IJAS Iranian Journal of Applied Animal Science

Egg Characteristics, Blood Parameters and Tibia Mineralization of Laying Hens Fed Varying Dietary Levels of Limestone and Periwinkle Shell

Research Article

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Received on: 30 Aug 2023
Revised on: 10 Dec 2023
Accepted on: 31 Dec 2023
Online Published on: Mar 2024

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Online version is available on: www.ijas.ir

ABSTRACT

The effect of feeding varying dietary levels of limestone (LS) and periwinkle shell (PS) on laying performance, egg characteristics, blood parameters and bone mineralization of hens was studied. A total of 108 sixteen-weeks-old pullets (weighing 1.2±2.00 kg) were allotted to 6 treatments with 3 replicate groups of 6 birds in each pen. The LS and PS were supplemented in the diets at three levels (3.00%, 3.75% and 4.50%) each for a period of 12 weeks. Birds fed 3.75% PS had the highest (P<0.05) feed intake and hen-day egg production with an improved feed conversion ratio. The inclusion levels of PS significantly (P<0.05) improved most of the external and internal egg quality parameters studied with the best result recorded among the laying hens fed 3.75% PS and 4.50% PS. Some of the blood parameters and bone mineralization were better (P<0.05) for hens fed 3.00% PS and 3.75% PS. On the other hand, the source of calcium and its levels had no significant effect on initial body weight, final body weight, total weight gain, average daily weight gain, egg weight, egg surface area, yolk height, yolk index, total protein, alanine aminotransferase, aspartate aminotransferase, bone phosphorus, tibia weight and dry defatted bone. Based on the results obtained from the present study, it was concluded that 3.75% PS was appropriate for laying hens without adverse effect on performance and the inclusion of PS in layer rations as calcium source is more beneficial than LS.

KEY WORDS calcium source, eggs, hen, performance.

INTRODUCTION

All across the world, commercial hybrids (layers and broilers) are bred for the production of eggs and meat. Traditional animal husbandry practices, which are known for their low productivity, are unable to satisfy the population's needs due to the acute shortage of animal proteins and the rapid population expansion (Oluyemi and Roberts, 2000). It is therefore, necessary to look into the various ways of enhancing animal production. The yields of animals can be increased as a result of large animal number, improvement of genotype and environmental conditions (Cilek and Tekin, 2005). To increase animal production, efforts must be geared towards boosting poultry production. This is because of the numerous merits of poultry compared to other livestock species. In addition to its economic worth (Zaman *et al.* 2004), chicken products are a valuable source of high-quality protein (eggs), which is crucial for human growth and other vital bodily functions (Mancinelli *et al.* 2022). Functionally and economically, calcium is regarded as the main mineral in the diets of laying hens due to their major contributions to the metabolism and the quality of the eggshell (Schreiweis *et al.* 2003). The amount of calcium excreted in eggs by highly productive laying hens during the egg-laying cycle is 20-30 times greater than the sum of calcium reserves in chicken body. Daily maintenance of a lay-

ing hen only for the shell formation is about 8-10 times higher (per 1 kg of body weight) than the daily requirement of a highly productive cow (Cilek and Tekin, 2005; Hester, 2017).

There has been a trend to a gradual increase in the calcium level in combined feeds for laying hens over the last decade (Fisinin *et al.* 2009; Adedokun *et al.* 2017). Although, NRC (1994) suggested a calcium requirement of 2.75-3.25% for laying hens without affecting either egg production or eggshell quality (Snow *et al.* 2004). However, requirements for this mineral are constantly changing because the commercial hybrid laying hens produce eggs at a higher rate due to increasing genetic potential, improvement in farming and nutrition strategies (Whitehead and Fleming, 2000). Therefore, a basic understanding of the potential solubility problems associated with the calcium salts themselves will allow for the preparation of feed using the most available forms. For the development of eggs with the best possible shell quality, a calcium supply in the digestive system is crucial (Keshavarz *et al.* 1993). This implies that the source of calcium and its solubility in the gizzard influence eggshell quality and hen productivity. According to Leeson and Summers (2005), slow solubilization is preferred to a very rapid one since the former more nearly fits the protracted time of demand for calcium delivery to the shell gland in laying hens. Slower calcium solubilization would increase calcium availability during the eggshell calcification process and reduce bone calcium mobilization (Skrivan *et al.* 2010). This scenario enables a progressive flow of calcium from the gizzard to the small intestine for absorption during the night when feed is not consumed, leading to an extended period of time during which the hen receives dietary calcium. The idea is to provide the bird with a steady supply of calcium to enhance the shell's properties, but not too much as this would reduce production. Additional sources of calcium have been introduced and are in practical use with birds, such as egg shell, oyster shell, limestone (LS) and others (Safaa *et al.* 2008) but there is a dearth of information on the use of periwinkle shell (PS) as calcium sources for laying hens which currently has added to the environmental waste menace (Anizoba *et al.* 2022). For sustainable development, wastes should be recycled, reused and channeled towards the production of value-added products (Abdulrahman *et al.* 2014). However, for the aim of satisfying the needs of new hybrids of greater genetic potential for production as a result of their high calcium requirements and the fact that the availability of the sources used will determine the quantity that is required in the diet as well as solving of continuously present problems of egg shell quality and bones of layer hens during mobilization (Whitehead and Fleming, 2000; Lukić *et al.* 2009), the knowledge on calcium sources that can replace or be used in association with calcitic LS is essential. Therefore, the present study was designed to investigate the effects of varying levels of LS and PS on performance, egg characteristics, blood parameters and bone mineralization of laying hens.

MATERIALS AND METHODS

Ethical consideration

This study was conducted in accordance with the recommendations of research ethics for scientific researchers involving animal subjects. The animals used were handled according to the principles of Animal Experimentation Ethics Committee University of Nigeria, Nsukka (Research Ethics Committee Recommendations, 2013).

Study site

The study was carried out at the Poultry Unit of the Department of Animal Science Teaching and Research Farm, University of Nigeria, Nsukka, Nigeria. Nsukka lies within longitude 6˚ 45′E and 7˚ E and latitude 7˚ 12.5 ′N and on the altitude 447 m above sea level. The climate of the study area is typically tropical, with relative humidity ranging from 65 to 80% and mean daily temperature of 26.8 ˚C (Okonkwo and Akubuo, 2007).

