Normal haematology and blood biochemistry of wild Nile crocodiles (*Crocodylus niloticus*) in the Okavango Delta, Botswana

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ABSTRACT

Wild Nile crocodiles (*Crocodylus niloticus*) of various size classes were captured in the Okavango Delta, Botswana. Blood was collected from the post occipital sinus and used for the determination of a wide range of haematological and biochemical parameters. These values were compared between the sexes and between 3 size classes. The values were also compared with the limited data available from farmed Nile crocodiles, as well as from other wild Nile crocodiles. The Okavango crocodiles were comparatively anaemic, and had comparatively low total protein and blood glucose levels. There was a high prevalence of *Hepatozoon pettiti* infection, however, there was no significant difference in haematological values between the infected and uninfected crocodiles. The values reported here will be useful in diagnostic investigations in both zoo and farmed Nile crocodiles.

Key words: biochemistry, *Crocodylus niloticus,* haematology, Nile crocodile, Okavango Delta.

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INTRODUCTION

Crocodile farming has developed into a large global industry over the past 25 years[14,30]. Since the promulgation of CITES, the proportion of crocodilian skins supplied to the industry from wild harvests has diminished dramatically, from over 99 % in 1983 to only 6 % in 1999. Of an estimated total of 1 182 469 skins, 70 381 originated from wild harvest, 255 945 from ranches and 856 143 were captive bred[15]. Wild harvesting refers to hunting or harvesting directly from a wild population, whereas ranching refers to crocodiles raised on farms, but collected in the wild either as eggs or hatchlings. Captive bred, or farmed crocodiles, are hatched from eggs originating from breeding stock kept on the farm. Eight of the 23 crocodilian species are currently utilised in the worldwide industry. The Nile crocodile (*Crocodylus niloticus*) is the only species utilised in Africa. Wild harvest still occurs in Tanzania, while ranching occurs in Botswana, Ethiopia, Kenya, Madagascar, Malawi, Mozambique, Namibia, Tanzania, Uganda, Zambia and Zimbabwe. Captive breeding occurs in Kenya, Madagascar, Namibia, South Africa, Botswana and Zimbabwe. In 1999, Nile crocodile skins accounted for 126 612 out of the total supply of 1 182 469 skins (10.7 %)[15]. As such it is the third most important crocodilian from an economic perspective.

Experience gained during the early years of crocodile farming in southern Africa has led to much improved husbandry, better survival rates and reduced disease prevalence. For example, hatchling mortality on Zimbabwe farms decreased from 31.2 % in 1983 to 11.7 % in 1991[8]. Nevertheless, certain diseases remain important[9,20,21]. Veterinary involvement on crocodile farms is vital to limit production loss due to disease. To date, diagnostics have relied primarily on *post mortem* examinations in the face of a disease outbreak. In veterinary medicine, clinical pathology is widely employed as a diagnostic tool in many species. The potential application of reference ranges for *Crocodylus porosus* as a basis in disease investigations has been demonstrated[19]. However, the usefulness of clinical pathology in Nile crocodiles is restricted by the lack of reference ranges for haematological and biochemical values. The limited studies to date have been on farmed Nile crocodiles, which due to their stressed metabolic state, do not necessarily represent true normal values.

The purpose of this study was to establish normal haematological and biochemical values for wild Nile crocodiles, to contribute towards the establishment of reference ranges for *C. niloticus*. Such ranges will facilitate the interpretation of laboratory results, making clinical pathology a viable diagnostic tool for Nile crocodiles in both zoos and farms. Furthermore, baseline information regarding haematology and biochemistry is essential for a species that may be used as an ecological indicator.

MATERIALS AND METHODS

Sample collection

Wild Nile crocodiles of various size classes were captured in the Panhandle region of the Okavango Delta in summer (February 2005). Capture was carried out at night, using a 4.8 m flat-bottomed aluminium boat propelled by a 60 hp engine. Crocodiles were located using a powerful spot-light which, once shone into the crocodile's eyes, reflected back a red glow due to the presence of a retinal *tapetum lucidum*. Crocodiles estimated to be smaller than 1.2 m total length (TL) were captured by hand. Crocodiles between 1.2 m and 2.3 m were captured using a swivelling noose (Animal Handling Co.) which was placed over the snout and pulled tight in the neck region. Crocodiles were then brought onto the boat, jaws were taped shut and the animals were physically restrained. Each crocodile was blindfolded and restrained in ventral recumbency. Fifty-three animals were randomly selected for blood collection. Blood was collected from the post-occipital sinus, on the dorsal midline and just caudal to the base of the head[4]. A 21 G or 23 G needle and a 3, 5 or 10 mℓ syringe was used, depending on the size of the crocodile, and the blood was transferred directly into a lithium heparin blood tube. Blood smears were made from whole blood using the cover slip method[12]. Following blood collection, the crocodile was measured: total length (TL) and snout-to-vent length (SVL) were recorded using a flexible measuring tape

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(±1.0 mm), and the animal was weighed using a harness around the forelimbs and a Pesola spring balance. Each crocodile was sexed by cloacal examination of the cliteropenis[11,13], and examined for clinical abnormalities, including bite wounds, skin lesions, conjunctivitis, and poor condition.

