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Original Research Article

Changes in food-derived minerals and molecules during different growth phases of freshwater tortoise (*Geochelone nigra*) under intensive management system: implication for public health

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ABSTRACT

Background: Freshwater tortoises (*Geochelone nigra*) are the most popular in the rainforest region. Their growth phases include hatchling growth phase (HGP), juvenile growth phase (JGP), sub-adult growth phase (SAGP) and adult growth phase (AGP). Objective of the study was to investigate the impact of minerals value on the different growth phases of the Nigerian freshwater tortoise.
Methods: This was an observational comparative study. The HGP, JGP, SAGP and AGP comprised four groups. 5-serial blood samples taken every two-months. The Ca^{2+}, Cl^{-1}, Na^{+}, and PO_4^{-2} were assessed as minerals; while blood glucose, lipids and proteins comprised food-derived molecules. Comparison was by simple ANOVA.
Results: Highest concentration of glucose and lipids were recorded at the SAGP (72.00 mg/dl and 64.10 mg/dl); while the concentration of protein showed gradient increase as the tortoises progressed from HGP to AGP. Growth phases significantly ($p<0.05$) differed in the mineral composition of the tortoises with SAGP showing highest values in Ca^{2+}, PO_4^{-2} and Na^{+}.
Conclusions: SAGP of *Geochelone nigra* has better serum biochemical values than HGP, JGP and AGP. This finding can be used to inform choice of the growth of tortoise when considering consumption and the desired relative nutritional value. Further implication on recreational and farmed are addressed.

Keywords: Nutritional values, Minerals, Health values, Growth phases, Freshwater tortoise, Biochemical values, Ecosystem

INTRODUCTION

The freshwater tortoise such as *Geochelone nigra* occurs commonly in the rainforest regions of Nigeria. Nutritional value or content problems in these species of tortoise are common, especially minerals imbalance.[1,2] One tool for the early diagnosis of health disorders being due to mineral imbalances is biochemistry tests of minerals in blood. Several studies have been conducted to set up minerals and serum biochemical values for the tortoises. In several studies, clinically healthy animals were examined to provide a glimpse into their physiological status.[3,4]

However, several samplings have been performed only in a limited number of studies.[5] Even in such cases, levels of some biochemistry can be influenced by demographic and environmental factors such as feeding, season, sex and/or species; as well as ecological systems that is known to

impact on nutritional values of animal meats.[6-9] The use of serum or plasma may also have impact on the value of the parameters.[10,11]

Yet, information about feeding and housing conditions of the tortoises has also often been limited or unknown. Therefore, the aim was to obtain basic serum biochemical data of Nigerian freshwater tortoises, establish preliminary baseline values for this species, and to investigate the impact of minerals and serum biochemical value on different growth phases of the Nigerian freshwater tortoise (*Geochelone nigra*).

Arguably more importantly, it is imperative to note that stress impacts growth and maturity. As is the case in humans, this is also known to be the case for tortoise.[12,13] Hence, considering tortoise farming for their food protein and medicinal values, this study on tortoise growth has implications for humans.[14-16]

METHODS

Study design

The research was designed to be an observational experimental study.

Place of study

The selected tortoise for the research were intensively managed at the Tortoise Unit of the Department of Animal Production Teaching and Research Farm, Delta State, University of Science and Technology (DSUST), Ozoro, Delta State, Nigeria. This was as previously published.[17] The test procedures were carried out at the Veterinary Clinic Section of Animal Production Laboratory, at DSUST.

Animal groups: 80 tortoises of different sizes were obtained and distributed equally into four groups according to growth phases including hatchlings (HGP), juvenile (JGP), sub-adult (SAGP) and adult (AGP). The tortoises were treated as described in previous publication; in the period of February 2023 to January 2024.[17]

Data

Chemical analysis of the serum biochemistries including colorimetric method for glucose, biuret method for proteins and mineral content (Na^+, K^+, Ca^{2+}, PO_4^{3-}, Cl^-), were accordingly validated as described in reported studies.[18,19]

Statistical analysis

The analysis was replicated twice. The data obtained were subjected to descriptive evaluations and one-way analysis of variance (ANOVA) All results were significant at p value of 0.05.

RESULTS

The morphometric traits of the experimental freshwater tortoises are shown in Table 1. The weight ranged from 41.00–848.00 g, while the number of scutes varied from 8.0–53.0. The chemical analysis of the serum biochemical revealed the presence of proteins, lipids and glucose in varying concentrations (Table 2). Growth phases significantly ($p<0.05$) influenced the concentration of the three organic substances. The serum biochemical of sub-adult had the highest values of glucose (72.00 mg/dl) and lipids (64.10 mg/dl) followed by the juvenile phase and hatchling phase for glucose and lipid respectively. However, highest concentration of protein was recorded by adult phase followed by the sub-adult stage.

