



Effect of Replacing Bone Ash with Fresh Water Snail (*Pila Ampullacea*) Shell Ash on Serum Biochemical Indices of Weaned Rabbits

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Authors' contributions

This work was carried out in collaboration among all authors. Author FBPA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SSE and SA managed the analyses of the study. Author Emmanuel SSE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Twenty five (25) mixed breed weaned rabbits were utilized in 84-days experiment to investigate the effect of replacing bone ash with fresh water snail (*Pila ampullacea*) shell ash on the serum biochemical composition of weaned rabbits. The experiment was conducted at the Teaching and Research Farm, University of Agriculture, Makurdi, Benue State, Nigeria for a period of 12 weeks. The rabbits were randomly assigned to the five dietary treatments and replicated five times giving a total of one rabbit per replicate in a completely randomized design (CRD). Rabbits were raised intensively in hutches of about 4ft x 4ft. Five experimental diets tagged T₁ to T₅ were formulated such that fresh water snail (*Pila ampullacea*) shell ash replaced bone ash at 0%, 25%, 50%, 75% and 100%. Feed and water were served *ad libitum*. Bio sanitary and bio security measures were strictly adhered to. At the end of the feeding trial, three rabbits per treatment were selected for the evaluation of serum biochemistry. The parameters assayed for were: total serum protein, serum

globulin, serum albumin, cholesterol, serum calcium, and serum phosphorus. The study showed that the serum biochemical indices were not influenced ($P>0.05$) by the dietary treatments except for calcium which was significantly ($P<0.05$) influenced, however, calcium values were within the normal reference values for rabbits. This study had shown that fresh water snail (*Pila ampullacea*) shell ash can serve as a substitute for bone ash in weaned rabbit diet up to 100% inclusion levels without adverse effect on the serum biochemical indices.

Keywords: Rabbits; fresh water snail shell ash; bone ash; serum biochemical indices.

1. INTRODUCTION

The cost of non-conventional feedstuffs of protein (fish meal, groundnut meal, soybean etc.), energy (maize, guinea corn, wheat etc.) and minerals (bone meal, limestone etc.) origin have been on the increase. The hike in cost of these feed stuffs have been translated into increased cost of animal products as feed cost constitutes 60-70% of total cost of production [1].

The non-conventional feedstuff and agro-industrial by-products is now being exploited because there are inexpensive, less competed for or not being competed for at all; since this will bring about sustainability of the livestock industry. Fresh water snail shell ash (*Pila ampullacea*) meets this demand.

Fresh water snail shell ash (*Pila ampullacea*) is made up of 92.45% ash, 41.60% calcium and 0.01% of Phosphorus. The calcium content of *Pila ampullacea* is higher than that of bone ash (34.64%), however, bone ash is richer in phosphorus [2]. There is dearth information on the use of fresh water snail shell ash in livestock feeding and as such, blood assays that act as pathological reflector of the status of exposed animals to toxicant and other conditions [3] should be investigated to ascertain if fresh water snail shell ash (*Pila ampullacea*) contains toxic substances. Blood assay are important tools that help detect any deviation from normal in the animal [4]. The examination of blood provides the opportunity to clinically investigate the presence of several metabolites and other constituents in the body of animals and it plays a vital role in the physiological, nutritional and pathological status of an organism [5-6].

2. MATERIALS AND METHODS

2.1 Experimental Site

The experiment was conducted in the rabbitry unit at the Teaching and Research Farm, College

of Animal Science University of Agriculture Makurdi Benue State. Benue State lies within the lower river Benue trough in the middle belt region of Nigeria. Its geographic coordinates are longitude 7° 47' and 10° 0' East. Latitude 6° 25' and 8° 8' North. The University is located on a land mass of 7,986.22 hectares [7] out of which less than half is occupied by buildings and crop farm, the rest is natural grassland on which cattle are grazed.

2.2 Source of Bone Ash

Bone ash was bought at God 4 Us Livestock Consult; beside SRS junction, new bridge road, north bank, Makurdi, Benue State, Nigeria.

2.3 Sources and Collection of Fresh Water Snail (*Pila ampullacea*) Shells

The test ingredient was sourced locally at Gbajimba and Iyeh in Guma Local Government Area and Makurdi metropolis, where the flesh is usually removed and the shells are thrown away by the consumers.

2.4 Processing of Fresh Water Snail (*Pila ampullacea*) Shell

The shells were thoroughly washed, dried and burnt for about 1 hour until they became whitish in appearance; they were then crushed into fine powder as shell ash and used in the diet. The mineral composition of the shell was analyzed by the procedure of Association of Official Analytical Chemists [8].

