Serum biochemistry of 4-day-old ostriches (*Struthio camelus*)[1]

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ABSTRACT.- Miranda R.L., Mundim A.V., Costa À.S., Guimarães R.V. & Silva F.O.C. 2008. **Serum biochemistry of 4-day-old ostriches (*Struthio camelus*).** P*esquisa Veterinária Brasileira 28(9):423-426.* Laboratório Clínico Veterinário da Faculdade de Medicina Veterinária, Universidade Federal de Uberlândia, Av. Pará 1720, Bloco 2T, Campus Umuarama, Uberlândia, MG 38400-902, Brazil. E-mail: renatavetufu@yahoo.com.br

An analysis was made of 30 four-day-old ostriches to evaluate their protein, metabolite, mineral, and serum enzyme profiles, to correlate them with the birds' sex. The values obtained were: Total proteins 3.59±0.72g/dL, albumin 1.04±0.14g/dL, globulins 2.51±0.56g/dL, A:G ratio 0.43± 0.07, total cholesterol 615.10±101.15mg/dL, high density lipoprotein cholesterol (HDL-C) 132.72±20.33mg/dL, low density lipoprotein cholesterol (LDL-C) 454.93±90.81mg/dL, very low density lipoprotein cholesterol (VLDL-C) 27.45±9.96mg/dL, triglycerides 137.23±49.78mg/dL, uric acid 6.24±2.15mg/dL, urea 18.27±12.33mg/dL, creatinine 0.30±0.04mg/dL, total calcium 9.38± 0.76mg/dL, ionized calcium 7.17±0.64mg/dL, phosphorus 6.96±0.91mg/dL, Ca:P ratio 1.37±0.21, iron 24.74±13.02µg/dL, sodium 142.03±6.17mEq/L, chlorides 109.59± 4.99mEq/L, aspartate aminotransferase (AST) 200.67±31.42 U/L, alanine aminotransferase (ALT) 3.90±1.92 U/L, γ-glutamyltransferase (GGT) 1.18±0.73 U/L, alkaline phosphatase (ALP) 597.30± 231.36 U/L, and creatine kinase (CK) 2348.30±755.60 U/L. Males and females showed significant differences in total proteins, globulins, alkaline phosphatase, A:G ratio, and uric acid.

INDEX TERMS: Serum biochemistry, ostriches, *Struthio camelus*.

RESUMO.- [**Bioquímica sérica de avestruzes (*Struthio camelus*) com quatro dias de idade.**] A fim de avaliar o perfil de proteínas séricas, metabólitos, minerais e enzimas séricas e correlacionar com o sexo das aves, este estudo analisou 30 avestruzes de quatro dias de idade. Os valores obtidos foram: proteínas totais 3,59±0,72g/dL; albumina 1,04±0,14g/dL; globulinas 2,51±0,56g/dL; relação A:G 0,43± 0,07; colesterol total 615,10±101,15mg/dL; lipoproteína de alta densidade colesterol (HDL-C) 132,72±20,33mg/dL; lipoproteína de baixa densidade colesterol (LDL-C) 454,93±90,81mg/dL; lipoproteína de muito baixa densidade colesterol (VLDL-C) 27,45±9,96 mg/dL; triglicérides 137,23±49,78mg/dL; ácido úrico 6,24±2,15mg/dL; uréia 18,27±12,33mg/dL; creatinina 0,30±0,04mg/dL; cálcio total 9,38±0,76mg/dL; cálcio ionizado 7,17±0,64mg/dL; fósforo 6,96±0,91mg/dL; relação Ca:P 1,37±0,21; ferro 24,74±13,02µg/dL; sódio 142,03±6,17mEq/L; cloretos 109,59±4,99mEq/L; aspartato aminotransferase (AST) 200,67±31,42 U/L; alanina aminotransferase (ALT) 3,90±1,92 U/L; γ-glutamiltransferase (GGT) 1,18±0,73 U/L; fosfatase alcalina (ALP) 597,30± 231,36 U/L; e creatina quinase (CK) 2348,30±755,60 U/L. Diferenças significativas existiram para proteínas totais, globulinas, fosfatase alcalina, relação A:G e ácido úrico entre machos e fêmeas.

TERMOS DE INDEXAÇÃO: Bioquímica sérica, avestruzes, *Struthio camelus.*

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INTRODUCTION

Originally from Africa, the ostrich (*Struthio camelus*) is a rustic flightless bird highly resistant to disease, which has been bred successfully in Canada, the United States, Australia, Spain, Italy and France. It is a monogastric herbivore whose diet is based on vegetable fibers.

