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PLASMA CHEMISTRY REFERENCE VALUES IN OSTRICHES

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ABSTRACT: Reference values for 18 plasma chemical variables in blue neck ostriches (*Struthio camelus australis*, $n = 60$, age 24–36 mo) were established for use in veterinary clinical practice using nonparametric statistics. The following values were established for the percentiles $P_{2.5}$ and $P_{97.5}$: sodium 147–157 mmol/L, calcium 2.4–4.8 mmol/L, inorganic phosphate 1.3–2.3 mmol/L, chloride 94–105 mmol/L, glucose 10.3–13.7 mmol/L, urea 0.5–0.8 mmol/L, uric acid 351–649 μmol/L, bile acids 8–33 μmol/L, total protein 39–56 g/L, albumin-globulin ratio 0.45–0.59, osmolality 304–330 mOsm/kg, alkaline phosphate 69–217 IU/L, aspartate aminotransferase 243–418 IU/L, gamma-glutamyltransferase 0–1 IU/L, creatine kinase 1648–4894 IU/L, glutamate dehydrogenase 8–17 IU/L, and lactate dehydrogenase 860–2236 IU/L. The plasma calcium concentration was significantly ($P < 0.001$; $r = 0.74$) related to the total protein concentration and an adjustment-formula for calcium was derived: adjusted Ca (mmol/L) = Ca (mmol/L) − 0.09 TP (g/L) + 4.4. The influence of blood sample treatment on the plasma potassium concentration as seen in other avian species was demonstrated in a separate experiment, emphasizing the need to separate plasma and cells immediately after collection in avian blood samples.

Key words: Ostrich, plasma chemistry reference value, potassium concentration, sample treatment, *Struthio camelus australis.*

INTRODUCTION

Ostriches are widely kept in zoos and private collections and are important as farm animals. Individual animals, especially breeding birds, have a high economic value and hence diagnosis and treatment of disease in individual animals is often requested. Interpretation of results of plasma chemistry in a particular species can only be achieved if reference values of that species, preferably established by the same methods, are available. Plasma chemistry reference values have been documented in ostriches, but either the number of sampled birds was statistically very small (Palomeque et al., 1991; Levy et al., 1989), the birds had not reached reproductive age (Van Heerden, 1985), or the age of the sampled birds varied greatly (Levy et al., 1989; Okothie-Eboh et al., 1992). In one study (Mushi et al., 1998) samples were collected in serum tubes which is not the preferred method in birds (Lumeij, 1997). Moreover, neither the influence of total protein on calcium concentration, nor the effect of blood sample treatment on potassium concentration was investigated in the publications mentioned. Selection of variables was based on previously established usefulness in other avian species (Lumeij and Overduin, 1990). The aim of this study was to establish plasma chemistry reference values of ostriches in the reproductive age for use in clinical avian practice.

MATERIALS AND METHODS

General plasma chemistry

Sixty apparently healthy 24 to 36-mo-old ostriches (20 males and 40 females) were sampled to establish plasma chemical reference values. The samples were taken from *Struthio camelus australis,* the most commonly kept subspecies outside the Republic of South Africa (Arts et al., 1995).

Ostriches were housed under identical conditions as one group for more than 8 mo. The diet was a mixture of a commercial ostrich pellet, 2 kg/bird/day (Struvo®, Kasper Faunafood, Veenwoude, The Netherlands) and alfalfa hay *ad libitum.* The diet was supplemented with vitamins and minerals (Totalin®, Virbac Nederland B.V., Barneveld, The Netherlands) and crushed bones. Water was provided *ad libitum.* Blood samples were taken from the jugular vein (Arts et al., 1995) and collected in heparinized vacuum tubes (lithium heparin). The samples were immediately placed in melting

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TABLE 1. Plasma chemistry reference values (inner limits of $P_{2.5}$–$P_{97.5}$ with a probability of 90%) for the ostrich (*Struthio camelus australis*) (n = 60).

