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Proximate, Mineral and Biochemical Evaluation of the Haemolymph of Growing Snails Fed Fresh Lettuce Waste, Whole Lettuce, Pawpaw Leaves and Cabbage Waste

[1]O.O. Babalola and [2]A.O. Akinsoyinu
[1]Department of Science Technology, Federal Polytechnic, Ado-Ekiti. Nigeria
[2]Department of Animal Science, University of Ibadan, Ibadan. Nigeria

Abstract: Disease states in experimental animals are usually accompanied by biochemical alterations. Thus a good working knowledge of reference values of some biochemical constituent of the haemolymph of snails (*Archachatina marginata*) is needed as reference point for future researchers on snail. It is also necessary to determine whether these values could be influenced by diets. Thirty six experimental snails of mean weight of 125.64±2.35g were randomly distributed into 4 dietary treatments of Pawpaw Leaves (PL), Whole Lettuce (WL), Lettuce Wastes (LW) and Cabbage Wastes (CW). Nine snails were used as control animals to determine the reference values. They were fed *ad libitum* for 60days. Parameters determined were, proximate composition, mineral profile, total protein, albumin, globulin, albumin:globulin, alanine amino transferase (ALT) and aspartate amino transferase. There was significant ($p<0.05$) reduction in crude protein of haemolymph from 24.14 to 21.20% for snails fed LW. The increase in ash from 0.79 to 0.84 in snails fed LW was significant. There were significant increases in all the minerals analyzed in all the treatment groups with Calcium recording the highest concentration of 170 mg/mL. There was also significant ($p<0.05$) decrease in albumin and albumin:globulin when snails were fed the experimental diets. Values obtained for the enzymes, ALT and AST between treatments were similar. The study established that the feedstuffs were safe for their consumption and could not lead to any tissue damage. Values of 165 unit/l for AST, 11 units/l for ALT, total protein, 7.1 g/dl, globulin, 3.9 g/dl, albumin, 3.2 g/dl, and albumin:globulin, 0.82 could serve as reference values.

Key words: Biochemical constituents, growing snails, haemolymph, mineral profile, proximate composition

INTRODUCTION

The blood of the snail is referred to as haemolymph. It is the fluid that baths the snails. Blood as a transport system of the body plays an active role in the sustainability and survival of organisms. Blood is the transport medium for the movement of nutrients to the cells and excretory products, which are an important means of maintaining the homeostasis of the body (Bentic-Smith, 1974). The blood contains a myriad of metabolites and other constituents which provide a valuable medium for clinical investigation and nutritional status of human beings and animals. Hence, World Health Organisation recommended the use of blood and biochemical parameters in medical nutritional assessment (Church *et al.,* 1984). The various blood functions are made possible by the individual and/or collective actions of its constituents (Aletor and Egberongbe, 1992). Church *et al.* (1984) reported that the dietary components have measurable effect on blood components hence blood constituents are widely used in nutritional evaluation and survey of animals.

The purpose of investigating blood (haemolymph) composition is to have a way of differentiating normal states of health from states of stress which could be nutritional, physical or environmental. Biochemical values measured in the blood of animals include serum total protein, albumin, globulin, and albumin:globulin. These values are useful in the assessment of the nutritional and health status of human beings and animals. Serum Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) in normal animals are low but after extensive tissue destruction, these enzymes are liberated into the serum (Mitruka and Rawnsley, 1977).

Disease states in the experimental animals are accompanied by biochemical alterations. Therefore use of the correct normal range of biochemical constituents in the blood of these experimental animals, established by quality controlled determination is required to avoid pitfalls in interpreting laboratory measurements. Thus the clinician should have a good working knowledge of reference values of the normal experimental animals with reference to the species of the animal (Mitruka and Rawnsley, 1977).

