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Effects of dietary supplementation with breadnut and breadfruit leaf meals and a commercial growth promoter on ovarian development and bone characteristics of laying hens

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Abstract: The use of commercial growth promoter and phytoadditives in poultry production is a growing phenomenon because of the expected positive impact on health and productive performance. This study focuses on the comparative effect of a commercial growth promoter and two phytoadditives on ovarian development and bone characteristics of laying hens. Ninety 18-week old ISA Brown layers were randomly allotted to five dietary treatments in a Completely Randomized Design. Each treatment consisted of three replicates with 6 birds per replicate (18 birds per treatment). The treatments included: T1, a basal diet without additives; T2, basal diet supplemented with Nufocare P, a commercial growth promoter at 1 g/kg feed; T3, basal diet supplemented with 6 g of breadnut leaf/kg feed; T4, basal diet supplemented with 6 g of breadfruit leaf meal/kg feed and T5, basal diet supplemented with a combination of 3 g breadnut leaf and 3 g breadfruit leaf per kg feed. At the end of the 12-week feeding trial, ovarian follicular parameters and bone characteristics, including mineral composition, density and breaking strength were determined. The results showed that all supplemented diets significantly ($p < 0.05$) enhanced ovarian follicular development compared to the control. Also, birds fed the combined phytoadditives (T5) had the highest relative ovarian weight (2.53%) and the highest number of pre-ovulatory follicles (5.67). Commercial growth promoter increased the number of small follicles. Dietary treatments significantly ($p < 0.05$) affected total mineral content, macro minerals and bone yielding load, whereas trace mineral content, bone density and bone breaking strength were not significantly affected. The results indicate that dietary supplementation with Nufocare P (1 g/kg feed, T2) and a combined inclusion of breadnut and breadfruit leaf meals (3 g each/kg feed, T5) can promote ovarian development and improve specific bone traits in laying hens, highlighting their potential as natural alternatives to synthetic growth promoters.

Keywords: commercial growth promoter; breadnut leaf; breadfruit leaf; ovarian follicles; bone; laying hens

INTRODUCTION

Poultry nutrition research has increasingly turned towards the use of non-antibiotic growth promoters, including phytogenic feed additives to support laying hen's health and productivity. The search for sustainable and natural alternatives to conventional antibiotic growth promoters in poultry production has stimulated interest in phytogenic feed additives due to their multiple biological activities, including antimicrobial, antioxidant, immunomodulatory, and digestive-enhancing effects (Abdelli et al., 2021).

Breadfruit (*Artocarpus altilis*) and breadnut (*Artocarpus camansi*), members of the Moraceae family, are tropical perennial trees widely cultivated in Africa, Asia, and the Pacific. Beyond their conventional use as food crops, their leaves

have recently been characterized as rich in secondary phytochemicals including flavonoids, saponins, tannins, alkaloids, phenols, carotenoids, and phytosterols with appreciable antioxidant and antimicrobial properties (Muhammad et al., 2021; Fatoki et al., 2025).

Tugiyanti et al. (2016) reported that inclusion of breadfruit leaf powder in the diets of ducks positively influence fat deposition, plasma protein profiles, and haematological parameters, further supporting its safety and efficacy as a phytoadditive source. Despite these promising findings, literature specifically addressing the use of breadnut and breadfruit leaves as phytoadditives in layers production are limited.

In poultry industry, egg-laying production efficiency is a significant economic trait. Excellent productivity is measured by the number of ovarian follicles heading for ovulation and the efficiency of the oviduct to convert the ova into a hard-shelled egg. A follicular hierarchy that is well-organized is important for improved egg laying performance (Huang et al*.,* 2015). The laying performance of chickens is determined by the growth, development and function of chicken ovarian follicles (Johnson, 2015). The laying of eggs starts with an orderly development of follicles in the chicken ovaries until the eggs are produced. Therefore, without the progressive stages of ovarian follicles' growth and development, the chickens' egg production performance will decline (Li et al*.,* 2019).