Table 1. Composition of fresh water snail (*pila ampullacea*) shell ash and bone ash

Minerals	(%Composition) Pasa
Ash	92.45
Calcium	41.60
Phosphorus	0.01

PASA = *Pila ampullacea* shell ash

2.5 Experimental Design

A Completely Randomized Design (CRD) was used for this experiment. A total of twenty five (25) weaned cross bred male rabbits (New Zealand white × Chinchilla) at five weeks of age with an initial average weight of about 664.00 – 667.00g were obtained from Dagwom Farm, National Veterinary Research Institute (NVRI) Vom, Jos Plateau State for the research. Male rabbits were used in all the treatments to avoid bias (homogeneous). The rabbits were allowed for a preliminary feeding period of seven days to enable them acclimatized after which they were randomly assigned to five (5) dietary treatments designated as T₁ to T₅. Each of the dietary treatment had five (5) rabbits with each rabbit serving as a replicate (R₁, R₂, R₃, R₄ and R₅).

2.6 Housing and Management of Experimental Animals

The rabbits were house individually in the hutches and labeled according to the treatment and replicate assigned to them. The dimension of the hutches was 40cm×30cm×30cm (this was to

enable it accommodate the feeders and drinkers). The initial weight of each rabbit was taken before assigning them to one of the five dietary treatments. Prophylactic medication was given against any infection before the commencement of the experiment. Agility, skin, fur, eyes and anus of each rabbit was observed daily to ascertain their health condition. A measured quantity of the treatment diet was served daily for each replicate and was provided *ad-libitum*, left over feed was weighed every week and the quantity consumed was determined by difference. Fresh clean water was also provided every morning. The experiment lasted for 12 weeks (84 days) because the study was to cover weaned to growing phases.

2.7 Experimental Diets

Five experimental diets were formulated tagged T₁ to T₅ respectively. T₁ served as a control diet. Fresh water snail (*Pila ampullacea*) shell ash replaced bone ash at 0%, 25%, 50%, 75% and 100% respectively. T₁ containing 100% bone ash while T₅ contained 100% fresh water snail (*Pila ampullacea*) shell ash. These were mixed with other ingredients as in the Table 2.

Table 2. Composition of experimental diets with fresh water snail (*Pila ampullacea*) shell ash as a replacement for bone ash (%)

Feedstuff	Experimental Diets				
	(0%Pasa)	(25%Pasa)	(50%Pasa)	(75%Pasa)	(100%Pasa)
Maize	30.00	30.00	30.00	30.00	30.00
Full fat soybean	20.00	20.00	20.00	20.00	20.00
Groundnut cake	12.00	12.00	12.00	12.00	12.00
Maize offal	14.00	14.00	14.00	14.00	14.00
Rice offal	20.05	20.05	20.05	20.05	20.05
Bone ash	3.00	2.25	1.50	0.75	0.00
Pasa	0.00	0.75	1.50	2.25	3.00
Methionine	0.20	0.20	0.20	0.20	0.20
Lysine	0.20	0.20	0.20	0.20	0.20
Table Salt	0.30	0.30	0.30	0.30	0.30
Vita/min. premix	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00

Analysed Nutrient:

(%).Crude protein	16.86	16.86	16.86	16.86	16.86
(%).Crude protein	2637.10	2637.10	2637.10	2637.10	2637.10

Note: Pasa = *Pila ampullacea* shell ash

2.8 Blood Collection for Serum Biochemical Analysis

At the end of the feeding trial at 84 days, three rabbits per treatment of live weight approximate to the average weight of the treatment were selected for the evaluation of serum biochemistry. This was done by fasting the rabbits for 12 hours over night and the jugular veins were cut with a sharp knife after hand stunning in the morning between 8.30 – 9.00 hours. 2ml of blood samples were collected in separate bottles without anti-coagulant for serum biochemical analysis. The blood was taken to University of Agriculture Veterinary Clinic laboratory for analysis. The serum biochemical indices include: total serum protein, serum globulin, serum albumin, cholesterol, serum calcium, and serum phosphorus were determined. The serum biochemical parameters were determined using procedure described by [9] using BC5380 Mindray analyzer.

2.9 Statistical Analysis

The data collected were subjected to one way Analysis of Variance (ANOVA) using Minitab statistical software version 16 [10]. The separation of means was effected using Duncan's Multiple Range Test (DMRT) as outline by [11].

3. RESULTS AND DISCUSSION

The result of the proximate analysis of fresh water snail (*Pila ampullacea*) shell ash is presented in Table 1. It was observed that fresh water snail (*Pila ampullacea*) shell ash contained high percentages of ash (92.45%) and calcium (41.60%) but has negligible amount of phosphorus (0.01%) when compared with bone

ash. The result showed that fresh water snail (*Pila ampullacea*) shell ash is a potential source of calcium in livestock feed.

