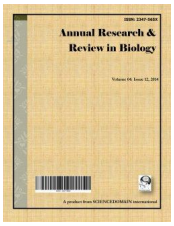

Annual Research & Review in Biology

21(1): 1-7, 2017; Article no.ARRB.32376
ISSN: 2347-565X, NLM ID: 101632869

Effect of Replacing Bone Ash with Fresh Water Snail *(Pila ampullacea)* Shell Ash on Haematological Indices of Weaner Rabbits

F. B. P. Abang[1*], S. Attah[1] and E. Zoo[2]

[1]Department of Animal Production, Federal University of Agriculture, Makurdi, Benue State, Nigeria.
[2]Department of Agriculture and Natural Resources, Gbajimba, Guma, India.

Authors' contributions

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

Article Information

DOI: 10.9734/ARRB/2017/32376
Editor(s):
(1) Tunira Bhadauria, Professor, Department of Zoology, Feroze Gandhi Post Graduate College, Kanpur University, U. P., India.
(2) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) B. O. Ajiboye, Afe Babalola University, Nigeria.
(2) Andell Edwards, University of Trinidad and Tobago, Trinidad and Tobago.
Complete Peer review History: http://www.sciencedomain.org/review-history/22279

Original Research Article

Received 23rd February 2017
Accepted 1st November 2017
Published 14th December 2017

ABSTRACT

Twenty five (25) mixed breed weaner rabbits were utilized in an 84 - day experiment to investigate the effect of replacing bone ash with fresh water snail (*Pila ampullacea*) shell ash on the haematological indices of weaner rabbits. Five experimental diets (T_1 - $T_{5)}$ were formulated such that fresh water snail (*Pila ampullacea)* shell ash replaced bone ash at 0%, 25%, 50%, 75% and 100% for treatments T_1, T_2, T_3, T_4, and T_5 respectively. The rabbits were randomly assigned to the five dietary treatments and replicated five times with one rabbit per replicate in a completely randomized design (CRD). The study showed that the haematological parameters were not affected ($P>0.05$) by the dietary treatments. This study revealed that fresh water snail (*Pila ampullacea*) shell ash can serve as a substitute for bone ash in weaner rabbits diet up to 100% without compromising their health status.

**Corresponding author: E-mail: abang.favour2@gmail.com, abang.favour@yahoo.com;*

Keywords: Rabbit; freshwater snail shell; bone ash and haematological indices.

1. INTRODUCTION

The alarming growing rate of the human population in developing countries such as Nigeria calls for the search for cheaper alternative feedstuffs and animal protein sources that will meet up the challenge [1]. [2] estimated the average animal protein consumption in Nigeria to be 7.4 g per capita/day, while 28 g per person per day was recommended by the British Medical Association [3]. [4] Stressed that the poor nutritional situation in Nigeria is very glaring as a result of the wide gap between the estimated protein requirement and the actual protein consumed. 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 [5]. 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 [6].

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 [7]. The cost of feeding constitutes about 60-70% of the total cost of production of livestock in Nigeria. [4,8] 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 [9]. In an attempt to search for alternative sources of calcium feedstuffs, there is an urgent need to explore the potentials of non-conventional calcium sources that do not compete with human food consumption. Fresh water snail *(Pila ampullacea*) shell ash meets this demand. Freshwater snails *(Pila ampullacea*) are abundance in River Benue and its tributaries. This is due to the availability of food, shelter and oviposition sites along the said river water body. One of the most successful methods of catching is hand picking done as a community effort on regular basis [10]. Snail shell is a mineral ingredient that contains about 98% of calcium carbonate [11]. 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 [12,13] and [14], but there is a lack of information on the use of snail shells especially fresh water snail *(Pila ampullacea)* shells in animal feeds. The blood contains several metabolites which provide useful information of nutritional status and clinical investigation of an individual, hence, WHO recommended the use of blood parameters for medical and nutritional assessments [15]. This paper therefore seeks to investigate the effect of replacing bone ash with fresh water snail *(Pila ampullacea*) shell ash on haematological indices of weaner rabbits.

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; 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 [16].

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.

2.3 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.

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 [17].

2.5 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 – 667.00 g were obtained from Dagwom Farm, National Veterinary Research Institute (NVRI) Vom, Jos Plateau State for the research in November 2013. 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, T_2, T_3, T_4, and 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).

