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Effects of Season and Sex on Some Blood Parameters of Snake Saw Scaled Viper *Echis carinatus* at Sennar State, Sudan

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ABSTRACT

Background and Objective: Snakes represent large group of reptiles and it has some values, the mean value of the snakes is that they form part of the environment and help preserve the balance of the nature, the snake eat many insects and rodents such as mice and rats. Their skins are used for making many leather products and their venom has several uses in medicine and biological research. The physiological mechanisms and behaviours of the snake show adaptability to environments and assist its survival. The study aimed to investigate some morphological and physiological parameters of saw scaled viper *Echis carinatus* for both males and females within the seasons of two years, 2008 and 2009. **Materials and Methods:** This study was carried out at Suki locality in Sinnar State Central Sudan. Statistical analyses were carried out for 30 snakes (15 males and 15 females), ANOVA was used to test the overall significance of the means for sex and seasonal effects. The bi-factorial design was used to test the effects of sex and season as well as their interactions. **Results:** The morphological aspects males and females looked the same except that males were longer and heavier in summer but the opposite was true in winter. All blood parameters, except hormones, showed a significant effect of season only, where haemoglobin levels were significantly higher in summer than in winter. Same observations were obtained for blood metabolites and minerals although hormones were not affected. **Conclusion:** The saw-scaled viper *Echis carinatus* blood heamatology, metabolites and minerals were affected by season as a poikilothermic animal where their activities follow ambient temperature. Hormone levels would follow diurnal changes.

KEYWORDS

Echis carinatus, morphological and physiological parameters, blood heamatology, metabolites, minerals and hormones

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INTRODUCTION

The saw-scaled viper (*Echis carinatus*) has an extensive geographical distribution from Senegal in the West, through the North of Africa, South as far Kenya and the Middle East to India[1]. According to this, Sudan is the main range of *Echis carinatus* which is found in Savanna regions, especially in Sinnar State. The physiological mechanisms and behaviours of the snake show adaptability to environments and assist its survival[2]. The physiological studies are used as part of conservation and management of populations and ecosystems. However, the physiological metrics are used for understanding the function of ecosystems as well as the factors that influence their structure[3]. Checking blood parameters in reptiles may guide the evaluation of physiological and health conditions of populations and may be used as an indicator in determining environmental conditions, since species are very sensitive to changes of habitat[4]. A system regulates blood pH in the snakes, which is important to eliminate the rapid rate of CO_2. The total plasma CO_2 or bicarbonate has a normal range between 20 and 30 mmol/L. The number of circulating eosinophils in normal reptiles varies with species and seasonal changes. For example, in reptiles, eosinophil counts are usually highest in the winter during hibernation, basophils numbers also vary with species but are usually low[5]. Lymphocytes are lowest in the winter and highest during the summer in reptiles[6].

Although studies of the effects of adrenal hormones on the reptilian immune system remain limited, reptiles exhibit seasonal patterns of variation in circulating white cell counts[7] and other measures of immune function that may reflect seasonal changes in circulating corticosteroids[8,9]. Corticosterone resulted in similar reductions in lymphocyte numbers[8]. Little studies were carried out concerning its ecological distributions, physiological responses within different seasons and venoms consistent. The study aimed to investigate some morphological and physiological parameters of saw-scaled viper *Echis carinatus* for both males and females within the seasons of two years, 2008 and 2009.

MATERIALS AND METHODS

The experiment was conducted at Elsuki locality which covers a total area of 5500 km^2 (Latitude: 12°-13°N, Longitudes: 33°-34°E and Altitudes 420 m above sea level). The locality belongs administratively to Sennar State, Sudan. The study was carried out for two consecutive years (2008-2009) that covered both dry (March to June) and wet season (July to September).

Snakes collection: Thirty snakes (15 males and 15 females) were captured using sticks and snakes holding tools. Saw scaled viper *Echis carinatus* was used and the snakes collected were transported in pillowcases (optimal method safety for holding snakes) to a laboratory of Faculty of Veterinary Sciences, University of Khartoum. All snakes were apparently healthy.

Blood sampling and blood parameters studies: Blood was obtained from the veins of the ventral tails[10] by needle (1 cm). Hematological parameters included packed cell volume (PVC), Hemoglobin (Hb) and total leukocyte count (TLC). These were determined according to the standard methods described by Schalm and Jain[11]. The TLC was performed microscopically under power, using an improved haemocytometer[11]. A micro-technique was used for PCV determination. The PCV was measured by plain capillary tubes using a microhematocrit centrifuge (Hawksly-London) operated for 5 min at 12000 rpm, special reader was used to determining PCV as a percentage of whole blood.