Collection of experimental feed ingredients

Periwinkle shell was sourced from the South-Eastern part of Nigeria. After collection, the PS was washed severally by agitating in a sink under continuously flowing tap water until they were freed from the dark outer coatings. The shells were oven dried at temperature of 40 ˚C to reduce the moisture content but not to destroy the chemical contents. The dried shells were pulverized in a Hammer Mill after which they were used to prepare the layer diet. The PS has 36.76% Ca (Ajakaiye *et al.* 1997) while the LS contains 38% Ca. The shell does not contain toxic factors, such as lead, cadmium, and arsenic (Ugoeze and Chukwu, 2015).

Experimental birds and management

A total of one hundred and eight (108) point- of- lay (POL) Isa Brown pullet of sixteen (16) weeks old purchased from a reliable farm were used for the study. They were placed on a commercial grower mash until they reached puberty i.e. age at first lay (20 weeks±9 days) before being placed on the experimental rations for twelve weeks. Six experimental diets were constituted in a 2 × 3 factorial arrangement containing varying inclusion levels of LS and PS in a completely randomized design. Each experimental diet (Table 1) was supplemented at three levels of inclusion (3.00%, 3.75% and 4.50%). The treatments include: 3% LS, 3.75% LS, 4.50% LS, 3.00% PS, 3.75% PS and 4.50% PS.

Table 1 Ingredient (%) and chemical composition (g/kg DM) of experimental diet

Ingredients (%)	Diets with Limestone inclusion			Diets with Periwinkle shell inclusion		
	3.00%	3.75%	4.50%	3.00%	3.75%	4.50%
Maize	45.96	45.96	45.96	45.96	45.96	45.96
Soybean meal (SBM)	15.44	15.44	15.44	15.44	15.44	15.44
Fish Meal	2.00	2.00	2.00	2.00	2.00	2.00
Wheat offal	19.68	13.33	7.64	18.67	12.57	6.45
Palm kernel cake (PKC)	6.64	10.79	14.73	7.38	11.44	15.53
Lysine	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25	0.25
Dicalcium phosphate	1.39	1.39	1.39	1.39	1.39	1.39
Limestone	7.89	9.87	11.84	0.00	0.00	0.00
Periwinkle shell	0.00	0.00	0.00	8.16	10.20	12.23
Vitamin and mineral premix[1]	0. 5	0. 5	0. 5	0. 5	0. 5	0.5
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100
Calculated composition						
Crude protein (%)	16.00	16.00	16.00	16.00	16.00	16.00
Metabolizable energy (kcal/kg)	2700	2700	2700	2700	2700	2700
Crude fibre (%)	6.35	6.35	6.35	6.35	6.35	6.35
Calcium (%)	3.00	3.75	4.50	3.00	3.75	4.50
Phosphorus (%)	0.25	0.25	0.25	0.25	0.25	0.25
Chemical composition (%)						
Crude protein	15.60	16.05	16.05	15.95	16.05	15.80
Metabolizable energy (kcal/kg)	2665	2665	2685	2670	2655	2690
Crude fibre	6. 45	6.50	6.55	6.70	6.75	7.00
Crude ash	10.11	11.45	12.55	10.65	13.55	11.25
Ether extract	4.80	4.50	4.80	5.00	4.40	4.90
Moisture	9.35	9.15	8.55	8.80	8.95	8.50
Calcium	2.99	3.73	4.47	2.98	3.74	4.49

[1] Composition of premix: 0.25 kg of premix contains: vitamin A: 1000 IU; vitamin D_3: 2500 IU; vitamin B_1: 900 mg; vitamin B_2: 1000 mg; Niacin: 15000 mg; vitamin B_{12}: 7.5 mg; vitamin K_3: 1000 mg; vitamin E: 9000 IU; Biotin: 500 mg; Folic acid: 500 mg; Pantothenic acid: 5000 mg; Choline chloride: 250000 mg; Manganese: 50000 mg; Copper: 5000 mg; Magnesium: 100 mg; Iron: 20000 mg; Zn: 50000 mg; Iodine: 500 mg and Selenium 100 mg.

The diets were prepared to meet the nutrient requirements of laying hens according to NRC recommendations (NRC, 2005) except calcium levels at 3.75% and 4.50%. The proximate composition of the experimental diet was carried out according to the standard method of association of officials' analytical chemist (AOAC, 1990). The metabolizable energy was calculated according to WPSA (1989). The pullets were randomly assigned to six (6) treatments with eighteen (18) birds per treatment. Each treatment was further divided into three (3) replicate groups with six (6) birds in each pen totaling eighteen (18) experimental units. Feed was given according to body weight and age twice daily in line with the Isa Brown management guide and water was supplied *ad libitum* to the birds. They were provided the same management conditions (floor space, temperature, light and vaccination program).

Performance evaluation

Initial and final body weight was obtained by weighing hens at the beginning and at the end of the experimental period. Daily weight gain was obtained by weighing birds individually from each replicate weekly using a 10.1 kg capacity precision weighing balance (models A and D Weighing GK-10K industrial balance) made in China. Mean of each group was taken (A) and that of the previous week (B) was subtracted from it (A-B). The difference between the two divided by seven days gave the daily weight gain for a particular day in a week i.e., (A-B)/7= daily wt gain (DWG).

Feed intake (FI) was determined by offering a known quantity of feed (X) to each replicate, morning and evening and the left over (Y) weighed the following morning. The difference between X and Y (X-Y) gave the quantity of

feed consumed. Average feed intake (g)= quantity of feed given – leftover feed (Olarotimi, 2021).
Feed conversion ratio (FCR=quantity of feed consumed/dozens of eggs produced) was calculated daily from these data but were presented as the averages for the complete 12-wk period.

Feed conversion ratio= quantity of feed consumed / dozens of eggs produced (Lelis *et al.* 2009).

Egg numbers were recorded daily and summarized on a weekly basis throughout the experimental period (*i.e.*, 20-32 weeks). Hen day egg production (HDEP) was evaluated by dividing the average number of eggs laid per bird per week by the average number of birds multiplied by seven, and the result was multiplied by 100 (Olarotimi, 2021).

Hen day egg production (%)= no of eggs produced per day / no of hens alive per day × 100

Age at first lay (AFL) was determined as the age at which the first egg was laid (Lawrence and Fowler, 2002).

