milking disinfectants, while zinc-based nanoparticles may additionally support tissue repair and skin protection. However, their biological effect depends strongly on particle stability, concentration, release, interactions with formulation components and safety toward animal tissues. Recent reviews emphasize both the potential of metal nanoparticles in mastitis management and the need to carefully evaluate their toxicity, stability and environmental safety before practical use.

The project will focus on the rational design of semi-solid formulations containing selected metal nanoparticles. Creams, ointments and gels may serve different purposes: protective topical preparations for udder and teat skin, hygienic formulations applied before or after milking, or intramammary-supportive preparations aimed at limiting microbial growth and supporting tissue recovery. A key scientific aspect will be to determine how the formulation matrix affects

nanoparticle stability, dispersion, release, antimicrobial activity and biocompatibility. The final formulation should not only act as a carrier, but also support the intended effect by maintaining nanoparticle activity, improving contact with the target tissue and reducing irritation.

The developed formulations will be evaluated using physicochemical, microbiological and biological methods. Particular emphasis will be placed on antibacterial and anti-biofilm activity against microorganisms relevant to udder infections, as well as on safety toward mammalian cells. Selected preparations may be further evaluated in application-oriented models related to teat skin protection, wound healing or intramammary use. The project will provide a basis for the development of new veterinary preparations supporting udder hygiene and care in goats.

1.3. Work plan

The project will start with the development of semi-solid veterinary formulations containing silver, zinc and copper nanoparticles, alone or combined with bioactive compounds such as plant extracts, probiotic bacterial hydrolysates, proteins or peptides. The preparations will be designed as creams, ointments or gels for topical udder care or intramammary-supportive use.

The formulations will be optimized and characterized for stability, pH, viscosity, rheology, texture, homogeneity, nanoparticle distribution, metal content, biological compounds content, antioxidant activity, etc. using selected microscopic, spectroscopic, spectrometric and instrumental techniques, including separation techniques such as GC and HPLC.

Microbiological studies will assess antibacterial and anti-biofilm activity against mastitis-associated microorganisms, including *Staphylococcus*, *Streptococcus* and *E. coli*, using viability assays, colony counts and fluorescence microscopy.

Biological evaluation will include standard cytotoxicity testing using L929 fibroblasts, followed by studies on more application-relevant models, including goat mammary epithelial cells and goat keratinocytes. Cytotoxicity, membrane integrity, morphology and regenerative potential will be assessed. The most promising formulations may be further tested in ex vivo or in vivo models related to teat skin protection, wound healing, udder hygiene or intramammary safety, after ethical approval.

1.4. Literature (max. 7 listed as a suggestion for a PhD candidate preliminary study)

1. Kalińska A., et al. *Silver and Copper Nanoparticles as the New Biocidal Agents Used in Pre- and Post-Milking Disinfectants with the Addition of Cosmetic Substrates in Dairy Cows*. International Journal of Molecular Sciences, 24(2), 1658, 2023. DOI:10.3390/ijms24021658.
2. Railean-Plugaru V., *Use of Lactobacillus paracasei isolated from whey for silver nanocomposite synthesis: Antiradical and antimicrobial properties against selected pathogens*. Journal of Dairy science, 104(3), 2480-2498, 2021. DOI: 10.3168/jds.2020-19049
3. Radzikowski D., et al. *In Vitro Evaluation of the Antimicrobial Properties of Nanoparticles as New Agents Used in Teat Sealants for Mastitis Prevention in Dry Cows*. Biomedicines, 11(8), 2291, 2023. DOI:10.3390/biomedicines11082291
4. Rodzik, A. et al. *Enhancing wound healing with zinc and silver nanocomposites synthesized with β -lactoglobulin: antimicrobial properties, collagen deposition, and systemic effects in a C57BL/6J mouse model*. Discover Nano 2024, 19(1), 150, doi: 10.1186/s11671-024-04091-9

1.5. Required initial knowledge and skills of the PhD candidate

The PhD candidate should be creative, scientifically curious and able to work independently and in a team. Basic knowledge of microbiology, veterinary hygiene, cell biology and analytical chemistry is expected. Experience in cultivation and identification of microorganisms, antibacterial testing and biofilm assays will be particularly valuable. The candidate should also be familiar with preparation and characterization of semi-solid formulations, as well as instrumental techniques such as UV-Vis spectroscopy, microscopy, ICP-OES or related methods for metal analysis. Basic experience in mammalian cell culture, cytotoxicity testing, data processing and English sufficient for scientific literature is required.

1.6. Expected development of the PhD candidate's knowledge and skills

During the PhD project, the candidate will gain interdisciplinary knowledge and practical skills in the development of veterinary preparations for udder hygiene, skin protection, wound healing and intramammary-supportive applications in goats. The candidate will develop competences in microbiology, analytical chemistry, veterinary formulation design and biological evaluation of metal nanoparticle-based systems. Skills in formulation design, physicochemical characterization, antibacterial and anti-biofilm testing, cytotoxicity assessment and data analysis will be developed. The results will be presented in scientific publications and at domestic and international conferences, and may contribute to patent-protected technological solutions for veterinary use.