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Gut Morphology and Internal Organs of Broiler Birds Fed Graded Levels of Bio-Detheobrominized Cocoa Bean Shell (CBS) Based Diets

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Authors' contributions

This work was carried out in collaboration between all authors. Author OGL designed the concept of the study. Author OO wrote the protocol and reviewed the experimental design. Author GOA supervised the feeding trials, wrote the first draft of the manuscript and all drafts of the manuscript. Author AOA carried out the feeding trials, managed the analyses of the study and was involved in contributing to the final drafts of manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The effects of feeding graded levels of bio-detheobrominized cocoa bean shell (CBS) based diets were studied in broilers. Detheobromination by fungi fermentation (bio-detheobromination) reduces the methylxanthine contents cocoa by-products when fermented with *Aspergillus niger*. 196 day old broiler chicks were used for the experiment in an eight week feeding trial. There were seven dietary treatments containing graded levels of CBS from 0 to 30% at the expense of maize and soyabean meal. The control group had no CBS in its diet. Parameters measured include feed intake, body weight gain, feed conversion ratio, carcass characteristics and gut morphology.
The results showed that total feed intake, average body weight gain, feed conversion ratio, weight of internal organs and gut morphology were significantly ($p<0.05$) different across dietary treatments while the carcass characteristics did not show any significant ($p>0.05$) difference. The result of the study revealed that there was a significant reduction in the weight gain and feed efficiency of the

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broilers fed CBS based diets as the level of dietary CBS increased beyond 10%. However, the duodenum villous height and crypt depths increased significantly as the level of CBS increased in the diets. It therefore suggested that the amount/quantity of anti-nutrients such as theobromine and tannins in the CBS based diets beyond 10% inclusion locked up essential nutrients thereby impairing their bio-availability and overall growth of the birds. Thus, additional research is needed to find further techniques of processing CBS so that its anti-nutrients could be further reduced for optimium use in broiler nutrition.

Keywords: Performance; internal organ; bio-detheobrominized cocoa bean shell; gut morphology.

1. INTRODUCTION

Cocoa bean shell (CBS), the thin husk immediately surrounding the cocoa bean, is a waste product from chocolate and cocoa milling industries. It is a potential tropical feed resource and its utilization in animal feeding will greatly reduce the disposal problem facing the cocoa processing factories [1]. It has been observed that the amino acid profile of CBS compares favourably with palm-kernel cake suggesting that it could be utilized as a medium protein source to substitute grain protein in livestock diets. CBS also has an intermediate buffer value between the protein and cereal sources of feed [2]. This suggests that animals consuming cocoa bean shell might not have difficulty in lowering the gastric pH thus, improving protein digestibility and utilization. Other waste products from cocoa such as cocoa pod husk, cocoa dust, and cocoa bean cake have been successfully utilized in livestock feeding [3,4]. The dried CBS contains 12-13% crude protein, 13.00% crude fibre, 8.71% ether extract, 9.15% ash [5]. Olubamiwa et al. [4] recommended that 10% dietary inclusion of physically/chemically treated CBS in replacement for maize was found suitable in layers diets. Cocoa bean shell and other by products from cocoa contains theobromine, an alkaloid poisonous to animals which limits their use for livestock feeding [6]. Olubamiwa et al. [4] recommended 10% inclusion of unprocessed CBS in the diets of broilers, cockerels and laying hens. However, [7] observed that boiling was a most efficient in de-theobrominising CBS. Detheobromination by fungi fermentation (Bio-detheobromination) however is a less expensive tested and proven [8]. It significantly reduced the methylxanhtine contents in cocoa by- product such as cocoa pod husk when fermented with a fungus, *Aspergillus niger* [9]. Pleurotus species (commonly known as giant mushroom) are fungi also provided with enzymes that degrade lignin present in vegetables. In favorable environments (temperature, relative humidity), they produce ligno-cellulose enzymes, 'mainly laccase (LAC)'' and Mn-peroxidase (MnP), which converts lingo-cellulosic residues to food. Thus the objective of this study was to determine the effect of feeding varied levels of detheobrominized cocoa bean shell as a partial replacement for maize and soya bean meal on the performance, primal cuts, and gut morphology of broiler birds.

