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Performance, egg quality characteristics, serum parameters, blood minerals and bone mineralisation of laying chickens fed bone dust as calcium source

Olayemi Wasiu Ajani[1], Oso Abimbola Oladele[2], Williams Gabriel Adedotun*[3], Ojo Oluwaseyi Temitope[4] and Omofunmilola Eunice Opeolouwa[1]

[1] Yaba College of Technology, School of Agriculture, Department of Animal Production Technology, Lagos, Nigeria

[2] Federal University of Agriculture, College of Animal Science and Livestock Production, Department of Animal Nutrition, PMB 2240 Ogun State, Abeokuta, Nigeria

[3] Lagos State University, School of Agriculture, Department of Animal Science, Lagos, Nigeria

[4] Animal Care Services Konsult Limited, Feed, Premix and Biotechnology Unit, Ogere, Ogun State, Nigeria

**Corresponding author*: gabriel.williams@lasu.edu.ng

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Abstract: The study investigated the effect of dietary inclusion of bone dust as a calcium source on performance, egg quality characteristics, serum parameters, blood minerals and bone mineralisation of laying chickens. A total of 128 point of lay birds (1470-1550g) were allotted on a weight equalisation basis to four dietary treatments. Each treatment consists of four replicates having 8 birds per replicate. The diets were designated T1, T2, T3 and T4 in which T1 is the diet with dicalcium phosphate (DCP), T2 is the diet with bone meal, T3 is the diet with 50% bone dust and 50% DCP and T4 is the diet with 100% bone dust. The experiment lasted for eight weeks; laying performance and egg qualities (internal and external) were evaluated on a weekly basis. Serum parameters, blood minerals and bone mineralisation were carried out at the end of the study. Data collected were subjected to a one-way analysis of variance using Minitab statistical software (17.0) and significant means were separated using Tukey's test. The inclusion of 50% and 100% dust resulted in increased ($P<0.05$) daily feed intake (DFI). The use of 100% bone dust in the diet of layers resulted in the highest ($P<0.05$) number of eggs laid (NEL) (293.00) and Hen-day egg production (HDEP) (118.33 %). Eggshell thickness (EST) was highest for layers fed T3 and T4 diets. The highest ($P<0.05$) albumin height (AH) (10.04 mm) and Haugh unit (Hu) (94.62 %) was observed for layers fed T4 diet. Layers fed T1 and T4 diets had higher ($P<0.05$) total protein and globulin than other treatments. Calcium and phosphorus content in blood was highest ($P<0.05$) for layers fed T1 diet. Bone calcium and phosphorus were highest ($P<0.05$) for layers fed the T3 diet but lowest for those fed the T1 diet. It was concluded that bone dust can be included in the diet of layers at 50 and 100% for better performance and egg quality without negative effects on blood and mineral composition.

Keywords: layers; bone dust; performance; egg quality; mineralisation

INTRODUCTION

The metabolism of laying hens is highly dependent on the availability and homeostasis of the minerals used in the formation of the eggshell, such as calcium and phosphorus (Runknke et al., 2021). Thus, hens require a substantial amount of calcium sufficient for eggshell formation throughout the whole laying cycle and to ensure the successful formation of properly min-

eralized eggshells, as well as the overall welfare and health of the hen. The bird's nutritional status plays a crucial role in the modulation of bone mineral homeostasis, influencing the mineralization and mechanical strength of the bones (Kubí́s et al., 2020; Olgun et al., 2018). Eggshell strength is characterized by its resistance to cracking, which can occur in the presence of external loads and it depends on the physical properties of the eggshell which is characterized on a macroscopic level by its shape, weight, thickness and mineralization (Narushin et al., 2004).

The source and level of calcium in the diet affect biomechanical properties or mineralization, which greatly affects shell deposition and shell quality. Apart from this, the dietary inclusion of calcium in poultry diet influences phosphorus synthesis, blood calcium mobilization and stimulation of parathyroid hormone (PTH), which promotes bone resorption to re-establish calcium homeostasis (Pelicia et al., 2009). In addition, poor eggshell quality resulting in broken and cracked eggs is considered the major economic loss to egg producers. Studies have shown the role of calcium on egg production, eggshell thickness, egg weight, egg-breaking strength, feed intake, feed conversion ratio and body weight (Chen & Chen, 2004). Thus, it is important that the right quantity of calcium is provided for laying hens to ensure that the eggs produced are of the right quality in terms of nutritional value and shell quality.

