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PLASMA CHEMISTRY REFERENCES VALUES IN PSITTACIFORMES

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SUMMARY

Reference values for 17 plasma chemical variables in African greys, Amazons, cockatoos and macaws were established for use in avian clinical practice. The inner limits are given for the percentiles $P_{2.5}$ and $P_{97.5}$ with a probability of 90%. The following variables were studied: urea, creatinine, uric acid, urea/uric acid ratio, osmolality, sodium, potassium, calcium, glucose, aspartate aminotransferase, alanine aminotransferase, gamma glutamyltransferase, lactate dehydrogenase, creatine kinase, bile acids, total protein, albumin/globulin ratio. Differences between methods used and values found in this study and those reported previously are discussed.

INTRODUCTION

The large parrots, belonging to the genera *Psittacus, Amazona, Cacatua* and *Ara* are highly prized pets. In studies, using the racing pigeon as an experimental model, it has been established that blood chemistry can be a useful tool in clinical avian medicine (Lumeij, 1987a, 1988). Although these studies have yielded some rules which can probably be applied generally in avian medicine, the interpretation of results of blood chemistry in a particular species can only be performed if reference values of that particular species, established by the same methods, are available.

Blood chemistry references values in psittacines are scarce and in general poorly documented with respect to materials and methods (Rosskopf and Woerpel, 1984; Hawkey and Gulland, 1988), which makes duplication of the values difficult. Furthermore, some variables which seem useful from a clinical viewpoint have hitherto not been studied in this order.

The present study was undertaken to establish references values for plasma chemical variables in the most commonly encountered pet psittacine species in veterinary practice, with methods used at the Utrecht University Department of

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Companion Animal Clinical Sciences. The selection of the enzymes mentioned in this study was based on previously established usefulness in pigeons (Lumeij *et al.*, 1988a, b).

MATERIALS AND METHODS

The birds were apparently healthy adult parrots of the following genera: *Psittacus* (n=103), *Amazona* (n=99), *Cacatua* (n=27) and *Ara* (n=16). The following (sub)species were involved: *Psittacus erithacus erithacus* (n=64), *P.e.timneh* (n=39), *Amazona aestiva aestiva* (n=44), *A.ochrocephala ochrocephala* (n=28), *A.o.oratrix* (n=11), *A.o.auropalliata* (n=2), *A.amazonica amazonica* (n=11), *A.farinosa farinosa* (n=3), *Cacatua sulfurea sulfurea* (n=3), *C.s.abbotti* (n=10), *C.s.citrinocristata* (n=3), *C.galerita triton* (n=1), *C.moluccensis* (n=3), *C. alba* (n=7), *Ara ararauna* (n=9, *A.chloroptera* (n=3), *A.macao* (n=5). All birds had been housed and fed under identical conditions for more than 6 months in a parrot shelter (Nederlands Opvangcentrum Papegaaien, Geldrop, The Netherlands). The food was a commercial parrot diet consisting of seeds supplemented with vitamins and minerals. Jugular blood samples (2.5 ml per bird) were collected in heparanised vacuum tubes and treated as reported previously (Lumeij, 1987b). Collection of the samples was performed by experienced handlers on one day between 10.00 and 18.00 h.

Reference values in plasma for the following variables were established: urea, creatinine, uric acid, urea/uric acid ratio, osmolality, sodium, potassium, calcium, glucose, aspartate aminotransferase (ASAT; EC 2.6.1.1), alanine aminotransferase (ALAT; EC 2.6.1.2), gamma glutamyl transferase (GGT; EC 2.3.2.2), lactate dehydrogenase (LDH; EC 1.1.1.27), creatine phosphokinase (CPK; EC 2.7.3.2), bile acids, total protein, albumin/globulin ratio. All enzymes were measured on a Multistat FL III centrifugal analyser, 'MCA', (Allied Instrumentation Laboratory, London, UK) at 30°C, according to the Recommendations of the German Society for Clinical Chemistry, as reported previously (Lumeij and Wolfswinkel, 1988). Total bile acids were measured with an enzymatic kit for spectrofluorometric end-point determination (Sterognost-3α Flu, Nyegaard & Co., Diagnostics Division, Oslo). Plasma urea, creatinine and uric acid concentrations and the urea/uric acid ratio were determined with methods published previously (Lumeij, 1987c). Total protein was determined on a 'MCA', with the 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, Mo 63178, USA). The albumin/globulin ratio was calculated as reported previously (Lumeij, 1987d): pre-albumin and albumin as determined by plasma protein electrophoresis (cellulose acetate membranes, barbital buffer pH 8.6, 20 min, 200 V, staining with Ponceau S) were combined as 'albumin' and all globulin fractions as 'globulin'. Methods for determinations of other variables have also been reported previously (Lumeij and de Bruijne, 1985).