The Brazilian herd consists of about 200,000 birds distributed through several regions of the country. In terms of the economy, ostrich breeding is an excellent option for Brazil, which has a favorable climate, plentiful food and labor and easily adaptable livestock infrastructure (Brazilian Ostrich Breeders Association 2007).

Ostriches raised in captivity are susceptible to health problems. Noninfectious diseases resulting from unskilled management due to the inexperience of most breeders are very common, involving environmental and nutritional stress (Quintavalla et al. 2001). Nutritional deficiencies, which are very frequent in commercial breeding, are caused by incorrect feed formulation, mixture and storage (Tullio 1998). These deficiencies lead to a high rate of deficient growth, bone deformities in the limbs and the loss of young birds (Brown & Jones 1996).

Blood biochemistry analysis is a common tool for the early diagnosis and correction of nutritional and metabolic disorders before the emergence of more serious symptoms, thereby preventing the premature loss and/or discard of birds. However, this is a scientific field still little exploited in Brazil, and studies are needed to define reference values. Such values are essential for the detection and interpretation of alterations in different clinical situations when evaluating the general health and well-being of these birds bred in captivity (Fudge 1997).

This study was motivated by the rise of ostrich breeding in Brazil, the lack of studies about the bird's clinical biochemistry, and the need to establish reference values for commercially bred animals in the country. Our objective was to evaluate the physiological variations and the influence of gender on the serum profile of proteins, metabolites, minerals and enzymes in 4-day-old ostriches.

MATERIALS AND METHODS

Our study involved 30 four-day-old ostriches, 12 males and 18 females, bred on Fazenda Pé Forte, a semi-intensive farm in the municipality of Uberaba, state of Minas Gerais, Brazil. The birds had been fasting from birth up to the moment of collection. Ostriches go without food for the first 5 days of life in order to absorb the yolk sac reserves.

We collected 3mL of blood from each bird by cardiac puncture, using 25x8 disposable needles and syringes. The samples were placed in tubes (Vacutainer™ SST™ Gel & Clot Activator 6ml Becton Dickinson, Juiz de Fora, MG) with separator gel and coagulation activator and were spun for 5 minutes at 4ºC. The resulting serum was then transferred to Eppendorf microtubes and stored at -20ºC until the moment of analysis.

Several parameters were analyzed (Table 1) using a multichannel automatic analyzer (Architect C 8000 Abbott Diagnostics, Abbott Park, Illinois, USA) and specific kits (Abbott Diagnostics, Abbott Park, Illinois, USA). We obtained the VLDL-C and LDL-C concentrations as described by Friedwald et al. (1972) and the ionized calcium according to Analisa Diagnóstica (2000).

Table 1. Parameters evaluated and methodologies used in the analysis

Parameters	Methodology
Total proteins	Biuret
Albumin	Bromocresol green
Globulins	Calculation: total protein - albumin
A:G ratio	Calculation: albumin / globulin
Urea	Kinetic urease method UV
Creatinine	Heinegard and Tiderstram modified method
Uric acid	Trinder uricase method
Total cholesterol	Trinder enzymatic method
HDL-C	Trinder enzymatic method
Triglycerides	Trinder enzymatic method
Total calcium	Cresolftaleine complexone method - CPC
Phosphorus	Phosphomolybdate method
Ca:P ratio	Calculation: total calcium / phosphorus
Iron	Ferrosine
Sodium	Selective ions electrode
Chlorides	Selective ions electrode
AST	Kinetic method UV-IFCC
ALT	Kinetic method UV-IFCC
Alkaline phosphatase	Optimized kinetic method
CK	Modified Okinada
GGT	Modified Szasz method

Sampling was random, and a calculation was made of the mean and standard deviation of each evaluated parameter. To compare the values by sex, we used the Mann-Whitney U test (Siegel 1975) for the nonparametric variables (GGT, uric acid, urea, creatinine and sodium) and Student's *t*-test (Graner 1966) for the parametric variables, both with a 5% level of significance.

Table 2. Mean, standard deviation (SD), minimum and maximum values of the serum biochemical parameters of 4-day-old male and female ostriches (*Struthio camelus*)

