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Bioconversion of Agrowastes as Calcium Source for Sustainable Pig Production

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ABSTRACT

Background and Objective: Globally, pork and chicken are the most widely consumed meats Unfortunately, their production is limited by the high cost of conventional feed ingredients, necessitating the need for the discovery of effective non-conventional feed ingredients. This study evaluated the effect of eggshells as a sustainable alternative to Dicalcium Phosphate (DCP) in weaner pig diets. **Materials and Methods:** Three diets were formulated: Diet 1: 100% DCP-based, Diet 2: 100% eggshell-based, replacing DCP and Diet 3: 50% DCP+50% eggshells-based. Fifteen healthy weaner pigs (Large White and Landrace) per treatment, with an average weight of 7.27±2.6 kg were used in this study. Growth performance, serum minerals, bone characteristics and histological parameters of the experimental animals were determined. Statistical analysis was performed using one ANOVA in SAS 2010, with Duncan's Multiple Range Test for mean separation and significance set at $\alpha = 0.05$. **Results:** The animals fed with 50% eggshell as a replacement for DCP (Diet 3) had the least value of body weight. However, body weight gain was not different. Feed intake and feed conversion ratio (FCR) were significantly ($p<0.05$) higher for pigs fed Diet 3 when compared with Diet 1 and 2. Calcium and phosphorus contents were significantly ($p<0.05$) higher for Diet 3. **Conclusion:** This study concluded that 50 and 100% inclusion levels of eggshell as a replacement for DCP did not negatively influence growth performance, serum minerals, bone minerals, bone characteristics and histology of weaner pigs. Eggshells regarded as agricultural waste materials successfully replaced DCP at 50 and 100% inclusion levels in pig nutrition which may benefit pig farmers.

KEYWORDS

Agricultural waste, eggshell, calcium, environment, pig

INTRODUCTION

Poultry and pork are the most widely consumed meats globally. However, the high cost of conventional feed ingredients limits production and drives the search for affordable alternatives that do not compromise animal performance[1]. In search of alternatives, one needs to ensure that essential nutrients and minerals are considered. Important minerals like calcium and phosphorus are crucial for swine nutrition, particularly during critical stages (such as weaning), influencing bone development, cellular processes and overall health while insufficient intake may result in fracture risks[2,3]. Proper mineral balance is essential to prevent deficiencies and health issues that can reduce productivity.

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To mitigate production costs, cost-effective and non-conventional feed ingredients are being evaluated, with ongoing research into alternatives that improve the general health and nutrition of weaner pigs. Traditionally, Dicalcium Phosphate (DCP) has been a primary source of these minerals in swine diets. However, rising costs and sustainability concerns have led to interest in alternative sources. Agriculture production is rising annually to meet the demand for food, arising from the increasing human population across the globe. Nonetheless, agricultural production generates by-products regarded as waste materials. In some places, they are allowed to accumulate in public places or burnt indiscriminately[4], adding to environmental hazards, although efforts are ongoing to utilize such by-products in livestock production[5-8]. The menace of agricultural waste materials such as eggshells in developing countries cannot be over-emphasized because their benefits are not known.

Eggshells, previously considered waste materials, offer a promising solution due to their calcium-rich composition, primarily calcium carbonate. Utilizing eggshells in swine nutrition may not only address economic challenges but may also support sustainable farming practices[9]. However, little is known about the detrimental effects of eggshells on livestock feeding in Nigeria, where they are mostly discarded as agricultural waste materials. Agricultural waste materials, in general, may pose health challenges to the public if not properly handled, because they may constitute an environmental nuisance if left to accumulate or burnt indiscriminately. They may also result in contamination of Salmonella-related illnesses, necessitating the need for studies such as this, although this is not a comprehensive study that aims to explore all aforementioned aspects, but a preliminary study to lay a foundation for more detailed and comprehensive experimental studies.