Haematology

Further processing of samples commenced immediately upon return to the field laboratory, resulting in a time interval ranging from 1 to 12 hours between sampling and processing. One millilitre of blood was transferred to an Epindorf tube for haematological analysis. The remaining blood was centrifuged using a manual desktop centrifuge, and the plasma supernatant was frozen for further biochemical analyses. If the volume of total blood was small it was allocated for either heamatological or biochemical analysis, but not both. Haematological analysis was performed on 39 samples.

Packed cell volumes (PCV) were determined using a Statspin MP microhaematocrit centrifuge. Blood was drawn into a standard microhaematocrit tube and spun for 5 minutes at 12 000 G.

Total red cell counts (RCC) were performed both manually and automatically using an electronic particle counter. The automated counts were determined using a Beckman Coulter Ac*T Series haematology analyser (Coulter SA). The manual counts were performed using Natt and Herrick's solution. A 1:200 dilution was made by drawing blood up to the 0.5 mark on a red blood cell diluting pipette, then filling the pipette to the 101 mark with Natt and Herrick's solution[4]. The diluted blood was then used to charge both counting chambers of an improved Neubauer haemocytometer (Hawksley and Sons, Lancing, UK). After 5 minutes in a damp chamber the red cells were counted in the 4 corner cells and central cell of the central large square of the counting chamber. This was repeated on the second chamber, and the average multiplied by 10 000 to obtain the total red cell count per microlitre.

Haemoglobin concentration (Hb) was determined using a Beckman Coulter Ac*T Series haematology analyser (Coulter SA).

Red blood cell indices were calculated using standard formulas[12] as follows:

Mean corpuscular volume: MCV (fl) = PCV/RCC.

Mean corpuscular haemoglobin: MCH (pg) = Hb (g/dℓ) × 10 / RCC.

Mean corpuscular haemoglobin concentration: MCHC (g/dℓ) = Hb (g/dℓ) / PCV.

Table 1: **Normal haematological values determined for wild Nile crocodiles captured in the Okavango Delta in Botswana** (n = 38).

Parameter	Mean	SD	Range
Total length (mm)	879.0		557–1930
Snout–vent length (mm)	426.0		255–1015
Mass (g)	2879.2		240–25000
PCV (%)	17.9	2.0	14–22
RCC (×10^6/μℓ)	0.59	0.12	0.35–1.00
Hb (g/dℓ)	7.11	1.00	4.7–9.5
MCV (fl)	312.2	60.6	200.0–465.1
MCH (pg)	123.2	25.1	83.8–220.9
MCHC (g/dℓ)	39.6	3.3	29.0–47.5
WBC (10^3/μℓ)	11.28	4.74	3.75–26.22
Heterophils %	20.5	8.7	4–39
Lymphocytes %	62.0	11.3	44–85
Monocytes %	0.9	1.8	0–10
Eosinophils %	4.9	4.8	0–17
Basophils %	5.9	4.5	0–16
Azurophils %	5.1	4.5	0–21
Heterophils (×10^3/μℓ)	2.09	0.75	0.45–3.66
Lymphocytes (×10^3/μℓ)	7.20	3.80	1.65–17.83
Monocytes (×10^3/μℓ)	0.09	0.18	0.0–0.79
Eosinophils (×10^3/μℓ)	0.53	0.56	0.0–2.14
Basophils (×10^3/μℓ)	0.69	0.66	0.0–2.90
Azurophils (×10^3/μℓ)	0.60	0.74	0.0–3.93

Total white cell counts (WBC) were obtained indirectly using the Unopette 5877 system (Becton-Dickinson, USA). The Unopette pipette was filled with blood (25 μℓ) and mixed with the phloxine B diluent in the reservoir. From this both counting chambers of an improved Neubauer haemocytometer were charged. After 5 minutes in a damp chamber all the pink-staining granulocytes were counted in both chambers.

Blood smears were stained with Diff-Quick stain (American Scientific Products, Illinois, USA)[3]. Differential leukocyte counts were carried out on the stained smears. The percentage of heterophils and eosinophils was calculated and used to calculate total WBC[4]:

Total WBC/μℓ = stained cells counted in chambers × 1.1×16×100 / percentage heterophils and eosinophils.

Thrombocytes were not counted. If included in a percentage differential count it tends to upset the other values.

The prevalence of haemoparasites was determined by examination of the Diff-Quick stained blood smears.

