Current data regarding the evolution of hematological profile in Broiler chickens: a review

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Abstract
In the last decades, along with the progress of poultry farming, avian medicine has seen remarkable progress. The use of improved breeds has raised the issue of their increased susceptibility to pathological factors and environmental conditions. Currently, the detection of diseases in birds, especially in poultry, is based on serological and necropsy investigations; complementary laboratory investigations, such as hematological and biochemical, are only used in companion birds. The assessment of the health of poultry through hematological and biochemical tests, especially those from intensive breeding farms, allows the early detection of signs of disease before it causes mortality and implicitly economic loss. Chickens were used as an animal model of research to establish physiological parameters for other avian species. However, little information has been published on the evolution of their hematological and biochemical parameters. Furthermore, the establishment of reference intervals for interpretation of results in birds in general and chickens in particular has proved extremely difficult due to their hematological and biochemical features. Hematology in birds partly mirrors health status can vary due to race, age, gender, stress, bacterial and viral infections. Considering the complexity of the animal organism and the complex interrelation established between its different systems, the administration of an exogenous substance will cause complex reactions.

Keywords: *hematology, poultry, broiler chickens, reference intervals.*

Introduction

Hematology is the discipline of medical science that studies blood and blood-forming tissues and is currently considered an integral part of clinical laboratory diagnostics in avian medicine. Hematological research rarely offers an etiologic diagnosis, but they are still indispensable diagnostic tools to assess the health and disease status of different individuals, to monitor the progress of different diseases, to evaluate the response to therapy and to provide a prognosis. The routine collection and processing of blood samples allows the evaluation of the hematological response to the disease. In addition, the creation of hematology databases is important in establishing benchmarks for different avian species. (Samour, 2006)

The interpretation of avian blood cells faces many challenges. Practitioners must be able to recognize the normal morphology and function of cells in order to be able to interpret the changes that these cells undergo (Campbell, 1995).

Analysis of the hematological profile in the broiler chicken

Blood sample collection. The total volume of blood in clinically healthy birds is between 6 and 11 ml per 100 g body weight. Thus, a bird weighing 250 g would have approximately 15 to 27.5 ml of blood, of which, in a clinically healthy individual, up to 10% (1.5-2.7 ml) can be safely collected without had a detrimental effect on the patient. However, an amount of 0.2 to 0.3 ml of blood is generally sufficient to perform a complete haematological examination on a bird. In birds, blood samples are usually harvested using the right jugular vein (v. *Jugularis dextra*), as this is generally larger than the left jugular vein in most avian species. Other preferred sites of choice include the basal vein (v. *Cutanea ulnaris superficialis*) and the caudal tibial vein (v. *Metatarsalis plantaris superficialis*) (Campbell, 1995; Samour, 2006; Yonas and Mersha, 2014).

It is not a correct method to collect blood samples from the claws, because cell distribution and cell content are invariably affected. The author prefers to obtain blood samples from most bird species from 200 to 4000 g, using the basilic vein while the bird is in the dorsal decubitus, although most US practitioners dealing with psittacine species prefer jugular venipuncture (Campbell, 1995; Samour, 2006; Yonas and Mersha, 2014).

In most avian species, the optimal area for collecting a blood sample from the basilic vein is along the median section of the vein. The right side is preferred if the doctor is right-handed, while the left wing is preferred if the doctor is left-handed. Venipuncture immediately above the elbow joint is not recommended, as haemostasis is difficult to achieve in this place in most cases. Applying pressure with the thumb at the proximal level of the humerus would help to elevate the vein, which becomes visible parallel to the external side of the humerus. After separating the feathers and preparing the place of election with an alcohol swab, the bent needle is easily inserted into the vein at an angle of approximately 45 °. The sample can now be collected, being careful not to exert too much negative pressure while withdrawing the blood with the syringe, as this will invariably lead to the collapse of the vein. While collecting the sample, it is recommended to continue maintaining pressure on the proximal humeral area to ensure a well-defined vein.