	Unit	$P_{2.5}$–$P_{97.5}$[a]	Mean	SD[b]	Median	Range	CV (%)[c]
Sodium	mmol/L	147–157	152.5	3.9	152	143–164	1.9
Calcium	mmol/L	2.4–4.8 (2.8–4.7)[d]	3.2	0.7	3.0	2.3–5.4	1.3
Phosphate	mmol/L	1.3–2.3	1.75	0.31	1.7	1.1–2.4	4.9
Chloride	mmol/L	94–105	100	4	100	87–107	1.1
Glucose	mmol/L	10.3–13.7	11.9	1.1	11.7	9.8–14	2.9
Urea	mmol/L	0.5–0.8	0.64	0.30	0.60	0.40–2.8	1.2
Uric acid	μmol/L	351–649	484	92	473	303–575	0.7
Urea/uric acid	—	0.9–1.8	1.3	0.56	1.3	0.79–5.1	0.6
Bile acids	μmol/L	8–33	14.4	9.8	12	8–66	
Total protein	g/L	39–56	47.4	5	47.5	35–60	0.4
Albumin/globulin	—	0.45–0.59	0.53	0.05	0.54	0.4–0.62	1.7
Osmolality	mOsm/kg	304–330	316	10	317	274–338	10.6
ALP	IU/L	69–217	126	60	116	56–381	2.6
AST	IU/L	243–418	321	56	316	143–471	2.4
GGT	IU/L	0–1	0.25	0.47	0	0–2	
CK	IU/L	1648–4894	2667	1041	2297	1268–5954	0.1
GLDH	IU/L	8–17	9.3	6.3	8	8–55	
LDH	IU/L	860–2236	1354	430	1336	638–2822	

[a] Recommended reference values.
[b] Standard Deviation.
[c] Day-to-day coefficient of variation of analytical method.
[d] Adjusted values.

ice and centrifuged (5000 rpm, 10 min) within 15 min after collection. Plasma were stored at 0 C during transport to the laboratory and stored at −20 C pending analysis. Neither hemolysis nor lipemia was detected in any of the samples used for further analysis.

Reference values for the following variables were established: sodium, calcium (Ca), inorganic phosphate, chloride, glucose, urea, uric acid, urea-uric acid ratio, bile acids, total protein (TP), albumin-globulin ratio, osmolality, alkaline phosphatase (AP; Enzyme Code [EC] 3.1.3.1), aspartate aminotransferase (AST; EC 2.6.1.1), gamma-glutamyltransferase (GGT; EC 2.3.2.2), creatine kinase (CK; EC 2.7.3.2), glutamate dehydrogenase (GLDH; EC 1.4.1.2), and lactate dehydrogenase (LDH; EC 1.1.1.27).

Sodium was measured by flame photometry (IL 943, Instrumentation Laboratory, London, UK). Calcium was determined using o-cresolphtaleïne-complex (without deproteinization). Inorganic phosphate was determined using molybdate (without deproteinization). Chloride was measured by an Ion Selective Electrode method (Beckmann Instruments, Synchron Analyser, Fullerton, California, USA). Urea was determined using the urease-GLDH method. Uric acid was measured by the amino-4-antipyrine (PAP) method. Bile acids were measured on a Multistat FL III Centrifugal Analyser (MCA, Instrumentation Laboratory, London, UK) using 3-a-hydroxysteroid dehydrogenase (3-a-HSD; EC 1.1.1.50). Glucose was measured using glucose oxydase and peroxydase. Osmolality was determined on a Gonotech-osmometer, Osmomat 030 (Gonotech GmbH, Berlin, Germany). The determination of total protein (TP) was performed on a MCA with a biuret method with blank correction using human protein as a standard (human protein standard containing 30 g/L albumin and 50 g/L globulin, no. 540-10, Sigma Diagnostics, St.Louis, Missouri, USA). Protein electrophoresis was carried out on a Beckmann Paragon system using agarose gels, staining with aminoblack. All enzymes were measured on a WAKO 20R analyzer (Sopar/Sopachem B. V., Nieuwegein, The Netherlands) at 30 C. The AP, AST, CK, and LDH activities were measured according to the recommendations of the International Federation of Clinical Chemistry. Gamma-glutamyl transferase (GGT) was analyzed according to the method of Szasz with glutamyl-P-nitroanilide as substrate. Glutamate dehydrogenase (GLDH) was analyzed according the recommendations of the German Society of Clinical Chemistry (Werner Schmidt and Werner Schmidt, 1987). The day-to-day coefficients of variation (CV) of the analytical methods were tabulated (Table 1). The albumin-globulin ratio was calculated from the pro-