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 40 cm × 30 cm × 30 cm (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 (from 1st December 2013 to February 22nd 2013 (84 days).

2.7 Experimental Diets

Five experimental diets were formulated using fresh water snail (*Pila ampullacea*) shell ash to replace bone ash at 0%, 25%, 50%, 75% and 100% for T_1, T_1, T_2, T_3, T_4 and T_5 respectively. These were later used with other ingredients to formulate test diets as presented in Table 1.

2.8 Blood Samples and Preparation for Haematological Indices

At the end of the feeding trial (at the 84th day), three rabbits per treatment of live weight approximate to the average weight of the treatments were selected for the evaluation of haematological indices and 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. 4 ml of blood sample were collected from the experimental animals into a well labeled sterile bottle containing a required quantity (1 ml) of ethylene diamine tetra acetate (EDTA) as an anticoagulant [18], for haematological analysis. The blood was taken to University of Agriculture Veterinary Clinic laboratory for analysis. The haematological indices determined were the total erythrocyte count (RBC), total leucocytes count (WBC), haemoglobin (Hb) concentration, pack cell volume (PCV), and mean corpuscular volume (MCV). The haematological indices were determined using procedure described by [19] 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 14 [20]. The separation of means was effected using Duncan's Multiple Range Test (DMRT) as outlined by [21].

3. RESULTS AND DISCUSSION

3.1 Results for Proximate Composition of Pasa, Proximate Composition of Experimental Diets and Haematological Indices are Presented in Tables 2 to 4 Respectively

3.2 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 for weaner rabbits across treatments.

The result of haematological indices is presented in Table 4. The result of the white blood cells (WBC) ranged from 4.80 to 7.23×10^9/ µl. these results were within the normal reference ranges of 4.00 to 10×10^9 µl reported by [19] and by [23] (5 to 13×10^9 µl). The white blood cells are known to protect the body from infections.

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 fat soyabean	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	0.75	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**
Calculated nutrients (%)					
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

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

Minerals	(%composition)	(%composition)
Parameter	**Pasa**	**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**
Magnesium	ND	**0.05**
Manganese	ND	**0.50**

Key: Pasa = Pila ampullacea shell ash; Ng = Negligible; ND =Not determined

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

Parameters	Treatments levels				
	(0% Pasa)	(25% Pasa)	(50% Pasa)	(75% Pasa)	(100% Pasa)
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
Analyzed nutrients (%):					
Crude protein	16.86	16.86	16.86	16.86	16.86
Crude Fibre	10.27	10.27	10.27	10.27	10.27
ME (Kcal/kg)	2637.10	2637.10	2637.10	2637.10	2637.10

Key: ME = Metabolizable Energy was calculated using the of method [22]
Pasa = Pila ampullacea shell ash

Table 4. Effect of replacing bone ash with fresh water snail *(Pila ampullacea)* shell ash on haematological indices of weaners' rabbits

Parameters	Treatment levels					
	(0%)	(25%Pasa)	(50%Pasa)	(75%Pasa)	(100%Pasa)	SEM
WBC (10^9/L)	4.80	6.00	4.47	5.20	7.23	0.52^{NS}
RBC(10^{12}/L)	6.57	4.67	5.23	4.73	5.53	0.25^{NS}
HB (g/dl)	11.73	10.57	10.87	5.20	11.53	0.30^{NS}
PCV (%)	40.67	42.00	43.67	43.00	47.67	1.67^{NS}
MCV (10^{15}/fl)	53.63	51.90	51.57	51.77	51.50	0.45^{NS}

Key: WBC = White Blood Cells; RBC = Red Blood Cells; HB = Haemoglobin; PCV = Pack Cell Volume; MCV = Mean Corpuscular Volume; SEM = Standard Error of Means; NS = Not Significantly Different (P>0.05);
Pasa = Pila ampullacea shell ash