This study was embarked upon:

- to determine the proximate and mineral composition of the haemolymph of snails

Corresponding Author: O.O. Babalola, Department of Science Technology, Federal Polytechnic, Ado-Ekiti. Nigeria

Table 1: Proximate composition (g/100 mL) of haemolymph of snails fed the experimental diets.

Nutrient (%)	Control	PL	WL	LW	CW	SEM
Dry matter	2.35^a	2.19^b	2.30^a	1.98^c	2.19^b	0.04
Crude protein	24.15^a	22.36^b	22.89^b	21.20^c	22.73^b	0.25
Ash	0.79^b	0.78^b	0.81^a	0.84^a	0.76^b	0.00
Ether extract	2.17^a	2.15^a	2.23^a	2.20^a	2.19^a	0.00

a,b,c: means along the same row with different superscripts are significantly different ($p<0.05$)
SEM - Standard error of means, PL – Pawpaw Leaf, WL – Whole Lettuce, LW – Lettuce Waste, CW – Cabbage Waste

Table 2: Mineral profile (mg/100 mL) of haemolymph of snails fed the experimental diets.

Mineral	Control	PL	WL	LW	CW	SEM
Calcium	120.00^e	150.00^c	130.00^d	170.00^a	160.00^b	4.98
Iron	2.30^b	1.90^c	2.00^c	2.60^a	1.70^d	0.08
Phosphorus	25.60^e	31.00^b	27.00^d	34.00^a	29.00^c	0.89
Magnesium	18.00^e	28.00^c	23.00^d	38.00^a	33.00^b	1.92
Copper	1.10^d	1.50^b	1.30^c	1.80^a	1.60^b	0.06

a,b,c,d,e: means along the same row with different superscripts are significantly different ($p<0.05$)
SEM – Standard error of means, PL – Pawpaw Leaf, WL – Whole Lettuce, LW – Lettuce Waste, CW – Cabbage Waste

- to obtain some biochemical values for the total protein, albumin, albumin:globulin, ALT, AST since these values were conspicuously absent in the book of reference values in normal experimental animals and could therefore serve as reference point for future researchers on snail and finally
- to determine whether these values could be influenced by diet (pawpaw leaves, whole lettuce, lettuce waste and cabbage waste).

MATERIALS AND METHODS

This study was conducted in April 2007. The test ingredients used in the trial were collected fresh on a daily basis. The lettuce and cabbage wastes were collected from the early morning trimming of vegetable sellers in Sabo area of Ibadan, Oyo State, Nigeria. Growing snails of *A. marginata* were purchased from three different markets within Ibadan metropolis in Oyo state, Nigeria. Forty five (45) growing snails of mean weight 125.64±2.56 g were used for the experiment. There were 4 dietary treatments with three replicates per treatment. Nine snails were allotted to each of the 4 treatments with 3 snails per replicate in a completely randomised design. The remaining 9 snails served as the control and were killed by striking iron rod on the shell carefully. The haemolymph was immediately drained and put in bottled container for chemical and biochemical analyses.

The 36 experimental snails were distributed into the 4 dietary treatments of Pawpaw Leaf (PL), Whole Lettuce (WL), Lettuce Waste (LW) and Cabbage Waste (CW). They were fed *ad libitum* for 60 days in wooden cages of $0.5x0.5x0.5m^3$ compartments. At the end of the feeding trial the snails were killed and the haemolymph collected for analysis as was done for the control snails.

Chemical analysis: Haemolymph collected were analysed for proximate composition (dry matter, Crude Protein (CP), ash and ether extract according to AOAC (2005). Iron, Magnesium and Copper were determined by Atomic Absorption Spectrophotometry (AAS) using Buck 200 (Buck Scientific, UK) at a wavelength of 324.7nm for copper, 285.2nm for magnesium and 495.8nm for iron. Calcium was by flame photometry using Jenway digital flame photometer and phosphorus determined using 210 digital UV spectrophotometer by Metrohm Inc. US at a wavelength of 470 nm. Total protein, albumin and globulin were determined by Biuret reaction method. Alanine amino transferase (ALT) and Aspartate amino transferase (AST) activity in Sigma-Frankel (SF) units/mL were determined after a calibration curve of Absorbance at 490-520 nm versus corresponding units of ALT and AST have been obtained.