Feed ingredients or additives intended for used in animal nutrition, especially non-ruminant animals must be free from chemical or bacterial contamination as well as being able to enhance growth performance, nutrient absorption and general wellness and health of the animals, in which gut health plays a key role. In developing countries where studies as this are needed, studies adopting eggshells in pig feeding are rare. Hence, this study aimed to evaluate the effect of eggshells as a sustainable alternative to DCP in weaner pig diets, focusing on growth performance, bone mineralization, bone characteristics, serum mineral contents and gut histology of weaner pigs. This study seeks to provide insights into the feasibility and effectiveness of eggshell-derived calcium in improving swine nutrition and production sustainability.

MATERIALS AND METHODS

Study duration and protocol approval: This study lasted for six weeks (April-May, 2024) and was conducted at the Piggery Unit of the Teaching and Research Farm, University of Ibadan, Southwest, Nigeria.

Ethical consideration: The experimental protocols were approved by the Department of Animal Science, University of Ibadan Animal Care and Welfare Ethics Committee (ANSUI/PS/04/24AOO).

Experimental diets: Fresh eggshells for the experiment used as a replacement for Dicalcium Phosphate (DCP) were collected from a local hatchery, cleaned, sun-dried and processed under hygienic conditions before being incorporated (powder) into the animals' diets in a completely randomized design. Three diets were formulated:

- **Diet 1:** 100% DCP-based (control diet)
- **Diet 2:** 100% eggshell-based, replacing DCP
- **Diet 3:** 50% DCP+50% eggshells-based

Table 1: Feed and nutrient composition of experimental diets of weaner pigs fed with eggshell as a replacement for dicalcium-phosphate

Ingredient (g/100 g)	Diet 1	Diet 2	Diet 3
Maize	45.00	45.00	45.00
Soybean meal	27.00	27.00	27.00
Groundnut cake	13.00	13.00	13.00
Palm oil	6.30	6.30	6.30
Wheat offal	6.00	6.00	6.00
Dicalcium phosphate	1.50	0.00	0.75
Salt	0.30	0.30	0.30
Methionine	0.30	0.30	0.30
Lysine	0.30	0.30	0.30
Premix*	0.30	0.30	0.30
Eggshell	0.00	1.50	0.75
Calculated nutrient composition			
Crude protein (g/100 g)	20.59	20.52	20.49
Crude fat (g/100 g)	12.67	12.71	A12.85
Crude fibre (g/100 g)	4.46	4.53	4.48
Calcium (g/100 g)	0.43	0.40	0.36
Phosphorous (g/100 g)	0.58	0.60	0.61
Metabolizable energy (kcal/kg)	3032.43	3033.51	3032.63

Diet 1 contained 100% DCP+0% eggshell, Diet 2 contained 100% eggshell+0% DCP, Diet 3 contained 50% DCP+50% eggshell. *2.5 kg contained 8,000,000 I.U. vitamin A, 1,600,000 I.U. vitamin D3, 15,000 I.U. vitamin E, 2000 mg vitamin K, 3000 mg vitamin B2, 20 g vitamin C, 20,000 mg niacin, 6000 mg pantothenic acid, 1500 mg vitamin B6, 10,000 mg vitamin B12, 500 mg folic acid, 400 mg biotin, 150,000 mg

Management of animals and data collection: Each treatment had three replicates with five healthy weaner piglets (Large White and Landrace breeds) each with an average weight of 7.27±2.6 kg. So, each treatment had 15 weaner piglets and a total of 45 weaner piglets were used for the study. Pigs in each replicate were housed together. The composition of the experimental diet is presented in Table 1.

Feed and water were provided twice a day. The animals had access to fresh feed and water *ad libitum*. Feed intake and body weight of pigs were measured weekly using a weighing scale. At the end of the experiment, the total body weight gain was determined by subtracting the initial body weight from the final body weight. Feed conversion ratio (FCR) was calculated from the obtained values for feed intake and body weight, as a ratio of feed intake and body weight[10].