The number of leukocytes (WBC) in the blood is often an indicator of disease. An increase in the number of leukocytes over the upper limits is called leukocytosis and a decrease below the lower limit is called leukemia. None of these were observed implying that the defensive mechanism of the rabbits was intact and so the rabbits were able to perform their phagocytic functions well [24]. The result of red blood cells (RBC) ranged from 4.67 to 6.57×10^{12}/ µl. the red blood cells (RBC) are known as erythrocytes and this was within the normal reference ranges of by [19] (3.50 to 5.50 ×10^{12} µl) and [23] (3.8 to 7.9 ×10^6µl). They are the principal means of delivering oxygen to the body tissues in the blood flow through the circulatory system [25]. The cytoplasm of erythrocyte is rich in haemoglobin, an iron – containing biomolecule that can bind oxygen and is responsible for the red colour of the cells. It infers that the blood of rabbits were not de – oxygenated. The haemoglobin (Hb) result ranged from 10.57 to 11.03 g/dl which were within the normal reference range reported by [26] (9.8 to 15.8 g/dl) and by [23] and [27] (9.4 to 17.4 g/dl). The implication is that, the rabbits across treatments performed their normal metabolic activities. Haemoglobin is known to carry oxygens from the respiratory organs to the rest of the body where it releases the oxygen to burn nutrients to provide energy to power the functions of the organism in the process of metabolism.

Pack Cell Volume (PCV) values obtained in this study ranged from 40.67 to 47.67% which were within the ranges reported by [26] (9.8 to 15.8 g/dl) and by [28] (33 to 50%). Pack cell volume (PCV) is also known as hematocrit or erythrocyte volume fraction (EVF). This is simply the percentage volume of red blood cells in the blood. The pack cell volume is a very useful measurement; if the concentration of red blood is low the animal is anaemic. If it is higher above upper limit of normal reference table, it causes dehydration with increase in total protein (splenic concentration) and absolute polycythemia. However, the rabbits did not experience any of these conditions mentioned. Result showed that

the Mean Corpuscular Volume (MCV) values ranged from 51.50 to 53.63×10^{15}/fl, these values were slightly lower than the lower limit of the normal reference values reported by [29] (58.0 to 76 × 10^{15}/fl) for rabbits. Similar low values were recorded across treatments implying that fresh water snail *(Pila ampullacea)* would not have accounted for these abnormalities.

4. CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

The values for haematological indices were comparable with the reference values revealing that the health status and performance of the rabbits were not compromised, hence fresh water snail *(Pila ampullacea)* shell ash can replace bone ash up to 100% in the diet without any adverse effect on weaner rabbits.

4.2 Recommendations

It was recommended that farmers, use fresh water snail *(Pila ampullac) shell* ash up to 100% in place of bone ash in rabbit diets to reduce the cost of production which will invariably reduce the cost of rabbit products and thus reduce the shortage of animal protein intake of Nigerians.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mailafia S, Onakpa MM, Owoleke OE. Problems and prospects of rabbit production in Nigeria. Bayero Journal of Pure and Applied Science. 2010;3(2):20–25.
2. Food and Agricultural Organization (FAO). Statistical Data base. Rome, Italy. 2006; 234-236.
3. British Medical Association. Basic food and nutrient requirement for Africa. In the World Book of Encyclopedia International. 1993;7:310-311.
4. Nworgu FC, Adebowale EA, Oredin OA, Oni A. Prospects and economic of broiler production using two plant protein sources. Tropical Journal of Animal Science. 1999; 2(1):159–166.
5. Owen OJ, Alawa JP, Wekhe SN, Isirimah NO, Chukuigwe EC, Aniebo AO, Ngodigha EM, Amakiri AO. Incorporating poultry litter in animal feed: A solid waste management strategy. Egyptian Journal of Animal Production. 2008;(In press).
6. Ajala MK, Balogun JK. Economics of rabbit production in Zaria, Kaduna State. Tropical Journal of Animal Science. 2004;7(1):1-10.
7. Biobaku WO, Oguntona EB. The effects of feeding multi nutrient mini blocks and pelleted diet on the growth of rabbits. Nigeria. Journal of Animal Production. 1997;24(2):147-149.
8. Apata DF, Ojo U. Efficiency of the *Trichoderma viride* enzymes complex in broiler starter fed cowpea testa based diet. In: Ukachuku, SN, Ibeauchi, JA, Ibe, SN, Ezekwe, G and Abasiekong, SF (Eds), Animal Production in the New Millennium: Challenges and Options. In Book of proceeding 25th Annual NSAP Conference. 2000;132-134.
9. Ansah T, Emelia AA, Deku G, Karikari PK. Evaluation of false Yam (*Icalina olviformis*) leaves on the growth performance of weaner rabbits (*Oryctolagus cuniculus*). Journal of Animal and Feed Research. 2012;2(1):76-79.
10. GISD. Global invasive species database. Invasive Species Specialist Group of the IUCN; 2012.
11. Cobbinah JR, Vink A, Onwuka B. Snail farm: production, processing and marketing. agrodok- series no. 47, Agromisa Foundation, CTA, Wageningen. 2008;84.
12. Omole AJ, Ogbosuka GE, Salaka RA, Ajayi OO. Effect of replacing oyster shell with gypsum in broiler finisher diet. Journal of Applied Sciences Research. 2005; 1(2):245– 248.
13. Safaa HM, Serrano MP, Valencia DG, Frikha M, Jimanez – Moreno E, Mateos GG. Productive performance and egg quality of brown egg – laying hens in the late phase of production as influenced by level and source of calcium in the diet. Poultry Science. 2008;87:2043–2051.
14. Saunders – Blades JL, Maclsaac JL, Korver DR, Anderson DM. The effect of calcium source and particle size on the