According to Wang et al. (2017), the bird's egg production performance depends largely on the progressive phases of growth and development of ovarian follicles, categorized into 2 classes' *viz*. pre-hierarchical and hierarchical follicles or preovulatory follicles. Folliculogenesis is the process of the development of ovarian follicles from primordial follicles to a well-developed follicular hierarchy, which includes growth and multiplication of cells and differentiation before ovulation (Yu et al., 2016). The pre-hierarchical follicles include many small and large white follicles, which are about 2–5 mm in diameter and 5 to 6 small yellow follicles (SYF), which are about 5 - 10 mm in diameter. Hierarchical follicles, also known as pre-ovulatory follicles, are about 5 - 6 in number and above 10 mm in diameter (Wang et al*.,* 2017). The ovary of layers consists of an order of yellow yolky follicles, known as the pre-ovulatory follicles from F5 to F1 and many thousands of small follicles from which large yolky follicles are conscripted (Apperson et al., 2017). Johnson (2015) reported that follicles develop from a category of small yellow follicles into the pre-ovulatory hierarchy approximately once a day during the laying period, with a process called follicle selection.

Non-antibiotic growth promoters such as probiotics and phytogenic feed additives enhance poultry folliculogenesis via antioxidant, anti-inflammatory and gut-ovary axis modulation (Li et al., 2023; Kim et al., 2025). Probiotics improve nutrient absorption, stabilize intestinal integrity and reduce oxidative stress thereby sustaining granulosa cell function and follicle recruitment (Zhang et al., 2024).

Laying hens are prone to osteoporosis and bone fragility due to prolonged calcium mobilization for eggshell formation resulting in thin cortical bone, trabecular thinning, and frequent fractures (Whitehead, 2004). Monitoring bone morphometry (cortical thickness, bone density, mechanical strength) is essential to evaluate interventions that enhance skeletal integrity. Phytoadditives with antioxidant, anti-inflammatory, or mineral-modulating properties have emerged as natural alternatives to antibiotics and synthetic supplements for improving poultry health (Seidavi et al., 2022) and bone structure. Given the increasing regulatory restrictions on antibiotics growth promoters and consumer demand for residue-free poultry products, exploring underutilized tropical plant resources such as breadfruit and breadnut leaves aligns with global efforts to enhance poultry health and productivity through sustainable feed strategies. Therefore, systematic evaluation of these botanicals as phytoadditives could advance both animal production and food safety objectives, particularly in tropical regions where these plant species are abundant and economically accessible (Aminullah et al., 2025). The objective of this study is to compare the effect of diet supplementation with a commercial growth promoter (Nufocare P), breadnut and breadfruit

leaf meals on the ovarian development and bone characteristics of brown-egg type layers.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Layer's Production Unit of the Teaching and Research Farm, Ladoke Akintola University of Technology, Ogbomoso, Southwest Nigeria. Ogbomoso is located in the derived savanna zone that lies on longitude $4^0$16 ' East of Greenwich meridian and latitude $8^0$8 ' north of the Equator. The latitude ranges from 300 m and 600 m above sea level while mean temperature and annual rainfall are 27^0C and 1247 mm (Google Earth Map, 2025).

Procurement and preparation of test ingredients

The commercial growth promoter (Nufocare P), containing citric acid, lactic acid, calcium formate, and calcium propionate, was purchased from Agricare STD Plus Limited, Oluyole, Ibadan, Nigeria, and included in the diet at the manufacturer's recommended dosage of 1 g/kg of feed. Breadnut and breadfruit leaves were harvested from villages within the Ogbomoso metropolis, Southeast Nigeria where the plants are abundant. The leaves were air-dried at room temperature until crispy and subsequently milled into powder for dietary inclusion.

Experimental diets and dietary treatments

Five experimental diets were formulated to represent five dietary treatments (T1 – T5) as follows:

T1: Control diet without inclusion of commercial growth promoter, breadfruit leaf, or breadnut leaf.