At the end of the experiment, blood samples were collected from pigs according to treatments, using the marginal ear vein method. Each sample was placed in plain bottles respectively and allowed to clot before centrifuging to obtain the serum. The separated sera were decanted into bijou bottles until analyzed[11]. Serum mineral contents were determined using a UV spectrophotometer while bone characteristics were assessed by defleshing the pigs and measuring tibia weight, length and breadth. Bone physical properties were analyzed by drying tibias at 105°C for 24 hrs and ashing at 600°C for 6 hrs.

For gut morphometry, 2 cm sections of the ileum and jejunum were harvested for histological analysis. Samples were fixed in formalin for 24 hrs, then in 10% neutral buffered formalin and processed in an automatic tissue processor. The tissues were processed with a tissue processor and dehydrated by passing them through different reagents. The tissues were eventually placed in wax baths. Having sectioned the tissue appropriately at 5 µm, mounted on glass slides. The slides were stained with hematoxylin and eosin[12,13] for examination with a light microscope. Villus height and crypt depth were measured at 40× magnification using computer software[14].

Statistical analysis: Statistical analysis involved One-way Analysis of Variance (ANOVA) in SAS 2010, with Duncan's Multiple Range Test used to separate significant means. Outliers were checked for and where there were none, the data were analyzed. The significance value, $\alpha = 0.05$ was used.

RESULTS AND DISCUSSION

The final body weight was significantly ($p<0.05$) higher for pigs fed 100% eggshell as a replacement for DCP (Diet 2) when compared with other treatments. The body weight gain was not significantly different among the treatments. Feed intake and FCR were significantly ($p<0.05$) higher for pigs fed Diet 3 when compared with Diet 1 and 2. However, the control diet and Diet 2 are statistically similar (Fig. 1).

The results indicate significant differences ($p<0.05$) among the three diets in terms of mineral concentrations in Table 2. Diet 3 showed the highest levels of calcium (10.33 mg/dL) and phosphorus (8.30 mg/dL), whereas Diet 2 had the lowest values for both minerals (9.47 and 6.63 mg/dL, respectively). Magnesium concentration was significantly higher in Diets 1 and 3 (2.83 and 2.93 mg/dL, respectively) compared to Diet 2 (2.50 mg/dL). Similarly, potassium levels were significantly higher in Diets 1 and 3 (5.47 and 5.50 mEq/L, respectively), with Diet 2 showing the lowest concentration (4.77 mEq/L). The observed variations emphasize the superior mineral content of Diet 3 over Diet 2, with Diet 1 showing intermediate values for most minerals.

The findings indicate that while bone weight, length, breadth, tibiotarsal index and robusticity showed no significant differences among the diets, notable variations were observed in ash content, calcium and phosphorus levels ($p<0.05$). Diet 3 had the highest ash content (25.73%), while Diet 2 showed the highest calcium (643.00 mg/dL) and phosphorus (55.33 mg/dL) levels. These results suggest that dietary composition significantly influences bone mineral content, particularly calcium and phosphorus, essential for bone quality in Table 3.

The findings indicate significant dietary effects on the ileum's morphological characteristics. Diet 3 resulted in the tallest villi (2649.82 µm) compared to Diets 1 and 2, with Diet 1 also showing higher villus height than Diet 2. Villus and cryptal widths were greater in Diet 1 than in Diets 2 and 3. Cryptal depth and muscle thickness were highest in Diet 1, followed by Diet 2 and lowest in Diet 3. The p-values indicate statistically significant differences ($p<0.05$) across all measured parameters, with relatively low SEM values supporting data reliability sown in Table 4.