Egg quality analysis

Ninety (90) eggs were randomly selected at the end of the experiment for egg quality analysis by then the hens had reached more than 50% of their laying performance. The eggs were collected daily and analyzed weekly for both the internal and external egg qualities with five (5) eggs randomly selected per replicate (i.e.15 eggs/treatment) for the assessments. Egg weight was taken for every egg collected for the hens and the weighing was done for all the collected eggs within one hour of collection using a sensitive electronic balance (D & G sensitive scale) to the nearest 0.01g and the measurement was expressed in grams.

The egg shells for each replicate were allowed to dry naturally at room temperature for 24 hours then weighed on an electronic sensitive balance to determine the shell percent.

The percent shell was calculated as: [shell weight (g) / egg weight (g)] × 100

The albumen and yolk heights were determined by utilizing the egg quality slide rule (Mohammed and Dei, 2013). Albumen and yolk width were taken as the maximum cross-sectional diameter of the albumen and yolk using a pair of calipers and read on a ruler calibrated in centimeter. Finally, the albumen index and yolk index were calculated as follows:

Albumen index= albumen height / albumen width

Yolk index= yolk height / yolk width

Egg shell thickness (mm) was determined by using a micrometer screw gauge. Three measurements were made on the sharp, blunt and equator of an egg and the average of the three values were calculated as described by Ehtesham and Chowdhury (2002).

Egg length which is measured as the distance between the broad end and narrow end of the egg and the egg width which is measured as the diameter of the egg at the widest cross-sectional region were determined using a pair of vernier calipers and read on a ruler calibrated in centimeter according to Olawumi and Ogunlade (2008). From the data obtained, the egg shape index was calculated according to Anderson *et al.* (2004) as shown below:

Egg shape index= egg width (cm) / egg length (cm) × 100

Surface area (cm^2) of each egg was calculated by using the formula of Carter (1975), ($3.9782W^{.7056}$), where W is the egg weight in grams.

Haugh Unit (HU) was estimated as HU= 100 log ($H+7.57–1.7W^{0.37}$)

Where:
H: albumen height.
W: egg weight (as reported by Oluyemi and Roberts, 2000).

Eggshell weight per surface area (ESWSA), expressed in mg/cm², was determined according to Abdallah *et al.* (1993) using the following formula:

ESWSA= {ESW/ [3.9782 × ($EW^{0.7056}$)]} × 1000

Where:
ESW= eggshell weight.
EW= egg weight.

Egg specific gravity (ESG) proposed by Kul and Seker (2004):

ESG= EW / (0.968EW–0.4759ESW)

Where:
EW: egg weight.
ESW: egg shell weight.

Determination of biochemical components

Blood samples were collected from (2) birds per replicate (i.e. 6 birds per treatment) at the end of the experiment on

plain bottles from the jugular veins and kept at room temperature for two hours in a slanting position before being transferred into a refrigerator and kept overnight at 4 °C. The samples were separated by centrifugation for 15 min at 3000 rpm in order to fix the blood and then the serum was kept frozen for processing. Serum total Protein was determined as described by Tietz (1995). Serum urea was estimated by Urease-Berthelot colorimetric methods, using a commercial kit (Randox Laboratories Ltd., U.K.). Also, the serum enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were obtained using the Randox Laboratories Ltd, UK test kits while serum calcium (Ca) and phosphorus (P) were determined according to the method of Elmer (1996).

Determination of tibia mineralization

At the end of the experiment (birds at 32 weeks of age), two birds (2) per replicate (after 12 h of feed deprivation) were randomly selected and slaughtered by cervical dislocation and the left tibia was collected for tibia mineral determination. The tibias were cleaned from adhering tissue and placed in boiling water for 5 min to complete the tissue removal; dried at 100 ˚C for 24 h, subsequently the weight was measured immediately. The tibia was kept frozen for the mineralized properties assessment. Tibia ash content was determined on dried defatted bones according to the method described by the association of official analytical chemists (AOAC, 1990). Tibias were defatted by immersion in a diethyl-ether solution for 24 h before ashing at 600 ˚C overnight.

Statistical analysis

All data were subjected to a 2 × 3 factorial analysis with the following model in a completely randomized design using SAS (2004) statistical package:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}.$$

Where:

Y_{ijk}: any of the response variables.

μ: overall mean.

a_i: effect of the i^{th} treatment (i=limestone and periwinkle shell).

B_j: effect of the j^{th} level (j=A, B and C).

$(\alpha\beta)_{ij}$: effect of the interaction between the treatment and the level.

ε_{ijk}: random error due to experimentation.

Mean separation, where applicable, was done with Duncan's multiple range test of the same statistical package and significance was accepted at a 5% level.

RESULTS AND DISCUSSION

The results of the performance of laying hens fed diets containing varying levels LS and PS are presented in Table 2. There was no significant treatment effect (P>0.05) on the productive performance parameters of laying hens. There were noticeable significant (P<0.05) effects of the varying levels of the two calcium sources in a dose-dependent manner in feed conversion ratio (FCR), feed intake (FI), hen day egg production (HDEP) and age at first lay (AFL) with the best results recorded among the birds fed diets containing 3% and 3.75% of calcium levels while initial body weight (IBW), final body weight (FBW) and total weight gain (TWG) had no significant (P>0.05) effect among treatments. The interactions of dietary calcium sources and levels on the productive performance of laying hens recorded no significant (P>0.05) differences on the IBW, FBW and TWG but FI, FCR, HDEP and AFL were significant (P<0.05) among treatments. The highest FI (P<0.05) was seen on birds fed 3.00% LS, 3.75% LS and 3.75% PS though, statistically similar with those that received dietary 3.00% PS while the lowest (P<0.05) value was observed in birds fed 4.50% LS and 4.50% PS. A better (P<0.05) FCR value was seen in birds fed 3.75% PS though statistically similar to 3% PS compared with those in other treatments. The highest HDEP (P<0.05) was seen on birds fed 3.75% PS while the lowest (P<0.05) value was observed in birds fed 4.50% LS and 4.50% PS. Birds on 4.50% PS had the highest (P<0.01) AFL values than the birds fed on other treatments. Birds on 3% LS and 4.50% LS had similar AFL values. However, birds on 3.75% PS had a lower (P<0.05) values different from 3.75% LS even though they are significantly similar to those that received 3% PS.

