Biological effects evaluation of transfusion therapy and determination of necessary blood for transfusion in Holstein calves anemia

SUMMARY OF THE PHD THESYS

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INTRODUCTION

Blood transfusion is a therapeutic act that involves the administration of whole blood or blood components to an animal. The first reports of this procedure date back to the 17th century, but the development of blood transfusion as a therapy in veterinary medicine began after 1960.

The main difficulties in developing as a therapeutic branch in veterinary medicine were the absence of organized structures to provide blood products, blood banks and high costs involved, despite the fact that transfusion therapy in veterinary medicine has the same indications as in the case of human medicine. Initially, blood transfusion was used for pets, only in recent years it has been used in field practice in cattle medicine. The major indications for blood transfusion are anemia (the most common being hemorrhagic), restoration of hemostasis function (especially in the case of rodenticide poisoning), restoration of plasmatic protein level and oncotic pressure through the supplied plasma macromolecules, neonatal diarrhea of calves and restoration of plasma antibody titer in calves in which passive colostrum transfer was inadequate.

There are currently very few studies that analyze in detail the biological effects of whole blood transfusion in cattle, both in terms of hematology and biochemistry and in terms of the impact on markers of plasma oxidative stress. Also, in bovine medicine there is no calculation formula for determining the need for transfused blood that can be used in field conditions, using body mass, patient hematocrit and donor hematocrit, as is the case of domestic carnivores.

THESIS STRUCTURE

The paper entitled “Biological effects evaluation of transfusion therapy and determination of necessary blood for transfusion in Holstein calves anemia" contains 115 pages and is structured in two parts.

The first part is structured in 6 chapters and presents the current state of knowledge. In this part we have summarized the main elements in the field of whole blood transfusion in cattle. Chapter 1 presents the physiology of erythropoiesis, with a brief description of the main stages and stages of hemato and erythropoiesis. Chapter 2 contains the most common erythrocyte disorders which are the main indications for blood transfusions. This chapter describes anemia (regenerative and non-regenerative), along with coagulation disorders in cattle and the role of blood transfusion in restoring the plasmatic protein level. Chapter 3 describes the main issues related to bovine blood groups and compatibility tests, and Chapter 4 presents the elements underlying the donor's choice and issues related to the technique of administering the whole blood transfusion. Chapter 5 presents the most common post-transfusion reactions and Chapter 6 summarizes some elements related to post-transfusion oxidative stress.

Part 2 is structured in 8 chapters and includes the personal contribution of this thesys. Chapter 7 presents the working hypothesis, and in chapter 8 are presented the objectives of the paper. Chapter 9 describes the general materials and working methods underlying the experimental protocol for all 3 studies presented in this paper, namely the biological material used, the construction of experimental lots, the protocol for inducing experimental anemia and the methods for assessing the blood compatibility. Chapter 10 is the first study in this research, entitled "Evaluation of the effect of whole blood transfusion on plasmatic oxidative stress in Holstein calves", followed by Chapter 11 which includes study 2 entitled "Evaluation of the efficacy of whole blood transfusions in Holstein calves” and Chapter 12 which presents the third study entitled

"Calculation of the transfused blood volume required to treat anemia in Holstein calves". The general conclusions are the subject of chapter 13, and in chapter 14 are presented the elements of originality and the innovative character of the thesys. The paper ends with the list of cited bibliography (146 bibliographic titles).

RESEARCH RESULTS

In the second part of the paper we studied the biological effects of whole blood transfusion in Holstein calves. The studies were carried in the SC Stazoo SRL farm, located in Galda de Jos, Alba County, whose main object of activity is the production of cow's milk. The main objectives we set for the thesys:

1) Evaluation of the level of plasmatic oxidative stress caused by whole blood transfusion in cattle;

2) Evaluation of the efficiency of whole blood transfusion by analyzing the hematological and biochemical parameters following whole blood transfusion;

3) Development of a mathematical formula to calculate the amount of blood needed for transfusion, using parameters determined in field conditions.

Chapter 9, entitled "General materials and methods", includes the selection of biological material for the construction of experimental groups, together with methods for assessing the health status of the animals that formed the groups, methods for taking biological samples and the intervals during which they sampled. The same chapter describes the protocol for inducing the experimental anemia, developed from a model used for rabbits described by Dunne et al., 2006, which induced a certain degree of anemia in isovolemic conditions. This protocol consisted of extracting approximately 38% of the total circulating blood volume of each calf, maintaining a constant volume of blood from the vascular system. Isovolemic conditions were essential to achieve moderate anemia and to avoid possible side effects due to hypovolemia. In the first stage, a venous catheter (12 G 80 mm) was fixed by suture at the jugular vein, followed by the placement of a venous catheter at the saphenous vein (12 G, 60 mm). The blood was extracted in 2 stages: in the first stage were extracted 20% from the volume of circulating blood without the need for i.v. fluid compensation. (Mudge, 2020), and in the second stage, the remaining 18% was extracted while fluids were administered through the catheter placed in the saphenous vein, simultaneously with the extraction of blood, in volumes equal to the volume of blood extracted. Thus, the volume of circulating blood remained constant. The blood was collected in 450 ml transfusion bags for human use (CPDA-1 whole blood bag, Lotus Global, London, UK). Blood compatibility was assessed by the Crossmatch method (major and minor). The major crossmatch consists in adding the patient's serum over the donor's red blood cells, followed by a specific protocol to determine the presence of hemolysis and / or agglutination. The minor crossmatch is performed by adding the donor serum over the patient's red blood cells, in a hemolysis tube, after which they are evaluated for agglutination (agglutination +, indicates incompatibility between the 2 blood samples, while lack of agglutination means that the 2 samples are compatible).