There are different sources of calcium used in poultry diets such as dicalcium phosphate (DCP), bone meal, oyster shell etc. The high cost of phosphate salts, such as monocalcium and dicalcium phosphates and the high exchange rate makes the use of dicalcium phosphate (DCP) as a calcium source expensive. This has led to an increase in the usage of substitutes such as bone meal, periwinkle shell, snail shell, eggshell etc. However, the limitations to the use of these alternative calcium sources are the methods of production and problem with impurities like stones, pebbles, wire, burnt latex and charcoal that affects its quality, which is also detrimental to poultry health.

Bone dusts are residues derived from filling and polishing of bone which involve processes such as washing, steam boiling, de-fatting, drying and filling. This production process makes the product (bone dust) safe as a calcium source. Therefore, this study investigated the effect of using defatted bone dust as a calcium source in layer diets on productive performance, egg qualities, serum parameters and bone mineralization.

MATERIALS AND METHODS

The study was carried out at the poultry unit of the Teaching and Research farm of Yaba College of Technology, Epe, Lagos State. It is situated at latitude 6.580N and longitude 3.90E. It is 42m above sea level along Epe-ijebu Ode Road. Epe lies in the low land rain forest vegetation zone within the Savannah agro-ecological zones of south Nigeria (Google Earth, 2022)

The feed ingredients such as maize, soybean meal, wheat offal, fish meal (72%), limestone, bonemeal, salt, premix, methionine and lysine were purchased from a feed mill within the South West in Nigeria. The bone dust was obtained from RIA Biotechnology Company Limited, Ota, Ogun state. The feed ingredients were crushed and mixed together to meet the nutrient requirements of the birds. The birds were offered growers mash till 10% production was recorded before introducing the experimental diets. Medications and vaccinations were offered to the birds as when due. Adequate feed and clean water were available to the birds.

A total of one hundred and twenty-eight (128) points of lay birds with body weight between 1470-1550g were used. The birds were allotted on a weight equalisation basis in a completely randomised design (CRD) to four dietary treatments with 32 birds per treatment consisting of 4 replicates having 8 birds per replicate. The four dietary treatments consist of the control diet (diet 1, with dicalcium phosphate (DCP)), diet 2 (diet with bone meal), diet 3 (diet with 50% bone dust and 50% DCP), diet 4 (diet with 100% bone dust). The diets were formulated to meet the nutrient requirement of the birds using the NRC (1994) recommendation (Table 1).

Table 1. Gross composition of experimental diets.

Ingredients	T 1	T 2	T3	T 4
Maize	50.00	50.00	50.00	50.00
Soybean meal	21.50	22.00	21.50	21.50
Wheat offal	14.00	15.00	14.50	14.50
Fish meal (72%)	1.00	1.00	1.00	1.00
Limestone	8.00	8.00	8.00	8.00
DCP	4.50	0.00	2.00	0.00
Bone meal	0.00	3.00	0.00	0.00
Bone dust	0.00	0.00	2.00	4.00
Salt	0.30	0.30	0.30	0.30
Premix	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20
Lysine	0.20	0.20	0.20	0.20
TOTAL	100	100	100	100
Determined Composition%)				
Crude protein	16.23	16.81	16.69	16.53
Crude fibre	3.08	3.15	3.44	3.24
Calcium	3.70	3.85	3.67	3.74
Phosphorus	0.81	0.50	0.70	0.70
Energy (kcal/Kg)	2904	3150	2872	2960
Ash	8.16	8.13	7.95	8.06
Moisture	1.26	1.32	1.55	1.31
Fat	2.65	2.56	2.46	2.84
NFE	68.58	67.91	67.81	66.68

Vitamin/mineral premix:vitamin A, 10,000,000 I.U.; vitamin D3, 2,000,000 I.U.; vitamin E, 16.0g; vitamin K, 1.0g; vitamin B1, 0.509 mg; Riboflavin, 2-4 mg; pyridoxine, 0.35 mg; niacin, 3.5 mg; biotin, 0.005 mg; choline chloride30.0 mg; folic acid 0.1 mg; vitamin B12, 0.002 mg; vitamin C, 2.50 mg; manganese, 10.0 mg; zinc, 4.5 mg; Copper 0.20 mg; iron 5.0 mg; methionine 2.0 mg; calcium panthothenate 1.0 mg; antioxidant 120,000 mg; selenium, 120mg.