Where practicable, reference values were established using a distribution free method (see Discussion). For each variable the inner limits of the percentiles $P_{2.5}$-$P_{97.5}$ are presented with a probability of 90%, except for macaws, where the ranges are given since the number of animals was too small to use the percentile method. Differences between (sub)species within one genus and differences between different genera were tested for significance using the Kruskal Wallis test, whereby significance was assumed when P=0.05. Only significant differences are mentioned under Results.

RESULTS

The values (Tables 1 to 4) are reported at the genus level, since differences between (sub)species within one genus were in general not significant. The only exceptions were creatinine in African greys and Amazons, ASAT in African greys and the albumin/globulin ratio in Amazons (Tables 1 and 2).

Differences between values of blood chemical variables between genera were often significant (Table 5). The only exception was plasma osmolality, for which variable no significant difference was seen between genera. Urea concentrations in macaws and Amazons were higher than values in cockatoos and African greys. Creatinine concentrations in African greys and macaws were higher than in Amazons and cockatoos. Uric acid concentrations in African greys and cockatoos were higher than those from Amazons, while the values from cockatoos were also higher than those from macaws. The urea/uric acid ratio was higher in macaws and Amazons than in cockatoos and African greys. Potassium concentrations in cockatoos were higher than those from the other genera. Calcium concentrations were lower in African greys than in any other genus, while glucose concentrations were slightly but significantly lower than in macaws. ASAT activities were lower in *P. erithacus erithacus,* than in any other (sub) species studied; this was not the case for *P. erithacus timneh.* ALAT activities were different between genera. GGT activities were higher in Amazons than in African greys and macaws. LDH activities were highest in cockatoos, followed by African greys, while the difference between Amazons and macaws was not significant. CPK activities were highest in African greys, followed by Amazons, while macaws and cockatoos showed no differences. Bile acid concentrations were highest in Amazons and lowest in African greys, while cockatoos and macaws were not different. Total protein concentrations were highest in macaws and lowest in African greys, while Amazons and cockatoos formed an intermediate group. The albumin/globulin ratio was highest in Amazons.

DISCUSSION

The findings clearly demonstrate that there are significant differences with respect to plasma chemistry between different genera of the order Psittaciformes and therefore reference values should not be given for the order as a whole, as was done recently (Hawkey and Gulland, 1988). Although for most of the variables the references values can be given for the genus, for some variables a subdivision into species and even subspecies seems warranted. Subdivision by sex and age-groups would have been impractical for clinical use, although variation due to these factors cannot be excluded. In the parrot species studied it is impossible to differentiate between male and female without performing endoscopic examination of the gonads. In future studies with parrot species in which differentiation between sexes is easy (*e.g.* eclectus parrots, cockatiels, budgerigars) it would be of interest to investigate the significance of sex differences.

