Erythrogram and erythrocytes measurement of ostriches (*Struthio camelus*) in São José do Rio Preto-SP, Brazil

Eritrograma e medição dos eritrócitos de avestruzes (Struthio camelus) em SãoJosé do Rio Preto-SP, Brasil

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Abstract

Ostriches' erythrocytic parameters help on the diagnosis of specific pathologies and serve as basic knowledge for studies in comparative avian pathology. To obtain reference values of erythrocyte indices for ostriches (*Struthio camelus*) raised in a commercial system in Brazil and verify if there are differences between gender and age groups, 240 healthy from both sexes animals were bled. Heparinized blood samples were analyzed using standard techniques to determine the red blood cell (RBC) count, hemoglobin concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) values, besides the red cell morphometry analysis using a computer software that calculates the greater and the smaller diameters of the erythrocytes. Prior to data analysis, ostriches were divided into three different age groups: from four to 13 months, from 13 to 23 months and from 23 to 30 months the. Younger ostriches presented lower erythrocyte indices than the older ones. The age group effect was only significant in females for the erythrocyte, hemoglobin, MCV, MCH, and MCHC variables. The female ostriches presented PCV, MCV, MCH, and MCHC values significantly higher than males in some age groups. It was observed that the erythrocytes of the female ostriches are more elongated and larger than males. It was concluded that erythrocytic parameters of ostriches in São José do Rio Preto-SP, Brazil are influenced by gender and age, highlighting the importance of consider besides these factors also the geoclimatic conditions to an adequate interpretation of the erythrogram.

Keywords: Avian influenza. Depopulation. Foam. Mechanical hypoxia. Poultry.

Resumo

Os parâmetros eritrocitários de avestruzes auxiliam no diagnóstico de afecções específicas, além de servir como conhecimento básico no estudo de patologia comparativa das aves. Para obtenção de valores de referência dos índices eritrocitários de avestruzes (*Struthio camelus*) criados em um sistema comercial no Brasil e verificar se existem diferenças relativas ao sexo e faixas etárias, foram colhidas amostras sanguíneas de 240 animais saudáveis e de ambos os sexos. Amostras sanguíneas heparinizadas foram analisadas utilizando técnicas-padrão para determinar a contagem de eritrócitos, concentração de hemoglobina, hematócrito, volume corpuscular médio (VCM), hemoglobina corpuscular média (HCM), concentração de hemoglobina corpuscular média (CHCM) além da análise morfométrica dos eritrócitos utilizando um programa de computador que calcula os diâmetros maior e menor dos eritrócitos. Para a análise dos dados os avestruzes foram divididos em três diferentes faixas etárias: de quatro a 13 meses; de 13 a 23 meses e de 23 a 30 meses. De modo geral, avestruzes jovens apresentaram índices eritrocitários inferiores aos dos animais mais velhos. Diferenças relativas à idade só foram significativamente relevantes em fêmeas para os valores de eritrócitos, hemoglobina, VCM, HCM e CHCM. As avestruzes fêmeas apresentaram valores de hematócrito, VCM, HCM e CHCM significantemente maiores que os machos em algumas faixas etárias. Os eritrócitos de avestruzes fêmeas são mais compridos e largos do que os de machos. Pode-se concluir que os parâmetros eritrocitários de avestruzes em São José do Rio Preto-SP, Brasil estão sob influência do sexo e da idade, ressaltando a importância de considerar além desses fatores, também as condições geoclimáticas para uma interpretação adequada do eritrograma.

Palavras-chave: Hematologia. Eritrócitos. Valores de referência. Avestruzes.

Introduction

Ostrich industry in Brazil is considered relatively recent (about ten years), the national flock has been increasing and its estimated number is about 200,000 birds. Even so, struthioculture in the region of São

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José do Rio Preto is one of the most important in the country[1]. As a result of high commercial value and great longevity of ostriches, there is an increasing interest in establishing hematologic reference values for the early diagnose of diseases and nutritional disturbances that affect this specie. Although clinical hematology is a useful diagnostic tool in avian medicine, interpretation is dependent on established baseline values for the species. Furthermore, these parameters may be influenced by climatic conditions prevalent in the particular geographic location and by different management systems[2]. Hematological parameters of birds are influenced by several factors such as specie, sex, age, diet and management systems[3].