At the onset of the experiment, the initial weights of the birds were taken to the nearest 0.01g. Records of daily feed consumption and egg production on a replicate basis were taken starting from two weeks in lay to 8 weeks, Weekly egg production per replicate was pooled and expressed as a percentage Hen–day egg production (%HDEP). It was expressed as a percentage of the ratio of the number of eggs laid to the number of hen days (NAPRI, 2000).

$$HDEP = \frac{\text{Number of eggs laid}}{\text{Number of hen-days}} \times \frac{100}{1}$$

Eight eggs (2 per replicate) from each treatment were selected on a weekly basis for eight weeks. Egg quality assessment was done within 24 hours of lay. The weight of each egg sampled was determined with a sensitive weighing scale (saltex® electronic balance) to the nearest 0.01g. The linear measurements were taken with Vernier calipers to the nearest 0.01cm and the length of the egg was taken as the longitudinal distance between the narrow and the broad ends. The egg width was taken as the diameter of the widest cross–sectional region.

The egg shape index (ESI) was calculated using the measured values of egg width and length from individual eggs sampled and multiplied by 100 as expressed below:

$$ESI = \frac{\text{Width of egg}}{\text{Length of egg}} \times \frac{100}{1}$$

The eggshell weight (ESW) was also determined by air-drying shells for 72 hours in egg trays at room temperature and the individual eggshells were weighed using an electronic weighing balance (sensitivity of 0.01g). The eggshell thickness (EST) was measured with a digital micrometer screw gauge to the nearest 0.01mm.

The internal egg characteristics were measured by using the destructive procedure in which the egg contents were poured into a flat plate and weighed. The albumen height (AH) was measured off the chalazae at a point above mid-way between the inner and circumference of the thick white with a spherometer having an accuracy of 0.01mm. Thereafter, the albumen was separated from the yolk using a smooth plastic egg separation funnel and the weight of the yolk weight (YW) was taken. The albumen weight (AW) was calculated as the difference between the egg weight and the combined weight of the yolk and dry eggshell for individual egg samples.

The yolk colour was evaluated and scored for individual egg yolk by comparison between the chips of a Hoffman-La Roche yolk colour fan rated 1- 15 with colour intensity ranging from pale yellow to deep orange (Hoffman-La Roche, 1984) and the colour of egg yolk obtained. The Haugh unit for individual eggs was calculated using the formula of Haugh (1973) for individual eggs sampled.

$$\text{Haugh Unit (Hu)} = 100 \text{ Log } (H + 7.57 - 1.7W^{0.37})$$

Where; H= Albumen height (mm), W= Weight of the egg (g) and the values 7.57 and 1.7 remain constant

At the end of the study, 2.5ml of blood samples were collected from a bird per replicate into non-heparinized tubes. The blood samples were centrifuged at 3000 rpm for 15 minutes and the serum obtained was stored at -20°C until analysis. The method of Colowick & Kaplan (1955) was used for total serum protein (TP) determination while serum albumin and globulin were determined using the bromocresol purple method of Varley et al. (1980). Serum creatinine was determined using the principle of Jaffe reaction as described by Bousnes & Tauslay (1945) while the serum uric acid was determined using the kit (Quinica Clinica Spam) (Wooton, 1964). Serum glucose was determined colorimetrically using the method described by Braham & Trinder (1972). Serum cholesterol was determined by the method described by Roeschlau et al. (1974) while minerals (calcium (Ca), phosphorous (P), iron (Fe), zinc (Zn) and magnesium (Mg)) were determined calorimetrically by using available commercial kits.