Plasma urea concentrations in the psittacines studied (Table 5) were considerably higher than those from racing pigeons (0.4 to 0.7 mmol/l), studied previously (Lumeij, 1987c), while uric acid concentrations (Table 5) were lower than those from pigeons (150 to 765 µmol/l; Lumeij and de Bruijne, 1985). It was interesting to note that those genera with the highest plasma urea concentrations (*Amazona* and *Ara*), had the lowest plasma uric acid concentrations. It seems that 'parrots' are 'less uricotelic' than pigeons, and Amazons and macaws are 'less uricotelic'

Table 1. Blood chemistry reference values (inner limits of $P_{2.5}$–$P_{97.5}$ with a probability of 90%) for the African grey parrot. Psittacus erithacus

Variable		$P_{2.5}$–$P_{97.5}$	*n*	Median	Mean	SD	Range
Urea	(mmol/l)	0.7–2.4	96	1.3	1.4	0.5	0.4–3.2
Creatinine	(μmol/l)	23–40	97	31	31	5.3	19–42
		23–39[a]	63[a]	29[a]	29[a]	5.1[a]	19–42[a]
		30–40[b]	34[b]	35[b]	35[b]	3.2[b]	28–41[b]
Uric acid	(μmol/l)	93–414	89	203	217	107	63–662
Urea/uric acid		2.4–15.6	65	6.7	8.0	4.6	1.4–31
Osmolality	(mOsm/kg)	320–347	70	332	332	8.8	316–356
Sodium	(mmol/l)	154–164	71	158	158	3.8	145–169
Potassium	(mmol/l)	2.5–3.9	71	3.1	3.2	0.5	2.1–4.4
Calcium	(mmol/l)	2.1–2.6	72	2.3	2.3	0.2	2.0–3.4
Glucose	(mmol/l)	11.4–16.1	74	13.7	13.8	1.4	10.4–17.5
ASAT	(IU/l)	54–155	101	88	96	35.6	42–230
		53–119[a]	63[a]	78[a]	82[a]	24.8[a]	42–173[a]
		68–193[b]	38[b]	116[b]	119[b]	39.0[b]	55–230[b]
ALAT	(IU/l)	12–59	101	37	36.8	14.3	9–87
GGT	(IU/l)	1–3.8	97	2.0	2.3	0.9	<1–4
LDH	(IU/l)	147–384	102	218	241	105	117–922
CPK	(IU/l)	123–875	99	242	335	234	96–1178
Bile acids	(μmol/l)	18–71	103	35	39	17.8	16–120
Total protein	(g/l)	32–44	71	36	37	4.4	28–49
Albumin/globulin		1.4–4.7	70	2.5	2.6	1.0	0.8–5.7

[a] *P. erithacus erithacus*

[b] *P. erithacus timneh.*

*Table 2. Blood chemistry reference values (inner limits of $P_{2.5}$–$P_{97.5}$ with a probability of 90%) for Amazon parrots (*Amazona *spp)*

Variable[a]	$P_{2.5}$–$P_{97.5}$	*n*	Median	Mean	SD	Range
Urea	0.9–4.6	99	2	2.2	1.3	0.4–9.9
Creatinine	19–33	99	27	26.6	4.0	15–37
	18–30[b]	44[b]	26[b]	24.9 [b]	3.9[b]	15–31[b]
	22–35[c]	41[c]	29[c]	28.3 [c]	3.7[c]	20–37[c]
Uric acid	72–312	96	150	176	103	10–630
Urea/uric acid	4.4–33	93	11.9	14.3	8.2	3.0–37.5
Osmolality	316–373	96	329	334	15.4	311–395
Sodium	149–164	97	155	156	4.8	143–174
Potassium	2.3–4.2	97	3.2	3.2	0.5	1.9–4.6
Calcium	2.0–2.8	99	2.5	2.5	0.3	1.9–4.2
Glucose	12.6–16.9	99	14	14.2	1.3	10.4–18.5
ASAT	57–194	99	109	114	44	27–279
ALAT	19–98	99	39	46	26.9	14–187
GGT	1–10	96	4	4.6	2.8	<1–15
LDH	46–208	99	95	110	54	28–313
CPK	45–565	99	121	202	220	36–1416
Bile acids	19–144	99	52	63	48	11–186
Total protein	33–50	99	40	41	5.1	23–53
Albumin/globulin	2.6–7.0	97	4	4.2	1.4	0.8–9.0
	2.7–7.1[b]	43[b]	4.5[b]	4.7[b]	1.6[b]	1.8–9.0[b]
	2.4–5.4[c]	41[c]	3.8[c]	3.9[c]	1.2[c]	0.8–7.6[c]

