(PhD THESIS SUMMARY)

Analysis of the hematological profile and hematopoietic potential of chickens during physiological stages and nutraceutical diets

PhD Student **Buta Andreea Mirela**

PhD supervisor **Prof. Univ. Dr. Ognean Laurenţ**

INTRODUCTION

Poultry industry is an important sub-sector of livestock production and plays an important role in economic growth, and an important aspect of raising healthy broiler chickens is the establishment of an effective stress management program for disease prevention. Also, the health status evaluation in poultry through comprehensive, hematological and biochemical tests, especially those from intensive breeding farms, allows the early detection of disease signs, before it can cause high mortality index and, implicitly, economic losses (DOUGLAS *et al.*, 2010).

Although chickens have been used as a research animal model in order to establish physiological parameters for other avian species, relatively little information has been published regarding the evolution of its hematological and biochemical parameters.

Establishing reference ranges for the interpretation of the hemogram in birds has proven extremely difficult due to the wide variations in the values of these indices, that depends on a multitude of internal and external factors (for example, sex, age, housing conditions, stress levels and so on).

In the last two decades, the number of domestic and wild birds that become patients in veterinary clinics and hospitals, as well as in wildlife rehabilitation centers, has increased considerably. In critical situations, and birds are usually brought in late, when the situation is already critical, it is important to provide the best emergency care, which, in the avian patient, also involves shock reversal and homeostasis restoration.

Therefore, a blood transfusion may be necessary to achieve this goal. Although there are variable differences between avian and mammalian physiology, such as the ability of birds to tolerate greater blood loss, transfusion therapy is an effective way to treat the anemic avian patient. Whole blood transfusions (autologous, homologous and heterologous) and the administration of oxygen-carrying hemoglobin-based solutions are the most commonly used treatments in birds.

Another great thing about birds comes from the fact that avian studies have played an important role in understanding hematopoietic events in mammals. However, some specific aspects of the ontogeny of the avian hematopoietic system, such as where hematopoietic cell expansion and differentiation occur, remain unclear.

According to the bibliographic studies conducted, this role is attributed to the fetal liver in mammals, and appears to be assumed by para-aortic foci in chickens (AHMED AL-NEDAWI, 2018). We believe that an integrated morphological study is important for the better understanding of the temporal and spatial distribution of hematopoietic sites during avian development.

An essential condition for the growth and health of birds, especially broilers, is the development of effective stress management programs, as well as their intercurrent and specific diseases, which can also resort to the use of nutraceuticals. Birds are very sensitive to stress, and its level can reach dangerous levels with severe consequences when it becomes decompensated.

PURPOSE AND MAIN OBJECTIVES OF THE THESIS

The main purpose of the documentation and investigations carried out in this PhD thesis consisted in the analysis of broiler chickens' medullary and extra-medullary hematopoietic potential, the basic hematological indices evaluation of birds in different conditions, intra and interspecific blood compatibility and the clinical testing of the probiotic effects of the nutraceutical product Biolactorom in Broiler chickens.

The achievement of this goal was based on the following **general objectives**:

- ✧ Updating the procedures for taking and processing samples of blood, bone marrow and other hematoforming organs in chickens in the stages of embryo, newly hatched chick and adult.
- ✧ Updating the tests used in the evaluation of hematological profile, hematopoiesis, blood compatibility and probiotic effects in chicken and other bird subspecies;
- ✧ Analysis of the evolution of the blood count and the development of some correlative models for evaluating the morphophysiological peculiarities of the progenitor and mature elements on the blood cell lines;
- ✧ The morphophysiological characterization of progenitor cell islands captured in blood, hepato-splenic and lung preparations in embryos and newly hatched chicks;
- ✧ Investigation of medullary and extramedullary hematopoietic potential in the embryonic and post-hatching period in broiler chickens;
- ✧ Investigation of intra- and interspecific blood compatibility on samples of birds belonging to the Orders Anseriformes, Galliformes and Columbiformes different in origin, maintenance conditions, age and sex;
- ✧ Analysis of the impact of some hematological indices in the investigation of the hematopoietic and probiotic influences of a nutraceutical based on Lactobacillus plantarum and deproteinized and glycerinated whey, in healthy broiler chickens and with intercurrent anemia;
- ✧ Evaluation of the opportunity to replace some growth promoters with nutraceuticals of interest in the intensive breeding of chickens.

