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Haematological variables in sport birds, common goose, domestic duck, Aztec duck, turkey and broiler chicken

Variables hematológicas en aves deportivas, ganso común, pato doméstico, pato azteca, guajolote y pollo de engorda

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ABSTRACT

Blood analysis in birds is an evidence that allows evaluating their health status and it is a diagnostic tool for the clinical veterinarian. The objective of the research was to know the hematological variables of common birds, sport birds, common goose, domestic duck, Aztec duck, turkey and broiler chicken by sex, species and the difference between species. A blood sample was taken from 88 birds by venipuncture of the ulnar vein using Ethylenediamine tetra-acetic acid (EDTA) as anticoagulant. Erythrocytes and total leukocytes were counted by the method of Natt and Herriks. Leukocyte differential was performed by blood smear, hemoglobin concentration was measured by the cyanmethemoglobin technique and microhematocrit percentage. Data were compared with an analysis of variance in a completely randomized design and differences were compared with Tukey's test ($P<0.05$). For eosinophils and basophils, the Kruskal-Wallis test was used because the data did not present normality. The hematological variables in sport birds, common goose, domestic duck, Aztec duck, turkey and broiler chicken were similar for females and males of the same species. But there were differences in white cells of the species studied.
Keywords: domestic fowl, hematology, Natt and Herriks.

RESUMEN

El análisis sanguíneo en aves es una evidencia que permite evaluar su estado de salud y es una herramienta diagnóstica para el médico veterinario clínico. El objetivo de la investigación fue conocer las variables hematológicas de aves comunes, deportivas, ganso común, pato doméstico, pato azteca, guajolote y pollo de engorda por sexo, especie y la diferencia entre las especies. Se tomó una muestra de sangre a 88 aves por venopunción de la vena ulnar usando ácido etilendiaminotetraacético (EDTA) como anticoagulante. Se contaron los eritrocitos y los leucocitos totales por el método de Natt y Herriks. El diferencial de leucocitos se realizó por medio de un frotis sanguíneo, se midió la concentración de hemoglobina con la técnica de cianometahemoglobina y el porcentaje de microhematocrito. Los datos fueron comparados con un análisis de varianza en un diseño completamente al azar y las diferencias se compararon con la prueba de Tukey ($P<0.05$). Para eosinófilos y basófilos se utilizó la prueba de Kruskal-Wallis debido a que los datos no presentaron normalidad. Las variables hematológicas en aves deportivas, ganso común, pato doméstico, pato azteca, guajolote y pollo de engorda fueron similares para hembras y machos de la misma especie. Pero hubo diferencias en células blancas de las especies estudiadas.
Palabras clave: aves domésticas, hematología, Natt y Herriks.

INTRODUCTION

In Mexico, sport birds, common goose, domestic duck, Aztec duck, turkey and broiler chicken are found in commercial farms, small backyard farms, animal reserves or they are even adopted as pets (Cuca-García *et al*., 2015; Pineda-Leyva *et al*., 2015). Knowledge of their hematological parameters is a general tool to know their health status with basic uses in avian clinic and scientific research (Mitchell & Johns, 2008).

Blood is a tissue that consists of a liquid part and cellular components; it is mainly responsible for capturing, transporting and distributing nutrients in the organism through blood vessels. Its evaluation is performed through a hemogram where the population of erythrocytes and leukocytes is expressed, in addition, it allows identifying morphological alterations and assessing its function (Campbell, 2013; Montalvo, 2017).

In birds and reptiles the hemogram is performed manually due to their nucleated erythrocytes and thrombocytes. In addition, leukocytes present multiple forms and cell size is different compared to mammals, so the total count of red (RCC) and white (WCC) cells is direct through a hematocytometer (Martínez-Silvestre *et al.,* 2011). To facilitate their counting, Natt and Herriks solution is used, which stains the cells improving their contrast; for leukocytes a general result is obtained and to know the specific population a blood smear is performed (Campbell, 2013). To identify heterophils (Het), eosinophils (Eos), basophils (Bas), lymphocytes (Lymp), monocytes (Mon) and thrombocytes (Throm) (Martinho, 2009).