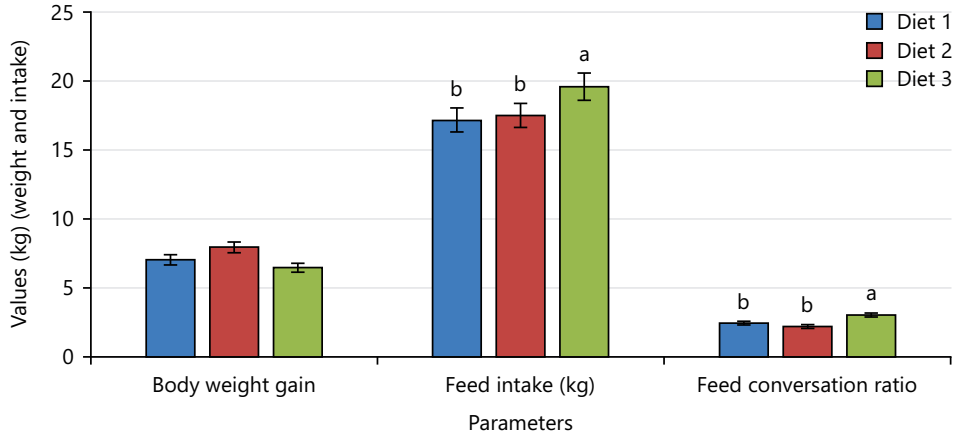

Fig. 1: Effect of eggshell as a replacement for dicalcium-phosphate on growth performance of weaner pigs

[a,b,c]Means with different superscripts are significantly ($p<0.05$) different

Table 2: Effect of eggshell as a replacement for dicalcium-phosphate on serum minerals of weaner pigs

Minerals	Diet 1	Diet 2	Diet 3	p-value	SEM
Calcium (mg/dL)	9.83[b]	9.47[c]	10.33[a]	0.015	0.02
Phosphorus (mg/dL)	7.67[b]	6.63[c]	8.30[a]	0.004	0.02
Magnesium (mg/dL)	2.83[a]	2.50[b]	2.93[a]	0.032	0.01
Potassium (mEq/L)	5.47[a]	4.77[b]	5.50[a]	0.042	0.02

[a,b,c]Means in same row with different superscripts are significantly (p<0.05) different. SEM: Standard Error of Mean, Diet 1 contained 100% DCP, Diet 2 contained 100% eggshell replacement with DCP, Diet 3 contained 50-50% DCP-eggshell and mEq/L: Milliequivalents per liter

Table 3: Effect of eggshell as a replacement for dicalcium-phosphate on bone characteristics of weaner pigs

Bone characteristics	Diet 1	Diet 2	Diet 3	p-values	SEM
Bone weight (g)	15.50	16.00	17.50	0.670	0.52
Bone length (cm)	11.75	10.50	10.50	0.082	0.10
Bone breadth (cm)	7.00	6.33	6.67	0.521	0.06
Tibiotarsal index	131.52	150.91	161.11	0.065	3.40
Robusticity	0.12	0.11	0.11	0.090	0.00
Ash content (%)	22.38[b]	11.18[c]	25.73[a]	0.024	0.07
Calcium (mg/dL)	533.93[b]	643.00[a]	416.69[c]	0.007	0.34
Phosphorous (mg/dL)	45.62[b]	55.33[a]	35.16[c]	0.005	0.15

[a,b,c]Means in same row with different superscripts are significantly (p<0.05) different. SEM: Standard Error of Mean, Diet 1 contained 100% DCP; Diet 2 contained 100% eggshell replacement with DCP and Diet 3 contained 50-50% DCP-eggshell

Table 4: Effect of eggshell as a replacement for dicalcium-phosphate on the histological performance of weaner pigs (ileum)

Ileum characteristics (µm)	Diet 1	Diet 2	Diet 3	p-values	SEM
Villus height	2505.28[a]	2170.09[b]	2649.82[a]	0.001	7.27
Villus width	286.00[a]	242.83[b]	235.84[b]	0.013	0.79
Cryptal depth	653.31[a]	505.30[b]	371.89[c]	0.006	4.86
Cryptal width	285.63[a]	244.18[b]	235.62[b]	0.026	0.80
Muscle thickness	405.40[a]	304.68[b]	257.37[b]	0.022	3.25

[a,b,c]Means in same row with different superscripts are significantly (p<0.05) different. SEM: Standard Error of Mean, Diet 1 contained 100% DCP, Diet 2 contained 100% eggshell replacement with DCP and Diet 3 contained 50-50% DCP-eggshell

Table 5: Effect of eggshell as a replacement for dicalcium-phosphate on the histological performance of weaner pigs (jejunum)