The dorsal puncture method of the basilic vein by entering under the adjacent tendon prevents the formation of the hematoma because the underlying tissue exerts pressure on the venipuncture site when the needle is withdrawn. It is essential to avoid sudden movements that can scare the bird and this can be fought, as this can easily break the vein and lead to a hematoma or, worse, severe bleeding. After collection, a small dry wool pad should be placed over the place of venipuncture, and the wings closed to maintain pressure for a few seconds. It is recommended to check the venipuncture site before releasing the bird back to ensure that no post-collection bleeding has occurred (Campbell, 1995; Samour, 2006; Yonas and Mersha, 2014).

An important aspect to consider when collecting blood samples from birds is gelling. This phenomenon is described in the literature by the total or partial transformation of the plasma, in a gelatinous mass similar to the clot, but without figurative elements. Gelling can be differentiated from coagulation by the lack of erythrocytes in the gelatinous mass, which are sedimented at the bottom of the tube. The phenomenon can be found in about 25% of the samples and can affect totally or partially plasma (Harr, 2006; Trânca, 2013)

Storage of blood samples. After collection, the needle is removed and the blood is easily deposited in a storage tube containing ethylenediaminetetraacetic acid (EDTA) anticoagulant (1.5 mg / ml of blood) or lithium heparin (1.8 mg / ml of blood). Passing the sample through the needle is not recommended, as it can cause severe damage to fragile blood cells.

For general hematological analysis, EDTA is the anticoagulant of choice, as it is not possible to estimate fibrinogen or to accurately calculate white cells in heparinized samples. However, in some avian species, storing blood samples in EDTA-containing tubes causes progressive red cell hemolysis and is not recommended; In these cases, heparinized tubes are preferred. This is the case of some species of *Corvidae*, such as the rock (*Corvus monedula*) and the crow (*Corvus corax*); *Gruidae* such as the black-crowned hornbill (*Balearica pavonina*) and the gray-crowned hornbill *(Balearica regulorum*); *Cracidae* such as black peacock (*Crax alector*); *Phasianidae* such as the Australian turkey (*Alectura lathami*); and the ostrich (*Struthio camelus*). It is recommended to store blood samples in sodium citrate when sending samples to a commercial processing laboratory using laser flow cytometry (Samour, 2006).

Hematology laboratory analysis. Ideally, laboratory tests should be performed within 3 to 4 hours after collection. Many US laboratories require that a smear be made immediately and sent

along with the EDTA tube. If this is not possible, the samples must be refrigerated at 8 to 12 ° C or in a suitable container for processing within 24 to 48 hours (Post et al, 2003).

Refrigerated samples are not ideal for hematological testing because the cells invariably undergo some changes. Only an experienced hematologist would be able to differentiate these changes from a true hemo-response to certain medical disorders. The amount of blood available for testing is very limited, which makes it impossible to complete a full range of tests. The clinician should take this into account and request the analyzes in order of priority (Samour, 2006).

1) Blood smear preparation and leukocitary formula. In clinical medicine, the morphological examination of the blood is frequently used on fixed and colored smears, the quantitative and qualitative evolution of the leukocyte population being investigated. Such an investigation, called a leukocyte formula (leukocyte array or leukogram) provides particularly useful data regarding the assessment of leukopoietic function and implicitly the defense capacity of the body. These data become even more conclusive if they correlate with the evolution of the total number of leukocytes, being able to obtain diagnostic value in some diseases specific to the leukocyte line. The leukogram technique includes the display, staining and examination of the smear, framing the identified cells and interpreting the frequency data by leukocyte types and subtypes.

There are two standard methods for performing blood smears on birds. It is the method of displaying with the help of two blades, respectively the method between blade and blade. These are the most commonly used, as they minimize morphological alterations due to display (Clark et al., 2009). Rubbing technique, using two microscopic blades placed at a 30 degree angle (as a standard method), can lead to various cellular alterations in the case of bird blood, as their figured elements appear to be more susceptible to breakage than mammals. (Pierson, 2000; Trâncă, 2013). In the case of the alternative method, which uses a microscopic blade for spreading, too thick areas may result, where the morphology of the figured elements cannot be determined precisely (Pierson, 2000).