Indirectly it evaluates the red series by measuring the percentage of hematocrit (Ht) which shows the proportion of red blood cells present in the blood, it also reports alterations in the serum such as hemolysis, jaundice or lipaemia, a low hematocrit may suggest anemia, dehydration or polycythemia (Agustí, 2015). Hemoglobin (Hb) is a protein contained in erythrocytes and gives the red color to blood, serves to transport 02 and if the amount is reduced it indicates (hypochromic), if it is normal (normochromic) and therefore its functioning (Gálvez *et al.*, 2009). In birds heterophilia and lymphopenia are indicators of stress, immunosuppression and active infections (Garbus *et al*., 2019). However, in birds hematological variables can be modified by different factors such as age, bird breed, species location and migratory behavior (Pistone *et al*., 2017; Bílcová *et al*., 2017). Therefore, it is necessary to know the hematological parameters by geographical area and the objective of the research was to know the hematological variables of the most common species of domestic birds in order to have clinical reference values.

MATERIAL AND METHODS

Location of the study

Blood samples were collected from birds obtained in Irapuato municipality, Guanajuato, Mexico, located at 1,715 m a.s.l, with a warm sub-humid climate and summer rainfall (INEGI, 2017). Samples were analyzed at the Poultry Laboratory of the University of Guanajuato, at the Life Sciences Division, Campus Irapuato- Salamanca, km 9 Irapuato-Silao highway in Irapuato, Guanajuato.

Blood sampling
Venipuncture of the ulnar vein was performed in all birds, with prior asepsis of the area with alcohol swabs, using 3 mL syringes and 23G gauge needles, 1 mL of blood was collected in vacutainer® tubes with EDTA.

Birds
A convenience sampling of 88 adult birds was carried out, of which 20 were sporting birds (*Gallus gallus*) 10 females and 10 males, 20 turkeys (*Meleagris gallopavo*) 10 males and 10 females, 8 Aztec ducks (*Cairina moschata*) 5 males and 4 females, 20 domestic ducks (*Anas platyrhynchos*) 10 males and 10 females, 20 geese (*Anser anser*) 10 females and 10 males, 20 broilers (*Gallus gallus domesticus*), 10 females and 10 males.

Blood smear
Commercial slides were used for the smearing by sliding the blood on the slide and air-drying for subsequent staining with Dip Quick stain®.

Method for counting blood cells
Blood cell counting was performed with the aid of a Neubauer chamber (Marienfeld, Germany) using Natt and Herriks solution. The following formulas were used to obtain the total number of cells:

$$RCC\ (mm3)\ =\ H\ (red\ cells\ counted)\ x\ 5\ x10\ x\ D\ (dilution\ factor\ 1{:}200).$$
$$WCC\ (mm3)\ =\ L\ (leukocytes\ counted)\ /\ 4\ x\ 10\ x\ D\ (dilution\ factor\ 1{:}20).$$

Leukocyte differential
To perform the leukocyte differential, 100 cells were counted, observing with the 100 x objective with immersion oil, following a zigzag pattern of observation, to obtain more accurate results of the white cell population.

Hematocrit
The capillary was placed in the centrifuge (TG12M, microhematocrit centrifuge) horizontally, leaving the sealed end facing out, programmed at 2500 rpm for 10 min and the following formula was used to obtain the percentage: $Ht\ (\%)\ =\ L2\ (red\ cells\ in\ mm)\ /\ L1\ (red\ cells\ +\ plasma\ in\ mm)\ *\ 100$ (Campbell, 2013).

Hemoglobin
5 mL of Drabkin (Hycel) reagent (cyanmethemoglobin) and 20 µL of blood with anticoagulant were placed in an amber bottle, mixed gently to homogenize, allowed to stand for 5 minutes and absorbance was measured at 540 nm in a spectrophotometer (Epoch, Biotech), the result was multiplied by 36.77 to obtain the hemoglobin concentration in g/dL (Samour, 2007).

Statistical analysis

An analysis of variance was performed with a completely randomized design (P<0.05), where erythrocyte, leukocyte, heterophil, lymphocyte, monocyte, thrombocyte, hematocrit and hemoglobin counts in birds were considered as dependent variables and sex and species as independent variables. The Kruskal-Wallis test was used for eosinophils and basophils because the data did not show normality. Subsequently, a multiple comparison of means was performed with the Tukey method (P<0.05) with the statistical program statgraphics centurion XV.

RESULTS

Hematological variables in sporting birds, common goose, domestic duck, Aztec duck, turkey and broiler chicken were similar for females and males of the same species (Table 1).