The result of mineral analysis indicated the presence of all the ions tested in the freshwater tortoise serum biochemical. Sub-adult stage recorded highest values in Ca^{2+}, PO_4^{3-}, and Na^+ (Table 3). It is noteworthy that the concentration of Na^+ ranked highest followed by Cl^- while PO_4^{3-} ranked least in the serum biochemical of the experimental freshwater tortoises.

Table 1: Averaged morphometric traits of the experimental freshwater tortoise.

Parameters	HGP	JGP	SAGP	AGP
Carapace length (cm)	5.6±0.03***	13.2±0.06**	17.6±0.04*	19.6±0.07*
Carapace circumference (cm)	4.6±0.01***	10.3±0.04**	12.8±0.06*	13.8±0.01*
Weight (g)†	41.0±0.08	363.0±1.06	760.0±0.50	848.0±0.58
No. of scutes	8.0±0.01	17.0±0.02	34.0±0.01	53.0±0.02

*Mean values in each row with the same superscripts are not significantly different ($p<0.05$), **mean values significantly lower than AGP and SAGP ($p<0.05$); ***mean values significantly much lower than AGP and SAGP ($p<0.05$); †Significant gradient difference in weights

Table 2: Mean levels of diagnostic bioactive compounds in blood of different *Geochelone nigra*.

Parameters	HGP	JGP	SAGP	AGP
Glucose (mg/dl)	36.00±0.03‡	48.00±0.04	72.00±0.02†	40.00±0.01
Protein (g/l)	43.50±0.11‡	48.03±0.12	58.20±0.15	72.06±0.25†
Lipid (mg/dl)	48. 40±0.05	59.50±0.20	64.10±0.24†	40.15±0.26‡

Statistically significant differences between groups at ($p<0.05$) – ‡lowest, †highest

Table 3: Mean levels of diagnostic bioactive minerals in blood of different *Geochelone nigra*.

Parameters	HGP	JGP	SAGP	AGP
Ca^{2+}	4.6±0.03*	6.4±0.02**	7.6±0.05***	4.5±0.02*
PO_4^2	0.05±0.01**	1.03±0.14*	1.08±0.02*	0.09±0.07*
Na^+	94.00±0.31*	96.30±0.34*	99.10±0.18*	87.09±0.12**
K^+	4.80±0.06*	5.04±0.01*	5.20±0.04*	7.00±0.04**
Cl^-	58.15±0.01***	47. 21±0.04**	43.10±0.3**	39.26±0.24*

*Mean values in each row with the same superscripts are not significantly different ($p<0.05$); **mean values significantly lower than AGP and SAGP ($p<0.05$); ***mean values significantly much lower than AGP and SAGP ($p<0.05$); †significant gradient difference in weights

DISCUSSION

The result of the present study revealed the growth phases significantly affect the morphometric trait of the freshwater tortoise, that is, the higher the age, the bigger the tortoises. The number of scutes also varied with the age and this observation agrees with a previous report, which had earlier observed that freshwater tortoise age can be determined by the number of scutes on its carapace.[20]

Sub-adult phase serum biochemical compounds indicate high concentrations of lipids and glucose than both juvenile and hatchling phases. Considering that lipids and glucose are the energy sources for energy supply to the cells, the observed higher concentration of these energy sources in sub-adult phase might be because this is the most active phase in freshwater tortoise life hence requiring higher energy resources to support and sustain their activities. On the other hand, adult phase because of its weight is not very active thus requiring less concentration of energy substrates.

We observed a directional correlation between protein concentration and the ages of the tortoises: protein concentration increases from 43.50 g/l during the hatchling age to 72.06 g/l in the adult age. Proteins are regulators of physiological processes such as growth and especially reproduction which is the main activity during adult stage.[21]

During the adult stage, protein is significantly ($p<0.05$) than the two other substrates (lipids and glucose). This is in agreement with the results of previous study.[22] Freshwater tortoises are known to be a good protein source which can be consumed to ameliorate the problem of protein deficiency in diets, a common phenomenon in Nigeria.[5]

The ionic analysis of the hematological showed that the highest values of Ca^{2+}, PO_4^{3-} and Na^+ were recorded by the sub-adult stage. The higher concentration of Ca^{2+} at this stage might probably be because of rapid increase in carapace size and the replacement of the lost scutes.

Phosphate plays an important role in the formation of ATP, a high energy form. Its higher concentration in the sub-adult stage is not unexpected as this is the most active phase.

The concentration of Na^+ and Cl^- were more than other minerals analyzed. This result contradicts the report of some other studies, which opined that freshwater tortoises must not be given salt.[8,23] Na^+ and Cl^- contribute significantly to the osmotic pressure of the animal tissues and are needed for nervous communication in animals.

Results from this study agree with findings of other researchers. It is speculated that medium size freshwater tortoises (i.e. SAGP) are more sexually active than other live weight groups and ecosystem factors constitute a determinant.[24] It implies that the sub-adult stage of freshwater tortoises has a better physiological status than both juvenile and hatchlings stages and thus should be recruited for breeding purposes.