Biochemistry

Epindorf tubes containing plasma were stored at –10 °C in a domestic gas freezer. On return from the study site 35 samples were submitted to the laboratory*. The interval between sampling and further processing ranged from 5 to 23 days. Biochemical analyses were performed on a Next/Vetex Alfa Wassermann Analyser (Alfa Wassermann B.V., Woerden, The Netherlands). Total protein was determined by a modified Wechselbaums biuret method and albumin by the bromocresol green method. Globulin and albumin:globulin ratio were calculated. Creatinine was determined by the picrate method, total calcium by the Arsenazo method, and glucose by the glucose oxidase method. Cholesterol was determined by enzymatic methods. Magnesium was measured by the zylidyl blue method, and uric acid by the uricase method.

Alkaline phosphatase (ALP), alanine aminotranferase (ALT) and aspartate aminotransferase (AST) were determined by optimised versions of the standard IFCC methods.

Sodium, potassium, chloride and ionised calcium were measured using an 865 pH/Blood Gas Analyser (Chiron Diagnostics Limited, Halstead), by means of ion selective electrodes.

Statistical analysis

All values were analysed for significant differences ($P < 0.05$) between sexes and between size classes by one way analysis of variance (ANOVA). The residuals were checked for normality of distribution with normal probability plots. Where data were not normally distributed, significance was tested by a Mann-Whitney test (gender comparison), and by a non-parametric Kruskall-Wallis test (size comparison).

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Table 2: **Comparison of haematological parameters between male and female crocodiles captured in the Okavango Delta, Botswana.**

Parameter	Males (n = 27)			Females (n = 9)		
	Mean	SD	Range	Mean	SD	Range
PCV (%)	17.7	1.9	14–22	19.0	2.1	16–22
RCC (×10^6/μℓ)	**0.57**[a]	0.10	0.39–0.74	**0.66**	0.15	0.43–0.72
Hb (g/dℓ)	**6.90**	0.85	4.7–8.2	**7.77**	1.23	6.0–9.5
MCV (fl)	319.8	57.9	216.2–461.5	298.4	72.4	200.0–465.1
MCH (pg)	124.3	20.4	83.8–182.1	122.6	39.0	89.0–220.9
MCHC (g/dℓ)	39.1	3.3	29.0–45.0	40.8	3.4	37.2–47.5
WBC (×10^3/μℓ)	11.31	5.02	6.13–26.22	10.95	3.63	3.75–15.53
Heterophils %	20.6	9.1	4–39	19.4	7.0	13–35
Lymphocytes %	63.0	11.3	39–85	58.2	11.7	45–72
Monocytes %	1.0	2.1	0–10	0.7	0.9	0–2
Eosinophils%	**3.8**	3.9	0–17	**9.1**	5.4	2–16
Basophils %	5.4	4.0	0–15	7.7	6.1	0–16
Azurophils %	5.3	5.0	0–21	4.6	2.9	0–8
Heterophils (×10^3/μℓ)	2.10	0.97	0.45–3.66	1.96	0.54	1.33–2.92
Lymphocytes (×10^3/μℓ)	7.31	4.30	3.3–17.83	6.59	2.82	1.65–10.02
Monocytes (×10^3/μℓ)	0.10	0.20	0.0–0.79	0.07	0.08	0.0–0.19
Eosinophils (×10^3/μℓ)	**0.42**	0.46	0.0–2.14	**0.97**	0.66	0.0–1.87
Basophils (×10^3/μℓ)	0.64	0.63	0.0–2.90	0.86	0.79	0.0–2.49
Azurophils (×10^3/μℓ)	0.63	0.82	0.0–3.93	0.47	0.32	0.0–0.99

[a]Mean values in bold differed significantly ($P < 0.05$) between males and females.

RESULTS

Haematology

One specimen had a severely elevated WBC (60.90 × 10^3/μℓ), 5.4 times the mean and was excluded from the results. This crocodile was not identified as sick on clinical examination, nor did its length to weight ratio (1.40) differ from the mean of its size class (1.44). The remaining 38 specimens used for haematology originated from crocodiles ranging in total length from 557–1930 mm, with a mean TL of 879 mm, and a mean SVL of 426 mm.

The haematology results are shown in Table 1. There was no difference between the mean RCC obtained from the manual and automated counts, indicating that an electronic particle counter can accurately determine crocodilian RCC's. The differential count includes azurophils. The exact nature of azurophils in reptiles is uncertain, and the existence of azurophils in crocodilians is controversial[17,32]. However, we found a line of cells distinct from monocytes that we could not classify except in their own category.

Table 2 compares haematological values between males and females. Males had significantly lower ($P < 0.05$) mean RCC and Hb than females. The mean eosinophil percentage and count was also significantly lower in males than females.

Table 3 compares haematological values of the different size classes, which were determined by SVL (yearlings 170–389 mm; juveniles 390–663 mm; subadults 664–1158 mm). There were a number of significant differences ($P < 0.05$). Mean PCV of the yearlings was lower than that of the subadults. Hb was lower in yearlings and juveniles than in subadults. Eosinophil percentage of both yearlings and juveniles was lower than that of

Table 3: **Comparison of haematological parameters between different size classes of crocodiles captured in the Okavango Delta, Botswana** (SVL: yearlings: 170–389 mm, juveniles: 390–663 mm, subadults: 664–1158 mm).