For bone mineralisation, the birds were randomly selected (two birds per replicate) and euthanized. The tibia bone was then removed, cleaned of adhering flesh, and stored at 20°C for further analysis. Fat was extracted from the bone samples by immersing in petroleum spirit for 24 h. The tibia bones were dried (105°C for 24 h) until no further changes in weight after which they were ashed in a muffle furnace at 600°C for 24 h. The ashed bone was digested and the digested solution was used for the analysis of Zn, Mn and Fe content of the bone using the atomic absorption spectrophotometer (Techtron model AA-10). The ashed tibia samples were also used to estimate the concentrations of Calcium (Ca) and Phosphorus (P). 10 ml of 6 M hydrochloric acid was added to the cooled bone ash, the solution was evaporated to dryness on a hotplate and the precipitate dissolved by adding 10ml of 6M hydrochloric acid and heated. The resulting solution plus washings from the crucible were filtered into a 100ml volumetric flask. The extracted bone solution was mixed with molybdovanadate to determine phosphorus colorimetrically using an automated analytical system.

Data generated from the study were subjected to one-way analysis of variance using the statistical analysis software Minitab 17.0 (2000) and where significant differences existed among

means, it was separated using Tukey's test in the same software.

RESULTS

Table 2 shows the performance traits of laying chickens fed the diet containing bone dust as a calcium source. Significant (P<0.05) effects of the different calcium sources on daily feed intake (DFI), total feed intake (TFI), number of eggs laid (NEL) and Hen-day egg production (HDEP) were observed. Result shows increased DFI in birds fed diet having 50% and 100% inclusion of bone dust (121.65g and 124.61g) respectively. The birds on 100% bone dust had the highest NEL (293) and the least was recorded in birds on the bone meal (T2) (205). HDEP was highest (P<0.05) (118.33 %) for layers on 100% bone dust while the layers on bone meal diet had the least (85.30%).

The effect of dietary inclusion of calcium from different sources on external and internal egg qualities is shown in Table 3. The result shows that EST and ESW were only significantly (P<0.05) affected by the dietary treatments under the external egg traits. Layer birds on diets T3 and T4 had increased (P<0.05) EST while those fed diets T1 and T2 had reduced EST. The ESW of layers fed diet T4 was higher (P<0.05) compared to other treatments.

The AH, AW and Hu were significantly (P<0.05) influenced by dietary treatments un-

Table 2. Effects of different calcium sources on performance.

Parameters	T1	T2	T3	T4	SEM
Daily feed intake (g/bird)	111.27^{b}	112.66^{b}	121.65^{a}	124.61^{a}	1.44
Total feed intake (g/bird)	6231.12^{b}	6308.96^{a}	6812.40^{a}	6978.16^{a}	648.64
Number of eggs laid	212.50^{b}	204.75^{c}	249.25^{ab}	293.00^{a}	12.10
Egg weight (g)	61.61	61.82	61.32	62.09	0.50
Hen-day production (%)	88.58^{c}	85.30^{d}	97.35^{b}	118.33^{a}	4.66

^{a,b,c,d}*Means on the same row having different superscript are significantly different (P<0.05).*

T1 = Dicalcium phosphate (DCP), T2= bone meal, T3= 50% bone dust plus 50% DCP and T4= bone dust at 100%.

Table 3. Egg quality traits of the hens fed different calcium sources.

Parameters	T1	T2	T3	T4	SEM
External traits					
Egg weight (g)	62.32	63.53	61.32	62.93	0.64
Egg length (cm)	50.57	51.27	51.08	51.15	0.20
Egg breadth (cm)	40.06	41.87	40.74	40.21	0.15
Shell thickness (mm)	0.44^{ab}	0.43^{b}	0.46^{a}	0.46^{a}	0.03
Shell weight (g)	6.12^{b}	6.32^{b}	6.39^{b}	6.62^{a}	0.07
Egg shape index	78.68	78.30	78.38	78.12	0.20
Internal traits					
Yolk weight (g)	12.74	12.59	12.77	12.95	0.10
Albumen height (mm)	7.42^{c}	7.75^{c}	8.38^{b}	10.04^{a}	0.25
Albumen weight (g)	37.49^{c}	38.38^{b}	39.84^{ab}	40.80^{a}	0.45
Yolk colour	6.25	7.0	6.75	6.67	0.50
Haugh unit (%)	88.75^{c}	91.99^{b}	92.89^{b}	94.62^{a}	0.61

