EFFECT OF REPLACING BONE ASH WITH FRESH WATER SNAIL *(Pila ampullacea)* SHELL ASH ON SERUM BIOCHEMICAL INDICES OF WEANED RABBITS

ABSTRACT

Twenty five (25) mixed breed weaneder rabbits were utilized in a 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. Five experimental diets comprising of 0%, 25%, 50%, 75% and 100% for treatments T_1 to T_5 respectively. The rabbits were randomly assigned to the five dietary treatments. Each treatment was replicated five times giving a total of one rabbit per replicate in a completely randomized design (CRD). 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 weaner weaned rabbits diet up to 100% inclusion levels without adverse effect on the rabbits' health.

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INTRODUCTION

The increasing demand for animal protein indicates the need to intensify livestock production. In order to achieve this objective, viable options need to be explored and evaluated [1]. Among such alternatives is the use of livestock species that are yet to play a major role in animal production within these countries. Rabbit production is a veritable way of alleviating animal protein deficiency in Nigeria [2].

The rabbit has immense potentials and good attributes which include fast growth rate, high efficiency in converting forage to meat, short generation interval, high prolificacy, relatively low cost of production, and high nutritional quality of rabbit meat which includes; low fat, sodium and cholesterol levels. Rabbit meat contains about 20.8% of protein and its consumption is bereft of cultural and religious biases [3].

The cost of feeding constitutes about 60-70% of the total cost of production livestock in Nigeria [4]. [5] stated that the high cost of production is largely due to the exorbitant prices and scarcity

of conventional feed ingredients. Therefore, in developing countries more important considerations would be to formulate cheap diets based on feedstuffs that are of little direct value as human food such as sweet orange peels, cassava peels and fresh water snail (*Pila ampullacea*) shells [6].

The abundance of freshwater snails *(Pila ampullacea*) in River Benue and its tributaries has been studied by [7]. Snail shell is a mineral ingredient that contains about 98% of calcium carbonate [8]. It is therefore a biological source of calcium that can be used in animal feeding. Investigations have been done on the use of many sources of calcium such as gypsum, limestone and oyster shell in layers and broilers diets [9], but there is a lack of information on the use of snail shells especially fresh water snail *(Pila ampullacea)* shells in animal feeds.

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MATERIALS AND METHODS

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; and shares boundaries with five other states namely: Nasarawa State to the north, Taraba State to the east, Cross-River State to the south, Enugu State to the south-west and Kogi State to the west. The state also shares a common boundary with the Republic of Cameroon on the south-east. Benue occupies a landmass of 34,059 square kilometers [10].

Source of bone ash

Bone ash was bought at **GOD 4 US LIVESTOCK CONSULT**, beside SRS junction, new bridge road, north bank Makurdi.

Sources and collection of fresh water snail (*Pila ampullacea*) shells

Freshwater snails are in abundance in River Benue and its tributaries. 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.

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 [11].

Experimental design

A Completely Randomized Design (CRD) was used for this experiment. A total of twenty five (25) weaned male rabbits of mixed breeds at five weeks of age with an initial average weight of between 664.00 – 667.00g were obtained from Dagwom Farm, National Veterinary Research Institute (NVRI) Vom, Jos Plateau State for the research. 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 T_1 to T_5. Each of the dietary treatment had five (5) rabbits with each rabbit serving as a replicate (R_1, R_2, R_3, R_4 and R_5).

Comment [AHS3]: Why male rabbit has been used in this study? Please describe in detail about the breed.

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. Each rabbit was observed daily to ensure good health. 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).

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Experimental diets

Five experimental diets were formulated tagged T_1 to T_5 respectively. T_1 served as a control diet. Fresh water snail (*Pila ampullacea*) shell ash replaced bone ash at 0%, 25%, 50%, 75% and 100% respectively.T_1 containing 100% bone ash while T_5 contained 100% fresh water snail (*Pila ampullaceal)* shell ash. These were mixed with other ingredients as in the table 1.

Table 1: 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 fatsoyabean	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
Vit/min. premix	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated nutrient composition (%).					
Crude protein	16.86	16.86	16.86	16.86	16.86
Crude fibre	10.27	10.27	10.27	10.27	10.27
M.E(Kcal/kg)	2637.10	2637.10	2637.10	2637.10	2637.10
Methionine	0.57	0.57	0.57	0.57	0.57
Lysine	0.58	0.58	0.58	0.58	0.58
Calcium	1.21	1.22	1.26	1.29	1.34
Phosphorus	0.44	0.43	0.42	0.41	0.40

Note: Pasa = *Pila ampullacea* shell ash

Blood collection for serum biochemistry 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 [12] using BC5380 mindray analyzer.

Comment [AHS5]: Why 84 days? Is there any review of using this duration for experiment period?

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Statistical analysis

The data collected were subjected to one way Analysis of Variance (ANOVA) using Minitab statistical software version 14[13]. The separation of means was effected using Duncan's Multiple Range Test (DMRT) as outline by [14].