The smear can be made either from blood that does not contain anticoagulant (especially if blood parasites are suspected) or from blood that contains EDTA. EDTA will cause red blood cell hemolysis in some birds. Prolonged exposure to EDTA may cause an increase in blood cell disruption in some species, so when an anticoagulant is used, a blood film should be made immediately after blood collection. Heparin should be avoided whenever possible for hematological studies because it contains artifacts (Campbell, 1995).

A variety of colors can be used to evaluate smear, such as Wright, Giemsa, Wright-Giemsa, Wright-Leishman or May-Grunwald and their combination (Campbell, 1995; Bounous and Stedman, 2000).

It should be noted that the use of the DiaQuick Panoptic staining technique allows a good assessment of the morphology of the figured avian elements, including facilitating the differentiation of heterophiles from eosinophils, differentiations that often pose problems for examiners.

The morphological assessments of the figured elements, in the case of birds, require the addressing of specific factors that refer to the techniques of making and coloring the smears, as well as the influence of the anticoagulants used. The smears once spread must be completely dried, thus there is the risk of artifacts (acidophilic granulations in the erythrocytes). After complete drying, they require immediate fixing, especially if they are done in conditions of excessive temperature or humidity. Exposure to direct sunlight or volatile substances, especially formol, should also be avoided, as they may affect cell morphology (Samour, 2006; Campbell et al., 2007)

It is recommended that the fixation be done by immersion in pure ethanol for 5-10 minutes; thus fixed the smears can be stored in stands for later staining, or they can be stained immediately after fixing. Friction fixation is essential to maintain the integrity of intracytoplasmic, eosinophilic (heterophilic) and basophilic granulations, as these structures are water soluble (Samour, 2006; Trâncă, 2013).

2) Packed cell volume estimation. The method of microhematocrit is advantageous through time, labor and blood economy. As a working technique, the tube is initially filled by capillary action, leaving about 5 mm of empty tube at the end; this end of the tube closes with plasticine or flame. Centrifugation for 3 minutes at 15000 rpm is sufficient to establish micro-hematocrit in most animal species. The readings are made with the help of a special device that is delivered with the micro-hematocrit centrifuge, or in the absence thereof, according to the procedure used in the case of the unread tubes for the determination of the hematocrit by macromethod. If a sufficient blood sample is available, the hematocrit can also be determined by the macro-hematocrit method (Ghergariu et al., 2000; Ognean and Cernea, 2006; 2011).

3) Total red blood cell count. The count of erythrocytes and leukocytes in the bird shows some differences from the count of these cells in mammals. The Prochaska-modified Natt-Herrik dilution fluid is used, which protects all figurative blood elements (erythrocytes, leukocytes, platelets). This important hematological test, usable in the evaluation of other parameters (hematocrit, mean red blood cells), was determined by the use of the hemocytometric method. Assessing the number of erythrocytes, using the Natt-Herrick fluid, is considered the standard method for evaluating avian samples (PIERSON, 2000; Campbell et al., 2007).

As working methods, there are several variants in the literature, but they are very similar and have the same basic principle. Thus, a suitable Natt-Herrick diluent and the blood needed to make a dilution of 1 to 200 are sucked into a properly labeled container. The dilution thus obtained is homogenized for 3 minutes. A hemocytometer is loaded with a sufficient amount of blood dilution and allowed to stand for 3-5 minutes for sedimentation (OGNEAN AND CERNEA, 2011; SAMOUR, 2006). The counting of the cells is done in the 4 cubes in the corners of the central network of the hemocytometer and in one of the middle ones, using the rule "L": the cells that reach the markings of the squares of the order of the 3 of the left hemocytometer will be counted and the cells on the right will be ignored. and above (OGNEAN and CERNEA, 2011; SAMOUR, 2006).

