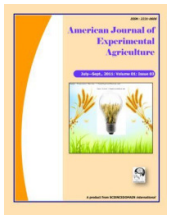

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Effect of Dietary Replacement of Soyabean Meal with Toasted Sesame Seed on Performance, Tibia Bone Mineralisation and Gut Morphology of Broilers Chicken

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Authors' contributions

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

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ABSTRACT

Aims: This experiment was conducted to assess the effect of dietary replacement of soybean meal with toasted Sesame seed on performance, tibia bone mineralization and gut morphology of broiler chicken.
Study Design: The design of the study was a completely randomized design.
Place and Duration of Study: The experiment was carried out at Poultry Unit, Teaching and Research Farm of University of Ibadan, Nigeria and lasted for 8 weeks.
Methodology: A total of 150 one-day old broiler birds were randomly allotted into 5 experimental treatments with 3 replicates each (10 birds per replicate) in a completely randomized design. Toasted sesame seed supplemented with 600 ppm microbial phytase was replaced in the dietary treatments at 0%, 10%, 20%, 30% and 40% for Soyabean meal both at the starter and finisher phases.
Feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were measured and calculated weekly throughout the experimental period. Six birds per treatment were selected at the

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end of the experiment, the birds were then slaughtered by cervical dislocation and the two tibia bones were removed and cleaned of all adhering muscle and cartilaginous caps for tibia bone mineralization, section of ileum and jejunum tissues were also collected for gut morphological parameters. All data obtained were analyzed with one way ANOVA using SAS 1990 software at 5% probability level while means were compared using Duncan Multiple Range Test.

Results: There were significant ($P<0.05$) differences in the performance characteristics across the treatments with birds fed 10% TSS having the highest weight gain of 54.36g/bird/day compared to the control of 38.71g/bird/day (0%TSS). The FCR in birds fed 10% TSS (1.62) and 20% TSS (1.72) revealed a better feed utilisation compared to birds fed diet 5 which recorded the highest FCR value of 3.72.The concentration of Ca, Mg, Zn, Fe and PO_4 in the tibia were significantly ($P<0.05$) different across the treatments. The villus height was highest in treatment 4(5930.2 µm) and the least in the control (2456.5 µm) in the ileum but in the jejunum, the control was the highest (4292.6 µm) and least in treatment 2 (2859.5 µm). Crypt depth were significantly ($P<0.05$) different across the treatments in both the ileum (1736.36 – 836.34 µm) and jejunum (1269 – 605.5 µm).

Conclusion: It can therefore be concluded that replacement of Soybean meal with toasted Sesame seed up to 20% inclusion level in broiler ration results in better performance and improved health condition of broiler birds.

Keywords: Soybean; toasted sesame seed; replacement levels; tibia mineralization and gut morphology.

1. INTRODUCTION

Legumes containing anti-nutritional factors in seeds are a major factor limiting the exploitation of alternative grain legumes as protein source for poultry [1]. Anti-nutritional factors have negative effects on digestion and performance of fowls [2].

Soybean meal (SBM) has long been considered the best source of supplemental protein in diets for poultry. In fact, it is often referred to as the "gold standard" among intact protein sources used in the animal feed industry [3]. However, SBM is becoming extremely expensive in the last decade especially in developing countries, and also the non availability of this SBM at an economically viable price, make the use of alternative protein source in poultry diets necessary. Therefore, search for alternative vegetable protein sources, which are cheap and locally available, has become an urgent subject to poultry nutritionists [4] and one of these available alternate protein source is Sesame (*Sesamum indicum*).

Sesame also known as benniseed is one of the ancient oil seed crops used by man and when used in the right proportions together with SBM which has a higher content of lysine; a balanced diet with respect to lysine and methionine will result [5]. Sesame seeds are also good source of manganese, copper, calcium, magnesium, iron, phosphorus, vitamin B1, zinc and other substances which limit their use in monogastric animal feed. Incidentally, both plants (Soyabean and Sesame) are high in phytic phosphorus which reduces phosphorus and calcium availability however, this adverse effect could possibly be overcome by dietary supplementation with exogenous phytase [6]. It has been demonstrated that the supplementation of poultry diets with exogenous phytases improves mineral retention, bone strength, feed conversion ratio (FCR), amino acid digestibility coefficients and other performance parameters [7].

Appropriate nutritional levels promote the normal development of bone tissue [8]; therefore, nutritional deficiencies can cause bone structure alterations. Calcium (Ca) is the most abundant mineral in the body of animals [9]. Well over 90% of Ca is found in the bones where it combines with phosphorus, the second most abundant mineral in bone.