Data analysis: All data were subjected to analysis of variance while the treatment means were separated using Duncan multiple range test (SAS, 1999).

RESULTS

Proximate analysis: The result of proximate analysis showed that haemolymph of snails contained dry matter 2.35%, crude protein 24.14%, ash 0.79% and ether extract 2.17% (Table 1). The various dietary treatments have significant effect on the proximate composition of the haemolymph. There was significant ($p<0.05$) reduction in crude protein of haemolymph from 24.14 to 21.20% for those fed LW diet. The increase in haemolymph ash from 0.79 to 0.84% in snails fed LW was significant. The values obtained for the ether extract were similar (Table 1).

Mineral profile of haemolymph: Table 2 showed the result of the mineral profile of haemolymph of snails. There were significant increases in all the minerals analyzed in all the treatment groups. Snails fed LW had highest Ca (170.0), Fe (2.6), P (34.0), Mg (38.0) and Cu (1.8) (mg/100 mL) as against 120.0, 2.3, 25.6, 18.0 and 1.10 (mg/100 mL) obtained for the control snails respectively. Of all the minerals tested for in the

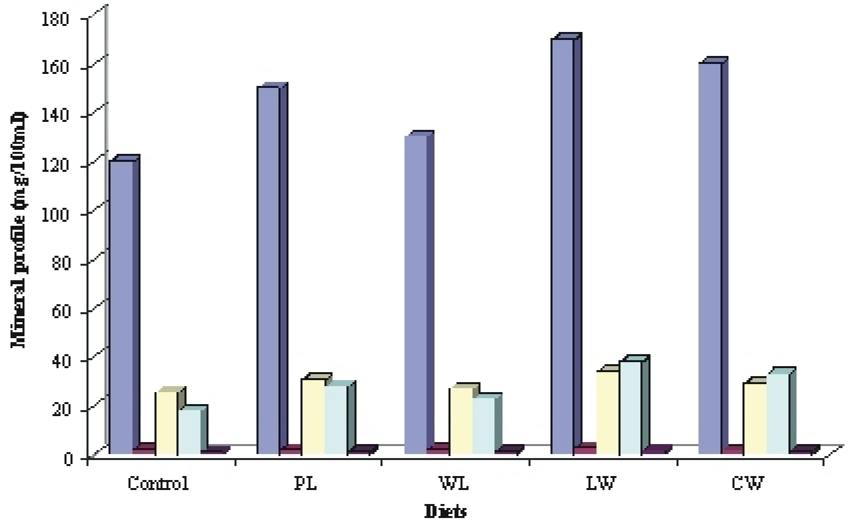

Fig. 1: Mineral profile of haemolymph of snails fed the experimental diets

Table 3: Biochemical parameters of haemolymph of snails fed the experimental diets

Parameters	Control	PL	WL	LW	CW	SEM
Total protein (g/dl)	7.10[a]	5.40[b]	5.60[b]	5.30[b]	5.50[b]	0.39
Globulin (g/dl)	3.90[a]	3.40[b]	3.40[b]	3.30[b]	3.40[b]	0.17
Albumin (g/dl)	3.30[a]	2.00[c]	2.20[b]	2.00[c]	2.20[b]	0.24
Albumin:globulin	0.85[a]	0.59[c]	0.65[b]	0.61[c]	0.65[b]	0.04
ALT (unit/l)	11.00	10.70	10.30	10.70	10.40	0.35
AST (unit/l)	165.00	162.70	161.20	166.30	161.50	2.20

a,b,c,d,e: means along the same row with different superscripts are significantly different ($p<0.05$)