There is a paucity of information about the hematology of ostriches[4,5,6,7,8,9], with divergent data probably due to the small number of analyzed samples without defining gender and geographic or climatic conditions of animals. Also, the lack of standardization in blood sample collection and analysis contribute for such divergences, since some authors used heparin[4,2], citrate[7], or EDTA[5,6]. Hematology is an essential tool to understand the physiology of animals; the hemogram reveals the animal general health and is crucial for follow-up and diagnoses of diseases[10].

The purpose of this study was to establish reference ranges for erythrocyte indices and verify age and gender influences in young ostriches (four to 30 months of age) raised on a commercial farming system in the region of São José do Rio Preto, São Paulo State, Brazil.

Material and Method

Ostriches used in this study were raised on eight different commercial farms in São José do Rio Preto region (-20°49'11" S; 49°22'46" N), the region is 489 m above sea level, the soil is podzol and latosol (arenaceous phase), open pasture vegetation, tropical climate with an average temperature of 25.4 ºC and annual rainfall of 200 mm from October to March[11]. The management system of raising birds in all farms was similar: balanced ration, growing birds were put on pasture (15 x 50 m) with maximum capacity of 60 birds; fattening birds were put on pasture (15 x 50 m) with maximum capacity of 30 birds. Adult females were not in the laying period. Only healthy birds with good corporal condition at the moment of the blood samplings were included in this study. Thus 240 ostriches (132 females and 108 males) were grouped according to gender in three different groups: from four to 13 months (92 birds); 13 to 23 months (80 birds), and 23 to 30 months (68 birds).

Animals were physically restrained and black hoods over its heads were used to avoid stress during blood collection, blood samples were withdrawn using sterile disposable syringe and needle (0,70 x 25 mm) by venipuncture from jugular, brachial or external radial veins. Hematological analyses were performed using seven milliliters of blood placed in siliconized and heparinized tube (Vacutainer, BD, São Paulo, São Paulo State, Brazil). Samples were kept cooled (4 ºC) until the laboratory analysis for a maximum period of five hours after collection. Packed cell volume (PCV) was determined by microhematocrit centrifugation for five minutes at 11,827× *g*. Hemoglobin concentration was performed using a commercial kit with cyanmethemoglobin release principle (Drabkin's Liquid, Newprov, Maringa, Parana State, Brazil), duplicated analyses were realized with previous centrifugation of samples before spectrophotometrical reading (S*pectrophotometer* CELM E205D, São Paulo, São Paulo State, Brazil) at a wave length of 540 nm. Centrifugation is needed to avoid interference of the suspended nuclear material on spectrophotometrical measurement[3]. Red blood cell (RBC) count was determined in a Neubauer chamber using Natt-Herrick solution[3]. Mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were calculated[9]. Morphometric determination of erythrocytes was performed in images from blood smears stained

with panoptic dye (Instant Newprov, Parana State, Brazil). Images of blood smear were obtained with an optic microscope (LEICA DMLS, Wetzlar, Germany) at 400 X connected to a digital camera (LEICA DC 300, Heerbrugg, Schweiz) and analyzed by a computer image analysis software (Image-Pro®Plus, version 4.1, Media Cybernetics, Maryland, USA) configured to calculate maximum and minimum diameters of elliptical structures. The morphometric parameters according to sex and age were determined from the analysis of at least 2,000 erythrocytes (100 erythrocytes/bird).

All statistical analyses were performed using a computer software package (SAS/Software, Statistical Analysis System Institute, 1997, USA). Data were normally distributed as indicated by the *Kolmogorov-Smirnov test and the different group* data were submitted to ANOVA followed by *Tukey-Kramer test* for multiple comparisons. Differences were classified as significant if $P < 0.05$.

Results

The erythrocytic parameters are shown (mean ± standard deviation) in table 1.

