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Haemolymph biochemical and mineral properties with morphometric parameters of reproductive organs of Archachatina marginata as affected by humid agro-ecological zones in Nigeria

[1]Osunkeye, O. J., [2]Abiona, J. A., [2]Onagbesan, O. M.[2] and [3]Omole, J. A.

[1]Department of Animal Science, College of Agriculture, Osun State University, Osogbo, Osun State, Nigeria.
[2]Department of Animal Physiology, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.
[3] Institute of Agricultural Research & Training, Obafemi Awolowo University, Moore Plantation, Ibadan, Oyo State, Nigeria

Corresponding Author: *osunkeye.olumuyiwa@uniosun.edu.ng* ***Phone number:*** *08036217578*

Target Audience: *Animal physiologist, Environmental physiologist, Animal conservationist, Scientist and Farmers*

Abstract

The study determined the effect of humid agro-ecological zones on the haemolymph mineral and chemical composition and morphometric parameters of snail (Archachatina marginata) obtained from two humid agro-ecological zones of Nigeria comprising South-South (Rivers and Delta States) and South-West (Osun and Lagos States). The results showed that haemolymph of snails from Rivers State had higher ($p<0.05$) pH (7.40 ± 0.04) and urea (1.88 ±0.06mmol/L) values than those from other locations. Similarly, haemolymph of snails from Osun State had higher ($p<0.05$) concentrations of total protein and bicarbonates (52.03 ± 2.92 g/L and 19.59 ± 0.16 mmol/L, respectively) than others. The highest concentration of glucose (21.27 ± 0.71mg/dl) and potassium (7.62 ± 0.13mmol/L) was recorded for haemolymph of snails obtained from Lagos State. No differences ($P<0.05$) were observed in the sodium, calcium and chlorine values across the four states. In addition, adult stage (>200g) snails from Lagos and Osun States had the highest ($P<0.05$) shell length compared to those from Rivers and Delta States. However, shell circumference was greater ($p<0.05$) for snails from Rivers and Delta. It is concluded that the ecological niche can have significant effects on growth pattern and some mineral and biochemical characteristics of snails, implying that ecological changes would likely effect physiological changes in the snails from the different ecological zones differently.

Keywords: *Agro-ecological zone, haemolymph, Archachatina marginata, biochemical parameters, mineral component*

Description of Problem

Micro and macro climatic conditions strongly determine the survivability, productivity and reproductive activities of snails and other animals. Slight differences in climate and environmental condition of various ecological zones are an important factor affecting the population and density of conspecific individuals (1, 2). The effect could either be negative or positive. In snail, it is most obvious that changes in the immediate climate induces environmental cue which influences their phenotypic characteristics. Also, according to (3), environmental cue can trigger seasonal regression of primary and accessory sex organs of mud snail (*Ilyanassa obsolete*). Because of the adverse conditions imposed by changes in the ecological parameters, animals inhabiting non-tropical environment undergo seasonal changes in reproduction in order to improve their survival and chances of successful reproduction. In this condition most snails aestivate (4). As reported by (5), the changes in the ecological

parameters such as temperature and humidity create some physiological and biochemical adjustments. Metabolic depression, regulated by specific protein involves the suppression of protein and glycogen synthesis (6). Also, according to (7), the micro-habitat of snails had significant effects on the productivities of breeder snails and their subsequent offsprings. Top soil and river sand are good micro-habitats for the hatching of the eggs of the giant African snail *Archachatina marginata* but for subsequent growth of the hatchlings, these microhabitats may not be adequate for growth (7). These imply that the agro-ecological zone of the country may impose a greater variation on the survivability, breeding, productivities as well as the physiological component of the snails, depending on the suitability and availability of materials that support their growth in such environment. Several studies have been carried out on the growth performance of matured snail. Little attention has been paid to the micro- and macro-habitat of the snails and their possible effects on constituents of the haemolymph, reproductive organs and carcass of the giant African land snail. Therefore, this study focuses on the effect of selected location within the humid agro-ecological zone of Nigeria on the biochemical and mineral components of snail haemolymph, reproductive organs and some morphometric parameters of giant African land snail, *Archachatina marginata*.