THESIS STRUCTURE

The doctoral thesis entitled, "***Analysis of the hematological profile and the hematopoietic potential during some physiological stages and diets with nutraceutical products in chickens***", is structured and drafted in accordance with the provisions of the PhD School of USAMV Cluj-Napoca and the recommendations of the Faculty of Veterinary Medicine. The work includes a total number of 164 pages, of which 52 (31.7%) belong to the first part, composed of 4 chapters, and 112 (68.3%) to the second part, spread over 8 chapters.

CURRENT STATE OF KNOWLEDGE

The first part compose a veritable bibliographic collection, which summarizes the main novelties and field on-going research and updates regarding blood tissue morphophysiology, hematological profile, hematopoietic functions, blood compatibility and clinical trial of a nutraceutical product. All this is summarized in the following four studies addressed in the thesis, represented by:

Chapter I is entitled " ***Actualities regarding blood tissue and hematological profile in birds***" and includes two sub-chapters, which abound in details regarding the peculiarities of the morphological components of the blood in birds and the evolution of the hematological profile in physiological and pathological conditions in chickens;

Chapter II, under the name "***Current aspects regarding the study of hematopoiesis in birds*** ", brings valuable data summarized in the following three sub-chapters - "The particularities of near hatching hematopoiesis phases observed in the selected tissues and organs samples", "The evolution of hematopoietic functions after hatching and during the adult life of Broiler chickens;

Chapter III is entitled "***Updates regarding blood compatibility testing in birds***" and summarizes, in two sub-chapters, the particularities of erythrocyte antigenicity in chickens and transfusion blood compatibility testing in birds;

Chapter IV is called "***Nutraceutical impact upon blood profile in chicken***" and addresses, in two sub-chapters, some legislative aspects regarding the use of nutraceuticals in animals and the hematopoietic and hematological impact of the use of nutraceuticals in birds.

PERSONAL CONTRIBUTION

Part II includes the personal contributions, which are grouped into six chapters, focused on the details presented in the pursued objectives.

Chapter V is called **"Materials and methods"** and consists of the synthesized presentation of the proposed main purpose and the general objectives formulated in order to obtain the estimated results.

Chapter VI (Study 1), entitled ***"Hemogram evolution in Broiler embryos and chickens".*** The results of the compared hematological study in birds included in the study, with a majority consisting of broiler chickens and domestic hens (*Gallus Domesticus*). During the research carried out, we encountered no particular problems and it was also possible to differentiate between heterophiles and eosinophils, aspect considered difficult by many well-known authors. It is important to mention that the data obtained in broiler chickens research, especially the hematological ones, are characterized by a wide variability, depending on age, sex, stress and many other factors, evolving dynamically even during a day.

Thus, the average values, between 2.35±0.22 - 2.83±1.04 T/L, of the RBC count in control group were slightly lower, compared to the average values of the experimental group with values between 2.27±0.38 - 4.07±5.15 T/L, both batches having normal values. As can be seen, the results obtained in the two batches show some individual differences, some being somewhat higher, including in the case of the control batch. The WBC count was situated within the physiological limits for the control group, with average values between 12.96±31.18 and 26.56±28.5 x109/L, and in the experimental group expressing values situated between 10.4±16 and 19.36±43.22 x109/L. We can see that the results of the two groups are different, with slightly higher values in individuals from the control group. Regarding the average values of heterophiles, for the control group they were determined to be situated between 42.8±12.63% and 48.6±8.35%, and between 42.4±4.27 and 50.8±5.21% in the experimental group they. Therefore, the two groups had similar results, which were within physiological limits, according to HOFFMAN (1961), although other authors consider these values to be slightly above the upper limit (PIERSON, 2000). Eosinophils recorded normal values for both groups, the control group having averages between 2.6±1.94 and 4.4±1.14 %, and, 2.6±1.51 and 4.2±3,03 %, the experimental group which are considered physiological. Basophils were found extremely sporadically in the smears of both batches, being below the normal limits, but without having a particular significance regarding the health status of the birds. Lymphocytes, with average values within 37.2±7.01-40.2±8.4 % in the control group and 35.4±4.72-45.4±8.64% in the experimental group, were also placed within normal limits by some authors, such as HOFFMANN (1961). The average values of monocytes in the control group, between 9.6±2.07 and 13.6±8.76%, and within 6±2.91 and 10.2±2.38 % for the experimental group, were evolving within standards, except for some individuals in the control group that expressed slightly above the physiological values, towards the end of the experiment. However, we can consider this values as being normal for the specificity of our study.