Regarding the determination of the number of red blood cells, we adopted the procedure of counting on 80 cubes of order 3, only in the case of uncertain samples I used the technique introduced by Samour (2006), consisting of counting 160 cubes. Finally, the calculation was made by dividing the number found by 100. Thus the total number of red blood cells in Tera / liter (T / l) was expressed, according to the formula: {*Number of cells obtained / 100 = Number of red blood cells}* (Trânca, 2013). To get the number of red blood cells per microliter of blood the number of red cells counted is multiplied by 10,000.

4) Total white blood cell count it is one of the most important hematological analyzes used in the assessment of animal health. In the case of birds, the same methods and materials are used as in the determination of the number of red blood cells (GHERGARIU et al., 2000; DOJANĂ et al., 2003; SAMOUR, 2006; OGNEAN and CERNEA, 2011, Johnson and Harison, 1996). Using the same hemocytometer, after counting the red blood cells, we count the leukocytes, arranged in the 4 squares of order I in the corners of the hemocytometer.

By applying the calculation formula {*Number of cells obtained / 100 = Number of erythrocytes}*, the total number of leukocytes is determined, dividing the sum of the cells found at 20, with the result being expressed in G / l (giga / liter) (GHERGARIU et al., 2000 ; SAMOUR,

2006; OGNEAN and CERNEA, 2011), or the total number of leukocytes is multiplied by 200 to obtain the number of leukocytes per microliter of blood.

5) Hemoglobin estimation is based on colorimetric methods that require erythrocyte lysis. This determination is made difficult by the presence of the erythrocyte nucleus in the case of blood samples from birds (HAWKEY and SAMOUR, 1988; SAMOUR, 2006; Campbell et al., 2007). In order to determine the hemoglobin concentration in birds, colorimetric method and semi-automatic or automatic methods can be used. Semi-automatic spectrophotometric method (OGNEAN AND CERNEA, 2011): 4 ml of ammonia solution is distributed in the analysis containers and 20 µl of blood will be sucked into the container. The sample thus prepared is homogenized for 3 minutes. About 3 ml of the resulting solution is decanted into the cuvette and the reading is made using the ammoniacal solution as a blank. The result obtained is multiplied by the correction factor 33 for the expression in g / dl as a unit of measure (SAMOUR, 2006).

6) Mean Corpuscular Values (Red cell absolute values) were made based on the use of the known calculation formulas.

MCV (mean corpuscular volume) is an index of cell size and represents the volume occupied by a single red blood cell. This index is calculated using the formula: *MCV = [PCV (%) / Nr. of red blood cells ($x10^6$ / µl)] x10}*

In addition to other red blood cell counts, MCV allows early detection of anemia. MCV depends on plasma osmolarity and erythrocyte division rate and has as a unit of measurement femtoliters (fl; 10–15 liters) (Duguy, 1970; Ghergariu et al., 2000; SAMOUR, 2006; Ognean and Cernea, 2011).

MCH (mean corpuscular hemoglobin) is a color index, which refers to the average amount of red blood cell hemoglobin. This parameter is determined using the formula: *{HEM = [Hemoglobin (g / dl) / Nr. of red blood cells (T / l)] x10}*

Together with MCHC and other erythrocyte indices it helps in the differential diagnosis of different types of anemia being expressed in pg (pico gram) as a unit of measure (Ghergariu et al., 2000; Samour, 2006; Ognean and Cernea, 2011).

MCHC (mean corpuscular hemoglobin concentration) represents the average hemoglobin concentration in a given volume of erythrocytes (or the ratio of hemoglobin quantity to red blood cell volume). This parameter is determined according to the formula: *{CHEM = [Hemoglobin (g / dl) / Hematocrit (%)] x100}*

As a unit of measure it is expressed in g / dl (grams / deciliter) (Ghergariu et al., 2000; Samour, 2006; Ognean and Cernea, 2011).