Blood metabolites: The plasma glucose concentration was determined by a colorimeter method using a photo/ colorimeter and commercial kit adopted by Osman and Gumaa[12]. Biuret reagent was used to determine serum total protein (TP) concentration as described by Lowry *et al.*[13]. The colorimeter method of Gyure and Raisys[14] was used to determine the serum albumin concentration.

Statistical analyses: Ecological analyses were performed using the Minitab package software[15]. The Analysis of Variance procedure (ANOVA) tested the overall significance of the means ($p<0.01$) for sex and seasonal effects. Separations were examined by Duncan's Multiple-Range Tests to detect statistical significance[16]. A bi-factorial design was used to test the effects of sex and season as well as their interactions[17].

RESULTS

Effect of season and sex on morphology of saw scaled snake (*Echis carinatus*): It could be shown that in summer, males were heavier and taller both in body and tail lengths. In winter, females showed heavier weight, taller body lengths and taller tails, however, no significant differences could be detected in Table 1. Similarly, males showed better parameters than females in summer, while females showed better parameters than males in winter as related to ventral, dorsal and rows of scales and number of spots Table 2.

Effect of season and sex on biochemical parameters scaled viper (*Echis carinatus*): Biochemical parameters showed that hemoglobin concentration was significantly lower in winter than summer for both sexes. The other parameters did not show significant differences as affected by season or sex, however, winter showed lowered levels than summer Table 3.

Effect of season on blood metabolites and hormones: Effect of season was also shown on glucose and minerals. Significant higher concentrations were obtained in summer compared to winter. Glucose showed higher levels in males than females, although significant differences could not be detected in Table 4. Thyroxine (T4) and cortisol concentrations were not affected by sex or season Table 5.

Table 1: Effects of season and sex on some morphology of saw-scaled viper (*Echis carinatus*) (Mean±SD)

	Summer		Winter	
Variables	Male	Female	Male	Female
Weight (g)	40.540±6.79	36.600±7.35	44.15±5.59	52.607±3.30
Total length (cm)	45.933±3.27	44.867±3.44	51.13±3.88	60.130±4.59
Standard length (cm)	41.000±2.87	40.460±3.29	46.60±4.77	51.470±13.56
Tail length (cm)	5.040±0.75	4.600±0.69	4.89±0.75	5.467±0.48

Table 2: Effects of season and sex on number of ventral and dorsal scales, number of rows of scales and number of spotted scales of saw scaled viper (*Echis carinatus*) (Mean±SD)

	Summer		Winter	
Variables	Male	Female	Male	Female
Number of ventral scales	185.20±2.46	184.20±2.7	182.60±1.99	184.73±2.76
Number of dorsal scales	3360.1±94.8	3335.7±66.8	3341.7±81.3	3372.9±87.6
Number of rows of scales	36.933±1.03	36.67±0.72	36.733±0.88	37.067±0.96
Number of spotted	552.20±8.06	553.80±8.91	547.8±7.5	550.33±8.59

Table 3: Effect of season and sex on some biochemical parameters of saw scaled viper (*Echis carinatus*) (Mean±SD)

	Summer		Winter	
Variables	Male	Female	Male	Female
HB (g/dL)	5.8 ± 1.612^{A}	5.6 ± 1.454^{A}	0.51 ± 0.15^{B}	0.44 ± 0.19^{B}
TWBCs $\times10^3$	38.07±21.5	$42.27\times10^3\pm20.8$	37.0	37.0
TRBCs $\times10^3$	$27.73\times10^3\pm11.3$	$27.87\times10^3\pm11.5$	21.53	16.0

^{AB}Values within the same row bearing different superscripts are significant at $p<0.01$, HB: Hemoglobin, TWBCs: Total white blood cells and TRBCs: Total red blood cells

Table 4: Effect of season and sex blood metabolites of saw scaled viper (*Echis carinatus*) (Mean±SD)

	Summer		Winter	
Variables	Male	Female	Male	Female
Glucose (mL/dl)	70.80±14.67^{A}	59.93±24.04^{A}	26.73±6.6^{B}	30.13±4.88^{B}
Ca (mg/dL)	8.18±0.731^{A}	8.72±0.65^{A}	3.23±0.75^{B}	3.05±0.72^{B}
Na (mg/dL)	141.87±10.01^{A}	138.00±11.59^{A}	9.00±2.20^{B}	9.6 2.849^{B}
K (mg/dL)	9.4±1.059^{A}	9.70±1.5 ^{A}	4.22±0.06^{B}	4.21±0.06^{B}
P (mg/dL)	6.067±1.13^{A}	5.56±1.04^{A}	2.66±0.37^{B}	2.72±0.44^{B}
Total proteins	20.49±1.13^{A}	19.51±2.95^{A}	4.72±1.39^{B}	4.45±1.48^{B}