T2: Control diet supplemented with commercial growth promoter (Nufocare P) at 1 g/kg of feed.

T3: Control diet supplemented with breadnut leaf meal at 6 g/kg of feed.

T4: Control diet supplemented with breadfruit leaf meal at 6 g/kg of feed.

T5: Control diet supplemented with a combination of breadnut leaf meal (3 g/kg) and breadfruit leaf meal (3 g/kg) of feed.

The gross composition of the experimental diets is presented in Table 1.

Experimental animals and management

Ninety birds (90) hybrid strain of ISA Brown layers at 18 weeks old purchased from a reputable farm with up-to-date vaccination and medication records were used for the study. At the commencement of the experiment the birds were weighed and distributed into five (5) treatment groups and the treatments were replicated three times with six (6) birds per replicate and eighteen (18) birds per treatment in a Completely Randomized Design. The birds were housed in a three-tier battery cage system and stocked at a density of three birds per cage cell (42 × 42 × 35 cm), providing a floor space allowance of about 600 cm² per bird. The birds were offered similar quantity of feed (120 g) and fresh water *ad libitum* on a daily basis.

Data collection

Ovarian development

At week 12 of the experiment, the live weight of the birds was measured, after which two birds per replicate were sacrificed following institutional animal care and use guidelines. The ovaries were excised, and all visible follicles were removed and weighed using a digital balance. The number of pre-ovulatory follicles, large follicles and small follicles were counted with the aid of a magnifying lens. The reproductive tracts were removed, weighed and lengths were measured with a measuring tape. The large and small follicles diameters were measured with a vernier caliper and then the values were recorded. The percentage ovarian weights were calculated by using the following formula:

$$\% \text{ Ovarian weight} = \frac{\text{Weight of Ovary (g)}}{\text{Live weight of the bird (g)}} \times 100$$

Bone minerals composition and bone density

The right femur was carefully excised from each carcass. All adhering muscles, connective

Table 1. Gross composition of experimental diets

Ingredients (%)	T1	T2	T3	T4	T5
Maize	45.00	45.00	45.00	45.00	45.00
Soyabean meal	19.80	19.80	19.80	19.80	19.80
Wheat offal	17.00	17.00	17.00	17.00	17.00
Fish meal (72%)	2.50	2.50	2.50	2.50	2.50
Palm kernel cake	3.00	3.00	3.00	3.00	3.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Limestone	9.00	9.00	9.00	9.00	9.00
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10
*Vitamin Mineral Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Nufocare P	-	0.10	-	-	-
Breadnut leaf meal	-	-	0.60	-	-
Breadfruit leaf meal	-	-	-	0.60	-
Breadnut + Breadfruit leaf meals	-	-	-	-	0.3 + 0.3
Calculated Analysis					
Crude protein (%)	17.16	17.16	17.16	17.16	17.16
ME (Kcal/Kg)	2598.50	2598.50	2598.50	2598.50	2598.50
Crude fibre (%)	3.72	3.72	3.72	3.72	3.72
Ether extract (%)	3.42	3.42	3.42	3.42	3.42
Calcium (%)	4.05	4.05	4.05	4.05	4.05
Phosphorus (%)	0.55	0.55	0.55	0.55	0.55
Lysine (%)	1.01	1.01	1.01	1.01	1.01
Methionine (%)	0.40	0.40	0.40	0.40	0.40
Price/kg of feed in Euros (€)	0.46	0.47	0.47	0.47	0.47

**Vitamin mineral premix supplied the following vitamins and trace minerals per kg diet: Vitamin A (14,000 IU),Vitamin D3 (3,500 IU), Vitamin E (20 IU), Vitamin K (2.4 mg), Vitamin B1 (1.8 mg), Vitamin B2 (5 mg), Vitamin B6 (1.8 mg), Vitamin B12 (0.012 mg), Niacin (18.4 mg), Pantothenic Acid (6 mg), Folic Acid (0.7 mg), Biotin (0.05 mg), Choline (240 mg), Manganese (96 mg), Zinc (60 mg), Iron (40 mg), Copper (8 mg), Iodine (1.4 mg), Selenium (0.24 mg), Cobalt (0.25 mg), Antioxidant (0.125 mg).*