Parameter	Yearlings (n = 22)			Juveniles (n = 11)			Subadults (n = 5)		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
PCV (%)	**17.3**a	1.8	14–21	18.2	1.5	16–22	**20.2**	2.0	17–22
RCC (×10^6/μℓ)	0.58	0.11	0.35–0.74	0.58	0.06	0.47–0.66	0.70	0.21	0.61–1.00
Hb (g/dℓ)	**6.73**	0.83	4.7–8.2	**7.16**	0.67	6.0–8.6	**8.62**	0.90	7.1–9.5
MCV (fl)	311.0	62.3	216.2–461.5	315.3	41.0	242.4–383.0	311.0	96.4	200.0–465.1
MCH (pg)	120.1	21.5	83.8–182.1	124.2	16.2	90.9–149.0	134.2	50.4	89.0–220.9
MCHC (g/dℓ)	38.9	3.6	29.0–45.0	39.4	1.6	37.2–42.2	42.8	3.2	39.5–47.5
WBC (×10^3/μℓ)	10.12	3.63	6.13–19.61	13.53	6.61	3.75–26.22	11.45	2.83	7.84–15.53
Heterophils %	22.0	9.0	4–39	19.4	9.3	8–35	16.8	4.8	13–25
Lymphocytes %	64.1	10.8	49–85	61.9	12.6	39–83	53.0	6.4	45–58
Monocytes %	1.2	2.2	0–10	0.4	0.9	0–3	0.6	0.9	0–2
Eosinophils%	**2.8**	3.1	0–11	**5.9**	4.9	1–17	**12.0**	3.7	7–16
Basophils %	**4.4**	3.3	0–13	**6.1**	4.6	1–15	**12.0**	4.2	7–16
Azurophils %	5.0	5.0	0–21	5.1	4.6	0–15	5.2	2.2	3–8
Heteroph. (×10^3/μℓ)	2.11	0.85	0.45–3.66	2.14	0.67	1.31–3.43	1.87	0.47	1.33–2.59
Lympho. (×10^3/μℓ)	6.64	3.08	3.30–14.71	8.79	5.25	1.65–17.83	6.19	2.11	3.53–9.01
Monocytes (×10^3/μℓ)	0.10	0.17	0.0–0.70	0.09	0.24	0.0–0.79	0.06	0.08	0.0–0.16
Eosinophils(×10^3/μℓ)	**0.27**	0.34	0.0–1.41	**0.68**	0.57	0.20–2.14	**1.35**	0.42	0.72–1.87
Basophils (×10^3/μℓ)	**0.45**	0.36	0.0–1.24	0.85	0.86	0.15–2.9	**1.38**	0.68	0.87–2.49
Azurophils (×10^3/μℓ)	0.51	0.52	0.0–1.92	0.81	1.18	0.0–3.93	0.56	0.20	0.37–0.89

[a]Mean values in bold differed significantly ($P < 0.05$) between size classes.

subadults, and the eosinophil count differed significantly between all 3 size classes. Basophil percentage of yearlings and juveniles differed from the subadults, and the basophil count differed between yearlings and subadults.

Haemogregarine parasites were present in 21 of 38 specimens (55.3 %). There were no significant differences between any of the haematological parameters in the infected group compared with the uninfected group. The mean PCV of the infected group was 18.2 %, compared with 17.6 % in the uninfected group. The mean SVL of the infected crocodiles was 463 mm compared to 382 mm for the uninfected crocodiles.

Biochemistry

The 35 specimens that underwent biochemical analysis were collected from crocodiles ranging in size from 593–1930 mm TL, with a mean of 916 mm, and a mean SVL of 449 mm. The results of the biochemistry are shown in Table 4.

When comparing males and females, mean total protein of males was significantly lower than that of females, and the albumin:globulin ratio in males was higher than in females. Potassium was significantly lower in males. The other parameters showed no significant differences between the sexes (Table 5).

The biochemical values of the different size classes are shown in Table 6. Mean total protein and globulin of yearlings and juveniles were both significantly lower than in the subadults. Albumin: globulin ratio was significantly higher in yearlings and juveniles compared to subadults. Aspartate aminotransferase of yearlings was significantly lower than that of subadults. Glucose was higher in yearlings than in juveniles. Sodium in yearlings was significantly lower than in subadults. Potassium was significantly lower in both yearlings and juveniles than in subadults.

Table 4: **Normal biochemical values of Okavango Nile crocodiles determined in this study.** (n = 35).