Females

In Table 1, the number of total erythrocytes of the common goose was lower when compared with that of the domestic duck, Aztec duck and turkey (P<0.05); likewise, the leukocyte count in sporting birds was higher (P<0.05) than in the common goose and domestic duck. In the leukocyte differential, the number of heterophils was similar among the six species. On the other hand, eosinophils presented a greater difference (P<0.05) between the common goose and turkey goose. The percentage of basophils was higher in domestic duck (P<0.05) in relation to sporting birds and broiler chickens. The lymphocyte index in sport birds and broiler chickens was higher than that recorded for domestic ducks, Aztec ducks and turkey (P<0.05). The number of monocytes in turkey was more than that observed in broiler chicken, sport bird and common goose (P<0.05). In addition, the highest hematocrit was observed in the Aztec duck (P<0.05) and the lowest in the broiler chicken, turkey and common goose. The presence of thrombocytes increased in the common goose and Aztec duck (P<0.05) compared to the sport birds, the hemoglobin concentration was higher in the Aztec duck (P<0.05).

Males

In Table 1 the red cell count in sport birds and domestic duck was higher (P<0.05) than that observed in common goose and broiler chicken, but in white cells the domestic duck and turkey were higher (P<0.05) compared to common goose and sport bird. On the other hand, in the leukocyte differential, the values of heterophils, basophils and monocytes were similar for the six species studied. Eosinophils were more frequent in the domestic duck (P<0.05) compared to the turkey. The number of lymphocytes was higher in sport birds (P<0.05) in relation to the common goose, domestic duck, Aztec duck and broiler chicken. The percentage of hematocrit was higher in sport birds (P<0.05) compared to the common goose, domestic duck, turkey and broiler chicken. Thrombocytes were higher in common goose, domestic duck and Aztec duck (P<0.05), followed by sport birds and turkeys, the lowest amount (P<0.05) was in broiler chicken. The hemoglobin concentration was higher in the Aztec duck (P<0.05) than in the other species.

Table 1. Hematological parameters in females and males of sport bird, common goose, domestic duck, Aztec duck, turkey and broiler chickens

