

STUDIES ON SEASONAL VARIATION IN BLOOD BIOCHEMICAL PARAMETERS OF CAPTIVE MUGGER CROCODILES (*CROCODYLUS PALUSTRIS*)

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Received: 25.10.2018; Accepted: 08.03.2019

SUMMARY

The present study was conducted to study the possible effect of season on blood biochemical parameters of mugger crocodiles (*Crocodylus palustris*) housed in captivity at Crocodile Conservation Park, Kotmi-Sonar (Chhattisgarh). The blood samples were collected from supra-occipital plexus and/or ventral tail vein (06 adult male crocodiles each) during winter, summer and rainy season. The findings of our study revealed a significant ($P < 0.05$) increase in uric acid, creatinine and sodium values in winter season while the values of AST, total protein, globulin and potassium were significantly ($P < 0.05$) increased during summer season. However, there was non-significant ($P > 0.05$) increase in ALT, CK and glucose during summer season while albumin and calcium levels increased non-significantly ($P > 0.05$) during winter season. The values of cholesterol and triglycerides increased non-significantly ($P > 0.05$) during rainy season.

Key words: Biochemistry, Crocodilians, *Crocodylus palustris*, Mugger crocodile

Biochemical analysis is an important method for measuring the physiological status of crocodiles that provides information for diagnosis and prognosis of various diseases. Environmental factors like temperature, season, geographic area, ecological habitat and wild versus captive status as well as physiological factors such as species, nutritional status, reproductive status, gender and age have been observed to affect the blood biochemical parameters of reptiles (Mader and Divers, 2014). Evidences suggest that a number of metabolic disorders occur in captive reptiles exposed to unhealthy conditions such as inadequate nutrition, improper environment and overcrowding. Hence, such factors need to be considered while establishing reference biochemical values to differentiate between normal health and disease in reptiles (Stacy and Whitaker, 2000). The present study was undertaken to establish the effect of different seasons (winter, summer and rainy) on blood biochemical parameters of captive mugger crocodiles at Crocodile Conservation Park, Kotmi-Sonar (Chhattisgarh).

Crocodiles were captured by free catch method using nets and restrained manually by securing the jaws and limbs and tapping the eyes (Combrick *et al.*, 2013) and were subjected to close inspection followed by physical examination. The body condition was judged on the basis of thickness of the neck, muscles of the supra-temporal fossa and tail position as per the procedure suggested by Huchzermeyer (2003). The crocodiles having good body condition ($n=18$, adult males) were selected for blood sampling. Blood samples were collected during winter (December and January), summer (April and May) and rainy (August and September) season. About 5 ml of blood was collected from supra-occipital plexus and/or ventral

tail vein of 06 crocodiles each during winter, summer and rainy season, using 20G sterile disposable syringe (Mader, 2014). The blood samples were immediately transferred to labelled heparinized vials and transported to laboratory. The heparinized blood samples were centrifuged at 448 RCF for 15 minutes for harvesting of plasma and stored in clean polypropylene vials at -20°C till further analysis. The biochemical parameters were estimated with Bayers's commercial diagnostic kits by using DiaSIL-100 Clinical Biochemistry Analyzer (Systronics India Limited) as per the standard methods and procedures given by manufacturer.

Analysis of Variance (ANOVA) was used to compare between seasonal differences in all parameters. Duncan's multiple range test (DMRT) was applied to test any significant difference in all parameter between different seasons (Snedecor and Cochran, 1994).

Mean \pm S.E. value of biochemical parameters during winter, summer and rainy season are presented in table 1. The values of plasma Alanine transaminase (ALT) were non-significantly higher ($P > 0.05$) in summer season as compared to winter and rainy season. However, plasma AST (Aspartate transaminase) values were significantly ($P < 0.05$) higher in summer season as compared to winter and rainy season. Higher ALT and AST activity in the plasma of healthy reptiles during summer season has also been reported by Campbell (2006), which might be related to exaggerated muscle activity and injury due to increased male aggression during the breeding season.

A non-significant ($P > 0.05$) higher plasma Creatine Kinase (CK) values were recorded in summer and rainy season as compared to winter season. Increased CK value might be due to higher muscular activity in reptiles and

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Table 1
Plasma biochemical parameters of *Crocodylus palustris* during different seasons (Mean \pm S.E.)