[a] Units as in Table 1.
[b] *A. aestiva.*
[c] *A. ochrocephala.*

*Table 3. Blood chemistry reference values (inner limits of $P_{2.5}$–$P_{97.5}$ with a probability of 90%) for cockatoos (*Cacatua *spp.)*

Variable[a]	$P_{2.5}$–$P_{97.5}$	*n*	Median	Mean	SD	Range
Urea	0.8–2.1	27	1.5	1.5	0.6	0.1–2.5
Creatinine	21–36	27	24	26.7	5.9	21–43
Uric acid	190–327	27	248	270	97	122–634
Urea/uric acid	2.7–8.9	27	6.0	6.1	3.0	2.0–17.0
Osmolality	317–347	27	331	330	11	317–360
Sodium	152–164	27	158	158	4.5	151–167
Potassium	3.2–4.9	27	4.3	4.2	0.6	2.9–5.2
Calcium	2.2–2.7	27	2.5	2.5	0.2	2.1–2.7
Glucose	12.8–17.6	27	14.7	14.7	1.8	12.7–19.7
ASAT	52–203	27	95	101	54	52–260
ALAT	12–37	27	22	23.4	8.2	9–40
GGT	2–5	27	3	3.1	1.8	<1–9
LDH	203–442	27	284	299	76	193–483
CPK	34–204	27	85	117	119	29–599
Bile acids	23–70	27	37	41	17.8	16–84
Total protein	35–44	27	38	39	3.7	31–47
Albumin/globulin	1.5–4.3	24	2.4	2.6	1.0	1.0–5.0

[a] Units as in Table 1.

*Table 4. Blood chemistry reference values (range) for macaws (*Ara *spp.)*

Variable[a]	*n*	Median	Mean	SD	Range
Urea	16	2.2	2.2	0.6	1.3–3.3
Creatinine	16	41.5	40.3	11.1	20–59
Uric acid	11	195	188	45	109–231
Urea/uric acid	11	12.6	13.6	6.1	5–28
Osmolality	11	334	341	18	319–378
Sodium	11	159	160	7.6	150–175
Potassium	11	2.3	2.7	0.8	1.9–4.1
Calcium	15	2.4	2.4	0.2	2.2–2.8
Glucose	16	15	15	1.7	12.0–17.9
ASAT	16	105	119	39	58–206
ALAT	16	60	64	19.6	22–105
GGT	15	3	2.3	1.8	<1–5
LDH	16	120	115	37.8	66–166
CPK	16	117	150	112	61–531
Bile acids	16	50	47	11.8	25–71
Total protein	11	44	43	5.1	33–53
Albumin/globulin	11	2	2.4	0.8	1.4–3.9

[a] Units as in Table 1.

than African greys and cockatoos, and excrete a substantial proportion of the waste products of protein metabolism in the form of urea. From these findings it can be expected that the incidence of gout would be higher in pigeons than in parrots and that African greys and cockatoos would be more susceptible to this condition than Amazons and macaws. The differences in concentrations of waste products of protein metabolism are even more clearly reflected in the urea/uric acid ratio. This ratio was found to be much higher in psittacines (Table 5) than in the pigeon (1.0 to 3.0; Lumeij, 1987c), especially in Amazons and macaws.

Table 5. Blood chemistry reference values for psittacine species, as used by the Utrecht University Department of Avian and Exotic Animal Medicine

Variable[a]	African Grey $P_{2.5}$–$P_{97.5}$	Amazon $P_{2.5}$–$P_{97.5}$	Cockatoo $P_{2.5}$–$P_{97.5}$	Macaw range
Urea	0.7–2.4	0.9–4.6	0.8–2.1	0.3–3.3
Creatinine	23–40	19–33	21–36	20–59
Uric acid	93–414	72–312	190–327	109–231
Urea/uric acid	2.4–15.6	4.4–33	2.7–8.9	5–28
Osmolality	320–347	316–373	317–347	319–378
Sodium	154–164	149–164	152–164	150–175
Potassium	2.5–3.9	2.3–4.2	3.2–4.9	1.9–4.1
Calcium	2.1–2.6	2.0–2.8	2.2–2.7	2.2–2.8
Glucose	11.4–16.1	12.6–16.9	12.8–17.6	12.0–17.9
ASAT	54–155	57–194	52–203	58–206
ALAT	12–59	19–98	12–37	22–105
GGT	1–3.8	1–10	2–5	<1–5
LDH	147–384	46–208	203–442	66–166
CPK	123–875	45–565	34–204	61–531
Bile acids	18–71	19–144	23–70	25–71
Total protein	32–44	33–50	35–44	33–53
Albumin/globulin	1.4–4.7	2.6–7.0	1.5–4.3	1.4–3.9