^{a,b,c,d}*Means on the same row having different superscript are significantly different (P<0.05).*

T1 =Dicalcium phosphate (DCP), T2= bone meal, T3= 50% bone dust plus 50% DCP and T4= bone dust at 100%.

der the internal egg traits. Layers fed the T4 diet had the highest (P<0.05) AH and those fed the T1 and T2 diets had the lowest AH while those fed the T3 diet had intermediate AH. The highest (P<0.05) AW (40.80 g) and Hu (94.62 %) were observed for layers fed the T4 diet and those fed the T1 diet had the lowest AW (37.49 g) and Hu (88.75 %) while those fed T2 and T3 diets were intermediate for AW and Hu.

Table 4 shows the effect of dietary calcium inclusion from different sources on serum indices of laying chickens. The TP and globulin were higher (P<0.05) for layers fed T1 and T4 diets than those fed T2 and T3 diets. The highest (5.17 g/l) (P<0.05) and lowest (4.85 g/l) albumin was observed for layers fed T2 and T3 diets respectively. Uric acid was highest for layers fed the T4 diet followed by those fed the T1 diet and those fed the T3 diet had the lowest uric acid content. The creatinine content was highest (P<0.05) for layers fed the T1 diet followed by those fed the T2 diet while those fed the T3 diet had the lowest. Alanine transferase (ALT) followed the same trend as serum creatinine. Layers fed the T2 diet had the highest (P<0.05) serum glucose followed by those fed the T3 diet while those fed the T1 diet had the lowest. Triglyceride, cholesterol and aspartate transferase (AST) followed the same trend in which layers fed the T1 diet was highest (P<0.05) followed by those fed the T2 diet while those fed the T4 diet had the lowest values for the serum parameters was highest for layers fed T1 diet

Table 5 shows the blood mineral composition of layers fed diets with different calcium sources. Zinc concentration was highest (P<0.05) for layers fed the T3 diet followed by those fed the T2 diet and those fed the T1 diet had the lowest zinc content. The highest (P<0.05) iron content was observed for layers fed the T4 diet and it was lowest for those fed the T2 and T3 diets. Layers fed the T1 diet had the highest (P<0.05) phosphorus content while those fed the T4 diet had the least. The blood calcium was highest for layers fed T1 diet while those fed T3 and T4 diets had the lowest. The blood magnesium of layers fed T3 diet was lower (P<0.05) compared to other treatments. The blood manganese was highest (P<0.05) for layers fed the T3 diet but lowest for those fed the T1 diet while those fed T2 and T4 were intermediate.

Table 6 shows the bone mineralization in laying chickens fed diets with different calcium sources. It was observed that dietary calcium sources significantly (P<0.05) affected all minerals determined. The result shows that calcium was highest (P<0.05) for layers fed the T3 diet followed by those fed the T4 diet and was lowest for those fed the T1 diet. A similar trend was also observed for iron, magnesium and zinc. Phospho-

Table 4. Serum indices of the hens fed different calcium sources.

PARAMETERS	T1	T2	T3	T4	SEM
Total protein (g/dl)	9.55^{a}	6.35^{b}	6.30^{b}	9.56^{a}	0.39
Globulin (g/dl)	4.75^{a}	1.18^{b}	1.45^{b}	4.82^{a}	0.40
Albumin (g/dl)	4.90^{ab}	5.17^{a}	4.85^{b}	4.74^{ab}	0.05
Uric acid (mg/dl)	13.25^{b}	12.55^{c}	10.75^{d}	14.49^{a}	0.34
Creatinine (mg/dl)	29.15^{a}	20.15^{b}	16.15^{d}	18.20^{c}	1.28
Glucose (mg/dl)	83.25^{d}	97.05^{a}	94.75^{b}	86.30^{c}	1.48
Triglycerol (mg/dl)	232.25^{a}	130.65^{b}	106.55^{c}	98.24^{d}	13.81
Cholesterol (g/l)	118.25^{a}	90.15^{b}	71.75^{c}	63.20^{d}	5.44
Alanine transferase (IU/L)	39.15^{a}	25.15^{b}	18.15^{d}	20.20^{c}	1.28
Aspartate transferase (IU/L)	108.22^{a}	103.17^{b}	93.55^{c}	76.18^{d}	3.15

^{a,b,c,d} Means on the same row having different superscript are significantly different (P<0.05)

T1 = Dicalcium phosphate (DCP), T2= bone meal, T3= 50% bone dust plus 50% DCP and T4= bone dust at 100%.