Parameters %	Sport bird	Common goose	Domestic duck	Aztec duck	Turkey	Broiler chickens	ES
			Females				
RCC mm^3	337.3 ± 49.1 ^{bc}	313.0 ± 55.6 ^{c}	437.9 ± 77.8 ^{a}	461.0 ± 83.9 ^{a}	417.8 ± 83.9 ^{ab}	361.7 ± 38.9 ^{abc}	20.6
WCC mm^3	565.9 ± 176.9 ^{a}	271.5 ± 92.2 ^{c}	356.2 ± 147.7 ^{bc}	405.2 ± 72.9 ^{abc}	442.2 ± 187.2 ^{abc}	540.5 ± 145.1 ^{ab}	47.9
Het	19.4 ± 7.6 ^{a}	27.8 ± 11.1 ^{a}	27.6 ± 8.5 ^{a}	31.7 ± 4.5 ^{a}	25.7 ± 5.9 ^{a}	22.4 ± 5.3 ^{a}	2.4
Eos	1.2 ± 1.6 ^{ab}	3.3 ± 2.6 ^{a}	2.9 ± 3.3 ^{ab}	1.0 ± 1.1 ^{ab}	0.2 ± 0.6 ^{b}	1.5 ± 1.9 ^{ab}	0.6
Bas	0.0 ± 0.0 ^{b}	0.2 ± 0.7 ^{ab}	1.0 ± 1.1 ^{a}	0.0 ± 0.0 ^{ab}	0.3 ± 0.9 ^{ab}	0.0 ± 0.0 ^{b}	0.2
Lymp	40.0 ± 11.5 ^{a}	28.3 ± 15.1 ^{ab}	19.4 ± 6.9 ^{b}	21.0 ± 5.9 ^{b}	21.4 ± 10.8 ^{b}	39.5 ± 8.4 ^{a}	3.3
Mon	39.1 ± 9.5 ^{bc}	40.2 ± 7.0 ^{bc}	49.2 ± 6.3 ^{ab}	46.2 ± 7.8 ^{abc}	52.4 ± 7.8 ^{a}	36.6 ± 8.2 ^{c}	2.5
Ht	46.6 ± 5.7 ^{ab}	41.8 ± 3.9 ^{bc}	47.4 ± 4.4 ^{ab}	54.6 ± 2.0 ^{a}	45.1 ± 4.0 ^{bc}	39.4 ± 4.1 ^{c}	1.4
Thromb µL	33.3 ± 5.9 ^{b}	43.2 ± 6.7 ^{a}	39.1 ± 6.7 ^{ab}	45.0 ± 7.6 ^{a}	39.8 ± 5.5 ^{ab}	40.7 ± 4.3 ^{ab}	1.9
Hb g/dL	11.9 ± 2.9 ^{c}	16.8 ± 5.5 ^{b}	15.0 ± 2.2 ^{bc}	32.4 ± 2.6 ^{a}	12.1 ± 0.9 ^{c}	14.0 ± 1.8 ^{bc}	0.9
			Males				
RCC mm^3	440.9 ± 89.8 ^{a}	318.5 ± 54.9 ^{cd}	426.7 ± 96.4 ^{a}	424.2 ± 73.0 ^{ab}	358.5 ± 55.6 ^{ab}	257.2 ± 48.3 ^{d}	22.7
WCC mm^3	234.8 ± 63.0 ^{bc}	221.7 ± 57.5 ^{c}	424.4 ± 96.8 ^{a}	335.2 ± 117.0 ^{abc}	434.6 ± 120.1 ^{a}	379.6 ± 167.4 ^{ab}	34.6
Het	22.6 ± 9.4 ^{a}	21.3 ± 8.3 ^{a}	28.9 ± 9.1 ^{a}	29.8 ± 3.1 ^{a}	23.9 ± 10.8 ^{a}	23.6 ± 8.8 ^{a}	2.8
Eos	0.4 ± 0.8 ^{ab}	2.3 ± 2.9 ^{ab}	2.5 ± 2.4 ^{a}	0.8 ± 1.0 ^{ab}	0.0 ± 0.0 ^{b}	0.3 ± 0.6 ^{ab}	0.5
Bas	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	1.2 ± 2.2 ^{a}	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	0.0 ± 0.0 ^{a}	0.2
Lymp	37.9 ± 12.1 ^{a}	27.1 ± 17.2 ^{b}	18.9 ± 8.4 ^{b}	22.4 ± 7.1 ^{b}	28.3 ± 12.5 ^{ab}	21.6 ± 6.3 ^{b}	3.6
Mon	38.9 ± 11.3 ^{a}	48.9 ± 16.1 ^{a}	48.6 ± 10.1 ^{a}	47.0 ± 6.0 ^{a}	47.9 ± 11.2 ^{a}	54.4 ± 12.2 ^{a}	3.8
Ht	54.1 ± 4.9 ^{a}	40.4 ± 3.4 ^{cd}	45.0 ± 3.3 ^{bc}	51.2 ± 4.3 ^{ab}	46.4 ± 4.9 ^{b}	34.6 ± 5.4 ^{d}	1.4
Thromb µL	34.5 ± 4.8 ^{b}	41.5 ± 3.6 ^{a}	42.4 ± 7.1 ^{a}	42.6 ± 1.8 ^{a}	33.8 ± 5.0 ^{b}	19.9 ± 2.1 ^{c}	1.4
Hb g/dL	16.1 ± 2.9 ^{b}	17.3 ± 6.0 ^{b}	14.3 ± 1.8 ^{b}	33.5 ± 4.1 ^{a}	14.0 ± 2.29 ^{b}	14.4 ± 1.2 ^{b}	1.0

$^{a-d}$ Different literals per row refer to significant statistical difference (P<0.05).
RCC mm^3= Total erythrocytes per mm^3, WCC mm^3= Total leukocytes per mm^3, Het= heterophils, Eos= Eosinophils, Bas= Basophils , Lymp= Lymphocytes, Mon= Monocytes, Ht= Hematocrit, Thromb µL = Thrombocytes per µL, Hb g/dL= Hemoglobin in g per dL.

DISCUSSION

Hematological variables are basic elements to know and evaluate the general health of birds, knowing the parameters by species and the differences that females have compared to males allows identifying imbalances in their cell populations (Fairbrother & O´Loughlin, 1990; Mitchell & Johns, 2008). Okeudo *et al*. (2003), Aengwanich & Tanomtong (2007), Azeez *et al*. (2011), Albokhadaim (2012), Lashev *et al*. (2015) report differences in total erythrocytes, hemoglobin and hematocrit related to the sex of the bird. In males the index increases and in females it decreases due to the level of testosterone which has direct effect on erythropoiesis Gonzales, 2011; Campbell, 2015). Fairbrother & O´Loughlin (1990), Moreira *et al*. (2009) and in the present study the hormonal effect was not significant, but showed similar trends. It is possible that there are bird species that can manifest in this way and others do not present the same effect as sexual dimorphism in birds.