ME: Metabolizable energy

Source: Authors' own elaboration

tissues and cartilage were gently removed without damaging the bone surface the weight of the right thigh bones were measured, and sun dried to a constant weight before taking to the laboratory for further analysis. Bone minerals and bone density were analyzed using the procedure of AOAC (2023).

Determination of bone breaking strength and yielding load

Bone mechanical properties were assessed using a dual-point support fixture. Each dried bone was weighed to the nearest 0.01 g and mounted horizontally. Compressive force was applied centrally. Breaking strength was determined using

calibrated weights and expressed in kilogram-force (kgf), while yielding load was measured with a load cell (500–1000 N capacity) and recorded in Newtons (N).

Statistical analysis

Data were processed using IBM SPSS Statistics, version 25.0. Results are presented as means ± SEM. Statistical differences were assessed using one-way ANOVA followed by Duncan's Multiple Range Test for post hoc comparisons. A p-value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The effect of dietary treatments on ovarian development of laying hens is shown in Table 2. Dietary treatments significantly ($p < 0.05$) influenced all the parameters measured except reproductive tract length and weight. The live weights of laying hens on dietary Treatments 2 to 5 were similar to the control (1.58 kg). Laying hens on T2 and T5 had the heaviest (35.33 and 37.33 g), whole ovary weight, while hens fed diet supplemented with 3 g of breadnut + 3 g of breadfruit leaf meal (T5) had the highest relative ovarian weight of 2.53 %. The number of pre-ovulatory follicles were significantly ($p < 0.05$) higher in Treatments 2 to 5 than the control (4.00). However, hens in the control group had the highest number of large follicles (17.00) while hens fed diet supplemented with commercial growth promoter (T2) had the highest number of small follicles (35.00). The diameter of large follicles significantly reduced in T3 (2.77 mm) while T4 had the largest diameter (5.40 mm). Pre-ovulatory follicle diameter was highest in T4 (20.40 mm).

Visual observations of ovarian follicles were documented for each treatment group. Figure 1 (A–E) presents representative images from hens fed the control diet (T1), the commercial growth promoter (T2), breadnut leaf meal (T3), breadfruit leaf meal (T4), and their combination (T5). These photographs illustrate the macroscopic appearance of the follicles and are intended solely for descriptive purposes.

Live weight was similar across the dietary treatments which suggest that the growth promoter and phytoadditives did not adversely affect the general health or body weight of the laying

Table 2. Ovarian development of laying hens fed diet supplemented with commercial growth promoter (Nufocare P) and two phytoadditives

Parameters	T1	T2	T3	T4	T5	SEM	p-value
Live weight (kg)	1.58^{ab}	1.56^{b}	1.60^{a}	1.57^{ab}	1.57^{ab}	0.01	0.01
Whole ovary weight (g)	26.00^{c}	35.33^{a}	32.67^{b}	29.00^{c}	37.33^{a}	1.52	0.05
% ovarian weight	1.67^{c}	2.18^{b}	2.03^{b}	1.88^{b}	2.53^{a}	0.10	0.01
Number of POF	4.00^{b}	5.00^{a}	5.00^{a}	5.00^{a}	6.00^{a}	0.17	0.02
Number of LF	17.00^{a}	11.00^{b}	10.00^{b}	10.00^{b}	11.00^{b}	0.89	0.01
Number of SF	26.00^{b}	35.00^{a}	17.00^{c}	28.00^{b}	27.00^{b}	1.72	0.05
LF diameter (mm)	5.00^{a}	4.33^{b}	2.77^{c}	5.40^{a}	3.80^{b}	0.32	0.02
POF diameter (mm)	15.43^{b}	17.60^{b}	12.57^{c}	20.40^{a}	17.40^{b}	0.87	0.03
RT length (cm)	74.00	75.33	70.67	68.33	76.00	2.10	0.36
RT weight (g)	57.67	59.67	51.00	62.00	53.00	2.18	0.18