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TABLE 2. Spearman rank correlation values between variables.

Calcium—Total protein	0.7362 ($P < 0.001$)
CK—AST	0.7265 ($P < 0.001$)
LDH—AST	0.5230 ($P < 0.001$)
LDH—CK	0.6754 ($P < 0.001$)
LDH—Bile acids	0.5551 ($P < 0.001$)

tein electrophoresis, whereby pre-albumin and albumin were combined as albumin and globulin-fractions as globulin.

Reference values were established using a distribution free method (Rümke and Bezemer, 1972a, 1972b). For each variable the inner limits of the percentiles $P_{2.5}$–$P_{97.5}$ are presented with a probability of 90%. The correlation between plasma chemical variables was investigated using the Spearman rank correlation test (Table 2; Zar, 1984). A least square regression line drawn from total calcium and total protein allowed derivation of an adjustment formula for calcium as reported previously (Lumeij, 1990). Significance was assumed at $P < 0.05$.

Plasma potassium concentration

Because plasma potassium concentrations in avian blood are strongly influenced by blood sample treatment (Lumeij, 1985) potassium concentration was studied separately. The influence of blood sample treatment on plasma potassium concentration in ostrich blood was studied in an experiment using six 24-mo-old ostriches. Housing and diet of the birds was as mentioned before. Blood samples were taken from the jugular vein and collected in heparinized vacuum tubes (lithium heparin). The uncentrifuged sample from each bird was immediately divided into two equal portions and stored at 0 C and 20 C respectively. Samples of each portion were centrifuged at 0, 10, 30, 60, and 120 min after collection. Immediately after centrifugation plasma was stored at 0 C pending analysis. Potassium concentrations were determined with an ion-selective device (Beckman Synchron CX-5CE, Beckman Instruments). The day-to-day CV of this method was 0.8%. None of these samples analyzed showed hemolysis or lipemia.

RESULTS

Plasma chemistry reference values are reported in Table 1. A significant correlation was found between calcium (Ca) and total protein (TP) ($r = 0.74$; $P < 0.001$). About 55% ($R^2 = 0.548$) of the variability in plasma calcium concentration was related to the total protein concentration. A least square fit to the values of calcium and total protein gave the equation: Ca = −1.24 + 0.09 TP (Fig. 1).

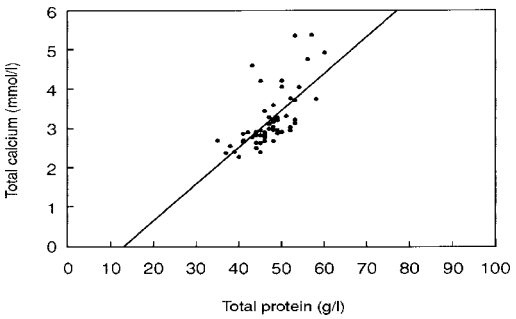


FIGURE 1. Significant relationship ($r = 0.74$; $P = 0.001$; $n = 60$) between total protein and calcium in plasma. The least square regression line is shown. About 55% of the variability in calcium was releated to the change in the plasma total protein concentration ($R^2 = 0.548$). The adjustment formula for calcium was derived by adding the difference between the y-intercept value from the regression line and the normal mean for plasma calcium. The y-intercept value for total protein was -1.2, the mean for total plasma calcium was 3.2 mmol/L and the difference was 4.4. The slope of the regression line (0.09) was multiplied by the absolute value for total protein. The final formula is: adjusted Ca(mmol/L) = Ca(mmol/L) − 0.09 TP(g/L) + 4.4.