Total erythrocyte count

Erythrocytes transport and distribute oxygen and carbon dioxide in the body (Scanes, 2015). Okeudo *et al*. (2013) report less RCC compared to our study in female and male Azure ducks. Campbell (2015) mentions that ducks have higher RCC values compared to geese due to photoperiod. Pineda-Leiva *et al*. (2015) obtained lower values in fighting males but Moreira *et al*. (2009) describe higher values than those observed in our study for female and male turkey geese. The amount of erythrocytes may be associated with the type of feeding in domestic species (Lashev *et al.*, 2015). In addition, variation may occur between species due to the breeding season and the relationship between their weight and metabolic rate (McKechnie, 2007).

Total leukocyte count

Leukocytes defend the body from pathogens or foreign agents Galvez *et al*., 2009). Lashev *et al*. (2015) observed variation of this cell line related to vaccines, diet or hygienic conditions in the poultry house. Pineda-Leyva *et al*. (2015) obtained lower WCC in fighting males. In our results both broiler females and males exceeded the average observed by Scanes (2015). On the other hand, Moreira *et al*. (2009) reported a higher WCC in females and males of turkey.

Azeez *et al*. (2011) have described high WCC counts in young birds as it is for broiler chicken in this study indicating the differences with the other species may be due to age and immature hematopoiesis. Foo *et al.* (2017) indicate that secondary sexual characteristics in species are developed by testosterone level which in birds has immunomodulatory effect. Therefore, the difference between species may be because of the development of plumage, their color and even the degree of aggressiveness they may manifest.

Leukocyte differential

It is a study that allows distinguishing the types of leukocytes to more accurately interpret the hemogram (Galvez *et al*., 2009). Lashev *et al*. (2015) did not observe differences between quail, combat birds, broiler chicken, turkey and pheasant. The parameters observed in the present study were lower than the averages reported by Scanes (2015), except, in monocytes which are present in higher amounts. Okeudo *et al*. (2003) report fewer heterophils in female and male Aztec duck, higher eosinophils and lymphocytes in higher numbers compared to ours. Oloyemi & Arewolo (2009) report higher parameters of lymphocytes, heterophils and lower monocytes and eosinophils in domestic ducks. Heterophils and lymphocytes are predominant

leukocytes associated with stressors, infections, inflammation or toxicity (Davis *et al*., 2008; Jones, 2015). The dominant behavior of the species can keep it under constant stress and determine its levels in the H:L ratio (Valdebenito *et al*., 2021). Differences between cares, feeding, deworming of each species can modify their hematological parameters (Campbell, 2015; Lashev *et al*., 2015).

Hematocrit

This variable reflects the volume occupied by red blood cells relative to whole blood (Galvez *et al*., 2009). According to Lashev *et al*. (2015) its quantity is directly related to erythrocyte and hemoglobin count, but it has Abdi-Hachesoo *et al*. (2011) reported difference between females of the same species associated with the height of residence. Okeudo *et al*. (2003) observed lower Ht in female and male Aztec ducks compared to our study. Moreira *et al*. (2009) described lower Ht in females and males of turkey than in the present study. Our parameters for this variable correspond to those established by Campbell (2015) for broiler chicken, turkey and domestic duck. Likewise our percentage observed in male sport bird is similar to the control group of Pineda-Leyva *et al*. (2015), also Oloyemi & Arewolo (2009) point out a similar amount of this variable in domestic duck in non-rainy season. It is possible that the percentage of Ht may be different between species due to their water consumption habits (Oloyemi & Arewolo, 2009), body mass, adaptation to environmental conditions and their metabolic rate (McKechnie, 2007).

Hemoglobin

Hemoglobin is a protein that transports oxygen to tissues. Okeudo *et al*. (2003) observed a lower concentration in Aztec duck than in our results. On the other hand, our parameters for broiler chicken, domestic duck and turkey are within Campbell (2015). Moreira *et al*. (2009) report in turkey a higher Hb concentration compared to this study, in the same way Pineda-Leyva *et al*. (2015) point out higher Hb concentration in fighting males. The aforementioned difference lies in that the Ht responds to acclimatization due to the difference in altitude above sea level and to the level of testosterone present in the different bird species that responds to erythropoiesis (Gonzales, 2011). Hemoglobin can vary according to the availability of iron in the diet and the intestinal capacity for its absorption (Tako *et al*., 2010).

CONCLUSION

Hematological variables in sport birds, common goose, domestic duck, Aztec duck, turkey and broiler chicken were similar for females and males of the same species. They showed differences in white cells among the species studied, because of the findings, it is necessary to continue studying blood cells between species due to locality, feeding and sexual dimorphism.

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