Table 5. Blood mineral of the hens fed different calcium sources.

Parameters (mg/dl)	T1	T2	T3	T4	SEM
Zinc	144.30[d]	227.25[b]	239.55[a]	194.30[c]	9.51
Iron	116.65[b]	101.75[c]	106.35[c]	155.30[a]	94.70
Phosphorus	9.55[a]	6.05[b]	5.67[b]	4.24[c]	0.50
Calcium	14.65[a]	12.15[b]	11.35[c]	11.70[c]	0.33
Magnesium	2.25[a]	2.25[a]	1.75[b]	2.23[a]	0.06
Manganese	0.76[c]	1.02[b]	1.25[a]	0.94[b]	0.04

[a,b,c,d] *Means on the same row having different superscript are significantly different (P<0.05)*

T1 = Dicalcium phosphate (DCP), T2= bone meal, T3= 50% bone dust plus 50% DCP and T4= bone dust at 100%.

Table 6. Bone mineral composition of the hens fed different calcium sources.

Parameters (mg)	T1	T2	T3	T4	SEM
Calcium	28.52[d]	33.23[c]	46.67[a]	39.31[b]	1.75
Iron	1.74[d]	1.91[c]	2.18[a]	2.10[b]	0.04
Magnesium	9.82[d]	12.6[c]	15.44[a]	13.84[b]	0.53
Phosphorus	68.63[c]	75.17[b]	84.21[a]	76.55[b]	1.52
Zinc	2.53[d]	2.82[c]	3.41[a]	2.94[b]	0.08

[a,b,,c,d] *Means on the same row having different superscript are significantly different (P<0.05)*

T1 = Dicalcium phosphate (DCP), T2= bone meal, T3= 50% bone dust plus 50% DCP and T4= bone dust at 100%.

rus was highest (P<0.05) for layers fed the T3 diet but lowest for those fed the T1 diet while those fed T2 and T4 diets had intermediate phosphorus content.

DISCUSSION

The performance traits observed in the present study showed that the inclusion of 100% bone dust in the diet of layers as a calcium source increased DFI, TFI, NEL, as well as HDEP. The results obtained could be as a result of the different chemical composition of the calcium source. The differences in feed intake could be attributed to the distinctions in the composition of the calcium sources as studies have revealed that hens have a specific appetite for calcium and therefore it may cause changes in feed intake to accommodate calcium needs (Safamehr et al., 2013). The significant increase in performance traits of laying birds fed the T4 diet is comparable with the studies of Menlonca et al. (2022), who observed increased performance in laying chickens fed a diet containing calcitic limestone, charru mussel shell, oyster shell and macuinm shell as calcium source. The Higher egg production recorded for layers on the T4 diet could also be attributed to the increased calcium bioavailability of the calcium source. The bioavailability of dietary calcium sources increases intestinal absorption of calcium and phosphorus while it also influences bone resorption (Buzinaro et al., 2006). In a previous study by Ahmed et al. (2013), it was reported that dietary inclusion of limestone instead of oyster shell increases egg production. The authors also observed that elevated egg weight, egg mass and improved feed conversion ratio are dependent on the quality of the test ingredient, which suggests that the improved quality of diet T4 as a result of the test ingredient influenced increased production.