Differentiation of the epithelial cells along the length of the gut continues to be a source of interesting information about the development and function of the digestive system.

2. MATERIALS AND METHODS

The experiment was carried out at the Poultry Unit, Teaching and Research Farm, University of Ibadan, Nigeria. A total of 150 one day-old broiler birds were used in this experiment. The birds were then divided into 5 dietary treatments, consisting of 3 replicates with 10 birds per replicate in a completely randomized design. The management of the birds was carried out according to the procedure of Oluyemi and Robert [10].

2.1 Experimental Diets

- Diet 1 =Diet contained 40% Soybean meal without toasted Sesame seed and Pyhtase (Control)
- Diet 2 = Diet contained 30% Soybean meal with 10% toasted Sesame seed and Phytase (600ppm)
- Diet 3 = Diet contained 20% Soybean meal with 20% toasted Sesame seed and Phytase (600ppm)
- Diet 4 = Diet contained 10% Soybean meal with 30% toasted Sesame seed and Phytase (600ppm)
- Diet 5 = Diet contained no Soybean meal with 40% toasted Sesame seed and Phytase (600ppm)

The sesame seeds were purchased from a local market and were toasted until a golden brown colour was achieved. It was then removed immediately from the heat source to prevent burning. The experimental diets are shown in Tables 1 and 2.

2.2 Data Collection

2.2.1 Performance parameters

The weekly feed intake and weight gain were recorded. Feed conversion ratio was calculated during the 8 weeks experimental period.

2.2.2 Histo-morphology of the small intestine

All birds were randomly selected, weighed and then slaughtered by cervical dislocation. Samples (section of ileum and jejunum tissues) were collected from two birds per replicate (a total of 6 birds per treatment). The contents of the jejunum and ileum were carefully hand-stripped. Segments measuring 2 cm in length from the mid-points of jejunum and ileum were excised, washed in physiological saline solution, and fixed in 10% buffered formalin. The tissue samples were later embedded in paraffin, and a 2 mm section of each sample was placed on a glass slide and stained with haematoxylin and eosin. Histological sections were examined with a phase contrast microscope coupled with an integrated digital imaging analysis system (Biowizard 4_2, Dewinter Optical Inc., Delhi, India). The variables measured were villus height, crypt depth and thickness of the muscularis mucosae. Villus height was measured from the top of the villus to the top of the lamina propria, and the crypt depth was measured from the base up to the region of transition between the crypt and villus. Altogether 10 measurements were taken per bird for each variable; for purposes of statistical analysis, the average of these values was used [11].

Table 1. Gross composition of the starter diet

Ingredient (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Maize	52.40	52.30	52.20	52.00	51.90
SBM	40.00	30.00	20.00	10.00	0.00
Fish meal	2.00	2.00	2.00	2.00	2.00
Toasted SS	0.00	10.00	20.00	30.00	40.00
Palm oil	2.90	2.90	2.90	2.90	2.90
Lysine	0.10	0.15	0.25	0.45	0.55
Limestone	1.00	1.00	1.00	1.00	1.00
DCP	1.00	1.00	1.00	1.00	1.00
Methionine	0.15	0.15	0.15	0.15	0.15
Salt	0.25	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**	**100**
Crude protein %	22.80	22.85	22.80	22.79	22.79
ME kcal/kg	3020	3016	3009	3000	2995
Calcium%	1.00	1.10	1.13	1.12	1.20
Total phosphorus%	0.53	0.54	0.56	0.56	0.60

SBM: Soybean Meal, DCP: Di-Calcium Phosphate, Toasted SS: Toasted Sesame Seed, ME: Metabolizable Energy

Table 2. Gross composition of the finisher diet

Ingredient (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Maize	50.20	50.20	50.20	50.20	50.20
SBM	40.00	30.00	20.00	10.00	0.00
Toasted SS	0.00	10.00	20.00	30.00	40.00
Palm oil	1.00	1.00	1.00	1.00	1.00
Wheat bran	5.00	5.00	5.00	5.00	5.00
Lysine	0.15	0.15	0.15	0.15	0.15
Limestone	1.50	1.50	1.50	1.50	1.50
DCP	1.50	1.50	1.50	1.50	1.50
Methionine	0.15	0.15	0.15	0.15	0.15
Salt	0.25	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**	**100**
Crude protein (%)	22.02	22.03	22.04	22.05	22.06
ME (kcal/kg)	2855	2853	2851	2849	2847
Calcium(%)	1.05	1.20	1.23	1.25	1.30
Total phosphorus(%)	0.50	0.60	0.61	0.62	0.65