Parameters	Unit	Mean	SD	Min		Max
Total proteins	g/dL	3.59	0.72	2.54	-	5.25
Albumin	g/dL	1.04	0.14	0.79	-	1.25
Globulins	g/dL	2.51	0.56	1.75	-	3.80
A:G ratio		0.43	0.07	0.29	-	0.58
Urea	mg/dL	18.27	12.33	5.00	-	67.00
Creatinine	mg/dL	0.30	0.04	0.20	-	0.40
Uric acid	mg/dL	6.24	2.15	4.70	-	15.10
Total cholesterol	mg/dL	615.10	101.15	468.00	-	843.00
HDL-C	mg/dL	132.72	20.33	90.00	-	180.00
LDL-C	mg/dL	454.93	90.81	311.40	-	660.00
VLDL-C	mg/dL	27.45	9.96	13.20	-	51.40
Triglycerides	mg/dL	137.23	49.78	66.00	-	257.00
Total calcium	mg/dL	9.38	0.76	7.90	-	10.80
Ionized calcium	mg/dL	7.17	0.64	5.90	-	8.10
Phosphorus	mg/dL	6.96	0.91	5.60	-	9.40
Ca:P ratio		1.37	0.21	1.00	-	1.70
Iron	µg/dL	24.74	13.02	6.10	-	54.10
Sodium	mEq/L	142.03	6.17	129.00	-	161.00
Chlorides	mEq/L	109.59	4.99	98.00	-	122.00
AST	U/L	200.67	31.42	157.00	-	276.00
ALT	U/L	3.90	1.92	0.00	-	6.00
GGT	U/L	1.18	0.73	0.50	-	4.00
Alkaline phosphatase	U/L	597.30	231.36	232.00	-	1056.00
CK	U/L	2348.30	755.60	1408.00	-	4267.00

Table 3. Mean and standard deviation (SD) of serum biochemical parameters of 4-day old ostriches (*Struthio camelus*), by sex

Parameters	Unit	Males (n=12)		Females (n=18)	
		Mean	SD	Mean	SD
Total proteins	g/dL	3.91a	0.68	3.38b	0.68
Albumin	g/dL	1.04 a	0.13	1.04a	0.15
Globulins	g/dL	2.76a	0.47	2.34b	0.56
A:G ratio		0.38b	0.07	0.45a	0.06
Urea	mg/dL	17.58a	8.88	18.72a	14.42
Creatinine	mg/dL	0.29a	0.03	0.30a	0.05
Uric acid	mg/dL	5.25b	0.47	6.91a	2.56
Total cholesterol	mg/dL	643.67a	98.89	596.06a	100.82
HDL-C	mg/dL	138.17a	12.65	129.09a	23.80
LDL-C	mg/dL	476.78a	90.42	440.37a	90,64
VLDL-C	mg/dL	28.72a	8.92	26.60a	10.76
Triglycerides	mg/dL	143,58a	44,61	133.00a	53.78
Total calcium	mg/dL	9,13a	0,53	9.55a	0.85
Ionized calcium	mg/dL	6.91a	0.44	7.34a	0.70
Phosphorous	mg/dL	7.19a	0.86	6.81a	0.93
Ca:P ratio		1.28a	0.15	1.43a	0.23
Iron	µg/dL	22.73a	15.64	26.09a	11.24
Sodium	mEq/L	142.42a	5.38	141.78a	6.78
Chlorides	mEq/L	109.86a	5,71	109.41a	4.62
AST	U/L	200.08a	28.26	201.06a	34.15
ALT	U/L	4.00a	1.86	3.83a	2.01
GGT	U/L	1.17a	0.64	1.19a	0.81
Alkaline phosphatase	U/L	710.92a	212.01	521.56b	216.88
CK	U/L	2174.08a	470.54	2464.44a	891.62

(a,b) Mean values followed by different letters indicate a statistical difference ($p<0.05$).
n = number of animals.

RESULTS

The mean, standard deviation and maximum values of each analyzed variable are shown in Table 2.

The results in Table 3 exhibit statistically significant differences in the values of total proteins, globulins and alkaline phosphatase, which were higher in the males, and in uric acid and A:G ratio, which were higher in the females.

DISCUSSION

Total protein values fell within the limits reported by Ben Romdhane et al. (2000), Quintavalla et al. (2001), Bouda et al. (2004) and Khazraiinia et al. (2006). However, albumin values were lower than those reported by Okotie-Eboh et al. (1992), Agaoglu et al. (2003) and Bouda et al. (2004). This difference was attributed to the high organic demand, low hepatic synthesis, and the fact that the birds had been fasting since birth. According to Thrall et al. (2004), albumin synthesis in the liver is influenced by nutrition, hormonal balance and stress. The globulin concentrations were similar to those reported by Agaoglu et al. (2003) and Bouda et al. (2004). The A:G ratio measured here could not be compared to other studies since the consulted literature lacks data on this parameter.