RESULTS AND DISCUSSION

The result of the proximate analysis of fresh water snail *(Pila ampullacea)* shell ash is presented in Table 2. 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.

The nutrient composition of the five experimental diets is shown in Table 3. The findings revealed that the proximate fractions were not different from each other significantly. The diets were observed to have met the nutritional requirements of weaned rabbits across treatments.

The total serum protein values ranged from 6.10 to 7.13g/dl. These result were within the normal reference range of 5.4 to 7.3g/dl by [15]. The serum protein is an indirect index for measuring nutritional protein adequacy [16]. The serum protein gives viscosity to the blood, helps in blood clotting, supplies antibodies and acts as amino acids reservoir [17]. 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 [18]. 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 antinutritional 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 [15]. Albumin is of great importance in regulating the flow of water between plasma and tissue fluid due to its effect on colloid osmotic pressure [19]. The albumin levels being a strong predictor of health status suggest that the rabbits were healthy during the experimental period.

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 [15]. High levels of serum globulin indicates the ability of the body to fight diseases [18]. There were no incidence of of ill health throughout the duration of the experiment implying that the diets boosted the natural resistance of the rabbits.

Table 2: Composition of fresh water snail (*pila ampullacea*) shell ash and bone ash.

Minerals	(%composition) Pasa	(%composition) bone ash
Crude Protein	Ng	ND
Crude Fibre	Ng	ND
Ash	92.45	ND
Crude Fat	Ng	ND
Nitrogen Free Extract	Ng	ND
Calcium	41.60	34.64
Phosphorus	0.01	16.98
Fluorine	ND	0.36
Zinc	ND	0.44
Manganese	ND	0.05
Magnesium	ND	0.50

Pasa = *Pila ampullacea* shell ash

Ng = Negligible

ND =Not determined

Table 3: **Proximate composition of the experimental diets (%).**

Parameters	**Treatments levels**				
	(0%)	(25%Pasa)	(50%Pasa)	(75%Pasa)	(100%Pasa)
Analysed composition					
Dry Matter	96.05	95.97	96.39	96.00	96.10
Crude Protein	17.50	17.94	17.50	17.50	17.50
Ether Extract	3.69	5.76	8.04	8.41	8.07
Ash	10.11	9.71	9.56	7.94	9.49
Crude Fibre	9.87	9.72	9.63	9.46	10.77
Nitrogen Free Extract	54.88	52.83	51.59	54.59	59.19
ME (Kcal/kg)	2897.58	2954.15	2955.16	2896.58	2963.43
Calculated nutrient composition					
Crude protein	16.86	16.86	16.86	16.86	16.86
Crude fibre	10.27	10.27	10.27	10.27	10.27
M.E(Kcal/kg)	2637.10	2637.10	2637.10	2637.10	2637.10

ME = Metabolizable Energy from the analysed was calculated from [20].

Pasa = *Pila ampullacea* shell ash

Table 4: Effect of replacing bone ash with fresh water snail (*pila ampullacea)* shell ash on serum biochemical composition of weaned rabbits.

Parameters	Treatment levels					
	(0%)	(25%Pasa)	(50%Pasa)	(75%Pasa)	(100%Pasa)	SEM
Total protein (g/dl)	6.10	6.23	6.73	7.10	7.13	0.21^{NS}
Albumin (g/dl)	2.80	2.50	2.63	2.73	2.23	0.89^{NS}
Globulin (g/dl)	3.37	3.73	4.27	3.70	4.90	0.19^{NS}
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^{NS}
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^{NS}

SEM= Standard Error of Means;

NS = Not Significantly Different (P>0.05)

^{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 [22]. Abnormal elevated cholesterol or triglyceride levels (hypercholesterolemia) in rabbits are likely to be most readily induced by dietary manipulation or hepatic impairment [23] 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 influenced the results.

The serum calcium levels ranged from 7.00 to 9.00mg/dl. There were significant differences (P<0.05) among the dietary treatments. However, the result agreed with the reference ranges of 7.0 to 15.50mg/dl by [15]. These results showed that the test diets did not have any effect on the rabbits as there was no sign of bone mal formation. The rabbit bones were strong.

Serum phosphorus ranged from 5.43 to 5.80mg/dl. This also falls within the reference range of 4.4 to 7.2mg/dl by [15]. These results equally indicated that the diet did not adversely affected bone formation in weaner rabbits as there were no sign of bone malformation throughout the duration of the experiment.

[24] reported that the biochemical components are sensitive to elements or factors present in the feed, including elements of toxicity. Most of the values obtained for serum biochemical indices in this study fall within the normal literature values for rabbits. It can therefore be concluded that up to 100% inclusion level of fresh water snail *(Pila ampullacea)* shell ash can be tolerated by rabbits without a deleterious effect on their health status.

Conclusion

The health status of the rabbits were not affected by the inclusion of fresh water snail *(Pila ampullacea)* shell ash in the diets of weaned rabbits. It was concluded that fresh water snail *(Pila ampullacea)* shell ash could replace bone ash up to 100% in weaned rabbit

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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