Materials and Methods

Experimental site

Snails sample were collected from four States (Rivers, Delta, Osun and Lagos) of the humid agro-ecological zone of Nigeria. This zone covers 21% of the landmass of Nigeria with annual rainfall and temperature ranging from 1,100 – 1,400 mmand18^{o}C–37^{o}C, respectively. These snails were then transferred to the Animal Science Laboratory, Department of Animal Science, Osun State University, Ejigbo Campus, Ejigbo, South Western Nigeria (7°30'N 4°30'E), and temperature ranging from 21.1^{o}C to 31.1^{o}C, though there are wide variations in these values from year to year. The snails were acclimatized for seven days before dissection.

Experimental animals

Giant African Land Snail (*Archachatina marginata*) was used for the experiment. Eighteen (18) snails were purchased from open markets in each of the four States, namely; Rivers, Delta, Lagos and Osun. Thus, 72 snails with an average weight of 143 g were used for the study. During the seven days of holding the snails before dissecting, the snails were housed in metal cages with adequate space and ventilation. The cage was also filled with loamy soil exposed to sunlight to get rid of harmful soil microorganism and moistened regularly for favourable humidity for the snail. Fresh feed and clean water were given to the snails *ad libitum* on a daily basis.

Biochemical analysis of the haemolymph

The apex of the snails' shell was broken and the haemolymph was collected using the method described by(10). The protein concentration of each sample was determined immediately using the Biuret method described by (11). Glucose content was determined by the colorimetric method of Baumniger (12) and haemolymph sodium (Na^{+}), potassium (K^{+}), calcium (Ca^{2+}), Hydrogen bicarbonate (HCO_3^{-}) and chloride (Cl^{-}) were determined by the method described by Association of Official Analytical Chemists (AOAC) (13) and (9).

Measurement of the reproductive organs, live weight and shell parameter

Three snails were selected from each treatment and dissected to remove the reproductive organs, the soft body mass and shell. The weight of these parts were

determined using a top-loading weighing scale (Mettler Toledo, Max – 210 g, d= 0.001 g). The shell length and shell circumference were measured using Vernier Callipers (0-150mm/0.05 Stainless Steel Vernier Callipers Gauge Micrometer).

Statistical Analysis

Data were subjected to analysis of variance using Statistical Package for Social Sciences (SPSS) version 16.0 and presented as means. Comparisons between different groups were done using Analysis of Variance (ANOVA) and Tukey Least Significant Difference (LSD).

Results and Discussion

Biochemical composition of the haemolymph of *Archachatina marginata* is presented in Table 1. Snails from Rivers and Delta States had the highest ($p<0.05$) haemolymph Ph (7.40 and 7.29 respectively) while those from Osun and Lagos had the lowest (7.07, 7.27, respectively). Osun State had the highest ($p<0.05$) total protein (52.03 g/L) while Lagos had the least (35.23 g/L). Lagos State had the highest ($p<0.05$) glucose value (21.27) while Delta State had the least (17.03 in Delta State. There were no significant differences ($p>0.05$) in the values of Sodium, Calcium, Chlorine, creatinine, albumin and globulin. Osun State recorded the highest ($p<0.05$) for HCO_3^-(19.594) while Delta had the least (17.73) value. Urea concentration was the highest ($p<0.05$) in Rivers State (3.95) and lowest in Osun State (3.64).

Table 1: Biochemical and mineral composition of the haemolymph of *Archachatina marginata*

Parameters	Rivers	Delta	Lagos	Osun	SEM	Significance
Biochemical compound						
pH	7.40[a]	7.30[a]	7.27[ab]	7.07[ab]	0.04	S
Total protein (g/L)	43.34[c]	44.85[b]	35.23[d]	52.03[a]	2.92	S
Glucose (mg/dl)	18.68[c]	17.03[d]	21.27[a]	19.32[b]	0.71	S
Urea (mmol/L)	3.95[a]	3.76[ab]	3.76[ab]	3.64[a]	0.06	S
Albumin (g/dl)	1.88	1.91	1.87	1.86	0.06	NS
Globulin (g/dl)	2.83	2.80	2.85	2.91	0.07	NS
Mineral						
Sodium (mmol/L)	119.78	121.87	124.16	123.57	3.37	NS
Calcium (mg/dl)	7.03	7.74	7.71	7.37	0.20	NS
Chlorine (mmol/L)	55.10	56.64	57.82	56.22	1.21	NS
Bicarbonate (mmol/L)	18.41[bc]	17.73[c]	18.86[b]	19.59[a]	0.16	S
Potassium (mmol/L)	7.24[ab]	6.82[c]	7.62[a]	7.35[ab]	0.13	S
Creatinine (µmol/L)	87.14	87.83	86.38	82.31	1.2	NS