Parameter	Mean	SD	Range
Total length (mm)	916.0		593–1930
Mass (g)	3208.0		305–25000
Total protein (g/ℓ)	41.2		28.9–57.1
Alb (g/ℓ)	14.7	1.8	11.1–19.4
Glob (g/ℓ)	26.5	6.8	16.5–42.6
A:G ratio	0.58	0.12	0.34–0.79
ALT (U/ℓ)	43.9	13.1	15–63
ALP (U/ℓ)	21.1	13.7	3–72
AST (U/ℓ)	66.5	56.4	14–211
Gluc (mmol/ℓ)	3.8	0.5	1.8–4.8
Na (mmol/ℓ)	147.9	8.3	122–164
K (mmol/ℓ)	4.88	1.03	3.30–7.65
Ca^{Total} (mmol/ℓ)	2.73	0.19	2.34–3.15
Ca^{2+} (mmol/ℓ)	1.35	0.12	1.08–1.61
Mg (mmol/ℓ)	1.15	0.26	0.65–1.72
Chol (mmol/ℓ)	5.49	2.08	0.0–9.86
Creat (µmol/ℓ)	34.0	10.2	17–56
Cl (mmol/ℓ)	120.3	9.6	97–135
UA (mmol/ℓ)	0.12	0.05	0.04–0.30

DISCUSSION

Haematology

The effect of gender and size, on haematological and biochemical values, has been studied in *Crocodylus palustris*[24]. There is no clear similarity between the parameters that differed in *C. palustris* compared with those that differed in *C. niloticus*. However, environmental conditions in the 2 studies were not comparable, as *C. palustris* were captive specimens. In *C. palustris* eosinophils differed between size classes, but subadults had lower counts than the juveniles and adults. In the Nile crocodile we found an increasing eosinophil count with increasing size. Causes of an increased eosinophil count in reptiles include parasitic infection and non-specific inflammation[4,32].

In the comparisons between gender and between size classes, the number of males and females in the sample size differed, as did the number of crocodiles in each size class. The distribution of sexes between the size classes of the captured crocodiles also differed. This made it impossible to eliminate the effect of gender when comparing size classes, and impos-

Table 5: **Biochemical comparison of various blood parameters between male and female Nile crocodiles captured in the Okavango Delta, Botswana.**

Parameter	Males (n = 27)			Females (n = 8)		
	Mean	SD	Range	Mean	SD	Range
Tot protein (g/ℓ)	**39.82**[a]	6.18	28.9–52.8	**45.83**	10.37	33.8–57.1
Alb (g/ℓ)	14.84	1.77	11.9–19.4	14.09	1.70	11.1–15.1
Glob (g/ℓ)	24.99	5.20	16.5–36.6	31.78	9.20	22.1–42.6
A:G ratio	**0.61**	0.11	0.43–0.79	**0.47**	0.11	0.34–0.61
ALT (U/ℓ)	45.2	12.14	23–69	39.3	16.14	15–63
ALP (U/ℓ)	22.7	14.34	3–72	16.0	10.42	3–32
AST (U/ℓ)	57.4	50.35	14–189	97.3	67.95	14–211
Gluc (mmol/ℓ)	3.87	0.60	3.4–4.8	3.71	0.29	3.3–4.1
Na (mmol/ℓ)	146.79	7.28	143–158	151.80	10.80	129–164
K (mmol/ℓ)	**4.65**	0.82	3.30–6.55	**5.68**	1.31	4.21–7.65
Ca^{Total} (mmol/ℓ)	2.72	0.17	2.38–3.05	2.78	0.26	2.34–3.15
Ca^{2+} (mmol/ℓ)	1.37	0.11	1.15–1.61	1.29	0.15	1.08–1.45
Mg (mmol/ℓ)	1.17	0.25	0.65–1.72	1.09	0.27	0.73–1.55
Chol (mmol/ℓ)	5.67	2.03	0–8.69	4.88	2.24	2.88–9.86
Creat (µmol/ℓ)	34.00	10.98	17–56	34.13	7.32	21–46
Cl (mmol/ℓ)	120.70	9.30	97–135	119.14	11.39	107–133
UA (mmol/ℓ)	0.13	0.05	0.04–0.18	0.11	0.04	0.05–0.15

[a]Mean values in bold differed significantly ($P < 0.05$) between males and females.

Table 6: **Biochemical comparison of various blood parameters between Nile crocodiles of various size classes captured in the Okavango Delta, Botswana.** (SVL: yearlings: 170–389 mm, juveniles: 390–663 mm, subadults: 664–1158 mm).