Jejunum characteristics (µm)	Diet 1	Diet 2	Diet 3	p-values	SEM
Villus height	2333.17	2212.86	2264.67	0.150	6.32
Villus width	251.50	252.51	249.98	0.086	1.33
Cryptal depth	491.42[b]	416.11[b]	673.99[a]	0.004	3.87
Cryptal width	255.14	241.99	243.86	0.201	1.07
Muscle thickness	379.81[a]	349.23[a]	248.32[b]	0.003	2.56

[a,b,c]Means in same row with different superscripts are significantly (p<0.05) different. SEM: Standard Error of Mean, Diet 1 contained 100% DCP, Diet 2 contained 100% eggshell replacement with DCP and Diet 3 contained 50-50% DCP-eggshell

The table shows significant differences in cryptal depth and muscle thickness among the three diets. Cryptal depth was significantly higher in Diet 3 (673.99 µm) compared to Diet 1 and 2, which had similar values. Muscle thickness was also significantly greater in Diet 1 (379.81 µm) compared to Diet 3 (248.32 µm). Villus height, villus width and cryptal width did not show significant differences across the three diets. These findings suggest that Diet 3 may promote deeper crypts, while Diet 1 may be more effective in increasing muscle thickness in the jejunum as shown in Table 5.

The findings in the present study concerning growth performance are corroborated by the previous findings of Makkar *et al.*[15], suggesting that eggshell supplements might have enhanced the growth performance of weaner pigs without adversely affecting other physiological parameters such as serum minerals and histology. Previous similar findings on eggshells replacing limestone in mink-feed diets revealed that eggshells significantly improved feed intake, feed efficiency and body weight gain in mink-fed diets[16].

Utilizing eggshell waste in animal feed may significantly reduce feed costs per unit of weight gain, especially in sub-Saharan Africa where food security is currently a challenge, although that is one of the limitations of this study, cost analysis was not evaluated. In previous studies, eggshells have been suggested to tend to reduce the cost of producing pork as well as being beneficial to growth performance[9], although the exact percentage has not been experimentally determined.

Calcium and phosphorus contents were higher for a 100% eggshell-based diet in bones and for 50% in serum and bone characteristics were similar to the control diets for most of the parameters, indicating the beneficial or non-deleterious effects of eggshells on bone mineralization (ash content for 100% DCP-based diet) and 50% DCP-based diets for Ca and P of weaner pigs. This suggests the potency of eggshells poorly recognized as a source of calcium for food and animal nutrition which can competitively replace dicalcium phosphate which is a conventional calcium source for livestock production, although a combination of both DCP and eggshell in serum minerals seems to better improve serum mineral than their individual use. It has been opined that eggshells may improve mineral utilization and enhance bone mineralization with no adverse effect on skeletal development[16]. Histology results for the jejunum in the present study showed positive relations with 100% eggshell-based and 100% DCP-based diets for most of the parameters measured, while for ileum, the similarity was mostly between eggshell-based diets. Literature is not informative about the use of eggshells in pig nutrition, particularly in developing countries like Nigeria, hence, limiting extensive literature comparison in this study. As interest in this area of research grows, it is believed that more data will be available for comparison and the functional mechanisms of this potential feed ingredient will be clearer.

The use of eggshells in foods as calcium supplements has been documented. However, studies involving the use of eggshells in pig nutrition are rare. As a result of limited findings in pig nutrition for eggshells, a comparison in this study is made with the use in human nutrition to have a glimpse of its potential benefits in animal nutrition. Fortification of white bread with about 2% eggshell powder has been recommended[17]. However, the modes of mechanisms are not yet fully understood. The findings of a previous study reported that eggshell (6%) supplementation did not negatively affect biscuit flavour[18].