[a,b,c] Means on the same row are significantly different ($p<0.05$)
Keynote: S = Significant, NS = Not significant

Effect of ecotype location on the reproductive organs, shell length, shell circumference, live weight, soft body mass and shell weight of African giant land snail is presented in Table 2. There were significant differences in the values of shell length, live weight, shell weight and little hermaphrodite. Delta State had the longest ($p<0.05$) shell length (12.76cm) while Lagos State had the shortest (12.17cm). Snails from Rivers State had the widest ($p<0.05$) shell circumference (17.58cm) while the smallest value (16.66cm) was recorded for Lagos State. The heaviest ($p<0.05$) snails (161.18g) were those from Rivers State while the least weight (143.81g) was recorded for snails from Osun State. Shell weight was found to be the

heaviest ($p<0.05$) for snails from Rivers State (47.58g), while those from Lagos State (23.74g) had the least shell weight. Soft body mass ranged from lowest (79.17g) in Delta State to highest (98.21g) in Lagos state. The whole reproductive organ also ranged from 6.76g in Rivers State to2.50g in Osun State while albumen ranged from 0.34 g (Osun) to 1.31 g (Rivers) There were no differences ($p>0.05$) in the soft body mass, whole reproductive organ and albumin weights. Little hermaphrodite had values of 0.80g, 0.12g, 0.08g, 0.35g in Rivers, Delta, Lagos and Osun State respectively. The highest weight of little hermaphrodite (0.80) was from Rivers State, followed by those from Osun State. The least weight of the little hermaphrodite was observed in snails from Lagos. Spermoviduct ranged from 0.08g in Rivers to 2.08 g in Delta State while ovo-testis ranged from 0.69 g in Delta State (0.69g) to 0.40 g in Osun State. However, both parameters showed no differences among treatment means.

Table 2: Effect of location on the reproductive organs, shell length, shell circumference, live weight, soft body mass and shell weight

Parameters	Rivers	Delta	Lagos	Osun	SEM
Shell length (cm)	12.76^{b}	12.79^{a}	12.17^{b}	12.47^{ab}	0.21
Shell circumference (cm)	17.58	16.86	17.46	16.66	0.35
Live weight (g)	161.18^{a}	150.74^{ab}	156.08^{b}	143.81^{b}	0.31
Shell weight (g)	47.58^{a}	41.89^{a}	23.74^{c}	31.82^{b}	0.26
Soft body mass (g)	86.29	79.17	98.21	87.94	0.66
Whole reproductive organ(g)	6.76	5.85	3.89	2.50	0.13
Albumen (g)	1.31	0.95	0.76	0.49	0.33
Little hermaphrodite (g)	0.80^{a}	0.12^{c}	0.08^{d}	0.35^{b}	0.02
Spermoviduct (g)	0.08	2.08	1.60	0.73	0.54
Ovotestis (g)	0.57	0.69	0.43	0.40	0.15

$^{a\,b\,c}$- Means on the same row differently superscripted are significantly ($P<0.05$) different.

The biochemical composition of haemolymph from A. marginata from different States in this study showed that snails from some States were richer in selected minerals a than others. Snails obtained from Rivers had higher pH and urea than other States in this study. This observation is an indication that the soil in the River and Delta states (7.40 and 7.30 respectively are slightly alkaline in nature than others, while the soils in other states fell within similar range. This is line with the report of (14) who stated that low pH treatments (4.6 and 4.7) resulted in significantly lower adult growth rates and reduced gross fecundity compared with circum-neutral pH treatments (7.2 and 7.4). This is because of the impact of soil pH on plant growth has implications for animal species that use those plants as their only source of nutrition. Also, snail body interact directly with the soil which is the reason for using it as bio-indicator reflecting the mineral and chemical substances present in the soil (15). According to (16), there was significant interaction ($P<0.001$) between soil moisture level and species on monthly weight gains of the snails. The feed intake and weight gain of snails (4.28 g and 6.43 g) in the high soil moisture treatment was significantly ($P<0.01$) higher than those in the low soil moisture treatment (3.07 g and 5.30 g).