7) Reticulocyte count. Reticulocytes are immature red cells that can be counted in blood smears stained with methylene blue (methylene blue), which stains residual cytoplasmic RNA in dark blue. Most mature avian red cells also have some residual cytoplasmic RNA, so in birds, the term "reticulocytes" should only be used for cells with a distinct ring of RNA surrounding the nuclei. The number of reticulocytes varies between 1 and 5% in healthy birds, and their increased number indicates red cell regeneration. This parameter also offers a more accurate idea of regenerative response than polychromacy (Duguy, 1970; Campbell, 1995; Baumann et al., 1983; Johnson and Harison, 1996).

8) Determination of platelet parameters

Trombocyte count - evaluation on smear. An estimate of platelet count can be obtained using blood smears prepared as for differential counting, as follows:

If PVC has a value in the range of 40-50%: *{No. Total platelets = no. of platelets found in 5 microscopic fields x 3500}*

If the hematocrit value is not in the range of 40-50%: *{No. Total platelet count = estimated TTC x normal Ht}*

Normally, there should be 2-3 platelets (10-15 / 1000 erythrocytes) on the microscopic field when viewed with the 100x objective under oil immersion. Increased platelet counts may indicate chronic disease (Duguy, 1970; Campbell, 1995).

Bibliographic references regarding the values of the hematological profile in broiler chickens

The analysis of the reference values found in the consulted bibliographic sources indicated wide physiological intervals for most of the erythrocyte parameters, known being their high degree of heterogeneity. Regarding leukocyte parameters, bibliographic sources also presented extremely varied reference intervals.

A number of physiological influences, including age, sex and season, can influence the haematological characteristics of birds.

The bibliographic data showed in the meat breeds, lower values of the hematocrit (30.10- 30.90%), compared to the white race Leghorn (31.90-33.90%). The average hematocrit of the Leghorn SPF strain (specifically pathogen free) within 5-42 days after hatching, ranged from 32.70% to 36.70% (Bounous and STEDMAN, 2000).

The hematocrit values in birds range from 35 to 55%. An Ht lower than 35% suggests anemia and more than 55% indicates dehydration or polycythemia. Within the same species, Ht varies according to age, sex, hormones and other physiological factors. For example, larger males and birds tend to have a higher Ht (Campbell, 1995; Kass et al., 2002; Campbell et al., 2004).

The presence of immature erythrocytes (eg rubricitis) in the peripheral blood together with an increase of polychromatosis indicates a marked regenerative response. Hypochromatosis may be associated with certain nutritional deficiencies in birds, especially iron deficiency. Polycythemia is rarely reported in birds and is usually associated with dehydration in birds; however, absolute polycythemia may also occur. Conditions often associated with absolute polycythemia in mammals are expected to be the cause of this disease and in birds (Campbell, 1995).

Table 1. Reference values for chickens found in the consulted bibliography

Sursa bibliografică	Ht (%)	Hb (g/dl)	Eritrocite (T/l)	Constante eritrocitare medii		
				VEM (fl)	HEM (pg)	CHEM (g/dl)
Pintea et al. 1982	-	8,00-9,50	-	-	15,53	29,00
Kolb, 1974	-	-	3,50 (3,00-4,00)	-	-	-
Gulland şi Hawkey, 1990	39,50 (30,00-49,00)	12,60 (10,20-15,10)	3,20 (2,50-3,90)	119,50 (104,00-135,00)	37,90 (32,00-43,90)	33,20 (30,20-36,20)
Bounous şi Stedman, 2000	22,00-35,00	7,00 – 13,00	2,50-3,50*	90,00-140,00	33,00-47,00	26,00-35,00
Ghergariu et al. 1999	32,00-48,00	10,20-10,60	3,00-4,50	-	-	-
Wallach şi Boever, 1983	24,00-43,00	8,90-13,50	2,20-3,30	120,00-137,00	-	-
*Trâncă, 2013	39,56	9,04	2,46	167,19 (72,25-285,71)	37,37 (18,48-96,56)	23,10 (10,27-53,63)