SEM – Standard error of means, PL – Pawpaw Leaf, WL – Whole Lettuce, LW – Lettuce Waste, CW – Cabbage Waste, ALT – Alanine amino transferase, AST – Aspartate amino transferase

Table 4: Proximate composition of the snail diet (g/100g dry matter)

Nutrients	Pawpaw Leaves (PL)	Whole Lettuce (WL)	Lettuce Waste (LW)	Cabbage Waste (CW)
Dry matter	25.43	5.96	7.04	10.10
Crude protein	33.25	11.20	7.35	9.80
Crude fibre	7.26	8.96	6.32	5.48
Ether extract	0.78	0.56	0.27	0.23
Ash	10.86	11.65	9.67	6.94
Nitrogen free extract	47.85	67.63	76.39	77.55

haemolymph, Ca recorded the highest concentration (Table 2 and Fig. 1).

Biochemical parameters of haemolymph: The results of the total protein, albumin, albumin:globulin, alanine amino transferase (ALT) and aspartate amino transferase (AST) analyzed are as shown on Table 3. There was a significant ($p<0.05$) decrease in the albumin, and albumin:globulin, when snails were fed the experimental diets. Values obtained for haemolymph enzymes, ALT and AST between treatments were similar (Table 3).

DISCUSSION

The least haemolymph crude protein was obtained for snails fed lettuce wastes. This is a reflection of the crude protein content of the feed (7.35), which was the lowest of all the treatment diets (Table 4). Church *et al.* (1984) observed that the hematological indices are reflections of the effect of dietary treatments on the animal in terms of the type and amount of feed ingested. The highest value obtained for haemolymph ash revealed that lettuce waste is a good source of minerals for snails of *A. marginata.*

The result of the mineral profile in which the peak values were obtained for snails on lettuce waste further confirmed adequacy of lettuce waste as a good source of minerals and this could be responsible for drinking of snail haemolymph by some pregnant women as reported by Adeyeye (1996). Haemolymph of *A. marginata* have been known to be fried and eaten like chicken egg in some parts of Southwest Nigeria. This could be due to the high contents of protein and mineral and where snail is processed on a large scale the haemolymph could be coagulated and used as animal feed.

The total protein determination is a measurement of serum protein, which is usually done with serum (Aniket, 2005). The total protein test is carried out: to evaluate liver and kidney functions since protein metabolism occurs in the liver and waste excreted in kidney, to determine whether a diet contains enough protein, to estimate the risk of developing an infection and also to evaluate nutritional status including malnutrition (Coles, 1986).

The general reduction in the total protein, albumin, globulin and albumin:globulin suggest that the snails might not consume enough protein in their diet, a clear indication of malnutrition or impaired protein digestion. The albumin concentrations were more affected than the globulin. The percentage reduction in albumin when compared with snails in the control was (30.33-39.39%) whereas that of globulin was (12.82-15.38%). This agrees with Ross *et al.* (1978) who observed that changes in the nutritional status are more readily reflected in the albumin than globulin fractions of the blood.

Dietary treatments have no appreciable effect on the haemolymph enzyme alanine amino transferase (ALT) and aspartate amino transferase (AST). Serum transaminases in normal animals are reported to be low but after extensive tissue destruction, these enzymes are released into the serum (Mitruka and Rawnsley, 1977). The fact that these enzymes were not elevated in the snails under the different treatments was an indication that the feedstuffs were safe for their consumption and could not lead to any tissue damage.

CONCLUSION

In conclusion, the study has been able to establish that the haemolymph of snail is rich in protein and that lettuce waste is a good source of minerals for growing snails. The diets were found to be safe for consumption by the snails but needs to be supplemented with a protein rich diet. Values of 165 unit/l for AST, 11 units/l for ALT, total protein, 7.1 g/dl, globulin, 3.9 g/dl, albumin, 3.2 g/dl, and albumin:globulin, 0.82 obtained for snails on control diets could serve as reference values. These values can serve as a reference point for future studies on snails.

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