^{abcd} Means within each row with different subscripts are significantly different ($p<0.05$) SEM: Standard Error of Mean
LF: Large follicles, SF: small follicles, POF: Pre-ovulatory follicles, RT: Reproductive tract
T1: Control diet (no inclusion level of breadnut and breadfruit leaf meal; T2: 1g of commercial growth promoter/kg of feed; T3: 6g of breadnut leaf meal/kg of feed; T4: 6g of breadfruit leaf meal/kg of feed; T5: 3g of breadnut + 3g of breadfruit leaf meal/kg of feed.
Source: Authors' own elaboration

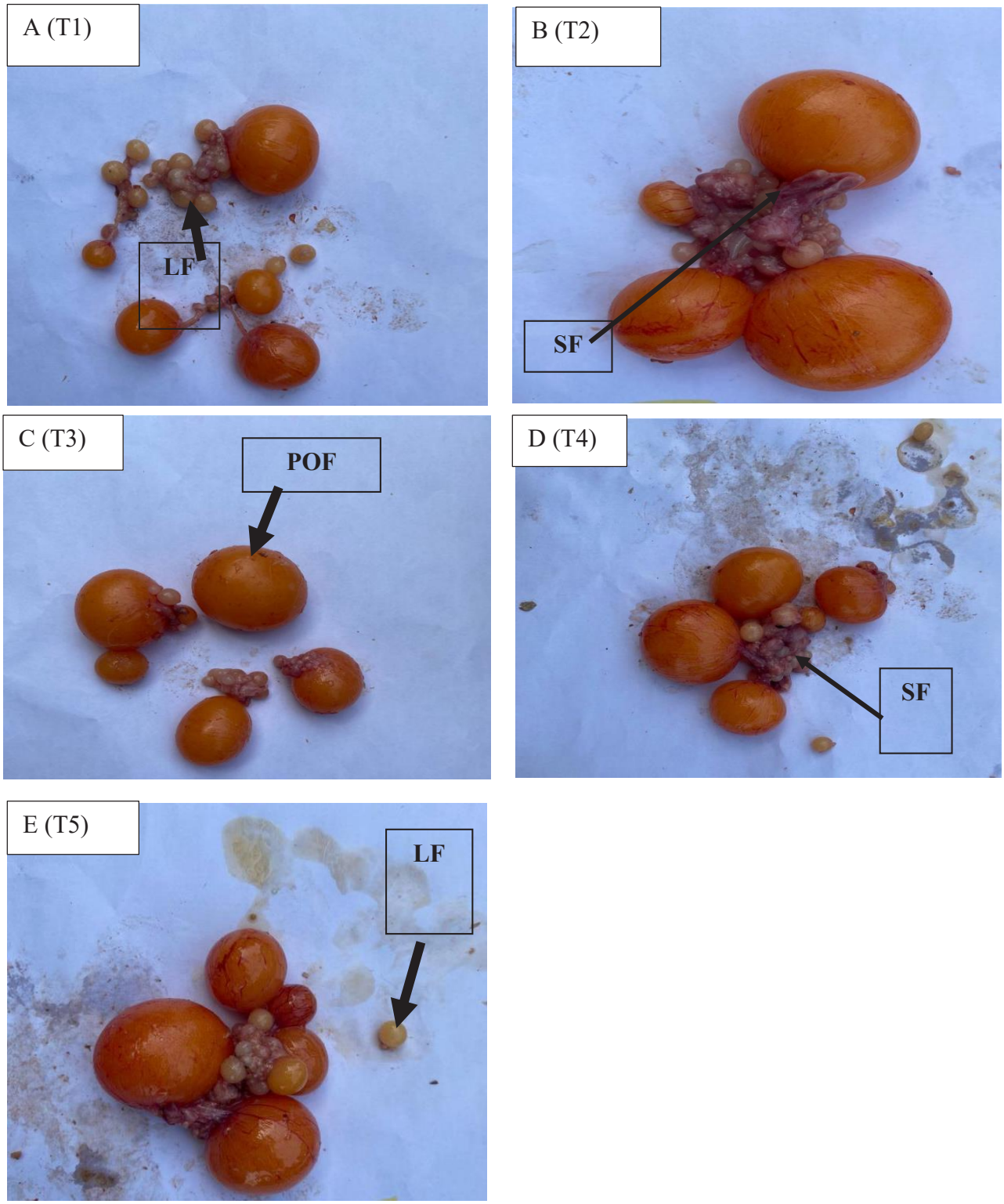


Figure 1 (A – E). Follicles of laying hens fed diet supplemented with commercial growth promoter and two phytoadditives

A (T1) - Control, B (T2) – 1 g of commercial growth promoter (Nufocare P) /kg of feed, C (T3) – 6 g of breadnut leaf meal/ kg of feed, D (T4) – 6 g of breadfruit leaf meal/kg of feed, E(T5) – 3 g of breadnut + 3 g of breadfruit leaf

Source: Authors' own elaboration

hens. This agrees with the report of Mehran et al. (2021), who reported that herbal additives do not negatively affect body weight but instead improve feed conversion and egg quality. The highest relative ovarian weight observed in Treatment 5 suggests a synergistic effect of combined phytoadditives (breadnut and breadfruit leaf meal). This agrees with the findings of Ahmed et al. (2021), who observed enhanced ovarian follicle development in hens fed with combined plant-based additives rich in flavonoids and saponins, which stimulate estrogen secretion and ovarian activity. The highest numbers of small follicles recorded in hens fed diet supplemented with commercial growth promoter implies that the commercial growth promoter may enhance early follicular