^{AB}Values within the same row bearing different superscripts are significant at p<0.01

Table 5: Effects of sex on T4 and cortisol of saw scaled viper (*Echis carinatus*) (mean±SD)

Traits	Male	Female	Level of significance
T4 (nmol/L)	3.36±1.128	2.60±0.998	Not significant
Cortisol (ng/mL)	50.80±3.834	45.20±8.672	Not significant

DISCUSSION

As far as the morphological characteristics are concerned, males showed better appearance than females whereas the opposite was true in winter, although significant differences could not be detected, this might be related to activities of feeding where males could be more active in looking for food than females. Generally, the blood parameters under study were affected by environment and not by sex. Significant higher values were obtained in summer than in winter. The blood hematology results with respect to hemoglobin showed values in the range of 7.4 and 4.2 g/dL which is comparable with those obtained in reptiles (8.15 and 8.7 g/dL), these results agreed with Hattingh and Willemse[18]. Effect due to season showed summer levels to be significantly higher than winter in both males and females, these were in line with those observed by Otis[19], who confirmed the environmental influence on normal biochemical aspects. The WBCs (37-42) and RBCs (27-16) reported in this study were lower than those obtained by Troiano *et al.*[20] for WBCs (9). Both RBCs and WBCs were higher in summer than winter although significant differences were not obtained. Similar results were obtained by Parida *et al.*[6].

For the blood metabolites, total protein levels ranged from 21.62 to 5.93, whereas in other studies reptiles were shown to have higher values (30-80)[21]. The effect of season was significant where summer protein levels were higher compared to winter. Similar observations for the effect of season were obtained in *Bothrops ammodytoids*[21]. Glucose concentrations were also significantly higher in winter than in summer (85.47 and 27.39). Similar observations for the effect of season were obtained by other authors but with lower values by Troieno *et al.*[20] in *Bothrops ammodytoids*.

Mineral levels were also higher in summer than in winter. Similar findings were obtained for sodium in *Bothrops ammodytoids*[21]. For potassium, in reptiles[5]. Results obtained for calcium were higher than those obtained by Chiu and Lam[21] for reptiles, as shown by *Bothrops ammodytoids* but less than those obtained by Lillywhite and Smits[5] for reptiles.

The higher values in all blood parameters in summer as compared to winter could be related to the fact the snake being cold-blooded animal activities is greatly affected by ambient temperature and so the snake is more active during summer than winter where its activity is greatly limited by the cold weather during winter. The variations obtained in this result related to other studies conducted elsewhere might be related to the genetic make-up although similar trends were observed for poikilothermic animals.

Thyroxin and cortisol hormones were neither affected by sex nor environment which could be related to the diurnal effect where the levels are increased as the temperature increases during the day due to the animal energy in searching for food as controlled by thyroxine hormone and by cortisol where animal anxiety is more active during the day than night. Sex seemed to have no effect on hormone levels,

although males tended to have higher levels for both hormones. However, other studies showed significant thyroxine levels in males than in females[21]. For cortisol, similar studies were obtained by de Weerth *et al*.[22].

The snake gains importance through its wide ecological distribution in Africa yet due to dynamic in human growth and threat imposed on the snake for leather and venom, a deep investigation is required for its distribution, behaviour, density abundance and richness. Effective essential conservation strategies should be undertaken to keep a balance for the snake and other taxa necessary for the existence of the snake. Further studies are required to investigate venom characteristics and leather quality.

CONCLUSION

Whereas morphological parameters would show the effect of sex, physiological differences could not be detected. Hematological and plasma biochemical parameters indicated a difference between seasons and some values were significantly different between the two genders. These hematological results provide a reference range for Sudanese population of adult *Echis carinatus*. The effect of season was apparent, where summer showed higher values except for glucose which showed an opposite trend. Hormone levels were not affected by season showing the maintenance of body mechanism control on metabolic rate and anxiety.

SIGNIFICANCE STATEMENT

The study has chosen one of the most important snakes widely distributed in the area of study which contributed to the balance of ecosystem as they eat insects and rodents and as they are themselves prey to birds and mammals. They are targeted by inhabitants for leather and venom which pose a threat to their distribution and existence. The physiological aspects would reflect the importance of ecological consequences. As shown by blood metabolites and minerals distribution could be nearer to existence of feed resources and where they could find better protection from predators. Hormones' level seemed to be controlled by effective mechanisms. These parameters may be used as indicators in determining environmental conditions since species are very sensitive to changes in habitat.

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