The hematocrit values in control group showed average values between 21.89±3.15-37.5±4.33 %, and for the experimental group the values were situated between 25% and 26%, being considered within the physiological limits. There were some individuals from the control group whose values were, however, towards the lower limit. Hemoglobin recorded average values of 8.62±1.37 and 21.76±6.2 g/L, for control group, and in the experimental group, this parameter values were confined between 10.69±3.44 and 19.86±2.73 g/dL, falling within the physiological standards, except for few chickens from the experimental group who slightly exceeded the upper limits. Regarding the values of erythrocyte constants, there were also some situations when they were outside the physiological ranges, but without pathological connotations, as daily clinically observed. Thus, the MCV averages were between 84.77±45.13 and 147.56±39.43 fl in the control group and ranged between 109.48±39.45-117.63±58.34 fl in the experimental group.

Regarding obtained values of MCH, this were within 37.04±7.54 and 82.3±14.65 pg respectively ranging from 46.8±9.59 pg to 61.79±27.93 pg. MCHC showed results comprised between 39.63 ± 6.38g/ dl and 71.87±52.65 g/dl, respectively 42.76±13.76 and 54.62±9.95 g/d.

These data revealed higher values of mean erythrocyte constants in control group than those of experimental group, exceeding the physiological upper limits discussed in the bibliographical research. According to some studies, there is a negative relationship between MCH, MCHC and RBC count and, it is somehow normal for a high amount of hemoglobin to lead to increased MCHC values, while the RBC values are located within normal limits.

Chapter VII (Study 2), called ***"Investigation of hematopoiesis in Broiler embryos and chickens"***, concentrates the microscopic investigation of the cytological profile of samples from embryos and newly hatched broiler chickens revealed some significant aspects for the morphophysiological characterization of cellular niches with hematopoietic functions diffused in various organs. The study of erythropoiesis in birds reveals that this process continues intra-vascular or intra-sinusoidal after hatching, which means that rubricytes can occasionally be found in the peripheral blood of healthy animals, and the percentage of reticulocytes in the peripheral blood of healthy chickens is commonly higher than in most species of mammals. Following the investigations carried out, we confirmed that in the avian blood and in some hematoforming organs, progenitor cell niches can be identified in the near hatching embryos and chickens which contribute to the completion of what it's known as definitive hematopoiesis (Fig. 1).

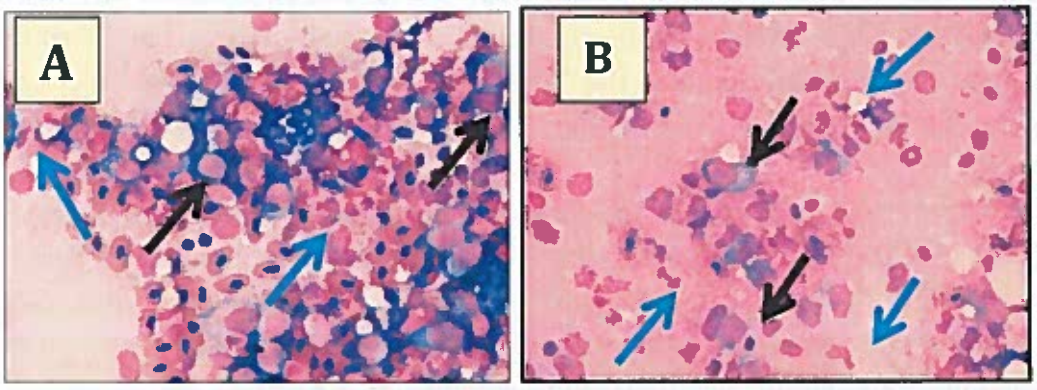

Fig. 1. Hematopoietic cell precursors in near-hatching broiler chickens. *Black arrows- eritropoietic cells aspects in different maturation stages; Blue arrows- granulopoietic line cells in different stages of development (Col. Diff-Quick, 100x) (Original)*

The embryonic bone marrow becomes the main eritropoietic organ starting from embryonic days 12-15. According to other similar research although the yolk sac maintains its eritropoietic function until the hatching period (NAGAI şi SHENG, 2008).