Of the various metabolites analyzed in this study, urea showed higher values than those reported by Van Heerden et al. (1985), Agaoglu et al. (2003), Bouda et al. (2004) and Khazraiinia et al. (2006). This difference is probably attributable to the large protein reserves contained in the yolk sac, allied to the birds' low water intake in the first four days of life. The creatinine concentrations remained within the intervals reported by Okotie-Eboh et al. (1992), Mushi et al. (1998), Quintavalla et al. (2001) and Bouda et al. (2004). Uric acid values were lower than those found by Palomeque et al. (1991), Bouda et al. (2004) and Khazraiinia et al. (2006), but similar to those reported by other researchers (Okotie-Eboh et al. 1992, Quintavalla et al. 2001, Moniello et al. 2005). This finding indicates adequate kidney function and low recycling of nucleic acids, since birds excrete uric acid via urine. The concentration of uric acid in blood varies directly according to the amount of food intake, protein percentage in the diet and the demand of amino acids for protein synthesis (Costa et al. 1993). The mean cholesterol concentration was higher than that reported by Okotie-Eboh et al. (1992), Mushi et al. (1998) and Ben Romdhane et al. (2000), possibly because ostriches use nutritional reserves from the yolk sac, which is rich in this metabolite. Bouda et al. (2004) also observed plasma cholesterol levels fivefold higher in 26-day-old ostriches than in older birds, ascribing this difference to the high reserves contained in the yolk sac. Triglyceride concentrations were similar to those reported by Okotie-Eboh et al. (1992), Mushi et al. (1998), Ben Romdhane et al. (2000) and Khazraiinia et al. (2006). Values of HDL-C, LDL-C and VLDL-C lipoproteins are not reported in the consulted literature.

As for minerals, total calcium fell within the limits mentioned by Brown & Jones (1996), Ben Romdhane et al. (2000), Quintavalla et al. (2001), and Simpraga et al. (2004). Ionized calcium and Ca:P ratio, however, could not be compared because they are not cited in the consulted literature. Iron levels matched those reported by Van Heerden et al. (1985) and Moniello et al. (2005). The mean phosphorus levels fell within the range reported by Okotie-Eboh et al. (1992), Brown & Jones (1996), Ben Romdhane et al. (2000), and Agaoglu et al. (2003). Sodium showed values similar to those mentioned by Mushi et al. (1998), Ben Romdhane et al. (2000), Quintavalla et al. (2001), and Agaoglu et al. (2003). Chloride concentrations were similar to those reported by Van Heerden et al. (1985), Mushi et al. (1998), and Quintavalla et al. (2001).

With regard to enzymes, the ALT values were lower than those found by Agaoglu et al. (2003), Bouda et al. (2004), and Khazraiinia et al. (2006), but fell within the limits observed by other researchers (Van Heerden et al. 1985, Levy et al. 1989, Okotie-Eboh et al. 1992). The low concentrations of ALT were possibly due to the non-intake of food in the first four days of life of these birds, with the resulting absence of hepatic overload. AST activity remained within the intervals mentioned by Quintavalla et al. (2001), Agaoglu et al. (2003) and Bouda et al. (2004). Alkaline phosphatase presented higher concentrations than those reported by Simpraga et al. (2004), Bouda et al. (2004), and Khazraiinia et al. (2006), which is ascribed to the release

of large amounts of bone isoenzymes during the intense process of bone formation and skeletal development of the birds in the embryonic period, according to Simpraga et al. (2004). GGT values were similar to those found by Mushi et al. (1998), Quintavalla et al. (2001), and Agaoglu et al. (2003). The CK activity, which exceeded the values reported by Palomeque et al. (1991), Mushi et al. (1998), Quintavalla et al. (2001), and Agaoglu et al. (2003), was characterized by the beginning of muscular activity in the newly hatched birds, which facilitates the release of muscle isoenzymes into the bloodstream.

In general, the young age of the birds, their use of yolk sac reserves, stress, and the birds' physiological adaptation explain the differences between the biochemical serum parameters of the ostriches in this study and those reported in the literature, which mostly involved adult birds. Some authors relates that variations in the biochemical serum of birds are age-related and very frequent, starting from the first months.

The large amount of globulins in the yolk sac is probably due to the higher concentration of total proteins and globulins and the lower A:G ratio observed in the male ostriches in this study. The ostrich's yolk sac represents 30 to 50% of the bird's live weight, and is the chick's main source of nutrients and water in its first week of life. On the other hand, Bouda et al. (2004) reported that total protein, albumin and globulin were lower in males. The higher alkaline phosphatase activity in males is possibly due to the fact that their embryonic osteogenesis is more intense, since they usually have a larger bone structure than females. The higher concentration of uric acid in females may be attributable to their high purine metabolism.

Because the ostrich is a domestic species whose breeding has been little studied in our country, any new information represents another step towards a better understanding of this bird. Notwithstanding of the small number of animals involved, the results of this study are valuable and contribute to the body of knowledge about the bird's physiology, the evaluation of the state of health, and the establishment of reference values for proteins, metabolites, minerals and serum enzymes of young ostriches bred commercially in Brazil.

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