production performance and bone quality of laying hens. Poultry Science. 2009;88:3338–353.
15. World Health Organization (WHO). Technical report series: Expert committee on medical assessment and nutritional status, Geneva. 1963;842.
16. Wikipedi. Benue State. Geology and Environmemt. Wikipedia the free encyclopedia; 2015.
Available:https://en.wikipedia.org
17. AOAC. Official Methods of Analytical chemists 16th Edition, Arlington, Virgininia, USA; 1995.
18. Banerjee GC. A Textbook of animal husbandry. (*Eight Edition).* Oxford & IBH Publishing Company PVT. Ltd. 133-B, Shahpur Jat. Asian Games Village Side, New Delhi 110049, India. 2013;130-1042.
19. Schenzhen M. Mindray bio-medical electronics Co. Ltd. Mindray Building, Keji 12th Road South, High-Tech industial park, Nanshan, Schenzhen 518057, P.R. China; 2012.
20. MINITAB Statistical Software. V.16, Minitab Inc. P.A.US; 2014.
21. Obi IU. Statistical methods of detecting differences between treatment means. 2ndEdition. Snaap Press. Enugu, Nigeria. 1990;25-85.
22. Pauzenga U. Feeding parent stock. Zootech. International. 1985;22:22-24.
23. Burke WN. Organization development: A process of learning and changing. Addison- Westly O D series. Wyatt Warner Burke 2nd Edition. Addison -Westly Publishing. ISBN (020150834549, 978020150852). 1994;214.
24. Alu SE, Ruma RSU, Umbugadu AA, Adua MM, Makinde OJ. The effects of different dietary fibre sources on the heamatological parameters and serum biochemical variables of growing rabbits. Proceeding of the 4th Annual Conference of Animal Science Association of Nigeria. Latoke Akintola University of Technolnology, Ogbomosho, Oyo State, Nigeria. 2009;274-276.
25. Lipowsky R, Sackmann E. Structure and dynamics of membrane, volume 1. From cells to vesicles, 1st Edition. 11. Generic and specific interaction. ISBN (97800080541914). 1995;1052.
26. Mitruka BM, Rawnsley H. Clinical biochemistry and hematological reference values in normal experimental animals. 1st Edition, New York, U.S.A. Masson Publishing Inc. 1977;106-112.
27. Fudge AM. Avian clinical pathology – haematology and chemistry, In: Altman, R. B., Clubb, S. L., Dorrestan, G M and Quesberry, K. (Edition). W. B. Saunders Company, Philadelphia, PA. Avian Medicine and Surgery. 1997;142–157.
28. Molly V. Text Book of Rabbit Medicine. 2nd Edition Hard Cover. B Vetmed CertZwmed med (Mamalian) MRCVS/; 2013.ISBN: 9780702049798
29. Hewitt CD, Innes DJ, Savory J, Wills MR. Normal biochemical and heamatological values in New Zealand white rabbits. Clinical Chemistry. 1989;35(8):1777-17.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/22279