We also found that, unlike in mammals, embryonic liver hematopoiesis in birds is predominantly carried out in the granulocyte line and less on the erythrocyte line. From the obtained images, it emerged that, along with the bone marrow, the liver and spleen (Fig. 1A;B; Fig. 2A;B) have minor contributions in definitive hematopoiesis and, we did not observed this characteristics in organs like the lung, kidney or pancreas , as recorded by other researchers in the field. Following the administration of the studied nutraceutical product, during a period of 45 days, in healthy and at risk of anemia Broiler chickens, we revealed statistically insignificant values of the erythrocyte indices, indicating the beneficial influences of Biolactorom on the hematological picture and hematopoietic functions. The evolution of the leukocyte profile was mainly characterized by oscillations towards the upper limit of the physiological standards, associated with no signs of pathological conditions, therefore indicating the involvement in stimulating and supporting the immunity of the studied product of broiler chickens.

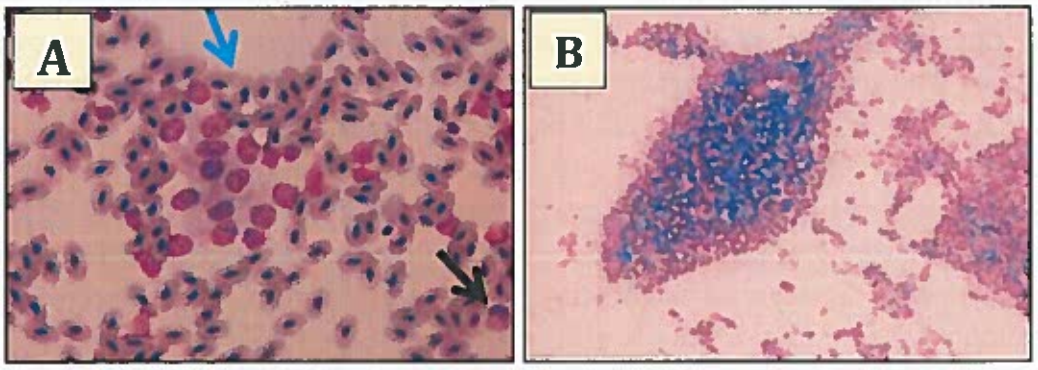

Fig. 2. ***Characteristic aspects of E20 hepatic (A) and splenic hematopoiesis (B)****- granulocyte progenitor cells (Black arrow); erythrocytes (Blue arrow) (Col. Diff-Quick, 100x) (Original)*

Chapter VIII (Study 3), entitled **"Evaluation of blood compatibility in tested birds".** The intra- and interspecific blood compatibility testing evaluations from our study were based on 110 cross-reaction tests, of which 55 were for the major Crossmatch test, where we've recorded 8 positive reactions (14.54%). Positive reactions showed different agglutination intensities, noted from 1+ to 4+ (Fig.3).

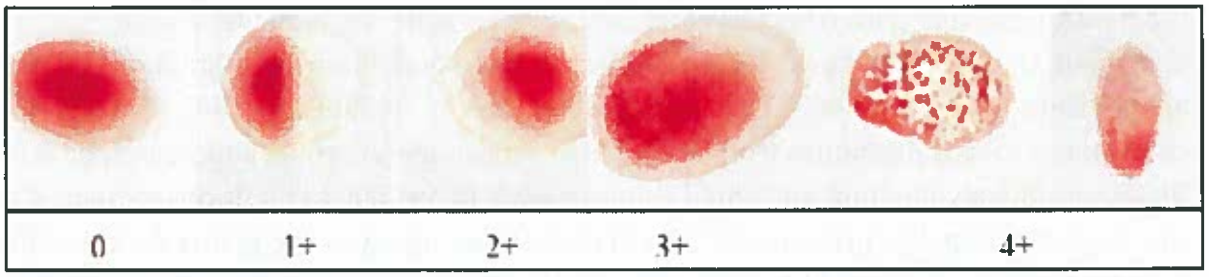

Fig. 3. Macroscopic aspects of agglutination reactions intensities *(Original)*

In contrast, during minor Crossmatch tests no positive reactions were registered, agglutinations being absent for all the investigated combinations.

According to the obtained results, the homologous combinations between the chickens did not show any incompatibility, the homologous combinations between the turkeys having the same evolution. Based on these results, it can be assumed that individuals of the same sub-species do not have preformed antibodies against the antigens of the different blood groups of the respective species (ALTMAN, 1982). In other words, regardless of their membership in a blood group system, individuals of the same sub-species should not show incompatibility during first blood transfusion OGNEAN, 2017). Simultaneously, we found that there was no positive link between blood compatibility and the taxonomic belonging to the same order (in our study *Galliformes, Anseriformes, Columbiformes).* It is also worth noting the compatibility between the blood of turkeys and geese, unlike the combinations between turkey/duck, turkey/chicken and duck/chicken showed different degrees of incompatibility (BOWLES *et al,* 2007; DEGERNES *et al.,* 2004). There was also an individual variation of the compatibility degree, with the heterologous incompatibility rate not being 100%.