The performance parameters such as FCR, TWG, FI, and HDEP are reliable indicators of feed economy in layers production. The different dietary calcium sources had no significant effect on the productive parameters (Table 2). This indicates that all sources used were able to supply the hens with sufficient calcium to meet the requirements of the laying hens. This agreed with the report of Scheideler (1998), who did not find any effect on performance when 25 or 50% fine LS in the diet was substituted with either oyster shell or large LS in laying hens. Moreover, Keshavarz *et al.* (1993) did not observe any effect on performance when 33% fine LS was substituted with oyster shell in the diets of laying hens. Saunders-Blades *et al.* (2009) reported that feed intake, body weight, egg production did not differ among hens fed the different calcium sources. However, Froning and Bergquist (1990) and Scheideler (1998) also reported that feed consumption was not affected by sources of dietary calcium.

Table 2 Performance of the laying birds fed diets containing varied levels of limestone (LS) and periwinkle shell (PS)

Treatment	Parameters							
	IBW (g)	FBW (g)	TWG (g)	ADWG (g)	FI (g)	HDEP (%)	FCR	AFL (days)
Source effect								
LS	1233.30	1835.30	602.30	4.79	7351. 80	73.63	3.29	145.78
PS	1235.00	1868.20	633.20	5.79	7012.00	69.51	3.12	147.33
SEM	24.87	17.62	6.33	0.33	12.48	1.12	0.14	3.79
P-value	0.821	0.215	0.224	0.229	0.076	0.117	0.45	0.291
Level effect								
3.00	1221.70	1835.30	613.00	5.12	7538.90^{a}	78.21^{a}	2.98^{b}	144.33^{b}
3.75	1242.50	1858.70	616.20	5.01	7326.90^{b}	76.54^{b}	2.78^{c}	139.00^{c}
4.50	1238.30	1861.30	623.00	5.12	6915.80^{c}	69.41^{c}	3.85^{a}	156.33^{a}
SEM	20.36	24.99	14.09	0.39	19.49	1.92	0.22	1.32
P-value	0.06	0.671	0.958	0.355	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Interactions								
LS × 3.00	1210.00	1801.70	591.70	4.62	7688.30^{a}	76.02^{b}	3.34^{b}	149.33^{b}
LS × 3.75	1250.00	1871.71	621.71	5.10	7498.90^{a}	74.32^{b}	3.28^{b}	135.67^{d}
LS × 4.50	1240.00	1832.70	592.70	4.64	6868.10^{b}	67.41^{d}	3.78^{a}	152.33^{b}
PS × 3.00	1233.30	1869.00	635.70	5.33	7089.40^{ab}	75.76^{b}	2.91^{bc}	139.33^{cd}
PS × 3.75	1235.00	1845.70	610.70	4.93	7555.00^{a}	79.41^{a}	2.52^{c}	142.33^{c}
PS × 4.50	1236.70	1890.00	653.30	5.60	6091.70^{c}	71.42^{c}	3.93^{a}	160.33^{a}
SEM	14.27	18.02	11.55	0.43	26.86	0.81	0.11	1.85
P-value	0.11	0.283	0.496	0.195	**0.026**	**0.023**	**0.045**	**< 0.001**

IBW: initial body weight; FBW: final body weight; TWG: total weight gain; ADWG: average daily weight gain; FI: feed intake; FCR: feed conversion ratio; EW: egg weight; HDEP: hen day egg production and AFL: age at first lay.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The difference in the FI of the hens fed different levels of LS and PS could be attributed to the fact that although energy is the largest determinant of feed intake, hens have a specific appetite for calcium and therefore may vary FI to accommodate calcium needs (Safamehr *et al.* 2013). A significant decrease in FI was evident with increasing calcium level and their interactions. Birds on 3% calcium levels had the highest FI than their counterparts. The result showed a negative impact on increasing dietary calcium on birds' intake which was most profound with 4.50% calcium. Excess calcium has a neutralizing effect in the intestines that caused a rise of intestinal pH. This caused deficiency by formation of insoluble calcium phosphate in the digestive tract (Keshavarz and Quimby, 2002) and impairs metabolic functions (Kheiri and Rahmani, 2006) that caused the birds to refrain from eating. The results obtained for the productive performance were not in agreement with Keshavarz *et al.* (1993) who found no differences in feed intake when feeding diets containing calcium levels from 3.5 to 5.5%. Whereas, Roland *et al.* (1996); Ahmad *et al.* (2003) and Frost and Roland (1991) reported a linear increase in feed intake when calcium intake increases. On the other hand, Araujo *et al.* (2003) found that feed intake was decreased when calcium level of diet increased from 3.5 to 4.2%. Also, Oliveira *et al.* (2002) observed a quadratic effect, when 3.6% calcium was added to diet, feed intake was decreased. FCR significantly improved by incorporating 3% and 3.75% calcium in the diet during the overall period as compared to the group fed diet containing 4.50% calcium.

Improved Feed conversion ratio with decreased dietary calcium was associated with the capacity of the birds to maintain optimal egg production with an increase in their feed intake. The increase in their FI for calcium translates into more nutrients for egg production. A dietary level of 3% calcium level was optimal and this corresponds with NRC (1994) which suggested that a calcium requirement of 2.75-3.25% for laying hens was effective without affecting either egg production or eggshell quality. Yakourt (2003) and Kadam *et al.* (2006) observed in their studies that calcium level maintained at 3.0% calcium with 0.35% phosphorus levels effected optimal feed conversion. HDEP was not different among calcium sources indicating that satisfactory amounts of calcium and other ingredients were available to support and sustain similar egg production. These results support researchers that also found egg production not to differ among hens fed LS or oyster shell (Guinotte and Nys, 1991; Grizzle *et al.* 1992). The overall period for hen day egg production showed better improvement by using 3% and 3.75% calcium inclusion compared to that of 4.50% calcium level. Along the same line, these results were in agreement with those reported by Rodrigues *et al.* (2005) who found that egg production was decreased when hens where fed diet containing 4.5% calcium compared to 3.8 % calcium in the diet. Higher egg production percentage and best FCR occurred by using the lower PS level 3.75% in this study than 4.50% calcium in the diet. It may be due to the fact that high calcium in the diet causes reduction in the biosynthesis of the protein which was a