Values recorded for urea were also attesting to the fact that those soils are rich in nitrogen which may boost the soil ability to produce better plant on which the snails can

feed on for better growth rate and shell development. Higher total protein and bicarbonates were recorded for snails from Osun State compared to other States is also an indication that the snails had access to protein rich diet and substance that can promote shell development in the state compared to others State considered in this study. In order words, higher glucose and potassium ion found to be higher in snails haemolymph from Lagos can also be related to plant rich in carbohydrate and organic salts known to be available as a result of sea salt found in Lagos.

The results of this study showed that location was a factor affecting the chemical constituency of the haemolymph of the giant African land snail found in the four states. Reports by (17) showed dietary variables have influence on the physiology of giant African land snails. Metabolite composition in the haemolymph reflects the mobilization of stored compounds for some specific metabolic activities as well as the transport of ingested nutrients to body sites for storage or functional use. Thus, the spectrum of metabolites is constrained by environmental factors that mold the availability and composition of food, the physiological adjustment to temperature and moisture which varies between geographical locations (18) Nicolai. Wide geographical distribution of a species exposes different populations to quite different environmental factors (19) (David et al.1996). It was therefore not unexpected that environmental and dietary variables could have contributed to the variations observed in the composition of snail flesh in snails obtained from the different locations. Studies by (20) recorded a strong relationship between the haemolymph concentration of glucose, lipid and protein and the modified environment. Since haemolymph is the fluid that bathes the flesh of snails, any physiological process that takes place in the body of the snails reflects in the haemolymph (21). Snails are ectothermic animals; their physiological processes are affected by environmental factors like temperature (22). It is therefore not unexpected that higher concentrations of total protein and HCO_3^- in the haemolymph of the snails from Osun state could have been aimed at overcoming the challenges that arose from the higher average daily temperature of the state.

The function of the haemolymph in the open circulatory system of snails is to serve as transport of nutrients to the various body parts (23). Hence, it is expected that the haemolymph should contain higher concentrations of minerals for absorption into body tissue. Haemolymph biochemical properties of giant African land snails have been reported to influence physiological processes (21) and growth performance (24, 20) of the land snail.

The concentration of Na^+ and Cl^- and Creatinine were high though not significant. Studies by (24) and (25) also reported that Na^+ and Cl^- were the most abundant ions in the haemolymph of the slug *Arion ater* and the land snail, *A. marginata*,. The role of Na^+ in nervous communication is significant. Therefore, higher concentrations of Na^+ recorded in the haemolymph of snails in this study could have been responsible for the control of the contraction of their muscular foot (23).

The study shows that values of whole reproductive organ were higher in snails found in areas with high soil moisture condition (Rivers and Delta), which may be due to the functional reproductive activity of the snails as a result of high soil moisture condition (26) in these are due to higher amount of rainfall pattern. *A. marginata* had higher values of albumen, which might be due to the effect of species variation on the reproductive tract. Values of spermoviduct were higher in the (River State and Delta State) compared to the (Lagos State and Osun State), which may be due to the fact that snails mate during the rainy

season.

The shell length was highest in snails from Delta State (12.79), though not significantly different from those from Osun State. However, the least shell length was recorded for snails from Rivers and Lagos States. Considering the shell length in this study, values recorded (10.29-14.42cm) were far higher compared to the reported values by (27). These differences may be as a result of calcium and phosphorus bio-availability in the soil and leaves consumed by the snails from different regions. Differences in rainfall pattern may also influence shell accumulation since these two elements are known to be present in plants that those snails consume in the wild.

Conclusion and Applications

It is clear from this study that ecological zone significantly influences haemolymph biochemical parameters, some selected reproductive indicators and live weight together with shell parameters.

1. Snails from coaster ecological area of Nigeria (Rivers and Delta States) had largest snails with longest shell length, and biggest hermaphrodite duct, which is one of the major ducts of reproduction in snail, compared with to those from other States.
2. The snails from Rivers and Delta States grow better which also influence the increase in the weight of hermaphrodite duct.
3. This research has therefore indicated the location from which snails (*Archachatina marginata*) can be selected, be it for farming, domestication, scientific research, medicinal or consumption purposes.

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