The adjustment formula for calcium was derived by adding the difference between the y-intercept value from the regression line and the normal mean for plasma calcium. The y-intercept value for total protein was -1.2, the mean for total plasma calcium was 3.2 mmol/L and the difference was 4.4. The slope of the regression line (0.09) was multiplied by the absolute value for total protein. The final formula is: adjusted Ca(mmol/L) = Ca(mmol/L) − 0.09 TP(g/L) + 4.4 Spearman rank correlation factors between Ca-TP, CK-AST, LDH-AST, LDH-CK and LDH-Bile acids are shown in Table 2.

Initial potassium concentration, measured in the separately performed experiment, ranged between 3.7 and 5.1 mmol/L. There were significant ($P < 0.05$) decreases compared to the initial potassium concentration at storage temperatures of

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TABLE 3. The effect of storage time and temperature of uncentrifuged blood samples on plasma potassium concentration in ostriches (*Struthio camelus australis* (n = 6).

		t = 1 min	t = 10 min	t = 30 min	t = 60 min	t = 120 min
0 C	Change (%)[a]	0	−4.38	−6.9	−8.28	−11.25
	SD[b]	0	2.04	3.49	3.76	4.43
	Mean (mmol/L)	4.47	4.28	4.15	4.08	3.95
	Range (mmol/L)	3.7–5.1	3.5–4.9	3.4–4.8	3.4–4.6	3.3–4.6
	SD (mmol/L)	0.53	0.48	0.44	0.38	0.39
20 C	Change (%)	0	+8.25	+12.88	+17.0	+19.78
	SD	0	3.15	3.78	3.16	4.05
	Mean (mmol/L)	4.47	4.82	5.03	5.22	5.33
	Range (mmol/L)	3.7–5.1	4.1–5.4	4.3–5.8	4.5–6.0	4.7–6.0
	SD (mmol/L)	0.53	0.51	0.54	0.56	0.50

[a] Change % = mean decrease (−) or increase (+) as % of initial potassium concentration.
[b] SD = standard deviation.

20 C and significant ($P < 0.05$) increases compared to the initial potassium concentration at storage temperatures of 0 C (Table 3). In Table 1 the potassium concentration is not reported.

DISCUSSION

Herein we present plasma chemistry reference values for ostriches. The inner limits of the percentiles $P_{2.5}$–$P_{97.5}$ with a probability of 90% are reported. For biological data which do not have a Gaussian distribution, non-parametric statistics should be used to establish reference values (Rümke and Bezemer, 1972a, 1972b). The following variables showed a nonparametric distribution: calcium, chloride, glucose, urea, uric acid, bile acids, GGT, CK, GLDH, and LDH. When parametric methods would be used to establish reference values for these variables (mean ± 2 SD), these values would fall outside the range of observed values (Table 1). Alternatively, the use of nonparametric methods to establish reference values which show a normal distribution has no great consequence for clinical use (Table 1).

The advantage of heparinized blood is that it can be centrifuged immediately after collection and changes in the plasma chemical profile can be prevented, e.g., potassium and glucose concentrations. Nearly all routine hematologic and biochemical investigations can be performed with blood placed in lithium heparin. When serum is prepared for blood chemistry it has to stand for a certain period to allow coagulation, which can cause changes in the sample (Lumeij, 1985; Hochleithner, 1997). Reclotting of serum after separating serum from the clot is another problem that frequently occurs in avian blood (J. T. Lumeij, unpubl. data), including ostrich blood (H. T. Arts, pers. comm.). For these reasons plasma was preferred above serum. Lumeij and MacLean (1996) demonstrated a highly significant correlation between plasma and serum TP in pigeon blood ($P < 0.001$; $R = 0.99$; $n = 50$). They showed that the concentration of total protein in avian plasma is about 1.5 g/L higher compared to serum because the former contains fibrinogen.