[a] Units as in Table 1.

For each variable the inner limits of the percentiles $P_{2.5}$ and $P_{97.5}$ are presented with a probability of 90%, except for macaws where the range is given.

Although differences between the reference values for plasma calcium concentration in the genera studied were not large, African grey parrots did have significantly lower mean and median values for this variable. Since the lower plasma calcium concentrations could only partially be explained by lower albumin bound calcium when compared with Amazons (Lumeij, 1990), the former species might have significantly lower plasma concentrations of free calcium. This could explain the fact that the hypocalcaemia syndrome is often reported in African greys (Hochleithner, 1989; Rosskopf and Woerpel, 1984), but rarely in the other psittacine species (Randell, 1981).

Comparison of data from the present study with those published previously is problematic, since considerable variation can be expected depending on the materials and methods used. Often there is no information available on the analytical techniques used and it is not always clear what the values given represent. For those biological data which do not have a Gaussian distribution it is incorrect to define the reference range as the mean plus or minus two standard deviations. In these cases non-parametric statistics should be used to establish reference values. When reference values are published for clinical use it is strongly recommended to use the inner limits of the percentiles $P_{2.5}$ and $P_{97.5}$ with a probability of 90% (Rümke and Bezemer, 1972), as was done in the present study. Examples of variables examined in this study where the 'reference values' would include values in the pathological range if mean plus or minus two times standard deviation had been used include plasma calcium concentration in African greys and Amazons, the albumin/globulin ratio in African greys, cockatoos and macaws and plasma potassium concentration in cockatoos (Tables 1 to 4).

When the inner limits of the percentiles $P_{2.5}$ and $P_{97.5}$ are used at least 2.5% of 'normal' individuals will have values $<P_{2.5}$ and at least 2.5% will have values $>P_{97.5}$. If the outer limits of the percentiles $P_{2.5}$ and $P_{97.5}$ are used maximal 2.5% of 'normals' will have values $<P_{2.5}$ and maximal 2.5% of 'normal' individuals will have values $>P_{97.5}$. For clinical use, where an early warning of developing abnormalities is important, the outer limits are less useful. For those variables in Tables 1 and 2 where $n>91$ the minimum and maximum values given are the same as the outer limits of the percentiles $P_{2.5}$ and $P_{97.5}$, with a certainty of 90%. The intervals between the outer and inner limits of the percentiles $P_{2.5}$ and $P_{97.5}$ can be regarded as the ranges of uncertainty between 'almost certainly normal' and 'almost certainly abnormal' (Rümke and Bezemer, 1972).

The lower limit of the plasma potassium concentration for 'psittacines' published by Hawkey and Gulland (1988) was extremely low (1.1 mmol/l) and may have been caused by experimental error. It has been demonstrated that plasma and cells should be separated within minutes of sampling to obtain reliable values for plasma potassium concentrations in avian blood, since there is a time dependent decrease of plasma potassium concentration due to contact between plasma and cells (Lumeij, 1985a).

As was reported previously (Lumeij *et al.*, 1990) considerable differences in total protein concentrations can be expected when different protein standards are used. Since 'parrot albumin' and 'parrot globulin' are not commercially available and because it would be impractical to use a species-specific protein standard when dealing with the different psittacine species, human protein was used, since this is the standard most widely used in commercial laboratories. Albumin and prealbumin concentrations were calculated from total protein concentrations and plasma protein electrophoresis, since it has been shown that the bromcresol green dye binding method for albumin determination is unreliable in avian blood (Lumeij *et al.*, 1990). Despite the fact that the refractometric method for total protein determinations is still being propagated for use in psittacines (Coles, 1988), it has been shown that this method is unreliable in avian blood (Lumeij, 1985b). Furthermore, various disease conditions are accompanied by a decreased albumin concentration and an elevated globulin concentration, while the total protein concentration remains in the normal range (Lumeij, 1987d), therefore the albumin/globulin ratio is of greater clinical significance than the total protein concentration.

