SUMMARY

RESEARCH ON THE USE OF HYPERIMMUNE PLASMA IN THE TREATMENT OF SPECIFIC PATHOLOGIES AND IMMUNOLOGICAL DEFICIENCIES IN CALVES

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KEYWORDS: colostrum, hyperimmune plasma, immunodeficiency, neonatal diarrhea, hyperimmunization

The doctoral thesis titled "**Research on the Use of Hyperimmune Plasma in the Treatment of Specific Pathologies and Immunological Deficiencies in Calves**" is written and structured according to the regulations in force at the time of its writing, into two main parts: the first part, represented by the "Bibliographic Study," which consists of 47 pages, and the second part, titled "Own Research," with a total of 91 pages. The doctoral thesis has 151 pages and is illustrated with 14 tables and 35 figures. The information consulted from the specialized literature in the first part of this work served as support for the interpretation of the results obtained during the study.

The first part called the "**Bibliographic Study**," is composed of 3 extensive chapters in which the immunological status of the mother and fetus is concisely described, both in the early stages of gestation and throughout its entire course, the immunological interaction between them, and the impact of maternal vaccination on the calf's immunity. Also, in the bibliographic study section of this thesis, notions about the immunological status of the newborn calf and the necessity of consuming quality colostrum to modulate the development of its immune system are highlighted. Furthermore, information on the evolution of neonatal pathologies in calves, with tropism for the respiratory or digestive system, and ways to correct the precarious immune status of newborns of this species is synthesized.

The second part, represented by "**Own Research**," is concentrated into 8 chapters in which the results of the research conducted during the doctoral stage are centralized, with the last chapter containing the conclusions and final recommendations of the conducted research. The chapters in this section are divided into subchapters that contain information about the experimental groups, the materials and methods used, the description and interpretation of the results obtained, and the partial conclusions drawn from each study conducted.

Chapter IV describes the "**Purpose and Objectives of the Research**," which are centered around the goal of providing an alternative therapeutic solution for neonatal pathologies in calves and the desire to obtain hyperimmune blood products to enhance the precarious immune status of newborn calves. To achieve this goal, a series of objectives were set, which involved obtaining hyperimmune plasma by hyperimmunizing cows free of infectious diseases and administering this plasma therapeutically to calves with diarrheal syndrome.

Chapter V focuses on the "**Evaluation of Colostrum Quality in Cows**" and involves quantifying the titer of colostral antibodies to determine the quality of colostrum from the farms under study. The study was

conducted on a sample of 20 dairy cows from two different breeds, Holstein (n=10) and Romanian Black Spotted (n=10), including both primiparous and multiparous cows. The cows belonged to farms in Ilfov County, located in southeastern Romania.

Colostrum is the lacteal secretion of females in the first days postpartum, rich in immunoglobulins and other constituents with immunomodulatory roles, and its quality is dictated by the concentration of immunoglobulins it contains. In this chapter, the concentration of gamma globulins was quantified using the spectrophotometric method, yielding very good results regarding the quality of the colostrum studied. Of the samples tested (n=20), 19 had high concentrations of these molecules, indicating good quality colostrum, with only one female recording colostrum immunoglobulin values below the acceptable limits.

Chapter VI describes the "**Evaluation of Cattle for Detecting Infectious and Contagious Diseases and Parasitic Infections by PCR Method**" and provides information on the epidemiological status of the females on the farm. Identifying carrier animals and eliminating them from the experimental group is important due to the influence these animals have on the study. Infected animals, whether latent or showing clinical signs, negatively affect post-vaccination antibody titers or influence the final study results due to post-infection reactions. PCR tests were chosen over conventional methods to obtain more conclusive results and because they minimize processing time by allowing work on larger samples simultaneously. The results presented in this chapter led to the exclusion of a single female from the study group, which tested positive for bovine herpesvirus type 1. After excluding the carrier bovine, the study continued with a sample of 19 females.

Chapter VII, titled "**Hyperimmunization of Cows in the Last Part of Gestation**," describes the selection stages of animals subjected to repeated immunization and the protocol used in this work to hyperimmunize pregnant animals. Healthy females (n=19) were selected for this purpose after being subjected to screening tests and clinical control, and vaccinated against the most common pathologies. The vaccine used is Bovilis Rotavec Corona, a polyvalent vaccine containing inactivated bovine Rotavirus strain UK-Compton, serotype G6 P5, inactivated bovine Coronavirus strain Mebus, and an agglutinated strain of E. coli F5 (K99), according to the manufacturer's prospectus. It contains mineral oil and aluminum hydroxide as adjuvants. The instituted protocol involves vaccinating the mothers with three doses of the vaccine at two-week intervals, starting around gestation days 220-235. The triple vaccination aimed to increase the antibody titers against the most common pathogens involved in neonatal calf diarrhea. The quantification results indicate successful hyperimmunization of the cows.