Although in the male groups was observed an age-dependent increasing in the RBC number, hemoglobin concentration and PCV, these values were not significantly different ($P > 0.05$). Female ostriches younger than 13-month-old showed significantly

Table 1 – Eryrthrocytic values and measurement of ostriches (*Struthio camelus*) raised in commercial farm system in São José do Rio Preto-SP, Brasil, 2008. Data are presented as means ± standard deviation (95% confidence interval)

Measurement	Female			Male		
	Age (months)			Age (months)		
	4 – 13 (n=57)	13 – 22 (n=40)	22 – 30 (n=35)	4 – 13 (n=35)	13 – 22 (n=40)	22 – 30 (n=33)
Erythrocyte count (x10^{12}/L)	1.77 ± 0.35 [a] (1.68 - 1.89)	1.57 ± 0.21 [b] (1.50 - 1.64)	1.58 ± 0.23 [b] (1.50 - 1.66)	1.64 ± 0.39 [a,b] (1.50 - 1.78)	1.73 ± 0.28 [a,b] (1.64 -1.82)	1.71 ± 0.26 [a,b] (1.62 - 1.80)
Hemoglobin (g/L)	95.86 ± 11.34[a] (92.80 – 98.80)	107.05 ± 12.07 [b] (103.19 – 110.91)	109.70 ±11.86 [b,c] (105.65 – 113.80)	100.32 ± 12.83 [a,b] (95.72 – 104.74)	103.55 ±16.05 [a,b,c] (98.42 – 108.68)	107.64 ± 14.01 [b] (102.42 – 112.61)
PCV (L/L)	0.39 ± 0.03 [a] (0.38 - 0. 40)	0.40 ± 0.03 [a] (0.39 – 0.41)	0.39 ± 0.03 [a,b] (0.38 – 0.40)	0.36± 0.03 [b] (0.35 – 0.37)	0.38 ± 0.05 [a,b] (0.37 – 0.40)	0.39 ± 0.4 [a,b] (0.37 – 0.40)
MCV (fL)	227.7 ± 31.8 [a,c] (219.4 – 236.0)	261.7 ± 38.7 [b] (249.3 - 274.1)	252.9 ± 39.3 [b] (239.4 - 266.5)	234.4 ± 53.7 [c] (216 - 252.9)	228.5 ± 37.7 [c] (216.4 - 240.6)	233.9 ± 46.3 [c] (217.5 - 250.4)
MCH (pg)	99.39 ± 9.64 [a,c] (92.83 –105.95)	69.05 ± 11.46 [b,c] (65.38 – 72.72)	70.77 ± 12.51 [b] (66.47 – 75.07)	64.64 ± 18.18 [c] (58.39 – 70.89)	60.91 ± 13.04 [c] (108.60 - 124.60)	64.19 ± 13.39 [b,c] (59.44 – 68.94)
MCHC (g/L)	243.10 ± 24.22 [a] (236.68 – 248.54)	263.93 ± 23.57 [b] (256.40 - 271.48)	279.76 ± 24.58 [b] (271.32 – 288.22)	274.06 ± 31.31 [b] (263.23 - 284.89)	267.12 ± 38.10 [b] (254.94 – 279.31)	278.04 ± 49.30 [b] (266.55 – 295.53)
Cell length (µm)	16.47 ± 1.03 [a] (16.43 - 16.50)	16.27 ± 1.11 [b] (16.23 - 16.30)	16.54 ± 1.17 [a,c] (16.48 - 16.60)	16.41 ± 1.08 [a,d] (16.43 - 16.5)	15.68 ± 1.24 [e] (16.36 - 16.45)	16.0 ± 1.95 [e] (15.9 - 16.05)
Cell width (µm)	9.75 ± 0.76 [a] (9.72 - 9.77)	9.79 ± 0.79 [a] (9.76 - 9.81)	9.98 ± 0.79 [b] (9.93 - 10.02)	9.66 ± 0.75 [c] (9.62 - 9.69)	9.20 ± 0.83 [d] (9.16 - 9.23)	9.27 ± 1.90 [d] (9.15 - 9.38)
Cell length/cell width (µm)	1.70 ± 0.13 [a] (1.69 - 1.70)	1.67 ± 0.14 [b] (1.66 - 1.67)	1.67 ± 0.17 [c] (1.66 - 1.67)	1.71 ± 0.14 [a,d] (1.70 - 1.71)	1.71 ± 0.15 [a,d] (1.70 - 1.71)	1.79 ± 0.38 [e] (1.76 - 1.81)

[a,d] Within a row for a given variable, values with different superscript letters differ significantly ($P \geq 0.05$; Tukey-Kramer test)

lower RBC count, hemoglobin concentration, MCV and MCHC values, but higher MCH values than older female birds (13-30 months).