It has been established that protein and mineral intake strongly influence the production performance of laying birds. It was observed in the current study that egg weight, egg length,

egg breadth and egg shape index were not significantly different across treatments. This suggests nutrient adequacy of the diets across treatments resulting in non-significant differences in these parameters. Adeyemo et al. (2012) stated that there is a direct relationship between the performance of laying hens and protein intake. The EST was significantly influenced by the different dietary calcium sources and layers fed T3 and T4 diets had increased EST. The increased EST observed is due to the increased availability of calcium from the diets consequently increasing eggshell deposition. The thickness of the shell is a reflection of the bird's metabolism of calcium and its efficiency in secreting calcium and associated minerals in shell formation. Leeson & Summers (2005) have earlier stated that the calcium source, as well as its solubility in the digestive tract is an important determining factor for egg formation with optimum shell quality and productivity. The availability of dietary calcium promotes an increase in its reabsorption from the kidney and absorption from the intestine for utilisation and re-utilisation in growth and production (Anwar, 2017). In general, the range (0.43 – 0.46 mm) of eggshell thickness obtained in the present study is higher than the minimum values (0.34 mm) recommended by Oluyemi & Roberts (2000). However, the values obtained in this study were less compared to the value (0.59 mm) reported by Rathnayaka et al. (2020) in commercial layers fed diet containing bone meal. The discrepancies could be differences in inclusion level and calcium source. The increased EST obtained for broilers fed T3 and T4 diets suggests that cracking and damage of eggs will be reduced. Olabode (2015) and Wang et al. (2021) also indicated that a thick eggshell prevents egg cracking during collection and reduces egg damage. The yolk weight and yolk colour were not influenced by the different dietary calcium sources which implies that the inclusion of bone dust did not alter these important egg parameters. The albumin height and weight were increased for the group of layers fed the T4 diet. This observation suggests that nutrient intake particularly protein was not impaired with the use of bone dust in the diet of layers. The inclusion of bone dust at 50% and 100% in the diet of layers resulted in increased Hu values compared to other treatments. The Hu measures the freshness of eggs and the values obtained across treatments in this study range from 88.75-94.62%, which is higher than 70% declared acceptable as golden standard egg quality by the United States Department of Agriculture. This implies that all the eggs obtained from this study across treatments were of high quality and acceptable to consumers. Sarmiento-García et al. (2022) reported higher values (104.67-106.79%) in Japanese quail fed a diet containing calcium pidolate as a calcium source. The differences observed could be due to differences in species and calcium source.

Serum total protein and globulin increased for layers fed T1 and T4 diets and it is important to note that the diets contain 100% DCP and 100% bone dust respectively. It therefore suggests that the diets support efficient protein utilisation which will consequently influence bone and eggshell formation. It has been reported that adequate dietary protein intake is quite important for maximum synthesis and maintenance of bone mass (Tsagari, 2020). The results revealed that crude protein levels in the diets were sufficient in supply for various physiological needs of the hens as data obtained were higher than the values (2.5 to 4.5 g/dL) reported by Café et al. (2012). The uric acid concentrations in this study ranged from 10.75 to 14.49 mg/dl. The values were higher than the values obtained in previous studies (Lumeij, 2008; Capitelli & Crosta, 2013). The layers fed diet T4 had increased uric acid. Increased serum uric acid levels have been linked to inefficient protein utilization and consequent increased deamination. This is not necessarily the case in this study as uric acid is normally synthesized in the liver and kidneys and excreted regardless of water reabsorption. Based on the report by Rajman et al. (2006) it could be suggested that the elevated uric acid may be due to the breakdown of excess protein.

The triglycerol and cholesterol content was reduced for broilers fed diets T2, T3 and T4 however, those fed T3 diet had the lowest. This implies

that the calcium source had an influence in reducing the accumulation of triglyceride and cholesterol in the blood. This observation indicates that triglycerides and cholesterol synthesized in the liver from the components of digestion and absorption of calcium varied depending on the source of calcium in the diet. It was stated by Rezende et al. (2017) that factors, such as diet, sex and hormone concentration affect triglyceride content. The cholesterol values observed in this study ranged from 63.20 to 118.25 mg/dl, which is lower than the upper and lower limit values (100 to 250mg/dl) reported by Lumeji (2008) for chicken. The reduced serum cholesterol concentration obtained in this study with the dietary treatments indicates a positive influence on cholesterol synthesis for better meat quality. The serum glucose concentration in this study ranges from 83.25 to 97.05 mg/dl, which is lower than the normal range (180-250mg/dl) reported for broilers (Hazelwood, 2000). Despite the low values, broilers fed diets T2, T3 and T4 had higher serum glucose compared to T1. Brelaz et al., (2021) reported that lower values in glucose may be associated with prolonged fasting, severe liver disease, septicaemia or endocrine disorders. However, the low values observed across treatments in this study did not indicate low nutrient availability since the birds were fed ad-libitum. The layers fed the T2, T3 and T4 diets had lower ALT and AST compared to those fed the T1 diet. The lowest ALT and AST were recorded for layers fed T3 and T4, respectively. The reduction indicates that the inclusion of alternative materials as calcium sources did not negatively affect the integrity of the liver as an increase in these liver enzymes in blood circulation suggests damage to the liver (Lu, 2017).