carrier of calcium in the duodenum which in addition to damaging the functionality of the protein through increase in the intestinal pH (Berggard *et al.* 2000) decreases the absorptive efficiency of this calcium mineral which directly affects the retention of this mineral by birds. In the calcium or phosphorus low diet, the parathyroid gland releases parathyroid hormone (PTH) which in turn stimulates the conversion of vitamin D_3 to the steroid hormone $1,25(OH)_2D_3$. Increased production of $1,25(OH)_2D_3$ in the kidney results in increased intestinal absorption of calcium and phosphorus and bone reabsorption and reduces calcium and/or phosphorus excretion by the kidney (De Vries *et al.* 2010; Proszkowiec-Weglarz and Angel, 2013) to maintain normal plasma calcium and phosphorus concentration. These results proved that in calcium low diets, there is higher calcium utilization due to higher efficiency of intestinal absorption. So, birds improve the utilization of dietary calcium within their physiological limits as a form of compensation. There is scarce information related to effect of calcium sources, calcium levels and their interactions on the age at first lay of laying hens. Hens supplemented with 3.75% calcium levels in the diet reached puberty earliest compared to the group fed diet containing 3% and 4.50% calcium. The best interaction for age at first lay resulted by using diet containing 3.75% LS (135.67 days) and 3% PS (139.33 days) compared to the other interactions. This could be attributed to the fact that birds have the ability to regulate their feed intake to meet their own needs for calcium and to adjust to low dietary calcium levels. By attempting to eat for the deficient nutrient (calcium), birds would over consume the other nutrients and energy. The over consumption of energy has been suggested to be responsible for the increase in body weight and the accelerated early attainment of puberty observed.

The results of the egg quality of laying hens fed diets containing varying levels of LS and PS are presented in Table 3. Significant ($P<0.05$) treatment effects were recorded in egg surface area (ESA), shell percentage (Shell %), egg shell weight per surface area (ESWSA), egg specific gravity (ESG), Haugh unit (HU), albumin index (AI), albumin height (AH), yolk height (YH) and yolk index (YI) while LS and periwinkle shell did not significantly ($P>0.05$) influenced the egg weight (EW), egg shell thickness (EST) and egg surface area (ESA). It was observed that the eggs from the hens fed diets containing PS had higher values in the parameters where significant differences occurred compared to those fed LS. The levels of the LS and PS significantly ($P<0.05$) had enhancing effects on the parameters such as EST, HU, AH and AI with hens on diets containing 3.75 and 4.50 % recording the highest values for the studied parameters.

The levels of the treatments, however, did not significantly ($P>0.05$) influenced EW, ESG, ESA, Shell %, EWSA, YH and YI among the hens fed varying levels of LS and PS. The interactions of dietary calcium sources and levels on the egg quality of laying hens recorded significant ($P<0.05$) differences on all the egg quality parameters studied in the present study except EW, ESA, YH and YI.

Physiologically, only an increase in age of birds increased EW resulting in decrease of EST and its weakness (Larbier and Leclercq, 1992). Therefore, lack of difference in EW by different calcium sources, levels and their interactions are in agreement with Cheng and Coon (1990) who concluded through a series of experiments that switching from LS to oyster shell in short-term laying trials caused no significant differences in layer performance including egg weight. These results are also in agreement with those of Anizoba *et al.* (2022) who found no significant differences in egg weight when hens were fed diets with PS levels from 0% to 100%. That is to say that longer period of feeding high calcium diets to younger hens may be required before a significant adverse effect on performance will be detected (Table 3) These results are also in agreement with those of Keshavarz (1998) who found no significant differences in egg weight when hens were fed reducing phosphorus levels from 0.46% to 0.24% with 3.5% to 5.5% calcium. However, this result is not in agreement with Sultana *et al.* (2007) who found that birds offered oyster shell laid heavier eggs. The present study showed that EW was 54.90-60.81 g and this is comparable to the estimates reported by Pelicia *et al.* (2009) who recorded 66-67g but higher than that found by Abd El-Maksoud (2010) who reported 46-54 g. Ahmad *et al.* (2003) and Safaa *et al.* (2008) found that calcium levels had no effect on feed consumption and egg weight. ESW, ESA, shell %, ESWSA, ESG, HU, AI, AH, YH and YI were significantly higher in the group with periwinkle shell in comparison with the diet containing LS. This might be related to the slower dissolution of the periwinkle shell in the upper digestive tract and the corresponding more uniform and sustained release of calcium. Thus, a slower solubilisation of sources of calcium would make calcium available during the time of the eggshell calcification and diminish bone calcium and phosphorus mobilization (Skrivan *et al.* 2010).

However, this is not in agreement with Saunders-Blades *et al.* (2009) who concluded that calcium particle size and different sources have no effect on eggshell quality. The result shows ESWSA increases or improves as dietary interactions levels of LS and PS increases. Oliveira *et al.* (2002) fed similar Ca levels (2.8, 3.2, 3.6, 4.0, and 4.4%) to commercial layers, but did not verify any effect on ESWSA.

Table 3 Egg quality of the laying birds fed diets containing varying levels of limestone (LS) and periwinkle shell (PS)