SBM: Soybean Meal, DCP: Di-Calcium Phosphate, Toasted SS: Toasted Sesame Seed, ME: Metabolizable energy

2.2.3 Bone mineralization

At 56, days two chicks from each of the 15 replicates were selected and slaughtered by cervical dislocation and their tibia were removed and cleaned of all adhering muscle and cartilaginous caps after which they were de-fatted by soxhlet extraction using petroleum ether. Fat-free dry tibias were ashed at 600±30ºC for 12hour. The fat-free tibias were ground and approximately 200 mg taken for analysis. The amount of calcium, phosphorus, magnesium and zinc in the tibia ash were determined spectrophotometrically using the standard curve according to AOAC [12].

2.2.4 Statistical analysis

All data obtained were subjected to statistical analysis of variance (ANOVA) using SAS [13] software at 5% probability level and significant treatment means were separated using Duncan's multiple Range test.

3. RESULTS

The result from Table 3 on the performance characteristics revealed no significant (P>0.05) differences in the initial weight. The final weight were significantly different (P<0.05) across the treatments with birds on 10% toasted Sesame seed (TSS) having the highest final body weight of 2800 g/bird and their counterpart on 40% SS having the least weight of 870 g/bird. The result of weight gain also showed significant (P<0.05) differences across the treatments with birds on 10% TSS having the highest weight gain of 54.36g/bird/day and birds on 40%TSS having the least of 14.97g/bird/day. However, no significant (P>0.05) differences were observed in the feed intake of birds fed Control (without toasted Sesame inclusion), 10%TSS and 20%TSS diets with mean values of 86.80, 88.03 and 85.5 g/bird/day respectively. The result also revealed significant (P<0.05) differences in the feed conversion ratio of the birds across the treatments with birds on 40% TSS having the highest value of 3.72 while birds on 10%TSS and 20%TSS had least significant values of 1.62 and 1.72 respectively.

The result of tibia bone mineralisation of birds fed dietary replacement of SBM with TSS as observed in Table 4 showed that the Calcium (Ca) level in the tibia of the experimental birds differed (P<0.05) significantly across the treatments with bone from birds on 20%TSS having the highest mean value of 552.75 mg/100g and the least in birds fed 40%TSS (464.25 mg/100g). The iron and zinc values obtained as shown in the table showed there were significant differences (P<0.05) in birds fed the control diet and the others. Birds on diets 1,2 and 3 were not significantly different for magnesium, the highest value was obtained in birds fed 10%TSS (26.83 mg/100 g) and the lowest mean value (18.05 mg/100g) was obtained in birds fed the control diet. The highest

value (375.00 mg/100g) was obtained in birds fed 10%TSS for phosphate while the lowest mean value (252.75 mg/100g) was obtained in birds fed 40%TSS. Generally, there was a decrease in the values across the treatment as the inclusion of TSS increases.

The result as shown in Table 5 indicated a significant (P<0.05) difference for ileum in the values recorded for villus height of birds fed the experimental diets, birds fed diets 3 and 4 that were replaced with 20% and 30% TSS were significantly higher (5561.1 and 5930.2 µm) respectively than others. Although birds fed diets 2 and 5 were not significantly (P>0.05) different from one another, they were significantly higher than the control. The trend observed for the crypth depth of the ileum showed a significant (P<0.05) difference with the highest value in birds fed diet 4(1736.36 µm). Birds on diet 3(1333.43 µm) were numerically higher but not significantly different from those on diet 2(1258.28 µm) and birds on diet 5(836.34 µm) had the least mean value. The smooth muscular width of ileum in this measurement showed highest mean value in birds fed diet 2(4763.4 µm).

Data obtained for jejunum revealed significant differences (P < 0.05) in all the parameters measured. The values recorded for villus height in birds fed diets 3 (3944.8 µm) and 4 (4075.1 µm) were not significantly (P > 0.05) different from the control (4292.6 µm), however, significantly (P < 0.05) lower value was recorded for birds on diets 2 and 5. Crypth depth showed significantly higher (P < 0.05) values in birds fed diets 2, 3 and 4 while the value recorded for those on diet 5 (605.5 µm) was not significantly different from the control (798.6 µ). Result recorded for the smooth muscular width in the jejunum also showed highest mean value in birds fed diet 2(4600.7 µm) and least mean value in birds on diet 5(1461.0 µm), though not significantly (P > 0.05) different from birds on diet 4 and the control.