The blood mineral of the hens in the present study indicated that Zn and Fe was highest for layers fed T3 and T4 diets. The increase in these trace minerals implies positive effect of dietary treatments. The trace minerals perform essential function in the poultry birds as iron deficiency results in anaemia, which predisposes animal to infection. The calcium source inclusion in layers diets through calcium metabolism resulted into increased Zn and Fe thereby promoting deposition in shell and increasing tibia strength. It was observed that layers fed T1 diet had increased blood calcium and phosphorus. The increase in blood calcium and phosphorus observed for the group of birds could be due to higher solubility of the Ca and P source. Nutrition is known to affect the mineral composition of the blood, so the calcium source will affect the mineral composition of the diet which reflects in the overall health status of the chickens (Attia et al., 2014). The layers fed T3 and T4 diets had reduced blood P and Ca. It is established that Ca, P and vitamin D are primary factors affecting eggshell formation and bone mineralization in layers (Attia et al., 2014). However, the reduced blood P and Ca did not result in poor egg shell formation for the group of layers which implies that P and Ca content were sufficient. High blood Mg was recorded for layers fed other diets except those fed T3 diet. The reduced Mg observed for layers fed T3 diet could be as a result of antagonistic effect due to increase in Zn in layers fed T3 diet. Arslan et al. (2018) observed interaction among minerals and the authors reported influence of boron supplementation on Ca, P, Mg and vitamin D metabolism.

The bone mineral composition shows that layers fed the T3 diet had the highest Ca, Fe, Mg and Zn followed by those fed the T4 diet. This result shows that bone dust as a calcium source can conveniently replace DCP in the diet of laying birds for good bone mineralisation. Zhang et al. (2020) indicated that reduced calcium and phosphorus levels in the serum which affects bone mineralisation are a consequence of reduced dietary calcium and phosphorus levels. It was also reported by Pelicia et al. (2011) that when serum calcium is inadequate due to poor intake, the hen reduces calcium synthesis in the eggshell and reduces its concentration in the bone. However, this is not the case in this study which implies that the inclusion of 50 and 100% bone dust in the diet of layers did not negatively affect calcium and phosphorus levels in the bone. The better Fe, Mg and Zn concentration in the bone of broilers fed T3 and T4 diets reveals that the dietary inclusion of bone dust as a calcium source did not cause an unnecessary rise

in calcium thereby preventing undue antagonistic effect on other minerals. Plumstead et al. (2008) stated that Ca has the potency to react with inorganic P in the intestinal lumen forming calcium orthophosphate which is insoluble and may cause unavailability of phosphorus for absorption. Yan et al. (2005) also indicated that elevated dietary calcium compared to phosphorus may result in competition for transporters by trace minerals. The outcome of the current study did not reflect any of these inadequacies as observed from the result of bone mineralisation

CONCLUSION

From the result of this study, the inclusion of bone dust in the diet of laying hens does not have a negative effect on the performance characteristics (HDEP, NEL) and egg quality traits (EST, albumin weight and height, HU), but rather it improved these parameters. The total protein and globulin increased while the triglyceride and cholesterol decreased with the inclusion of bone dust at 50 and 100% in the layers diet. The use of DCP increased Ca and phosphorus in the serum for layers but the use of 50 and 100% bone dust resulted in reduced Ca and P. Bone mineralisation was better for layers fed T3 and T4 diets. It can be recommended based on the result obtained that bone dust can be included in the diet of the hen as a suitable calcium source either in combination with DCP or its inclusion at 100%.

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