Treatment	Parameters												
	EW (g)	ESW (g)	EST (mm)	ESI (%)	ESA (cm^2)	Shell (%)	ESWSA (mm/cm^2)	ESG	HU (%)	AH (mm)	AI (%)	YH (cm)	YI (%)
Source effect													
LS	58.09	4.97^{b}	0.36	76.81	72.48^{b}	8.21^{b}	66.11^{b}	1.06^{b}	89.17^{b}	7.38^{b}	6.26^{b}	1.45^{b}	39.91^{b}
PS	57.70	5.20^{a}	0.36	77.16	75.56^{a}	9.01^{a}	71.96^{a}	1.09^{a}	92.97^{a}	8.22^{a}	6.94^{a}	1.55a	41.06^{a}
SEM	0.75	0.08	0.01	1.05	0.818	0.180	1.12	1.08	1.24	0.24	0.25	0.02	0.68
P-value	0.118	**< 0.001**	0.666	0.462	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Level effect													
3.00	60.00	5.29^{a}	0.35^{c}	70.48^{c}	74.95	8.81	70.77	1.09	88.08^{b}	7.33^{b}	5.60^{b}	1.51	39.39
3.75	59.75	5.16^{a}	0.46^{b}	78.60^{a}	74.78	8.63	69.19	1.08	94.54^{a}	8.50^{a}	7.38^{a}	1.46	39.63
4.50	57.68	4.81^{b}	0.58^{a}	76.91^{b}	72.33	8.33	67.23	1.08	90.49^{b}	7.63^{b}	6.82^{a}	1.55	41.68
SEM	1.07	0.09	0.01	0.44	1.02	0.23	1.38	0.01	1.45	0.28	0.27	0.02	0.77
P-value	0.112	**< 0.001**	**< 0.001**	**< 0.001**	0.104	0.377	0.081	0.394	**< 0.001**	**< 0.001**	**< 0.001**	0.106	0.09
Interactions													
LS × 3.00	60.81	4.51^{c}	0.38^{b}	74.26^{c}	75.88	7.41^{c}	60.23^{c}	1.06^{c}	86.94^{cd}	7.11^{cd}	5.26^{b}	1.47	39.14
LS × 3.75	60.19	4.95^{b}	0.36^{b}	77.57^{b}	75.20	8.22^{bc}	66.15^{b}	1.06^{c}	86.18^{d}	6.71^{d}	5.91^{b}	1.37	37.66
LS × 4.50	60.46	5.10^{ab}	0.30^{c}	76.44^{b}	75.53	8.43^{b}	69.47^{ab}	1.05^{d}	94.72^{ab}	8.55^{ab}	7.73^{a}	1.53	40.84
PS × 3.00	59.20	5.13^{ab}	0.36^{b}	76.59^{b}	74.05	8.66^{b}	72.00^{a}	1.08^{bc}	89.29^{bcd}	7.44^{bcd}	5.94^{b}	1.54	40.35
PS × 3.75	59.31	5.36^{a}	0.36^{b}	79.73^{a}	74.23	9.03^{ab}	72.12^{a}	1.08^{bc}	91.99^{abc}	8.00^{abc}	6.94^{a}	1.55	41.21
PS × 4.50	54.90	5.45^{a}	0.41^{a}	77.31^{b}	69.15	9.92^{a}	74.25^{a}	1.09^{a}	96.90^{a}	9.00^{a}	7.83^{a}	1.57	43.28
SEM	0.91	0.19	0.01	0.53	1.32	0.25	1.46	0.01	1.66	0.28	0.32	0.03	0.99
P-value	0.115	**< 0.001**	**< 0.001**	**< 0.001**	0.111	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.276	0.582

EW: egg weight; ESW: egg shell weight; EST: egg shell thickness; ESI: shape index; ESA: egg surface area; Shell %: shell percentage; ESWSA: egg shell weight per surface area; ESG: egg specific gravity; HU: haugh unit; AH: albumen height; AI: albumen index; YH: yolk height and YI: yolk index.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

A possible reason for this difference may be the layer age, Oliveira *et al.* (2002) used 72- to 88-week-old hens, whereas in the present experiment, birds were younger (20 weeks of age) at the beginning of the experimental period, and therefore, due to their higher Ca requirements, responded better to dietary Ca in terms of ESWSA. These differences among studies demonstrate the importance of determining the nutritional requirements of commercial layers as a function of bird age. The increase in ESWSA in group fed PS may be explained by the study of Vicenzi (1996) who found that excessive dietary Ca increased Ca deposits in the egg, thereby increasing ESWSA. Another hypothesis is that the increase in ESW and ESWSA observed in the present study is that the high plasma Ca concentrations stimulated biochemical reactions (increase of $1,25(OH)_2D_3$ in the kidneys), which increase blood Ca^{++} level, and this additional blood Ca^{++} may be used for eggshell calcification. Independent of dietary Ca level, a fraction of Ca will always be removed from the bones; in case of Ca deficiency in the diet, more Ca will be removed, resulting in osteopenia. SG is an indirect tool for eggshell evaluation because it is related to EST and shell %. Generally, the higher SG value observed in group fed 4.50% PS (1.09) is related to thicker eggshell and shell weight per unit of surface area which is a desirable characteristic in the egg industry (Keshavarz and Quimby, 2002).

These results were not in agreement with the findings reported Keshavarz and Nakajima (1993) who noted no effect of 3.5% or 4.5% dietary calcium on egg specific gravity. However, Clunies *et al.* (1992) found an increase in eggshell weight and egg specific gravity when layer hen fed diets containing 3.5% and 4.5% calcium. Also, Pelicia *et al.* (2009) reported that increasing the dietary calcium level up to 4.5% enhanced the eggshell percentage and eggshell weight per surface area as well as egg specific gravity. There were effects of calcium levels and the interaction between the main effects (calcium sources and levels) on EST. The highest EST was obtained from eggs of hens fed the diet containing 4.50% PS. The increase in EST may be because of the presence of the coarse particles of PS. This shows that the larger particles should remain in the gizzard (an acidic environment) longer than LS, thereby having greater calcium retention than the small particle. Therefore, the acidity would help to dissociate the $CaCO_3$ into ionic calcium, hence producing more available calcium for absorption. However, the longer period of digestion of PS in the gastrointestinal tract due to its particle size supplied high amount of the body's requirements for calcium. The more calcium that is available to the shell gland during egg shell synthesis, the thicker the egg shell will ultimately be. This result is consistent with the results obtained from Safaa *et al.* (2008) who found an improvement in EST for

brown egg layer hens fed diets containing 4.0% calcium compared to those fed diets containing 3.5% calcium from 58 to 73 weeks of age. Conversely, Pelicia *et al.* (2009) found no significant differences in EST and ESG for brown egg layer hens fed 3.0, 3.5. 4.0, 4.5% calcium levels in the second laying cycle. There were improvement of calcium levels and the interaction between the main effects (calcium sources and levels) on ESW, HU, AH and AI. AH and AI are in the range of 6.71-9.00 mm and 5.26-7.83% respectively. Shell weight was found to be 4.95-5.36 g and this is lower than the estimates reported by Mankpondji *et al.* (2012) who recorded 6.58-6.05 g. Variations in this response could be due to the type of the diet, breed, and age of the bird or the prevailing environmental conditions. From the result, the highest ESW was obtained from eggs of hens fed the diet containing 3.75 and 4.50% PS. The improved ESW caused a decrease in the high gas interchanges through egg shell pores which finally improved albumen quality and haugh unit which is significant in diets containing periwinkle shell. According to the United States Department of Agriculture (USDA), a HU of 72 and above (score AA) is acceptable and indicates freshness in egg (Durunna *et al.* 2007). Similarly, haugh unit is a desirable characteristic of a numerical expression of albumen quality. Thus, the Haugh Unit obtained from all the group is an indication that eggs produced by hens fed both LS and PS were of standard quality. This result is in line with the findings of Anizoba *et al.* (2022) who found significant differences in ESW and HU when periwinkle shell was fed to birds. However, this result is not concomitant with the findings of Richter *et al.* (1999) who reported no significant differences in egg shell weight and haugh unit when hens were fed with 70% oyster shell.