Significant differences in erythrocyte length were found between age and sex of the ostriches. Female ostriches had longer erythrocytes than males in the 13-30-month-age birds, in these groups was also possible to observe the higher length of the females erythrocytes. Considering the difference between age groups in females, the mean values for cell length in 13-22-month-age birds (15.68 μm) was significantly lower than others two age groups. In contrast, 22 to 30-month-old female birds had the longest erythrocytes (16.54 μm).

The erythrocyte width value was significantly higher in females than in males. With age, differently from males, the females showed a increasing in the erythrocyte width. Wider erythrocytes were observed in the female groups older than 22 months of age (9.98 μm), whereas the male group aged between 13-22 months had narrower erythrocytes (9.20 μm).

Discussion

Erythrogram values described in table 1, in general, are different from those values previously described[2,5,6]. The comparison of our results with those obtained in previous studies was compromised because authors had not considered sex[2,5] or age[8] of the birds.

RBC count and hemoglobin concentration in ostriches from five to 12-month-age birds were lower than those reported by others authors[4]. Lower PCV and higher MCV, MCH and MCHC values in 12-month-age birds were also observed.

RBC values were similar to those previously observed for ostriches aged between one to ten months, whereas PCV and MCV values were higher, and MCH and MCHC values were lower than the reported ones[2]. Compared with the same study, all erythrocytic values obtained were higher than those described for birds between 11 and 18-month-old, except for MCV values. RBC count, PCV and MCV values were similar to those observed in ostriches with age between ten and 60 months[5], however greater hemoglobin concentration, MCH and MCHC values were observed, that could be due to the lack of centrifugation of the samples before reading. On the other hand, in another study, RBC counts, PCV and MCV values were similar, while hemoglobin concentration, MCH and MCHC values were greater than those reported for ostriches of both sexes, and age varying from two to eight-month-old[6]. Unfortunately, gender influence in the different age groups on erythrocytic parameters was not evaluated in previous studies, which compromise comparisons among other findings in the literature.

Young ostriches presented lower erythrocytic values than adults, similar to others studies[2,4,6]. The increase in erythrocyte number and hemoglobin concentration according to age that were observed in some studies can be related to a crescent demand of oxygen during the growth phase[6]. Contrary to these findings, in the present study, RBC counts were significantly higher only in younger females than in older birds. This increased RBC count could be a compensatory effect due to the lower hemoglobin levels found in young female ostriches. In fact, young female ostriches showed significantly lower MCHC values than other age groups in both sexes.

Considering the study performed with one hundred 30-months-old birds in another region in Brazil[8], similar results were obtained for RBC count, hemoglobin concentration, MVC and MCHC values, furthermore no difference between gender was observed. This similarity reaffirms the orientation that the geoclimatic differences must be considered to an adequate interpretation of ostriches' erythrogram.

Length and width values of erythrocytes in a previous study[6] were lower than those found in the present study. Probably, these morphometric differences are, at least in part, related to the use of EDTA as anti-

coagulant on that study. Recently in our laboratory, we demonstrated[9] that blood samples from ostriches collected with EDTA presented erythrocytes with bigger diameter and surface area, when compared with samples collected with heparin, as previously demonstrated in birds and reptiles[12].

In this study, length of erythrocytes as similar to those reported in another study[2], whereas width values were lower than those in farmed ostriches[2]. It is important to mention that in previous studies[2,4,6], gender of ostriches was not considered and a lower number of erythrocytes were counted using a manual morphometry, which can induce more errors than computerized morphometry used in the present study. Such differences in methods, beyond those related to the breed, climate and farming management, can explain the differences found in morphometric data of our study.

