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PhD THESIS SUMMARY

Management of reproduction and the puerperal period in the cow

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INTRODUCTION

The most challenging period for dairy cows is certainly around parturition, particularly during the puerperal period, where metabolic changes are at their peak due to milk secretion and the reparative processes in the reproductive system. The multitude of pathologies that can be triggered immediately following parturition have been extensively discussed in the literature. However, no exact solution has been identified for each deviation from the physiological norm. Consequently, many presumptive hypotheses for ameliorating puerperal pathology remain to be explored through updated knowledge and the development of new protocols that could add significant value to the field.

The degree of impact of puerperal pathologies is represented by varying percentages due to the multitude of specific or non-specific factors originating from the genetic base of the animals, nutrition, and maintenance practices. Because of this, studying incidence is beneficial, as an overview can provide considerable value to practicing veterinarians and farmers by implementing prophylactic or therapeutic protocols for individuals or groups and correcting deficiencies related to farm management.

The increased energy demand of dairy cows during the first 60 days post-partum and the excessive mobilization of bodily resources lead to considerable metabolic imbalances through the development of ketosis, which subsequently disrupts reproductive physiology. This aspect necessitates monitoring ketone bodies in the bloodstream or through non-invasive methods, such as in milk, a practice that is gradually advancing in Romania. The benefits of determining ketone bodies are not only short-term, through the application of therapy, but also long-term, involving the genetic pool and enabling the selection of animals.

The necessity of adopting prophylactic protocols that are accessible from all perspectives is indispensable for dairy cows during the puerperal period. Currently, certain prophylactic techniques are employed, such as selecting animals based on the pathologies developed during their productive life, using feed recipes that closely match physiological needs, controlling infectious diseases through biosecurity measures, avoiding parturition for extended lactations, and various hormonal protocols. However, there is still no exact standardization of prophylaxis. Considering the impact of ketosis on reproductive function, it is likely that the primary underlying issue lies in metabolic disturbances.

The phenomenon of bacterial resistance to antibiotics and the increased demand for products free from drug residues or as natural as possible is a current issue. This necessitates the study of treatments that meet these requirements. Zeolite, in this context, is suitable due to its natural occurrence and the minimal processing required to activate its properties. Its current utility is vast, as it can act as a detoxifying agent, antioxidant, hemostatic, anti-diarrheal, growth stimulant, antiviral, antibacterial, and immunostimulant.

The present study aimed to develop prophylactic and therapeutic protocols to improve reproductive function. The steps undertaken were as follows: determining the incidence of puerperal pathologies, identifying bacterial species present in the uterus, measuring the level of beta-hydroxybutyrate in milk, analyzing the evolution of the puerperium, administering activated zeolite in cattle feed, determining the main indicators of uterine health, establishing the optimal dose of micronized zeolite for intrauterine administration, applying it intrauterinely in cattle, and monitoring its evolution, followed by its application in cases of puerperal pathologies. The research was conducted in four farms and at the university's biobase. After the treatments were applied, the groups were compared to establish protocols that offer the best results. Keywords: puerperal period, zeolite, beta-hydroxybutyrate.

THESIS STRUCTURE

The doctoral thesis entitled "Management of Reproduction and the Puerperal Period in Cows" contains 138 pages and is structured into two parts.

The first part represents the current state of knowledge, divided into 5 chapters, and spans 32 pages. This section covers general concepts about cattle farming at the global and national levels, a description of the cattle breeds used in the studies, information on the anatomy and physiology of the bovine reproductive system, a brief analysis of the main causes involved in puerperal pathology, and a detailed discussion of the most common pathologies. Additionally, it presents various strategies for preventing puerperal pathologies and a concise overview of the applications of natural zeolite in the medical field.

The second part contains 85 pages and represents the personal contribution. It is structured into 6 chapters, which include the working hypothesis and objectives, the specific characteristics of the natural/artificial environment where the experimentation took place, materials and methods, results and discussions, general conclusions and recommendations, with the final chapter being dedicated to originality and innovative contributions.

Each chapter includes data from the 5 studies, illustrating the specifics of each chapter. The studies are numbered from 1 to 5, with the following titles: Study 1 - Incidence of Puerperal Pathology in the Studied Farms and Identification of Pathogenic Bacterial Species; Study 2 - The Relationship Between the Clinical Evolution of the Puerperium and the Level of Ketone Bodies in Milk; Study 3 - The Effect of Oral Administration of Zeolite on Energy Metabolism and Reproductive Health in the Postpartum Period; Study 4 - The Effect of Intrauterine Administration of Zeolite on the Puerperal Period in Dairy Cows; Study 5 - Analysis of Therapeutic Protocols for Improving Puerperal Pathology.