***Ahmed M. Al-Nedawi, 2018**	**34,70 (25,00-45,00)**	**10,38 (5,58-15,14)**	**3,14 (2,40-3,90)**	**113,44 (72,97-173,85)**	**36,84 (19,60-58,69)**	**32,29 (21,48-34,84)**
Douglas et al. 2010	**22,00-35,00**	**7,00-13,00**	**2,50-3,50**	**90,00-140,00**	**33,00-47,00**	**26,00-35,00**

***** In the experiment conducted by Trâncă, the studies were carried out on a number of 10 experimental lots, each containing 15 broiler chickens from the Ross 308 line. The birds included in the study came from 2 commercial intensive breeding farms, from Hunedoara and Mures counties. Chickens aged 9-14 days and weighing at least 250 grams were selected, which were maintained and fed under conditions similar to those from the farms of origin.

***** In the experiment conducted by Ahmed M. Al-Nedawi, the studies were performed on 80 broiler chickens, Ross 308, aged 35 days. The chickenss received water and food *ad libitum*.

There is a wide variation in normal leukograms in birds of the same species. Preparing normal reference values for healthy individual birds is the best method for evaluating the parameters of a bird's blood during the disease (Campbell, 1995).

General causes of leukocytosis include infections (general or localized), trauma, toxicities, bleeding in the body cavity, fast-growing neoplasms and leukemias. Because leukocytosis is often caused by inflammation, heterophilia is usually present. Idiopathic eosinophilia occur sporadically in birds and further research is needed to clarify the significance of this condition. As with avian eosinophils, the exact function of basophils in birds is not known. Because basophils appear to play a role in early inflammation and possibly hypersensitivity reactions in birds, a basophilia in peripheral blood may suggest the presence of these conditions (Campbell, 1995, Kass et al., 2002; Campbell şi colab, 2004).

Avian platelets play a major role in hemostasis in a manner similar to mammalian platelets. They may also have a phagocytic function and may participate in the removal of foreign material from the blood. A normal platelet count of between 20,000 and 30,000 / µl of blood or 10-15/1000 red blood cells can be used as a general reference for most birds (Campbell, 1995, Campbell şi colab, 2004).

Table. 2. Reference values for leukocyte parameters in chickens

Sursa bibliografică	Leuc. (G/l)	Heter (%)	Eoz. (%)	Bazof. (%)	Limfo. (%)	Mono. (%)
Gylstorff, 1983	**19,80-32,60**	**19,80-32,60**	**1,50-2,70**	**1,70-4,30**	**45,00-75,00**	**8,10-16,50**
Hoffmann, 1961	**20,00-30,00**	**13,00-49,00**	**2,00-14,00**	**1,00-17,00**	**31,00-72,00**	**1,00-4,00**
Pîrvu et al. 1984	**13,00-32,00**	**20,00-31,00**	**1,00-3,50**	**1,50-4,00**	**50,00-70,00**	**6,00-11,00**
Ghergariu et al. 2000	**25,00**	**25,00**	**4,00**	**2,00**	**62,00**	**4,00**
Uray, 1992	**-**	**13,00-35,00**	**1,20-2,50**	**2,10-3,10**	**58,00-76,00**	**1,10-6,50**
Bedáňová et al.2007	**-**	**14,50**	**0,60**	**0,50**	**83,70**	**0,80**
Peebles et al. 2004	**-**	**24,00**	**0,60**	**1,40**	**72,00**	**2,00**
Branton et al. 1997	**-**	**30,10**	**2,50**	**1,30**	**63,00**	**3,10**
Latimer et al. 1988	**-**	**17,80**	**2,80**	**2,30**	**70,10**	**7,10**
Altan et al. 2000	**-**	**7,50**	**3,70**	**2,30**	**73,50**	**2,90**
Trâncă, 2003	**18,28**	**48,85**	**2,40**	**0,39**	**36,77**	**11,59**
Douglas et al. 2010	**12,00-30,00**	**-**	**-**	**-**	**-**	**-**

According to the synthesis of the reference works in the consulted literature, there is still much controversy regarding the physiological values of the biochemical indices in broiler chickens, the available data mainly referring to adult chickens and less to chickens.

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