The results of the blood parameters of laying hens fed diets containing varying levels of LS and PS are presented in Table 4. Varied calcium source did not significantly ($P>0.05$) affect all the serum biochemical indices studied. There were no significant ($P>0.05$) differences on the serum urea, TP and Ca whereas serum P, ALT and AST were highly significant ($P<0.05$) among treatments. Moreover, serum urea, Ca, ALT and AST of the treated birds were not significantly ($P>0.05$) influenced by the varied inclusion levels of the treatments used in the present study. However, the serum TP and P concentrations of the treated birds were significantly ($P<0.05$) affected by the varied inclusion levels of the LS and PS. The varying inclusion levels of LS and PS significantly ($P<0.05$) increased the serum TP concentrations of the treated birds. Furthermore, the serum P concentrations decreased with birds fed 3.00 % diet of the LS and PS recording the lowest significant ($P<0.05$) values compared to the other treatment levels.

The interactions between the LS and PS each with their respective levels of inclusion did not have significant ($P>0.05$) influences on the serum TP, ALT and AST concentrations. The interactions between LS and PS treatment and varied inclusion levels showed significant ($P<0.05$) difference for serum urea, Ca and P. However, the treatment interaction with the level of inclusion at 4.50 % LS diet significantly ($P<0.05$) lowered the serum urea, Ca and P concentrations of the treated birds. Furthermore, the interactions between the PS treatment and the varied inclusion levels followed the same trend as reported for the interactions between the LS treatment and the varied inclusion levels.

The improved treatment effects observed in urea among the hens fed PS supplemented diet over those fed LS was suggestive of the better bioavailability of PS in the diets (Table 4). Urea as a product of protein catabolism increases as the egg laying percentage increases (Agraharkar, 2008). The increase that occurred between the egg laying percentage and the level of uric acid shows enhancement of the protein catabolism which is related to the enhancement of egg production. The present study recorded a significant increase in ALT and AST when the inclusion levels of calcium were increased to 4.50% diet. The non-significant effect observed in the calcium sources for TP concentration of the treated birds indicated that calcium source did not interfere with protein metabolism with the inclusion levels employed in this study. It also, was suggestive that there were no problems in the liver or kidney. Blood proteins are primarily synthesized in the liver, and hence, the increased concentration of TP recorded among the birds on different calcium level is indicative of normal hepatic function and unimpaired protein synthesis. Hepatic cell damage is characterized by a significant rise in blood enzyme activities such as ALT and AST and thus causes alterations in liver function (Kim *et al.* 2008).

Hence, the significant treatment increases in the serum concentrations of these enzymes indicated that high levels of calcium in the diets of laying birds may predispose them to liver damage. In this study, the higher value of the serum Ca level observed in interaction between the main effects (calcium sources and levels) at 3.75% PS corresponds to a higher production of eggs. It appears that a higher production of eggs induces an increase in the serum concentration of calcium showing a more elevated turnover of the calcium in deposits. Furthermore, the increase from 3% to 4.50% showed a significant decrease in calcium blood concentration. These results were in line with Pelicia *et al.* (2009) who reported that animal fed calcium deficient diets increase absorption levels whereas high dietary levels of calcium reduced absorption.

Table 4 Blood parameters of the laying birds fed diets containing varying levels of limestone (LS) and periwinkle shell (PS)

Treatment	Parameters					
	Urea (mg/dL)	T.P (mg/dL)	Ca (mg/dL)	P (mg/dL)	ALT (U/L)	AST (U/L)
Source effect						
LS	10.72	5.88	9.58	0.41^{a}	178.40^{b}	40.58^{b}
PS	11.24	6.02	9.77	0.33^{b}	219.83^{a}	45.77^{a}
SEM	0.19	0.07	1.05	0.02	6.85	1.22
P-value	0.062	0.167	0.465	**< 0.001**	**< 0.001**	**< 0.001**
Level effect						
3.00	10.64	5.92^{b}	10.57	0.46^{a}	191.65^{b}	46.55^{b}
3.75	11.12	6.19^{a}	9.50	0.35^{b}	177.43^{b}	44.71^{b}
4.50	11.18	5.74^{a}	8.97	0.30^{c}	225.81^{a}	53.28^{a}
SEM	0.294	0.120	0.9	0.03	1.36	0.99
P-value	0.191	**< 0.001**	0.149	**< 0.001**	**< 0.001**	**< 0.001**
Interactions						
LS × 3.00	10.94^{ab}	6.24	11.43^{ab}	0.38^{b}	162.78	42.33
LS × 3.75	10.92^{ab}	5.84	10.04^{b}	0.37^{b}	163.34	42.97
LS × 4.50	10.37^{b}	5.57	9.98^{bc}	0.28^{c}	208.95	40.64
PS × 3.00	11.42^{a}	6.14	11.95^{ab}	0.59^{a}	220.55	43.36
PS × 3.75	11.86^{a}	6.01	12.71^{a}	0.35^{b}	191.06	40.21
PS × 4.50	10.38^{b}	5.91	8.65^{c}	0.33^{bc}	242.82	44.51
SEM	0.39	0.01	0.87	0.01	3.15	0.17
P-value	**< 0.001**	0.082	**< 0.001**	**< 0.001**	0.576	0.239

TP: total protein; Ca: calcium; P: Phosphorus; ALT: alanine aminotransferase and AST: aspartate aminotransferase.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

In this study, as dietary calcium levels increased, the birds used part of it and excreted the excess resulting to a decrease in blood calcium concentration. This is explained by the fact that there are two calcium absorption pathways; one is saturable and the other not saturable (Shkembi and Huppertz, 2022).