Parameter	Yearlings (n = 17)			Juveniles (n = 13)			Subadults (n = 5)		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Tot protein (g/ℓ)	**37.73**[a]	6.05	28.9–52.8	**41.41**	5.63	33.4–52.5	**52.42**	6.51	41.4–57.1
Alb (g/ℓ)	14.42	1.75	11.9–17.4	14.79	2.09	11.1–19.4	15.18	0.64	14.3–15.9
Glob (g/ℓ)	**23.34**	4.73	16.5–36.4	**26.62**	4.84	19.5–36.6	**37.24**	6.95	25.7–42.6
A:G ratio	**0.63**	0.09	0.45–0.79	**0.57**	0.12	0.43–0.77	**0.42**	0.11	0.34–0.61
ALT (U/ℓ)	45.35	10.62	26–63	45.54	14.20	23–69	34.40	16.80	15–54
ALP (U/ℓ)	20.82	11.59	3–54	22.31	17.56	5–72	19.20	11.08	3–32
AST (U/ℓ)	**36.59**	33.67	14–149	79.23	55.87	14–189	**135.00**	54.05	59–211
Gluc (mmol/ℓ)	**4.12**	0.37	3.4–4.8	**3.54**	0.64	1.8–4.5	3.60	0.29	3.3–4.0
Na (mmol/ℓ)	**146.17**	4.47	137.9–157.0	146.81	10.79	122.0–158.0	**156.88**	6.39	148.0–164.0
K (mmol/ℓ)	**4.44**	0.73	3.30–5.96	**4.92**	0.80	3.89–6.55	**6.30**	1.28	4.21–7.65
Ca^{Total} (mmol/ℓ)	2.69	0.16	2.38–2.90	2.74	0.20	2.34–3.05	2.86	0.24	2.63–3.15
Ca^{2+} (mmol/ℓ)	1.33	0.09	1.14–1.49	1.39	0.14	1.15–1.61	1.31	0.16	1.08–1.44
Mg (mmol/ℓ)	1.10	0.26	0.65–1.72	1.19	0.28	0.73–1.57	1.24	0.19	1.03–1.55
Chol (mmol/ℓ)	6.36	2.40	0.00–9.86	4.74	1.42	2.88–7.80	4.47	1.07	2.91–5.49
Creat (µmol/ℓ)	31.82	11.16	17–50	36.77	10.30	19–56	34.40	3.91	29–40
Cl (mmol/ℓ)	122.71	6.19	112–135	118.17	12.19	97–133	118.50	11.68	107–130
UA (mmol/ℓ)	0.13	0.04	0.05–0.20	0.12	0.07	0.04–0.30	0.09	0.01	0.08–0.10

[a]Mean values in bold differed significantly ($P < 0.05$) between size classes.

sible to eliminate the effect of size when comparing genders. A future study will require a larger sample size in order to evaluate the differences between genders and size classes more accurately.

A comparison of haematological ranges with ranges reported previously for Nile crocodiles is difficult because of the limited data available (Table 7). The packed cell volume of the Okavango population was substantially lower than that reported from farmed Nile crocodiles. Likely causes of a low PCV include erythrolysis due to haemoparasites, poor nutritional plane or poor collection techniques[32,34]. Over half of the specimens were infected with *Hepatozoon pettiti.* However, there was no significant difference in PCV between the infected and uninfected group. The nutritional plane of wild Okavango crocodiles is undoubtedly lower than that of farmed crocodiles, which frequently become obese. In a comparative study involving wild and farmed American alligators (*Alligator mississippiensis*), the wild alligators were relatively anaemic, having a lower PCV of 24.44 %, compared with 27.29 %, and a RCC of 0.42 × $10^6/\mu\ell$ compared with 0.47 × $10^6/\mu\ell$ in the farmed alligators[2]. The wild alligators were transferred into captivity and under captive conditions the alligators became less anaemic.

The WBC of Nile crocodiles in the Okavango Delta was higher than that reported from farmed Nile crocodiles in Zimbabwe[16]. Causes of increased WBC include inflammatory response to microbial infection, parasites and non-specific inflammation[4,32]. In some species of reptiles there is a seasonal fluctuation (increase in summer)[34].

Haematological values for various other crocodilian species are shown in Table 8. The haematological values reported in this study are generally within the ranges recorded for other crocodilians, although the mean PCV obtained in this study is lower than those reported for other species. The total leukocyte count varies widely between the species.

Table 7: **Comparison of haematology parameters between wild and farmed Nile crocodiles.**

Parameter	Okavango (wild)		Zimbabwe farms[16]		Zimbabwe farms[7]		SA farms[26]
	Mean	Range (n = 38)	Mean	Range (n = 44)	Mean	Range[a]	Mean (n = 5)
PCV (%)	17.9	14–22	27.2	24–31	22	13–27	
RCC (×$10^6/\mu\ell$)	0.59	0.35–1.00	0.92	0.6–1.31			
Hb (g/dℓ)	7.11	4.7–9.5	8.7	7.8–9.5	7.4	6.4–8.7	
MCV (fl)	312.2	200.0–465.1	306.7	206.1–440.7			
MCH (pg)	123.2	83.8–220.9	97.9	65.3–153.3			
MCHC (g/dℓ)	39.6	29.0–47.5	31.9	29.0–38.3			
WBC (×$10^3/\mu\ell$)	11.28	3.75–26.22	6.4	4.0–11.5			
Heterophils %	20.5	4–39	13.4	6–20			50
Lymphocytes %	62.0	44–85	82.2	73–95			21
Monocytes %	0.9	0–10	2.5	1–7			5
Eosinophils%	4.9	0–17	4.4	2–8			2
Basophils %	5.9	0–16					22
Azurophils %	5.1	0–21					
Heterophils (×$10^3/\mu\ell$)	2.09	0.45–3.66					
Lymphocytes (×$10^3/\mu\ell$)	7.20	1.65–17.83					
Monocytes (×$10^3/\mu\ell$)	0.09	0–0.79					
Eosinophils (×$10^3/\mu\ell$)	0.53	0–2.14					
Basophils (×$10^3/\mu\ell$)	0.69	0–2.90					
Azurophils (×$10^3/\mu\ell$)	0.60	0–3.93					