WORK HYPOTHESIS/OBJECTIVES

The main objective of this work was to develop a protocol to improve reproductive function during the puerperal period, primarily through prophylactic means, to minimize economic losses during this period.

The secondary objectives of this work were:

- Monitoring cattle to diagnose puerperal pathologies;
- Determining ketone bodies in milk using the Milkoscan Foss device;
- Establishing the daily dose of zeolite for oral administration in cattle;
- Determining beta-hydroxybutyrate and glucose levels in bovine blood;
- Collecting uterine biopsies and vaginal secretions from cows in the puerperal period and analyzing them;
- Establishing the dose and impact of zeolite on uterine tissue – applied in laboratory rats;
- Intrauterine administration of zeolite in cattle and monitoring key parameters;
- Prophylactic administration of prostaglandin F₂ α in the early puerperal period;
- Diagnosing and treating puerperal pathologies using conventional methods;
- Statistical analysis of the obtained data and formulating general conclusions.

RESEARCH RESULTS

Study 1, titled "Incidence of Puerperal Pathology in the Studied Farms and Identification of Pathogenic Bacterial Species," aimed to determine the extent of puerperal pathologies across four farms and identify the bacterial species comprising the uterine microbiome.

To achieve this goal, several steps were undertaken: consulting the farm databases, monitoring parturition and the puerperal period through weekly farm visits, and comparing the collected data. Regarding the uterine microbial flora, it was necessary to collect uteri from slaughtered animals, followed by the collection of uterine secretions, laboratory processing, and interpretation of results.

The incidence rates of each pathology showed a consistent trend across all farms. For primiparous cows, there was a minimal increase in incidence, while multiparous cows exhibited variations that did not significantly indicate that parity influences puerperal incidence. The overall percentage for each pathology among the studied cattle was as follows: dystocia - 2.02%; retained placenta - 8.54%; hypocalcemia - 4.66%; puerperal metritis - 12.27%; clinical endometritis - 15.84%; and pyometra - 0.93%.

Based on the bacterial population research of the uterus from the 24 samples, a higher prevalence of Gram-positive bacteria was observed, accounting for 63.63%. The identified bacteria were not pathogenic, with many being commensal organisms of the genital tract. Their pathogenicity is exerted only under conditions of local immune suppression and systemic defense breakdown of the host organism.

The major species identified through biochemical testing were *Micrococcus* spp., *Brevundimonas vesicularis*, *Shewanella putrefaciens* spp., *Aeromonas hydrophila*, and *Bacillus* spp. Enrofloxacin, a broad-spectrum antibiotic effective against Gram-negative and Gram-positive bacteria, as well as numerous commensal bacterial species of the bovine uterus, exhibited the most extensive action in the antibiogram readings.

Study 2, titled "The Relationship Between the Clinical Evolution of the Puerperium and the Level of Ketone Bodies in Milk," includes research on the levels of beta-hydroxybutyrate (BHB) and the protein-fat ratio in milk, relative to key reproductive indicators.

Data from 176 cows, collected between March 2019 and July 2020, were used. These cows were in the 4-80 days postpartum period and came from four different farms that varied in terms of management, feeding, and breed. Based on clinical data, the cows were divided into two groups: the control group, which had reproductive system pathologies, and the reference group, which were clinically healthy without reproductive system pathologies. The collected data were statistically analyzed along with the biochemical parameters provided by the laboratory.

BHB levels, service period, and the protein/fat ratio in milk were statistically analyzed in relation to season, breed, and the farm of origin. No significant correlation was found between these variables, suggesting that certain reproductive pathologies during the puerperal period and the diagnosis of ketosis are not influenced by factors independent of the individual.

According to the literature, the animals in the study were classified based on the clinical evolution of ketosis, relative to the concentration of beta-hydroxybutyrate. The results were as follows: for the reference group, 86.2% were negative, 3.44% were suspect, and 10.34% were positive. For the control group, 55.68% were negative, 9.10% were suspect, and 35.22% were positive.

The average number of inseminations required for a new pregnancy was 1.9 for the control group and 1.36 for the reference group, indicating a statistically significant difference. The significance of the relationship between BHB levels and the number of inseminations was 0.72, suggesting a non-significant correlation; however, the mathematical difference was not large enough to be statistically considered, necessitating further research in the future.

The mean values of BHB, service period, and the protein/fat ratio reported for the two groups—control and reference—showed statistical significance. The control group had higher mean values for these three variables compared to the reference group, indicating that ketosis influences the puerperium in cows.