PARAMETER	WINTER n=6	SUMMER n=6	RAINY n=6
ALT (IU/L)	52.52 \pm 0.19	53.02 \pm 0.54	52.35 \pm 0.2
AST (IU/L)	52.04 \pm 0.26 ^b	53.04 \pm 0.26 ^a	52.21 \pm 0.28 ^b
CK (IU/L)	8.71 \pm 0.07	8.74 \pm 0.09	8.78 \pm 0.1
Total protein (g/dl)	3.11 \pm 0.03 ^b	3.45 \pm 0.02 ^a	3.09 \pm 0.04 ^b
Albumin (g/dl)	1.22 \pm 0.01	1.13 \pm 0.08	1.18 \pm 0.07
Globulin (g/dl)	1.88 \pm 0.03 ^b	2.32 \pm 0.08 ^a	1.9 \pm 0.07 ^b
Glucose (mg/dl)	63.06 \pm 1.17	66.72 \pm 1.38	64.72 \pm 1.38
Uric acid (mg/dl)	6.76 \pm 0.09 ^a	5.76 \pm 0.09 ^b	5.8 \pm 0.05 ^b
Creatinine (mg/dl)	1.35 \pm 0.04 ^a	0.35 \pm 0.04 ^b	0.38 \pm 0.02 ^b
Cholesterol (mg/dl)	259.14 \pm 15.84	258.80 \pm 16.29	260.05 \pm 16.86
Triglycerides (mg/dl)	372.28 \pm 22.37	382.28 \pm 22.37	373.61 \pm 22.23
Calcium (mg/dl)	12.87 \pm 0.15	12.79 \pm 0.11	12.77 \pm 0.15
Potassium (mg/dl)	7.91 \pm 0.14 ^b	8.91 \pm 0.14 ^a	7.93 \pm 0.15 ^b
Sodium (mg/dl)	145.45 \pm 0.12 ^a	144.45 \pm 2.92 ^{ab}	142.95 \pm 0.7 ^b

Values with different superscripts differ significantly ($p < 0.05$) among the groups in different season

commonly observed during active period of year viz. summer and rainy season (Dickenson *et al.*, 2002; Chung *et al.*, 2009).

During summer months, the plasma total protein (TP) values were significantly ($P < 0.05$) higher as compared to other two seasons. Divers (2000) also reported that captive reptiles might exhibit higher plasma TP concentrations during the period of increased food intake which might be the probable reason for higher TP level during summer season. The present study revealed non-significant ($P > 0.05$) changes in plasma albumin values throughout the observation period. However, plasma globulin values were significantly ($P < 0.05$) higher during summer and rainy season as compared to winter season. Comparison between albumin and globulin values revealed higher globulin values in crocodiles throughout the study period. Higher values of albumin are found in active species with higher metabolic rates and in those living in dry, hot environments. Albumin was found to be responsible for 50 percent of the colloidal osmotic pressure in reptiles. Lower level of plasma albumin has also been reported in fresh water turtles (Campbell, 2006).

A non-significantly ($P > 0.05$) higher plasma glucose level was recorded in summer and rainy season as compared to winter season which might be due to lower activity level and metabolic rate of crocodiles in winter season. Silva *et al.* (2011) have reported that an increase in temperature produces hyperglycemia in reptiles.

Reptiles tend to have higher blood uric acid concentration and their plasma uric acid level generally gets doubled the day after a meal and a higher level of nitrogenous waste is discharged during warmer season

(Campbell, 2006). The decreased tubular function at lower ambient temperature, as observed in reptiles also leads to higher plasma uric acid level (Silva *et al.*, 2011). In the present study also, the plasma uric acid concentrations were significantly ($P < 0.05$) higher in winter season as compared to summer and rainy season. During the winter season, the values of plasma creatinine was significantly ($P < 0.05$) higher as compared to summer and rainy season. Protein serves as an energy source during brumation in reptiles during winter. The concentration of uric acid and creatinine as well as osmolarity gets increased to prevent water loss from body. However, after hibernation, these values become lowered as water intake increases (Wallace *et al.*, 2008; Chung *et al.*, 2009).

The level of triglycerides in reptiles has been reported ranging from 300 to 1670 mg/dl and fat accounts a major share. The fat level changes slowly during starvation, cold torpor or hibernation (Silva *et al.*, 2011). Plasma cholesterol and triglyceride levels also revealed non-significant ($P > 0.05$) alterations throughout the present study.

Plasma calcium level revealed non-significant ($P > 0.05$) changes during our study. The normal plasma calcium concentration for most of reptile ranges between 8 to 11 mg/dl and varies with the species and the physiologic status of the reptile (Campbell, 2006). Plasma potassium were significantly higher ($P < 0.05$) in summer season as compared to other two seasons. Higher potassium levels in summer might be due to greater food intake during the warmer period (Campbell, 2006). Analysis of plasma sodium level revealed significantly ($P < 0.05$) higher sodium values in winter as compared to rainy season.

Campbell (2006) explained that the higher sodium level in winter might be due to lower water intake during the winter because crocodiles spend less time in the water and more time on land during cold season.

The present study provided an essential much needed information on plasma biochemistry in captive muggers. However, for a species specific reference range to be developed for captive muggers, future studies are warranted with some additional parameters and large sample sizes to evaluate other differences between genders and age classes along with habitat evaluation to establish reliable relationships between these parameters.

ACKNOWLEDGEMENTS

The authors are highly grateful to the authorities of Chhattisgarh Forest Department and staff members of Crocodile Conservation Park, Kotmi-Sonar (Chhattisgarh) for their kind support and cooperation during the tenure of our study.

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