[a]n was not specified.

Table 8: **Comparison of haematology parameters between various crocodilian species.**

Parameter	*Crocodylus niloticus*[a]		*Crocodylus rhombifer*[6]	*Crocodylus porosus*[5]	*Crocodylus porosus*[18]	*Crocodylus johnstoni*[5]	*Alligator mississippiensis*[10]	*Crocodylus palustris*[24]	*Caiman latirostris*[28]	*Caiman crocodilus*[28]
	Mean	Range								
PCV (%)	17.9	14–22	23.6–25.8	20–22	17–41	18–21	18.6	24.9	22	27
RCC (×10^6/μℓ)	0.59	0.35–1.00		0.86–0.98	0.6–1.3	0.71–0.93	0.4	0.69	0.56	0.69
Hb (g/dℓ)	7.11	4.7–9.5	8.1–8.9	6.2–7.7	4.7–12.2	5.7–7.5	7.2	8.3	9.4	12
MCV (fl)	312.2	200.0–465.1			240–311		516.0	362.4		
MCH (pg)	123.2	83.8–220.9			72–92		185.0	120.7		
MCHC (g/dℓ)	39.6	29.0–47.5			26.1–31.9		36.1	33.4		
WBC (×10^3/μℓ)	11.28	3.75–26.22		39.6–44.2	6.4–25.7	26.4–48.8	5.3	8.71	22.7	16.4
WBC (×10^3/μℓ)				5.33[31]			6.4[17]			
Heterophils %	20.5	4–39					37.4, 54.7[17]		3	5
Lymphocytes %	62.0	44–85					50.6, 23.9[17]		65	60
Monocytes %	0.9	0–10					3.0, 0.7[17]		5	5
Eosinophils%	4.9	0–17					5.5, 10.4[17]		19	21
Basophils %	5.9	0–16					3.5, 12.7[17]		1	0
Azurophils %	5.1	0–21							8	9
Heterophils (×10^3/μℓ)	2.09	0.45–3.66		3.08[31]	0.8–7.4			5.6		
Lymphocytes (×10^3/μℓ)	7.20	1.65–17.83		1.69[31]	4.5–21.6			2.48		
Monocytes (×10^3/μℓ)	0.09	0.0–0.79		0.05[31]	0.0–1.2			0.09		
Eosinophils (×10^3/μℓ)	0.53	0.0–2.14		0.35[31]	0.0–0.7			0.53		
Basophils (×10^3/μℓ)	0.69	0.0–2.90		0.15[31]	0.0–0.4			0.01		
Azurophils (×10^3/μℓ)	0.60	0.0–3.93								

[a]This study: $n = 38$; [5]Canfield, $n = 4$, subadults (ranges); [6]Carmena-Suero *et al.*, $n = 19$, subadults (range of means); [10]Glassman *et al.*, $n = 45$ (means); [17]Mateo *et al.*, $n = 35$ (means); [18]Millan *et al.*, $n = 29$, yearlings (ranges); [24]Stacey and Whitaker, $n = 24$, juveniles (means); [28]Troiano *et al.*, (means); [31]Turton *et al.*, $n = 140$, hatchlings (means).

Biochemistry

Effect of gender and size on biochemical parameters again seems variable when compared with *C. palustris*[24]. Adult *C. palustris* had significantly lower AST than the smaller sizes. In contrast, we found that AST of subadults was higher than that of yearlings. Both species showed increasing blood glucose with increasing size. Subadult *C. palustris* had lower potassium concentrations than adults and juveniles, while *C. niloticus* showed an increasing potassium concentration with increasing size.

Biochemical values reported for Nile crocodiles in other localities are shown in Table 9. Total protein determined in this study is lower than that reported in farmed Nile crocodiles, which may be caused by a lower nutritional plane or parasitism[4]. Significantly lower total protein was also recorded in wild alligators when compared to farmed alligators[1,2]. Mean globulin concentration of the Okavango crocodiles was lower than that in Nile crocodiles in the Kruger National Park, while albumin levels were similar[25]. This may reflect greater antigenic stimulation of the Kruger specimens[4].