Study 3, titled "The Effect of Oral Administration of Zeolite on Energy Metabolism and Reproductive Health in the Postpartum Period," aimed to mitigate ketosis and consequently puerperal pathologies in dairy cows during the puerperium.

The puerperal period is the most critical phase in the life of dairy cows, as it involves the intersection of accelerated energy metabolism needs with the reproductive system's recovery functions. This study involved 82 pregnant cows, divided into two groups: control and reference. The control group received 400 g of zeolite daily, starting from 14 ± 3 days before parturition until 28 ± 3 days postpartum. The cows were monitored for parturition progress, and beta-hydroxybutyrate (BHB) and glucose levels were measured weekly from the start of administration. At 21 ± 2 days postpartum, vaginal secretions were collected to determine the total germ count and Enterobacteriaceae. The service period was determined, and the number of inseminations required for a new pregnancy was recorded.

The data collected were correlated for all the cows in the study, showing a significant correlation between the monitored parameters. To assess the impact of additional zeolite administration, the statistical relationship of the parameters for the two study groups was analyzed. The control group achieved pregnancy on average 12.78 days earlier, and the average number of doses used for a future pregnancy was 0.44 less than the reference group. The number of pathogens present in the uterus was lower in the control group compared to the reference group, and the uterine score was more favorable for the control group ($p < 0.05$).

Regarding metabolic parameters (BHB and glucose), no significant differences were observed during the antepartum period or on the day of parturition. However, postpartum data indicated significant differences in favor of the animals that received additional zeolite. Despite the uterine score being in favor of the group that received additional zeolite, no statistical significance was established.

Therefore, based on the results, clinoptilolite zeolite can be considered a prophylactic method for addressing the main pathologies developed during the puerperal period.

Study 4, titled "The Effect of Intrauterine Administration of Zeolite on the Puerperal Period in Dairy Cows," aimed to demonstrate that intrauterine administration of zeolite can improve indicators related to the development of puerperal pathologies. This is based on the extensive use of micronized zeolite in medicine, where it has various properties, including antibacterial effects.

To determine the appropriate dose and potential toxic effects on the uterine mucosa, a preliminary study was conducted on rats. Different doses were administered on the second day postpartum, and ultrasound measurements of the uterus, as well as bacteriological and uterine tissue samples, were collected at 7, 14, and 21 days. The results were satisfactory regarding the effects on uterine tissue, with the 20% dose yielding the best results for the monitored parameters.

Following the preliminary study, cows were grouped into two groups (n=32), divided equally. Intrauterine administration of zeolite was performed on the second day postpartum, and the stage of uterine involution was monitored on days 7, 14, and 28 of the puerperium. On day 14, samples were collected to determine the number of germs, and uterine tissue was sampled. The data indicated that the group receiving intrauterine micronized zeolite (n=16) had an average of 15,890.38 CFU, while the control group had an average of 17,661.53 CFU ($p < 0.05$), showing a significant decrease in bacterial population for the treated group.

Throughout the study period, uterine involution was more accelerated in the group receiving intrauterine micronized zeolite. Although the results were satisfactory in terms of the average score obtained, statistical significance was observed only for days 21 and 28 of monitoring ($p < 0.05$). The percentage difference between the two groups increased over time: 4.29% on day 7, 6.09% on day 14, 16.31% on day 21, and 25% on day 28.

Histologically, the uterine score proposed by Chapwanya et al. (2009) showed an average score of 0.4231 for the treated group (n=26) and 0.7308 for the control group (n=26) ($p < 0.05$). The lesions observed included mild hyperplasia of the uterine glands, diffuse mild infiltration of the superficial lamina propria with mononuclear cells, and multifocal hyaline degeneration of the uterine arteries.

Following the puerperium, it is necessary to conceive a new calf to ensure the economic viability of dairy cows. Therefore, the finalization of the research occurred when subsequent pregnancy was diagnosed. The average service period recorded was 59.73 days for the treated group (n=26) and 65.03 days for the control group (n=26) ($p < 0.05$).

The number of inseminations required for a new pregnancy ranged from 1 to 3, with an average of 1.23 for the treated group and 1.46 for the control group. Statistically, there was no significant difference in the number of inseminations used.

Study 5, titled "Analysis of Therapeutic Protocols for the Improvement of Puerperal Pathology," aimed to evaluate multiple therapeutic protocols to determine their efficacy.

Among dairy cows with retained placenta, the lowest incidence of subsequent pathologies was recorded in animals treated with povidone-iodine. Regarding reproductive indicators, the treatment with povidone-iodine yielded the best results: the number of days until the first estrous cycle was the shortest, as were the service period and the number of insemination doses used.