The findings of research focused on the use of chicken eggshells reported the potential of eggshell powder to more effectively alleviate bone loss than inorganic calcium carbonate in the ovariectomized animal model of osteoporosis[19], linking the variation in the results to their bioavailability[20]. Eggshell has also been reported to increase bone mineral density and lower pain in the elderly population with osteoporosis. Also, it has been documented that eggshells obtained equally good apparent absorbability of Ca as the one obtained from $CaCO_3$ in piglets[21]. A recent study reported that eggshell powder significantly enhanced the minerals of biscuits (mainly calcium contents) and reduced the calorie value. It was reported that 5% inclusion in biscuits resulted in a higher calcium absorption rate, leading to no unwanted sensory changes up to 10% inclusion level[22]. However, a lot remains unexplored about their functional mechanisms. Chicken eggshells are mostly thrown away in sub-Saharan Africa. They are treated as waste materials because little is known about their importance, constituting environmental nuisance and affecting public health as a result of environmental pollution arising from their accumulation and other so-called agricultural waste materials.

Despite the potential benefits inherent in eggshells, in developing countries like Nigeria, it seems the beneficial effects of this considered-waste-nutrient-rich resource are not known, little wonder why it is sparingly used in livestock feeding, where rising costs of conventional feed ingredients are affecting both the farmers and the populace who depend on these animal products. The results of this study may enlighten and awaken swine farmers' interest in the use of poorly recognized eggshells for pig production, thereby helping them to maximize their profits through reduced costs of production and by extension improving food security through the provision of affordable animal products for the region already plagued with food insecurity, with an accompanying benefit of reduced environmental pollution that may arise from indiscriminate burning or accumulation of such agro-by-products regarded as waste materials.

CONCLUSION

The use of eggshells in livestock feeding in Nigeria is rare, despite the nutritional benefits inherent in them. This study concluded that 50 and 100% inclusion levels of eggshell as a replacement for DCP did not negatively influence growth performance, serum minerals, bone minerals and characteristics and histology of weaner pigs. It may thus be concluded that pig farmers have a lot to benefit by replacing DCP with eggshells, regarded as agricultural waste materials, although many studies may be required to establish their full potential including sustainably improving environmental health their limitations and safety concerns, if any. Also, it should be noted that the possibility of *Salmonella* contamination was not considered in this study, therefore, it was recommended that future studies that will address such safety concerns of eggshells in animal nutrition. Eggshell is poorly recognized in the region as a calcium source but its inclusion in swine production may benefit livestock production in the region.

SIGNIFICANCE STATEMENT

The study demonstrates that eggshells can effectively replace DCP in pig diets without negatively affecting growth, serum minerals, bone minerals and histology of weaner pigs. The findings suggest potential benefits for pig farmers, especially in utilizing eggshells as an agricultural waste material. However, further research is needed to explore their full potential, safety concerns and environmental impacts, including the risk of *Salmonella* contamination.

REFERENCES

1. Akpojo, A.C., H.A. Buhari, I.O. Adejumo and A.T. Adesola, 2020. Response of weaned pigs to different dietary protein sources and diet types. J. Agric. Sci. Environ., 20: 56-64.
2. Cano, A., P. Chedraui, D.G. Goulis, P. Lopes and G. Mishra *et al*., 2018. Calcium in the prevention of postmenopausal osteoporosis: EMAS clinical guide. Maturitas, 107: 7-12.
3. Brozek, R., M. Kurpisz and R. Koczorowski, 2018. Application of stem cells in dentistry for bone regeneration. J. Physiol. Pharmacol., 69: 23-33.
4. Adejumo, I.O. and O.A. Adebiyi, 2020. Agricultural Solid Wastes: Causes, Effects, and Effective Management. In: Strategies of Sustainable Solid Waste Management, Saleh, H. (Ed.), IntechOpen, London, United Kingdom, ISBN: 978-1-83962-560-2.
5. Adebiyi, O.A., M. Sodeke, O.O. Adeleye and I.O. Adejumo, 2018. Effects of extruded rice bran based diets on the performance, intestinal microbiota and morphology of weaned pigs. Agricultura Trop. Subtrop., 51: 13-19.
6. Adebiyi, O.A., H.O. Awotale, I.O. Adejumo, O.A Osinowo, M.A. Muibi and O.B. Nwaodu, 2019. Performance, serum and haematological indices of pigs fed watermelon waste based-diet. Trop. Anim. Prod. Invest., 22: 10-16.
7. Adejumo, I.O., K.A. Badmus, A. Maidala, O.J. Makinde, A.D. Maina, I.C. Mohammed and A. Bomoi, 2020. Locally-processed cowpea husk improved body weight gain of on-farm raised rabbits in Northeastern Nigeria. Niger. J. Anim. Sci., 22: 199-208.
8. Adebiyi, O.A., U.T. Oboli, I.O. Adejumo, O.A. Osinowo, M. Olumide and A.T. Adeshola, 2020. Nutritive value of palm oil sludge and its influence on fat composition and deposition in grower pigs. Niger. J. Anim. Sci., 22: 253-261.
9. Faridi, H. and A. Arabhosseini, 2018. Application of eggshell wastes as valuable and utilizable products: A review. Res. Agric. Eng., 64: 104-114.
10. Famakinwa, A.A., O.A. Adebiyi and I.O. Adejumo, 2021. Growth pattern and behavioural response of weaned pigs to multiple enrichment objects (polyester ropes). Slovak J. Anim. Sci., 54: 94-98.
11. Adebiyi, O.A., T.A. Ajayikolade, I.O. Adejumo, O. Abiola and O.A. Adediran, 2021. Effect of levels of energy-protein value on growth performance, blood profile and nutrient digestibility of weaned pigs. Nigeria Agric. J., 52: 179-183.