Implications on humans

Animal farming including the common poultry and cattle as well as uncommon tortoise constitute major sources of occupation, nutrition and livelihood.[17,25,26] The animal farming practice has public health significance.[27,28] The hygiene practices is one of the factors animal farming productivities, hence phenomenon of research interest.

The relevance in the public health concept of poultry farming with regards to family nutrition are quite well discoursed, while there is dearth of discussion on tortoise.[25,27,29-31] Therefore, this work focuses on tortoise farming, and advances on previous report.[17]

Perhaps, it is pertinent to remind about oxidative stress, which impacts growth in animals regardless of species.[13] The findings of this study on the serum biochemical values and mineral concentrations in freshwater tortoises (*Geochelone nigra*) have several potential implications for human health and nutrition. Specifically, the insights gained from this research can inform dietary and health management strategies in several ways.

Nutritional value tortoises

The high protein content observed in the adult phase of freshwater tortoises suggests that these animals could be a valuable source of dietary protein. In regions where protein deficiency is prevalent, incorporating tortoise meat into the diet could help address nutritional gaps. The progressive increase in protein concentration from hatchling to adult phases indicates that the nutritional

value of tortoise meat may vary with age, which is important for optimizing its use as a food source.[5]

Medicinal and recreational activity values

The apparently healthy tortoises have their own levels of antioxidants that are both medicinal and nutritional.[32] Nevertheless, zoos constitute economic, recreational and tourist activities for countries. On this discourse, zoo tortoises subject to breeding as well as prone to infection, both of which may benefit from diagnostic blood tests.[33] What this report advances are that the conservation and health concerns for tortoises would contribute to sustainability of its medicinal use.

Yet, there are two phenomena of interest worthy of note. First is the evidence that trauma vis-a-vis chronic stress causes increased plasma protein fractions relative to the apparently healthy ones. Second is that there are potential variations in reference values, which may be due to the anthropogenic factors, or analytical method.[6,34]

Educational and community health programs

The knowledge gained from this research can be incorporated into educational programs aimed at improving community health. Teaching communities about the nutritional benefits of tortoise meat and how to properly raise and manage these animals can lead to improved health outcomes. Furthermore, sharing best practices in animal husbandry and nutrition, based on scientific research can empower local communities with knowledge of ecosystem factors to enhance their livelihoods and health.[35] That is, giving considerations to long-established determinants of ecological systems' impact on animals' health and the significance of conservation.[36]

Implications for tortoise health management and conservation

Tortoise has been recognized among the animals that would benefit from conservation efforts in developing countries including West Africa. Over the decades, anthropogenic factors have been known for their roles in animal extinction vis-à-vis the need for conservation.[36] Within the past five years, several reports have reiterated this knowledge.[4,8,37-39] One of the major advancements is suggestion of biochemistry and haematology tests that could be used in the clinical diagnosis.[4,37,40,41] With this regard to these clinical laboratory tests, reference intervals have been developed for diagnostic purposes.[6,7,42]

Health monitoring and diagnostic tools

The study's methodology in analyzing serum biochemical values provides a framework that could be adapted for human health monitoring. Regular monitoring of blood glucose, lipid, and protein levels, as well as essential minerals such as calcium, phosphorus, and sodium, is crucial for diagnosing and managing various health conditions in humans including cognizance of ecological systems.[43] The techniques used in this study can be applied to develop better diagnostic tools and protocols for tortoise health assessments, particularly in regional ecosystems with limited access to advanced medical technologies.[44]

Mineral imbalances and dietary recommendations

The findings highlight the importance of maintaining balanced mineral levels for healthy growth and development. For humans, this underscores the need for a diet that provides adequate amounts of essential minerals to prevent deficiencies and related health issues. The study's results could inform dietary recommendations and interventions aimed at preventing mineral imbalances, particularly in vulnerable populations such as children and the elderly.[2]

Limitations

It is known how blood samples are processed can affect the quality of biochemistry tests.[45,46] This study had limited affordances to carry out external quality control, except by doing tests in duplicates. Therefore, this is acknowledged as a limitation. Cognizance is taken that the tortoises in this experiment have been intensive management system: hence test results may not be comparable to the wildlife range system. Considering potential differences due to intensive management relative to free/wildlife range species, this is acknowledged as a limited-scope study and calls for further research.[47]

CONCLUSION

This report advances the potential levels of biochemical compounds in blood of *Geochelone nigra* at different growth phases. The primary significance of this report lies in the medicinal and nutritional values of tortoise in human food security. Hence, upon translation of this report into practical applications, there is potential to make significant contributions to human health and nutrition, particularly in regions where dietary deficiencies and access to medical resources are major challenges. In conclusion, the implications of this study extend beyond the ecological and biological understanding of freshwater tortoises to include food values and ecosystems.

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