Some values could not be compared with those from the literature since they have not been reported previously for psittacines, such as plasma urea and bile acid concentrations. Both variables, however, are of great clinical significance. It has been shown for the racing pigeon, *Columba livia domestica* (Lumeij, 1987c) that, contrary to common belief, plasma urea concentration is a useful variable for detecting prerenal renal failure in birds, while plasma uric acid concentration is not useful for this purpose. In prerenal renal failure there is an increase in the urea/uric acid ratio. Bile acid concentration is the single most useful variable in plasma for detecting liver disease in birds, since it is both specific and sensitive (Lumeij, 1988).

The data presented in this paper form a basis for further studies with regard to plasma biochemistry as a result of experimentally induced or spontaneously occurring disease in parrots. When using these reference values the avian clinician should carefully take materials, methods and sample preparation into consideration and discuss these with the laboratory to avoid errors due to differences in methodology.

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RESUME

Constantes sanguines chez les Psittaciformes

Les valeurs de référence pour 17 constantes chimiques du plasma chez des perroquets gris du Gabon, amazones, cacatoes et aras ont été établies pour être utilisées en pratique clinique aviaire. Les limites sont données en percentiles $P_{2,5}$ et $P_{97,5}$ avec une probabilité de 90%. Les constantes suivantes ont été étudiées: urée, créatinine, acide urique, rapport urée/acide urique, osmolarité, sodium, potassium, calcium, glucose, aspartate aminotransférase, alanine aminotransferase, gamma glutamyltransferase, lactate dehydrogenase, creatine kinase, acides biliares, protéines totales, rapport albumine/globuline.

Les différences entre les méthodes utilisées et les valeurs trouvées dans cette étude et celles rapportées précédemment sont discutées.

ZUSAMMENFASSUNG

Referenzwerte für die Plasmachemie bei Psittaciformes

Referenzwerte von 17 chemischen Plasmaparametern von Afrikanischen Graupapageien, Amazonen, Kakadus und Aras wurden für den Gebrauch in der klinischen Vogelpraxis aufgestellt. Die inneren Grenzen wurden für die Werte $P_{2.5}$ und $P_{17.5}$ mit einer Wahrscheinlichkeit von 90% angegeben. Folgende Parameter wurden untersucht: Harnstoff, Creatinin, Harnsäure, Harnstoff/Harnsäureverhältnis, Osmolalität, Natrium, Kalium, Calcium, Glukose, Aspartataminotransferase, Alaninaminotransferase, Gammaglutamyltransferase, Laktatdehydrogenase, Creatinkinase, Gallensäuren, Totalprotein, Albumin/Globulin Verhältnis.

Die Unterschiede zwischen angewandten Methoden und zwischen den in diesen Untersuchungen gefundenen und früher berichtenen Werten werden diskutiert.

RESUME

Valores plasmáticos de referencia en las Psittaciformes

Se obtuvieron valores de referencia de 17 variables plasmáticas para su empleo en la prática clínica aviar de cacatúas y araraunas grises africanas y amazónicas. Los límites interiores fueron dados por los percentiles $P_{2.5}$ y $P_{97.5}$ con una probabilidad del 90%. Se estudiaron los siguientes valores: urea, creatinina, ácido úrico, valor urea/ácido úrico, osmolalidad, sodio, potasio, calcio, glucosa, aspártico aminotransferasa, alanin aminotransferasa, gamma glutamiltransferasa, láctico deshidrogenasa, creatin kinasa, ácidos biliares, proteínas totales, valor albúmina/globulina. Se discuten las diferencias encontradas entre los métodos empleados y los valores encontrados y aquéllos descritos previamente.