conscription. However, the control group had the highest number of large follicles (17) while other treatments ranged between 10 and 11, this could be attributed to a delay in follicle recruitment or turnover in the control group while supplemented groups might have progressed further into ovulation cycles leaving fewer follicles in the large follicle category as at the sampling time. This result is in line with the report of Olarotimi et al. (2024), who suggested that phytoadditives may accelerate follicular maturation, causing a faster transition through the follicular hierarchy. The largest diameter observed in Treatment 4 suggests that breadfruit leaf meal may support follicle growth better than breadnut leaf meal alone. According to the findings of Li et al. (2019), dietary antioxidants from leafy plant materials enhanced blood supply and nutrient delivery to ovarian follicles which leads to improved follicle development. There were no significant differences in reproductive tract length and weight across the treatments, indicating that the additives primarily influenced ovarian growth without markedly affecting the gross anatomy of the reproductive system. This observation is consistent with the work of Li et al. (2019), who noted that while phytobiotics improved ovarian function, they had limited impact on reproductive tract size unless administered over prolonged periods.

The total minerals, macro minerals and bone yielding load were significantly ($p < 0.05$) affected by the dietary groups while the trace minerals,

Table 3. Bone minerals composition, bone density and strength of laying hens fed diets, supplemented with commercial growth promoter (Nufocare P), Breadnut and Breadfruit leaf meals