The differences in erythrocyte diameters due to sex and age, although statistically significant, cannot be detected by optic microscopy. However, these small variations in erythrocyte diameter, when considering the total number of erythrocytes, certainly contribute for significant differences in the PCV, and consequently MCV and MCHC values.

Conclusions

The erythrocyte parameters of ostriches in São José do Rio Preto region are different from those cited in the literature for birds raised in different geoclimatic conditions and farming management. Also, erythrocytic parameters of ostriches are influenced by sex and age range. Finally, it is important to consider these factors, mainly when evaluating the RBC count and the hemoglobin levels, for an accurate clinical interpretation of the erythrogram.

References

1. ASSOCIAÇÃO DOS CRIADORES DE AVESTRUZ DO BRASIL (ACAB). **Estrutiocultura**. São Paulo, Disponível em: <http://www.acab.org.br/?md=estatico&pag=estrutiocultura/index>. Acesso em: 25 jul. 2005.
2. MUSHI, E. Z.; BINTA, G. M.; CHABO, R. G. ; ISA, J. F. W.; KAPAATA, R. W. Selected hematologic values of farmed ostriches (*Struthio camelus*) in Botswana. **Journal Veterinary Diagnostic Investigation**, v. 11, p. 372-374, 1999.
3. CAMPBELL, T. W. Hematology of birds. In: THRALL, A. M.; BAKER, D. C.; CAMPBELL, T. W.; DENICOLA, D.; FETTMAN, M. J.; LASSEN, E. D.; REBAR, A.; WEISER, G. **Veterinary hematology and clinical chemistry**. Philadelphia: Lea & Fabiger, 2004. p. 225-258.
4. PALOMEQUE, J.; PINTÓ, D.; VISCOR, G. Hematologic and blood chemistry values of the masai ostrich (*Struthio camelus*). **Journal of Wildlife Diseases**, v. 27, n. 1, p. 34-40, 1991.
5. BEN-ROMDHANE, S.; ROMDANE, M. N.; MHIRI, S.; BEN-MILED, M. A.; KORTAS, M. Les paramètres biochimiques et hematologiques chez l'autruche (*Struthio camelus*) dans un élevage tunisien. **Révue de Médicine Vétérinaire**, v. 3, n. 3, p. 231-238, 2000.
6. DURGUN, Z.; KESKIN, E.; ÇÖL, E.; ATALAY, B. Selected haematological and biochemical values in ostrich chicks and growes. **Archiv fuer Gefluegelkunde,** v. 69, n. 2, p. 62-66, 2005.
7. RAUKAR, J.; SIMPRAGA, M. Haematological parameters in the blood of one day old ostriches. **Israel Journal of Veterinary Medicine**, v. 60, n. 4, p. 112-116, 2005.
8. BONADIMAN, S. F.; STRATIEVSKY, G. C.; MACHADO, J. A.; ALBERNAZ, A. P.; RABELO, G. R.; DaMATTA, R. A.. Leukocyte ultrastructure, hematological and serum biochemical profiles of ostriches (*Struthio camelus*). **Poultry Science**, v. 88, n. 11, p. 2298-2306, 2009.
9. SABINO, A. J.; TREVELIN, S. C.; CIARLINI, P. C. Comparação do efeito do ácido etilenodiaminotetracético (EDTA) e da heparina sobre os eritrócitos de avestruzes (*Struthio camelus* L.). **Revista Ceres**, v. 57, n. 3, p. 338-342, 2010.
10. JAIN, N. C. **Essentials of hematology.** Philadelphia: Lea & Fabiger, 1993.
11. SÃO JOSÉ DO RIO PRETO. Secretaria Municipal de Planejamento e Gestão Estratégica. **Conjuntura econômica de São José do Rio Preto**. Cooredenação de O. Bolsoni. 2006. 94 p. Disponível em: <http://www.riopreto.sp.gov.br/externos/smplanejamento/conjunturaeconomica/2006.pdf>. Acesso em: 20 maio 2007.
12. HATTINGH, J.; SMITH, E. M. Anticoagulants for avian and reptilian blood: heparin and EDTA. **Pflugers Archives**, v. 363, n. 3, p. 267-269, 1976.