Results of serum biochemical indices nutrient are shown in Table 3. Total serum protein values ranged from 6.10 to 7.13g/dl. The results were not influenced by the dietary treatments. The results were within the normal reference range of 5.4 to 7.3g/dl by [12]. Serum protein is an indirect index for measuring nutritional protein adequacy [13]. The serum protein gives viscosity to the blood, helps in blood clotting, supplies antibodies and acts as amino acids reservoir [14]. Reduction in serum protein as well as the concurrent increase in blood urea has been attributed to poorly utilized feedstuff due to the presence of anti-nutrients [12]. This result indicated that protein was adequate in the diets and that the protein in the feeds were fully utilized by the rabbits due to the absence of anti-nutritional factors / toxic factors in fresh water snail (*Pila ampullacea*) shell ash. The result of serum albumin ranged from 2.23 to 2.80g/dl. There were no significant ($P>0.05$) differences across treatments. These results were within the normal reference range of 2.4 to 4.5g/dl reported by [12]. Albumin is of great importance in regulating the flow of water between plasma and tissue fluid due to its effect on colloid osmotic pressure [15]. The plasma and tissue fluid of the rabbit was balanced. Serum globulin (a salt soluble protein) values ranged from 3.37 to 4.90g/dl, which were within the normal reference values of 2.90 to 4.90g/dl by [12]. High levels of serum globulin indicates the ability of the body to fight diseases [16]. There were no incidence of ill health throughout the duration of the experiment implying that the immune system of the rabbits were not compromised.

Table 3. Effect of replacing bone ash with fresh water snail (*pila ampullacea*) shell ash on serum biochemical composition of weaned rabbits

Parameters	Treatment levels					SEM
	(50%Pasa)	(0%)	(25%Pasa)	(75%Pasa)	(100%Pasa)	
Total protein (g/dl)	6.10	6.23	6.73	7.10	7.13	0.21
Albumin (g/dl)	2.73	2.50	2.46	3.40	2.23	0.89
Globulin (g/dl)	3.37	3.73	4.27	3.70	4.90	0.19
Glucose (mg/dl)	23.07 ^a	22.03 ^a	15.40 ^b	17.43 ^b	15.63 ^b	1.10
Cholesterol (mg/dl)	67.17	87.63	71.43	77.13	75.70	3.61
Calcium (mg/dl)	9.00 ^a	7.07 ^{bc}	7.33 ^{bc}	7.77 ^b	7.00 ^{bc}	0.23
Phosphorus (mg/dl)	5.80	5.43	5.60	5.73	5.70	0.07

SEM= Standard Error of Means; a, b, c= Means in the same row with different superscripts are significantly different ($P<0.05$); Pasa= *Pila ampullacea* shell ash

Serum cholesterol ranged between 67.17 to 87.63mg/dl. These results were within the normal reference range of 77.9 to 134.4mg/dl by [17]. Abnormal elevated cholesterol or triglyceride levels (hypercholesterolemia) in rabbits are likely to be most readily induced by dietary manipulation or hepatic impairment [18] with wide variations possibly occurring between rabbits. The diets were safe for consumption by the rabbits. Fresh water snail (*Pila ampullacea*) shell did not negatively influence the results. The results revealed that, the rabbits were not associated with hypothyroidism (underactive thyroid gland) also their liver and kidney were not diseased. This is a pointer that Pasa is void of toxic substances. The serum calcium levels ranged from 7.00 to 9.00mg/dl. Rabbits served Pasa had significantly ($P<0.05$) lower values of calcium in their blood. However, these values were within the normal reference range of 7.0 to 15.50mg/dl by [12]. Calcium assist in teeth and bone formation. Calcium is mostly stored in the bones and is critical for muscle contraction, nerve signaling, blood clotting, maintaining normal heart function, absorption and utilization of vitamin B12 and inhibits cell membrane permeability of sodium. The implication is that, the bones and teeth of the rabbits were well developed. Serum phosphorus ranged from 5.43 to 5.80mg/dl. This also falls within the normal reference range of 4.4 to 7.2mg/dl by [12]. The result indicated that the bone growth was normal, the kidney was functional, cell growth adequate and the body's acid- alkaline was balanced. Phosphorus is needed for bone growth, kidney function and cell growth. It equally plays a role in maintaining the body's acid- alkaline balance. [19] reported that the biochemical components are sensitive to elements or factors present in the feed, including elements of toxicity. The values obtained for serum biochemical indices in this study fall within the normal literature values for rabbits.

4. CONCLUSION

Serum biochemical indices were not adversely affected by the test material (*Pila ampullacea*). It was concluded that fresh water snail (*Pila ampullacea*) shell ash could replace bone ash up to 100% in weaned rabbit.

5. RECOMMENDATION

It was recommended that for optimum metabolic activities, supplemental levels of calcium be included in weaned rabbit's diet as the level of

calcium decrease following a numerical pattern across the treatments. However, the values were within the normal reference range for rabbits.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee"

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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