Parameters	T1	T2	T3	T4	T5	SEM	p-value
Total Minerals (%)	37.71^{a}	38.20^{a}	34.71^{ab}	32.70^{b}	35.42^{ab}	0.62	0.05
Calcium (%)	40.74^{a}	41.31^{a}	39.05^{b}	40.45^{ab}	41.33^{a}	0.31	0.03
Phosphorous (%)	14.80^{a}	14.68^{a}	13.84^{b}	14.15^{a}	14.99^{a}	0.16	0.05
Potassium (%)	1.69^{a}	1.72^{a}	1.36^{b}	1.52^{b}	1.70^{a}	0.14	0.02
Magnesium (%)	0.60^{a}	0.60^{a}	0.39^{c}	0.46^{b}	0.59^{a}	0.32	0.04
Iron (µg/g)	158.95	158.36	142.94	148.56	160.65	2.68	0.06
Zinc (µg/g)	165.18	165.79	154.86	153.89	167.52	2.31	0.09
Sodium (µg/g)	0.60	0.62	0.55	0.57	0.64	0.01	0.06
Copper (µg/g)	14.32	14.19	12.94	13.43	14.24	0.21	0.06
Bone density (g/cm^{3})	0.81	0.81	0.78	0.79	0.80	0.01	0.12
Bone breaking strength (kgf)	32.23	31.70	30.02	28.70	31.51	0.56	0.08
Bone yielding load (N)	320.86^{b}	393.45^{a}	398.20^{a}	312.41^{c}	328.64^{b}	3.56	0.01

^{abc} Means on the same row with different superscripts are significantly different ($p<0.05$) SEM: Standard Error of Mean
T1: Control diet (no inclusion level of breadnut and breadfruit leaf meal; T2: 1g of commercial growth promoter/kg of feed; T3: 6g of breadnut leaf meal/kg of feed; T4: 6g of breadfruit leaf meal/kg of feed; T5: 3g of breadnut + 3g of breadfruit leaf meal/kg of feed.

Source: Authors' own elaboration

bone density and bone breaking strength were not significantly affected as shown in Table 3. Laying hens fed the control (T1) and diet supplemented with commercial growth promoter (T2) were similar ($p > 0.05$) and had higher total minerals (37.71 and 38.20%) than others while hens fed diet supplemented with breadfruit leaf meal (T4) had the lower value (32.70). Calcium, phosphorus, potassium and magnesium were significantly ($p < 0.05$) higher in T1 (40.74, 14.80, 1.69, 0.60%), T2 (41.031, 14.68, 1.72, 0.60%) and T5 (41.33, 14.99, 1.70, 0.59%) while hens fed diet supplemented with breadnut leaf meal (T3) had the least values (39.05, 13.84, 1.36, 0.39%). However, laying hens fed diet supplemented with commercial growth promoter (T2) and breadnut leaf meal (T3) had the highest ($p < 0.05$) bone yielding load (393.45 and 398.20 N) while hens fed dietary breadfruit leaf meal (T4) had the lowest bone yielding load (312.41 N).

The higher total mineral content and bone yielding load observed in laying hens supplemented with the commercial growth promoter (Nufocare P) suggest improved mineral retention. However, since bone density and breaking strength remained unaffected, the skeletal integrity was not compromised across treatments. These findings are consistent with previous studies on alternative feed additives (Hossain et al., 2021). Breadnut leaf meal reduced macro mineral levels but was associated with increased bone yielding load, possibly due to phytochemical effects. In contrast, breadfruit leaf supplementation led to the lowest mineral retention and yielding load, potentially due to its high fibre content, which may impair mineral absorption (Olukosi & Adebiyi, 2013; Bello et al., 2021). The combined inclusion of breadnut and breadfruit leaf meal appeared to mitigate individual limitations, resulting in mineral profiles closer to those of the control group.

CONCLUSION

The findings of this study demonstrate that dietary supplementation with a combination of breadnut and breadfruit leaf meals (3 g each/kg feed) effectively supported ovarian development and bone mineral composition, yielding outcomes comparable to the commercial growth promoter (Nufocare P). While individual supplementation with breadfruit leaf meal negatively affected mineral retention, the combined inclusion mitigated these effects, suggesting a beneficial associative action. Therefore, the combined use of 3 g/kg each of breadnut and breadfruit leaf meal is recommended as a viable natural alternative to synthetic growth promoters for supporting the physiological status of laying hens.

Conflict of interest

Absence of conflict of interest

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