Chapter VIII, titled "**Evaluation of the Serum Immunoglobulin Levels of Hyperimmunized Cows**," aims to quantify the amount of serum immunoglobulins in cows subjected to the hyperimmunization protocol. In this stage, it was necessary to collect blood samples from the 19 females included in the study and process them using Bovine IgG ELISA kits. The 65% increase in antibody titers in the serum of cows immunized in the last part of gestation, compared to the amount of immunoglobulins present in the serum before the first vaccination, confirms that immunizing females during gestation has positive effects on antibody titers.

Chapter IX describes the framework and stages of "**Obtaining Hyperimmune Blood Products and By-products in Cows**". The blood products obtained consist of hyperimmune plasma and erythrocyte concentrate, separated from blood units collected from hyperimmunized cows. In the first stage, the blood units collected from disease-free cows were subjected to centrifugation, followed by the separation of the two products facilitated by a semi-automatic plasma expressor, ultimately resulting in the segregation of plasma concentrate and erythrocyte concentrate. Each blood unit weighed between 480-650 grams, yielding hyperimmune plasma in the proportion of 63-64%, with the remainder being erythrocyte concentrate. Two blood units were obtained from each female.

The recommendation of hyperimmune plasma in neonatal pathologies supports the establishment of therapy in species whose placenta does not allow the transfer of antibodies from mother to fetus or when, in the absence of quality colostrum consumption, immune defense is deficient. The applicability of this therapy as an alternative to antibiotics is of increasing interest, given the alarming rate of bacterial resistance emergence

against antimicrobial substances. Additionally, administering hyperimmune plasma to calves with low post-colostral antibody titers enhances the body's defense against environmental pathogens.

The final study chapter, **"Evaluation of the Efficacy of Hyperimmune Blood By-products Used in Calves with Digestive Disorders**," is the most extensive part of this thesis. Neonatal digestive pathologies in calves are a major cause of economic losses in the bovine sector due to the high rates of morbidity and mortality in the first weeks postpartum. Neonatal diarrhea occurring within the first 2-4 days of a calf's life is often caused by E. coli (K99), while between days 5 and 21, rotavirus, coronavirus, and Cryptosporidium spp. also contribute to its occurrence.

First-line therapy for treating diarrheal syndrome in calves focuses on antibiotics and fluid therapy. The increasing rate of antimicrobial resistance drives research toward finding alternative solutions to these medications, which is the goal behind this study.

Before the intravenous administration of hyperimmune plasma, fecal samples were collected from the calves to confirm the diagnosis of diarrhea of infectious or parasitic origin. The etiological diagnosis was made using ELISA kits, and all calves in the research group tested positive for at least one targeted pathogen. After selecting the calves for the study group, plasma was transfused intravenously through the jugular vein, with each animal being monitored for allergic reactions.

To highlight the effects of hyperimmune plasma administration, two groups of calves were formed: a control group treated with antibiotics (n=10) and an experimental group (n=20), with the latter undergoing hyperimmune plasma transfusion. Blood samples for antibody titer quantification were collected before any form of therapy, 1 hour and 24 hours after plasma transfusion, and 48 and 72 hours after antibiotic administration.

The study results demonstrated the efficacy of hyperimmune plasma transfusion. The serum antibody titer in calves receiving this hyperimmune product was double that of the control group calves treated with antibiotics. Additionally, the increase in antibody concentration occurred rapidly; within just one hour of hyperimmune product administration, the circulating antibody concentration was 9.5 times higher than before administration. Clinically, the disease duration was shorter in the plasma-treated group compared to the antibiotic-treated group. Furthermore, the survival rate was higher in the experimental group, at 98%, compared to only 50% in the control group, with half of the control group calves not surviving the diarrheal disease.

In Chapter XI, a series of **conclusions and recommendations** were drawn from the research conducted in this doctoral thesis. Additionally, the thesis includes results published in articles indexed in international databases.

In the **bibliography references**, a total of 287 titles from international and national literature are included, 38 of which were published before the year 2000, 50 between the years 2000-2010, and the rest of the research conducted from 2010 onwards until the time of writing the thesis. Of the total cited titles, 20 are specialized books and 267 are articles from prestigious journals.