Chapter 10, entitled "Evaluation of the effect of whole blood transfusion on plasmatic oxidative stress in Holstein calves" aimed to analyze some oxidative stress parameters from plasma: total antioxidant capacity (TAC), total antioxidant status (TAS), nitric oxide availability (NO), malon dialdehyde (MDA), thiols and oxidative stress index (OSI)) in the context of the use of blood transfusions in Holstein calves. To perform this study, 3 experimental groups of 3 animals each (category 100.1 ± 3.1 kg) were used. After induction of experimental anemia, group 1 (control) received fluids i.v. (saline, Duphalyte and Calcium Borogluconate) 18 ml/kc body weight. Group 2 (heterotransfusion) received compatible blood in the same amount (18 ml/kg MC), and group 3 (autotransfusion) received their own blood. The rate of administration was 20-40 ml/kg/h, not exceeding 1000 ml/h (Hubans-Belkilani, 2001). This study started from the premise that oxidative stress plays an important role in the biological deterioration and phagocytosis of erythrocytes, or even in inefficient erythropoiesis (Fibach et al., 2008). Following transfusion therapy in anemia, no statistically significant variations were observed in any of the 6 parameters evaluated during the 14 days posttransfusion. These results are consistent with the findings of literature data, oxidative stress having an important role in erythrogenesis, and later in life and mechanisms of erythrocyte destruction. According to the results obtained in our studies, transfusion therapy does not have the ability to generate significant oxidative stress in the plasma.

Chapter 11, entitled "Evaluation of the efficacy of whole blood transfusions in Holstein calves" aimed to verify in vivo the efficiency of whole blood transfusion in cattle, hematologically and biochemically, using an experimental protocol under controlled conditions. To perform this study, 3 experimental groups of 3 animals each (category 100.1 ± 3.1 kg) were used. Following the protocol for inducing experimental anemia, the values obtained (day 2- before transfusion) are representative for moderate anemia (HGB 7.22 ± 0.45 g / dL; RBC 7.48 ± 0.36 106 / µL; HCT 21.99 ± 1.42%). After induction of experimental anemia, group 1 (control) received fluids i.v. (saline, Duphalyte and Calcium Borogluconate) 18 ml/kc body weight. Group 2 (heterotransfusion) received compatible blood in the same amount (18 ml/kg MC), and group 3 (autotransfusion) received their own blood. The rate of administration was 20-40 ml/kg/h, not to exceed 1000 ml/h. The hematological parameters of the animals (subject to AIP) included in the study were compared with individuals who received transfusions with compatible blood, with the control group (who received saline) but also with a group in which autotransfusions were performed. This experimental design was chosen to test the hypothesis that the short life of transfused red blood cells in the circulatory system of the recipient organism is due only to immune-mediated mechanisms, or other mechanisms are involved in the rapid elimination of transfused red blood cells. The results obtained indicate that whole blood transfusion has a limited efficacy in cattle, erythrocyte parameters starting to decrease at only 2 days after transfusion (heterotransfusion group), and after another 3 days to be relatively at the same level as the control group, at which was not administered blood. In the following days when the animals were monitored in terms of haematological parameters, the blood transfusion did not influence the recovery of the animals, the erythrocytes registering a similar dynamic in all three groups. Post-transfusion hemolysis did not significantly influenced the studied biochemical parameters, and within the white blood cells there were no significant variations except for basophils and eosinophils. But even in their case, they were transitory, returning to levels similar to those at the beginning of studies. Autotransfusion was less effective than expected, with erythrocyte counts decreasing after day 5. The mechanisms involved in the eradication of red blood cells can only be presumptive, and factors may be involved in sampling and/or conservation and/or administration procedures rather than compatibility.

Chapter 12, entitled "Calculation of the transfused blood volume required to treat anemia in Holstein calves", aimed to develop a formula in order to eliminate subjective factors from the therapeutic process and to determine more accurately the volume of blood needed for transfusion in Holstein calves, in a shorter time interval, using as parameters the donor's hematocrit and patient's hematocrit, body weight and a constant (CC) characteristic for each species. To perform this study, 2 experimental groups were used (category 100.1 ± 3.1 kg and 151.1 ± 3.0 kg), of 10 individuals each. After the induction of experimental anemia, each group was treated by autotransfusion: individuals in the first group received 1800 ml, and those in the second group received 2700 ml of blood, with a flow rate of 20-40 ml/kg/h, not exceeding one liter per hour. This amount was administered to increase HCT by a maximum of 10% and to avoid post-transfusion circulatory overload. Starting from the formula described by Godinho-Cunha et al., (2011) and Helm (2014), to calculate the value of the CC constant for cattle with specific weight ranges, we replaced all the variables used in the formula with known values: body weght, transfused blood volume, donor HCT, desired HCT (HCT_Fin) and patient HCT (HCT_ini), the CC constant remaining the only unknown parameter in the equation. The values of the CC constants obtained from the experiments were 80.6 for the group of 100.1 ± 3.1 kg and 76.5 for the group 151.1 ± 3.0 kg and it was statistically validated (by linear regression with fixed point), the mean values of the CC constants calculated by proving the “flatness” of the ordinal series in correlation with the values of body weight (MC). It can also be stated (based on the ANCOVA test, P = 0.05) that these two weight groups have different values of CC constants, with different value distributions and the same coefficients of variation. This study analyzed only two weight groups separately (100.1 ± 3.1 kg and 151.1 ± 3.0 kg) and generated two different statistical values of CC constants. The difference in body weight (MC) between the analyzed groups can be considered wide, but does not cover the entire weight range encountered in clinical practice. The proposed method applies to two specific weight categories and, in order to mitigate this experimental limitation, future work should consider analyzing a wider weight range and larger groups.

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