Blood glucose was lower than that reported in both the farmed and other wild Nile crocodiles. Blood for glucose determination was not collected in fluoride tubes and this may have resulted in lower readings. However, both total protein and glucose were within the ranges reported for certain other species (Table 10). Watson[33] reported a very high mean cholesterol level (35.8 ± 5.4 mmol/ℓ) from 5 captive Nile crocodiles, but the value determined in this study was similar to that reported in other species. Cholesterol levels can be influenced by the timing of blood collection relative to a meal. This is usually an unknown factor when collecting blood from wild crocodiles.

There is considerable variation of both haematological and biochemical values between the different crocodilian species. Clearly a species-specific reference range is required if clinical pathology is to be employed as a diagnostic tool.

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Table 9: **Comparison of biochemical values for Nile crocodiles from various localities.**

Parameter	Okavango		Kruger NP[25]	St. Lucia[23]	SA captive[33]	SA farms[26]	Zimbabwe farms[7]
	Mean	Range (n = 35)	Range of means (n = 14)	Range of means (n = 80)	Mean ± SD (n = 5)	Mean (n = 5)	Mean (n not sp.)
Tot. prot. (g/ℓ)	41.2	28.9–57.1			50 ± 3	50.2	53.0
Alb (g/ℓ)	14.7	11.1–19.4	9.8–16.38				19.0
Glob (g/ℓ)	26.5	16.5–42.6	30.7–47.35				31.0
A:G ratio	0.58	0.34–0.79					
ALT (U/ℓ)	43.9	15–63					13.1
ALP (U/ℓ)	21.1	3.–72				64.2	
AST (U/ℓ)	66.5	14–211					16.6
Gluc (mmol/ℓ)	3.8	1.8–4.8	3.2–11.45		5.9 ± 0.9	5.9	4.57
Na (mmol/ℓ)	147.9	122–164	141.5–154.5	141.8–160.7	154.0 ± 1.00	153.8	
K (mmol/ℓ)	4.88	3.30–7.65	2.53–5.35	3.2–5.8	3.8 ± 0.50	3.8	
Ca (mmol/ℓ)	2.73	2.34–3.15	2.6–3.98		2.97 ± 0.09	2.97	2.63
Mg (mmol/ℓ)	1.15	0.65–1.72	1.51–2.24		0.9 ± 0.10	0.52	
Chol (mmol/ℓ)	5.49	0–9.86			35.8 ± 5.4		
Creat (µmol/ℓ)	34.0	17–56	36.5–97.0				
Cl (mmol/ℓ)	120.3	97–135	88.5–120.5	86.0–118.6			
UA (mmol/ℓ)	0.12	0.04–0.30					0.24

Table 10: **Comparison of biochemical values for various crocodilian species.**

Parameter	*Crocodylus niloticus*[a]		*Crocodylus porosus*[18]	*Crocodylus palustris*[24]	*Alligator mississippiensis*[1]	*Tomistoma schlegelii*[23]	*Caiman atirostris*[29]	*Crocodylus moreletii*[22]
	Mean	Range (n = 35)	Range (n = 120)	Mean (n = 24)	Mean (n = 24)	Mean	Mean	Mean
Total protein (g/ℓ)	41.2	28.9–57.1	41–70	31.2	37.8	37	50.1	93.7
Alb (g/ℓ)	14.7	11.1–19.4	14–23	11.4	10.3		24.2	
Glob (g/ℓ)	26.5	16.5–42.6	27–50	19.8	27.5		31.0	
A:G ratio	0.58	0.34–0.79	0.3–0.7					
ALT (U/ℓ)	43.9	15–63	11–51	52.63	34.35			
ALP (U/ℓ)	21.1	3.–72	31–180	52.75	22.82	17.8		
AST (U/ℓ)	66.5	14–211	23–157	52.13	141.42			
Gluc (mmol/ℓ)	3.8	1.8–4.8	4.5–12.1	3.63	3.6	4.2	5.72	2.97
Na (mmol/ℓ)	147.9	122–164	143–161	143.17	140.99	155.9		
K (mmol/ℓ)	4.88	3.30–7.65	3.8–7.2	8.0	5.93	4.4		
Ca (mmol/ℓ)	2.73	2.34–3.15	2.41–3.45	3.18	2.31	2.55	2.53	2.63
Mg (mmol/ℓ)	1.15	0.65–1.72	0.8–1.4					
Chol (mmol/ℓ)	5.49	0–9.86	1.1–7.2	6.4	1.71	2.86	6.0[27]	
Creat (µmol/ℓ)	34.0	17–56	20–51	35.4	13.26	18.6	31.8	93.7
Cl (mmol/ℓ)	120.3	97–135	88–127	119.71	112.1	120.0		
UA (mmol/ℓ)	0.12	0.04–0.30	0.17–0.99	0.35	0.25	0.19		0.49

[a]This study.

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