Table 3. Performance characteristics of birds fed dietary replacement of soybean meal with toasted sesame seed supplemented with phytase

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM
Initial weight (g/bird)	140.00	136.67	123.33	126.67	136.67	5.37
Final weight (g/bird)	2036.67^{c}	2800.00^{a}	2556.67^{b}	1573.33^{d}	870.00^{c}	64.24
Weight gain (g/bird/day)	38.71^{c}	54.36^{a}	49.66^{b}	29.52^{d}	14.97^{c}	1.34
Feed intake (g/bird/day)	86.80^{a}	88.03^{a}	85.50^{a}	73.95^{b}	55.51^{c}	2.54
FCR	2.25^{b}	1.62^{c}	1.72^{c}	2.51^{b}	3.72^{a}	0.09

^{abcd} Means along the row with the same superscript are not significantly (P>0.05) different SEM = Standard Error of Means FCR = Feed Conversion Ratio. Diet 1 =Diet contained 40% Soybean meal without toasted Sesame seed and Pyhtase (Control) , Diet 2 = Diet contained 30% Soybean meal with 10% toasted Sesame seed and Phytase (600ppm), Diet 3 = Diet contained 20% Soybean meal with 20% toasted Sesame seed and Phytase (600ppm), Diet 4 = Diet contained 10% Soybean meal with 30% toasted Sesame seed and Phytase (600ppm), Diet 5 = Diet contained no Soybean meal with 40% toasted Sesame seed and Phytase (600ppm)

Table 4. Tibia bone mineralization of birds fed dietary replacement of soybean meal with toasted sesame seed supplemented with phytase

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM
Ca(mg/100g)	484.33^{b}	552.75^{a}	531.75^{a}	478.75^{b}	464.25^{b}	13.28
Fe(mg/100g)	8.42^{a}	7.10^{b}	7.10^{b}	7.01^{b}	6.95^{b}	0.16
Zn(mg/100g)	1.98^{a}	1.73^{ab}	1.62^{ab}	1.52^{b}	1.33^{b}	0.14
Mg(mg/100g)	18.05^{b}	26.83^{a}	26.65^{a}	22.95^{ab}	21.48^{ab}	1.74
PO_4(mg/100g)	323.50^{ab}	375.00^{a}	329.75^{ab}	260.63^{b}	252.75^{b}	25.85

^{abcd} Means along the row with the same superscript are not significantly (P>0.05) different, SEM = Standard Error of Means FCR = Feed Conversion Ratio. Diet 1 =Diet contained 40% Soybean meal without toasted Sesame seed and Pyhtase (Control) , Diet 2 = Diet contained 30% Soybean meal with 10% toasted Sesame seed and Phytase (600ppm), Diet 3 = Diet contained 20% Soybean meal with 20% toasted Sesame seed and Phytase (600ppm), Diet 4 = Diet contained 10% Soybean meal with 30% toasted Sesame seed and Phytase (600ppm), Diet 5 = Diet contained no Soybean meal with 40% toasted Sesame seed and Phytase (600ppm), Ca: Calcium Fe: Iron Zn: Zinc Mg: Magnesium PO_4: Phosphate

Table 5. Histological parameters of birds fed dietary replacement of soybean meal with

Parameters	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	SEM
Ileum (μm)	2456.5^{c}	3383.2^{b}	5561.1^{a}	5930.2^{a}	3264.3^{b}	207.14
Villus height						
Crypt depth	1108.17^{c}	1258.28^{bc}	1333.43^{b}	1736.36^{a}	836.34^{d}	50.22
SMW	1675.3^{c}	4763.4^{a}	2779.8^{b}	1986.3^{c}	1580.9^{c}	116.77
Jejunum (μm)	4292.6^{a}	2859.5^{b}	3944.8^{a}	4075.1^{a}	3227.7^{b}	220.48
Villus height						
Crypt depth	798.6^{b}	1147.5^{a}	1264.9^{a}	1269.4^{a}	605.5^{b}	77.92
SMW	1711.3^{cd}	4600.7^{a}	3122.9^{b}	2662.2^{bc}	1461.0^{c}	301.66

^{abcd}: Means along the row with the same superscript are not significantly different (P>0.05)

SEM: Standard error of mean SMW: Smooth muscular wall. Diet 1 =Diet contained 40% Soybean meal without toasted Sesame seed and Pyhtase (Control) , Diet 2 = Diet contained 30% Soybean meal with 10% toasted Sesame seed and Phytase (600ppm), Diet 3 = Diet contained 20% Soybean meal with 20% toasted Sesame seed and Phytase (600ppm), Diet 4 = Diet contained 10% Soybean meal with 30% toasted Sesame seed and Phytase (600ppm), Diet 5 = Diet contained no Soybean meal with 40% toasted Sesame seed and Phytase (600ppm)