The saturable pathway requires calcium-binding proteins where the amount is constant in the epithelial cells. Therefore, when low calcium levels are fed, the ratio between the binding protein and calcium is higher promoting higher calcium digestibility due to higher efficiency of intestinal absorption. From this study, serum P decreased with increasing dietary levels of calcium. This might be attributed to the fact that high dietary calcium levels reduced the availability of phosphorus (Driver *et al.* 2005). Calcium is known to form insoluble complexes with phytate phosphorus which may hinder phytate activity (Angel *et al.* 2002). Calcium has the capacity to interact with inorganic phosphorus in the gut lumen to form insoluble calcium orthophosphate (Plumstead *et al.* 2008) which may make inorganic phosphorus less soluble and available for absorption in excess of pH 5.0. It also reduces the need for bone resorption thereby reducing phosphorus needs and subsequently increased environmental pollution. This result is in agreement with Hartel (1990) who reported that high calcium levels in layer hen diets decreased phosphorus utilization. This effect could explain our results that the lowest performance was observed with high calcium diet at 4.50%. The results of the tibia mineralization of laying hens fed diets containing varying levels of LS and PS are presented in Table 5. There was a significant treatment effect (P<0.05) only in the bone Ca and P. The hens fed diets supplemented with PS had higher bone calcium and magnesium (Mg) than those on LS supplemented diets. All other bone mineralization indicators such as tibia weight (TW), bone phosphorus, tibia ash (TA) and defatted bone (DB) were not significantly (P>0.05) influenced by the two-calcium source supplemented in the experimental diets. There were noticeable significant (P<0.05) effects of the varying inclusion levels of the two calcium sources in a dose-dependent manner in some of the bone mineralization parameters considered with the best results recorded among the birds fed diets containing 3.75 % calcium. The interactions between LS and PS treatment and varied inclusion levels showed significant (P<0.05) difference for bone calcium, magnesium and tibia ash.

Table 5 Tibia mineralization of the laying birds fed diets containing varying levels of limestone (LS) and periwinkle shell (PS)

Treatment	Parameters					
	TW (g)	Ca (ppm)	Mg (ppm)	P (ppm)	TA (%)	DB (%)
Source effect						
LS	12.55	720.00^{b}	57.67^{b}	80.05	33.50	72.66
PS	12.60	833.33^{a}	66.67^{a}	74.71	34.67	73.50
SEM	0.38	20.79	2.48	1.96	1.23	0.81
P-value	0.081	< **0.001**	< **0.001**	0.067	0.368	0.495
Level effect						
3.00	11.86	750.00^{b}	69.41^{a}	73.42	37.95^{b}	70.41
3.75	11.56	860.26^{a}	60.13^{a}	81.43	38.75^{b}	70.15
4.50	10.91	720.23^{b}	57.50^{b}	77.28	43.25^{a}	70.10
SEM	0.67	36.91	1.83	2.41	1.16	0.72
P-value	0.319	< **0.001**	< **0.001**	0.062	< **0.001**	0.160
Interactions						
LS × 3.00	12.66	600.31^{d}	72.30^{b}	77.28	30.57^{b}	70.50
LS × 3.75	12.46	760.23^{c}	48.57^{d}	85.57	34.88^{ab}	72.15
LS × 4.50	12.76	640.11^{d}	51.86^{d}	77.28	36.50^{a}	75.44
PS × 3.00	12.06	900.54^{b}	77.59^{a}	69.56	32.57^{b}	69.50
PS × 3.75	12.66	960.77^{a}	61.44^{c}	77.28	36.28^{a}	71.86
PS × 4.50	12.06	800.09^{c}	64.73^{c}	77.44	35.44^{a}	80.57
SEM	0.73	23.54	2.59	3.98	1.87	1.44
P-value	0.127	< **0.001**	< **0.001**	0.384	< **0.001**	0.068

TW: tibia weight; Ca: calcium; Mg: magnesium; P: phosphorus; TA: tibia ash and DB: defatted bone.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

However, the treatment interaction with the level of inclusion at 4.50 % LS diet significantly (P<0.05) lowered the bone calcium and magnesium but increased tibia ash. Furthermore, the interactions between the PS treatment and the varied inclusion levels followed the same trend as reported for the interactions between the LS treatment and the varied inclusion levels.

The Significant effect of calcium sources and levels on bone mineralization was noted (Table 5). Birds fed on 4.50% calcium levels had higher percentage TA than those fed on other levels. Analysis of the significant interaction showed that superior relative ash values were attained with 4.50% LS, 3.75% PS and 4.50% PS level. However, comparable percentage ash values with this group was noted for diet with 3.75% LS. The result showed that increasing amount of calcium in the diet resulted to higher amount of tibia ash with 4.5% calcium values as optimal. The fact that the tibia ash value increased with increased amount of dietary calcium means that the birds were able to deposit calcium rather than having it used for shell formation and other calcium needs. This implies that when sufficient calcium is obtained from the diet, calcium from the medullary bone is not released for eggshell formation (Skrivan *et al.* 2010).

The results found for the deposition of calcium in the tibia showed that calcium content of the bone is highest with 3.75% PS having the highest mean values. Some studies proved that low calcium level in the diets of layers promote bone mobilization of calcium to meet the needs of the birds and maintain the shell quality (Schreiweis *et al.* 2003; Almeida Paz *et al.* 2009).

Therefore, considering the results obtained, it is possible to affirm that the calcium level obtained was enough to meet the requirements of the layers. However, this result did not agree with Pastore *et al.* (2012) who reported no significant effect of calcium: phosphorus ratios (9.76:1; 10.81:1 and 12.12:1) on the contents of calcium (g/kg) or phosphorus (g/kg) in the tibia of white egg layers in second production cycle. From this study, the Mg content of the bone decreased with increasing dietary levels of calcium. This may be due to the fact that an excess in the level of dietary calcium may interfere with the availability of other minerals including phosphorus, copper, manganese, magnesium and zinc (Maenz *et al.* 1999).

The solubility of mineral complexes decreased when calcium is supplemented at high levels. This high level can increase ileal pH and reduces the absorption of other minerals (Shafey, 1993).

CONCLUSION

Since the varied inclusion levels of the two calcium sources used in the present study positively enhanced productive performances of the laying hens, serum biochemistry, bone mineralization as well as improved the qualities of their eggs without any residual effects recorded on the health status of the birds, it is, therefore, concluded that 3.75% calcium level was appropriate for laying hens and that periwinkle shell can conveniently replace the use of LS in the laying hens' diet as a satisfactory calcium source while simultaneously lessening the environmental burden of landfills. Thus, it is possible to formulate a diet that is less expensive, better suited for birds, and contains a highly digestible calcium source at a lower dietary concentration while still retaining the quality of the egg as the ultimate product.

ACKNOWLEDGEMENT

The authors wish to acknowledge the Department of Animal Science, University of Nigeria, Nsukka Campus for making available the facilities for this research work. Professor S.O.C. Ugwu and Mr. N.E. Ikeh are well appreciated for their scientific clarifications during the preparation and submission of the present paper.

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