There are significant differences between TP concentrations when different standards are used, such as human, bovine, pigeon, and chicken standards, although there is a high correlation between the results obtained with the various standards (Spano et al., 1988; Lumeij et al., 1990). When a pigeon standard was used to determine serum TP concentration with the biuret method, values were significantly higher than values found with the biuret method using the human standard, but there was a high correlation (Lumeij et al., 1990). Spano et al. (1988) on the other hand, found consistently lower TP

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values in chicken serum with the use of a chicken standard than with a bovine standard. Because the use of a species-specific standard for all avian species is not realistic and because a high correlation exists between the results obtained with the various standards in the present study, recommendations of Kaneko et al. (1997) were followed. Total protein reference values for various avian species should be established using the most commonly used standard, namely the human standard (Lumeij, 1997).

A formula for the calculation of the calcium − total protein relation is presented. The findings show that it is useful to apply a correction formula when low or high plasma total protein values are found. Although plasma calcium concentration can be interpreted more precisely with this adjustment formula, it should be realized that changes in total protein in diseased birds may not always reflect the protein portion that binds the calcium and therefore the correction formula is not flawless.

Age of the animals strongly influences some values, especially total protein (Levy et al., 1989). Total protein concentration is highly correlated to calcium concentration as shown in this study (Table 2). The higher calcium concentration measured in our study compared to the calcium concentrations presented in previous articles is most likely caused by age related factors in total protein concentration. The correction formula can be used to correct the calcium concentration for total protein.

Glucose concentration in most studies, including ours, was higher compared to the study of Mushi et al. (1998), most likely due to the fact that they stored the serum blood sample for one hour at 20 C.

It has been well documented that avian plasma potassium concentrations are strongly affected by storage time and temperature (Lumeij, 1997). Because of the strong influence of storage time and temperature the plasma potassium concentrations are not reported in Table 1. Results of the separately performed experiment are reported in Table 3. This study showed that ostrich blood also should be centrifuged immediately after collection. Compared to the study of Levy et al. (1989), the potassium level in our study was higher. Exact time between sampling and separating plasma from cells in the study of Levy et al. (1989) is unknown. Changes in plasma potassium concentrations are caused by a shift of potassium into or out of the blood cells. The latter is especially prominent with lysis of red blood cells, although hemolysis was not seen in the samples from this study. The need to use plasma rather than serum to enable immediate separation of plasma from the blood cells seems not to be completely realized by the veterinary community, as can be illustrated by a recent article on plasma chemistry in mourning doves (*Zenaida macroura*) in this journal where blood was collected by cardiac puncture in euthanazed animals and plasma and cells separated within 4 hours after collection (Schulz et al., 2000). Plasma potassium concentrations up to 14 mmol/L were reported as reference values in this species. Considering the above arguments, reevaluation of these reported values seems appropriate.

In Table 2 Spearman rank correlation factors are shown. Especially CK-AST and CK-LDH are highly correlated to each other. This is probably because CK, AST and LDH are all related to muscle mass. There is no correction formula for these enzymes because half-time-values (= $t_{1/2}$) differ considerably. For example, $t_{1/2}$ of LDH in birds has been shown to be much shorter than $t_{1/2}$ of AST in birds and therefore the time of blood collection is of great influence on the values (Lumeij et al., 1988).

Plasma AST and CK concentrations were high in our study. This might have been caused by high muscle activity and capture-induced stress. Collection of blood was performed without penetrating skeletal muscle during venipuncture. There is a strong correlation between AST and CK as shown in Table 2.

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Urea in our study was higher compared to other studies. In general dehydration is the most likely cause of higher urea levels in birds (Lumeij, 1997). No explanation could be found for the higher urea concentrations in this study: both groups were apparently healthy and had *ad libitum* access to water.

Birds at reproductive age are the largest and most valuable group. As the birds in this study were a large, uniform group at reproductive age the values are likely to be of considerable use in ostrich clinical practice. In further studies it would be of interest to investigate possible differences between reference values established in different age-groups.

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