4. DISCUSSION

The highest values obtained for body weight gain in diets 2 and 3, (10%TSS and 20%TSS respectively) suggests the possible influence of effective substitution of SBM with TSS on the weight gain. Sesame has been suggested as an acceptable alternative to SBM in broiler rations when substituted at level 15% or less [14,15]. Broilers performance was depressed by feeding a diet containing 30% Sesame seed meal or more [16,17]. This claim can also be attributed to the use of phytase enzyme used in this study because several authors had reported that supplementation of exogenous phytase is effective for optimum growth in poultry.

The values recorded for feed intake may suggest a possible influence of the presence of some anti-nutritional factor in Sesame seed. This is an agreement to the reports by Al-Harthi and El-Edeek [18] that birds consumes less of feed containing high amount of Sesame. Although the seed was toasted to reduce the anti-nutritional factor in it, Abeke and Otu [19] reported that heat treatment can only reduce the effect of anti-nutritional factors not totally remove it. Other factors that influence feed consumption include texture, taste and odour [20].

Diets 2 and 3 containing 10% and 20% TSS inclusion had a better feed utilization than the control (Diet 1) that had no inclusion of TSS, a lower value of FCR is an indication of better performance and feed conversion into flesh. However diet 5 with 40% inclusion of TSS had poor feed utilization. Kaneko et al. [17] had reported a depressed performance in broiler chickens when Sesame seed totally replaced SBM.

There was an increase in calcium, and phosphorus retention in the tibia of birds fed diets containing 10% and 20% TSS when compared with control and a decrease in value as inclusion of TSS increased in the diets. This implies a reduction in the inhibitory effect of phytic acid on mineral absorption through its hydrolysis by phytase as well as toasting of the seeds as the sesame seeds inclusion increased. This agrees with the previous study of Sebastian et al. [21] where increased retention of phosphorus and calcium in bone were observed from the tibia of birds fed diets supplemented with phytase. Also there was a decrease as the inclusion of TSS increases in Fe, Zn and Mg and this is in agreement with the work of Zanini and Sazzad [22] who reported an increase in the concentration of Fe and Zn in the tibia by phytase supplementation. Removal or reduction of phytate in food generally improves zinc bioavailability [23].

Most of the digestive and absorptive processes of ingested feeds occur in the intestine. It has been reported that intestinal villus and epithelial cell morphology is associated with intestine function and growth rate [24]. The increase in the villus height as the inclusion of TSS increases to 30% inclusion rate showed that TSS has the ability of increasing the villus height and thus the intestinal surface area for absorption. Caspary [25] confirms that Increase in the villus height suggests an increased surface area capable of greater absorption of available nutrients. Large-sized villi have been associated with activated cell proliferation [26]. The crypt depth in this

study showed corresponding increase as the villus increases because long villi indicate a faster multiplication of the base of the crypt which migrated faster to the tip of villi [27]. However, as the villus height and crypt depth increases with increase in inclusion rate of TSS to 30%, there was no corresponding increase in the growth rate. This showed that though there was increase in villus height, its functions were not well activated because greater villus height and numerous epithelia cells are indicators that the function of the intestinal villi is activated [28,29] and this is because the mucularis which contain the epithelia cells when measured in this study became shorter as the villus height increases. This agrees with the findings of Nordstrom and Dahlqvist [27] who reported that long villi indicate a faster multiplication of the base of the crypt which migrated faster to the tip of villi and the turnover of the epithelial cells would therefore be shorter. The function of the epithelial layer is fundamental to the digestion and absorption of nutrients from the intestinal lumen. The epithelium is covered by a layer of mucus composed of mucin glycoproteins that are synthesised by goblet cells. The mucous layer acts as a layer of protection, lubrication and transport between luminal contents and epithelial cells. Therefore, changes in the properties of this barrier could affect the absorption of both dietary and endogenous macromolecules and ions such as in nutrition, the same trend was observed in the ileum and jejunum in this study.

5. CONCLUSION

Based on the findings of this study, replacing Soybean with toasted Sesame seed above 20% has adverse effects on broiler performance and predisposed the birds to serious health hazards. It can therefore be concluded that replacement of Soybean meal with toasted Sesame seed up to 20% inclusion level in broiler ration results in better performance and improved health condition of broiler birds. In view of the findings of this study, it is recommended that toasted Sesame seed supplemented with phytase can replace Soybean meal up to 20% inclusion level.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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