Chapter IX (Study 4), called **"Analysis of the hematological and symbiotic action of Biolactorom".** Following the administration of the nutraceutical product Biolactorom, in healthy and at risk of anemia chickens. The obtained results revealed relatively important and statistically significant evolution of the erythrocyte indices, correlated with the beneficial influences of Biolactorom on the hematological picture and the hematopoietic functions. The evolution of the leukocyte profile was also characterized by statistically significant oscillations, towards the upper limit of the physiological standards. These were associated with the absence of pathological symptoms, indicating the involvement of nutraceuticals in stimulating and supporting immunity in broiler chickens (SUGIHARTO, 2016).

The evolution of the leukocyte profile was characterized by insignificant oscillations towards the upper limit of the physiological standards, associated with the absence of pathological signs, suggesting the involvement of Biolactorom in stimulating and supporting the immune system of Broiler chickens. In the coproparazitological evaluation of control group chickens from the the microbiological examinations revealed the predominance of bacteria of the genus Bacillus, the Gram negative ones reaching 80%, and the Gram positive ones 40% (BUTA, 2019). To these were added filaments, lactose-negative bacteria and coliforms. Colonies formed were large, medium and small, smooth or mucous. Colony counts yielded a maximum of 860 million UFC and a minimum of 430 million UFC, with an average of $645{,}08 \times 10^6$ UFC (Fig. 4). The coproparazitological samples from chickens in experimental group, supplemented with Biolactorom, only medium and small colonies were identified. In addition, compared to the control group, the presence of thin filaments belonging to the genus *Lactobacillus* was reported at the level of the colonies. In all cultures, Gram-positive bacilli (100%) were identified, as well as Gram-negative bacilli or cocobacilli, but in a much lower proportion (60%) and devoid of pathogenic germs.

Regarding the number of colonies, a maximum value of 688 million UFC was noted in the experimental group, respectively a minimum of 113.4 million UFC, the average of 315.28 million UFC, being much lower than in the control group.

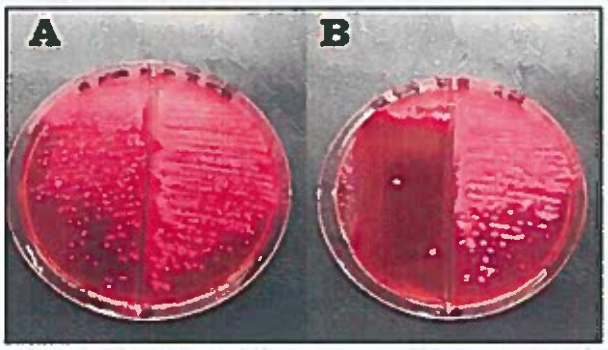

Fig. 4. Colony counting on McConkey agar: *experimental* ***(A)*** *and control group* ***(B)*** *(Original)*

The experimental batch therefore stood out, with predominantly superior microbiological results, with a beneficial character, compared to those of the control batch. As already confirmed, the commensal gut microbiota is particularly important for innate and acquired defense mechanisms in birds. The clinical and coproparazitological examinations have relevant good general condition and maintenance, with negative the coproparasitological examination, in the chickens tested. At the end of the experimental studies of the Biolactorom product, it was also implemented in a mini hall for the intensive growth of broiler chickens, with the major impact coming from the evolution of the main productive performance indices: average slaughter age, average weight, average daily gain, mortality, RCF (Rate of Feed Conversion) and IEE (European Efficiency Index) (SUGIHARTO, 2016). This study revealed that the flock of chickens supplemented with Biolactorom showed superior values of performance parameters compared to the control flock, but very close to the average levels on the farm (BUTA, 2019). Thus, the average daily growth and mortality registered slightly increased levels in the control herd compared to the experimental one, but the IEE exceeded the minimum threshold of 370, in the case of both samples, reaching a value of 396.

The general conclusions and recommendations formulated following the analysis and grouping of the obtained results, constituted a succinct assembly, which completes the content of the thesis.

The originality and innovative contributions of the thesis, finalize the personal contribution by synthesizing the main novelties and elements of originality, analyzing the innovative character of the investigations and tests carried out, as well as the contribution of the results obtained to enriching the data from the specialized literature.

The bibliographic references group an important number of titles (266), appropriate to the documentation and investigations carried out and, above all, to the discussions and results obtained.

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