Two cows from each differently treated group, diagnosed with puerperal metritis, required a second treatment. The best results were observed in the group treated with povidone-iodine combined with PGF 2α , likely due to the potentiating effect of these treatments.

In cases of clinical endometritis, similar to puerperal metritis, the potentiating effect of combining an intrauterine treatment with PGF 2α administration proved to be the most beneficial.

The intrauterine administration of micronized natural zeolite showed satisfactory results compared to widely used conventional treatments. The data suggest that micronized zeolite is not inferior to other treatments; rather, it stands on equal footing with them.

The administration of PGF2 α during the early puerperium resulted in an improvement in reproductive parameters, leading to the first estrous cycle 6.28 days earlier, a service period shortened by 13.20 days, and 0.27 fewer doses of semen needed for successful conception.

CONCLUSIONS

1.The percentage of animals diagnosed with at least one pathology at each farm was as follows: Farm A - 31.02%; Farm B - 43.83%; Farm C - 37.20%; Farm D - 26.81%.

2.The average incidence of pathologies among all animals included in the study was as follows: dystocia - 2.02%; retained placenta - 8.54%; hypocalcemia - 4.66%; puerperal metritis - 12.27%; clinical endometritis - 15.84%; and pyometra - 0.93%.

3.Gram-positive bacteria comprised the largest proportion (63.63%) of all uterine samples studied.

4.The predominant species identified through biochemical testing were *Micrococcus* spp., *Brevundimonas vesicularis*, *Shewanella putrefaciens* spp., *Aeromonas hydrophila*, and *Bacillus* spp.

5.Enrofloxacin exhibited the broadest spectrum of action in the antibiograms.

6.The development of ketosis cannot be attributed to factors such as breed, farm of origin, or season.

7.Elevated levels of beta-hydroxybutyrate and an increased protein-fat ratio are associated with a higher number of artificial inseminations and a longer service period.

8.Regarding parameters monitored following oral administration of zeolite, it was determined that there are correlations among these parameters.

9.A pregnancy was achieved on average 12.78 days earlier in animals that received supplemental zeolite, and the average number of doses used for a future pregnancy was 0.44 fewer compared to the control group.

10.The number of pathogenic agents present in the uterus was lower in cows that received supplemental zeolite compared to the control group, with a favorable average uterine score ($p < 0.05$).

11.Metabolic parameters (BHB and glucose) did not show significant differences in the antepartum period or on the day of parturition, but postpartum data indicated significant differences in favor of animals that received supplemental zeolite.

12. Following intrauterine administration of zeolite in female *Rattus Norvegicus*, it was observed that uterine involution, bacterial population, and uterine scores were most favorable at a 20% concentration.

13. Intrauterine administration of micronized zeolite in bovines resulted in a lower number of CFUs and an improved uterine score compared to the control group.

14. Regarding uterine involution in animals treated with intrauterine micronized zeolite, no significant difference was found between groups in the early puerperium, but in the later part, involution was more pronounced and statistically significant.

15. The average service period and the number of semen doses for future pregnancy were 5.3 days and 0.23 doses of semen fewer, respectively, for the control group.

16. Treatment of retained placenta showed the best reproductive results following the administration of povidone-iodine.

17. In cases of puerperal metritis and clinical endometritis, the most successful results were obtained with intrauterine treatment supplemented with intramuscular PGF 2α .

18. Intrauterine administration of micronized zeolite in cases of puerperal diagnosis yielded results similar to conventional treatments.

19. Administration of prostaglandin F 2α in the early puerperium has positive effects on the service period and repeat breeding syndrome in dairy cows.

THE ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE TESIS

The studies conducted within this thesis introduce a novel element of originality by examining the relationship between ketone bodies in milk and the evolution of the puerperal period. The results obtained in this context form the basis for future research by identifying predisposing factors for puerperal disturbances.

Another original element is the oral administration of activated zeolite for the prophylaxis of ketosis by balancing ruminal pH, which has yielded significant results on uterine health and the improvement of reproductive indicators. This innovative approach to enhancing reproduction, to the best of our knowledge, has not been implemented or described in the existing literature.

A major contribution to the originality of the thesis is the implementation of a protocol for the intrauterine administration of micronized zeolite. This was achieved through laboratory application of various doses to rats, leading to conclusions that facilitated the extrapolation of the study to bovines. A groundbreaking aspect of this study was the intrauterine inoculation of micronized zeolite to improve reproductive indicators, followed by its application to various pathologies, providing a new perspective on treatments for dairy cows facing postpartum difficulties. Thus, this work represents a first step towards alternative treatments, as there is currently no natural product available on the market that can be safely applied in this context.