12. Adetunji, C.O. and I.O. Adejumo, 2018. Efficacy of crude and immobilizedenzymes from *Bacillus licheniformis* for production of biodegraded feather meal and their assessment on chickens. Environ. Technol. Innovation, 11: 116-124.
13. Adetunji, C.O. and I.O. Adejumo, 2018. Potency of agricultural wastes in mushroom (*Pleurotus sajor-caju*) biotechnology for feeding broiler chicks (*Arbor acre*). Int. J. Recyl. Org. Waste Agric., 8: 37-45.
14. Adeyemo, G.O., K.T. Oyinlola and O.G. Longe, 2013. Carcass characteristics and gut histomorphology of marshall broilers fed maxigrain supplemented diets. Niger. J. Rural Ext. Dev., 7: 66-70.
15. Makkar, S., N.C. Rath, B. Packialakshmi, W.E. Huff and G.R. Huff, 2015. Nutritional effects of egg shell membrane supplements on chicken performance and immunity. Poult. Sci., 94: 1184-1189.
16. Ahlstrøm, Ø. and A. Skrede, 2020. Eggshell as a calcium source replacing limestone meal in mink (*Neovison vison*) diets. J. Anim. Feed Sci., 29: 338-344.
17. Platon, N., V.A. Arus, A.M. Georgescu, I.D. Nistor and N. Barsan, 2020. White bread fortified with calcium from eggshell powder. Rev. Chim., 71: 299-306.
18. Hassan, N.M.M., 2015. Chicken eggshell powder as dietary calcium source in biscuits. World J. Dairy Food Sci., 10: 199-206.
19. Omelka, R., M. Martiniakova, M. Babikova, K. Svik and L. Slovak *et al*., 2021. Chicken eggshell powder more effectively alleviates bone loss compared to inorganic calcium carbonate: An animal study performed on ovariectomized rats. J. Physiol. Pharmacol., 72: 873-879.
20. Meiron, O.E., E. Bar-David, E.D. Aflalo, A. Shechter, D. Stepensky, A. Berman and A. Sagi, 2011. Solubility and bioavailability of stabilized amorphous calcium carbonate. J. Bone Miner. Res., 26: 364-372.
21. Schaafsma, A. and G.M. Beelen, 1999. Eggshell powder, a comparable or better source of calcium than purified calcium carbonate: Piglet studies. J. Sci. Food Agric., 79: 1596-1600.
22. Shahnila, S. Arif, I. Pasha, H. Iftikhar, F. Mehak and R. Sultana, 2022. Effects of eggshell powder supplementation on nutritional and sensory attributes of biscuits. Czech J. Food Sci., 40: 26-32.