2. MATERIALS AND METHODS

2.1 Fermentation of Cocoa Bean Shells with *Pleurotus tuberigium*

The dried cocoa bean shells used for the fermentation were milled into a uniform particle size of 2mm using a hammer mill after which composting took place. During composting, the substrate (cocoa bean shells) was spread on black polythene sheet, water was sprinkled and turned inside-out to achieve appreciable moisture content. The substrate was then heaped to a height of 1.5m [8] and covered with the polythene to protect it from direct effect of sun and rain. It was left for four days with intermittent turning over every two days to ensure equal distribution of water and heat throughout [9]. This also ensured uniform degradation and replenishing of oxygen. The composted substrate was then supplemented with 4% calcium carbonate to control the pH of the materials during fermentation. The composted substrate was wrapped in aluminum foils in 200g portions and arranged in a pressure pot suspended in water and tightly covered under pressure. Sterilization was achieved by applying heat (100ºC) for 15 minutes. The sterilized substance was allowed to cool for one hour thereafter, the bottle containing the spawn was shaken to loosen the grains and 2 grams of the grains was introduced into 200 grams of the substrate in each foil.

2.2 Inoculation and Incubation of CBS with *Aspergillus niger*

The aluminum foils with the inoculated substrates were kept under room temperature condition undisturbed to allow the growth of the mycelia through the substrates. The mycelia were grown for eight weeks on the substrate after which the substrate were dried and milled to homogeneity. One hundred grams of sterile CBS powder (Substrate) were inoculated with 1 gram *Aspergillus niger* spore. Substrate inoculated with the *A. niger* spores were kept in a room temperature condition for seven days after which diets containing graded levels of fermented CBS were formulated and compounded.

2.3 Experimental Design and Management

One hundred and ninety six (196) Day old Ross broiler chicks were randomly allotted to seven (7) dietary treatments in a completely randomized design with four replicates and seven birds per replicate. The diets shown in Tables 2 and 3 contained graded levels of CBS at 0, 5, 10, 15, 20, 25 and 30% respectively. Diet one had no CBS and served as the control. The birds were reared on deep litter. The usual conventional brooding practices and poultry management were observed during the experimental period. Routine vaccination schedule were properly observed and the health of the birds were well taken care of.

2.4 Data Collection

One bird per replicate in each of the seven treatment diets (Tables 2 and 3) were randomly selected at eight weeks old, slaughtered, dressed and weighed to determine warm dressed weight. The carcass was eviscerated and the internal organs separated and weighed (expressed as percentage of live-weight). Duodenum and ileal sections of each sacrificed bird were cut to determine and compare their villus height, crypt depth & villus width.

2.5 Gut Morphological Studies

The entire intestine of each sacrificed bird was removed and 5cm intestinal sections from the duodenum (after the duodenal loop) and ileum (5cm before the cecal tonsils) were collected. Sections were cleared and embedded in paraffin where they were initially fixed in 10% phosphate-buffered formalin for 24 hours, transferred to 50% ethanol solution for 15 minutes, and immersed in 70% ethanol. Formalin-fixed sample were embedded in paraffin and 5-um section were sliced and stained with heamatoxylin. The samples were analyzed under a standard light microscope with special emphasis on gut architecture as described by Brady et al. [10]. The villous height, villous width and crypt depth were determined using computer aided light microscope image analysis as described by [11]. Sections from one bird per replicate in each of the seven treatments were placed on each slide. A total of 5 digital images were taken from each tissue using a microscope with digital camera. From each slide, 5 areas were chosen, and 10 villi from each of these areas were evaluated for villus height and width. In addition, 10 crypts were evaluated for crypt depth at each of these areas.

2.6 Proximate Composition of CBS and Diets

Proximate composition of the fermented CBS and experimental diets shown in Table 1 (crude protein, crude fiber, either extract, ash) were determined using the procedure of AOAC [12]. The anti-nutritional factor (theobromine) was determined using gravimetric method by Holmes [15].

2.7 Statistical Analysis

All data obtained were analyzed using analysis of variance of the completely randomized design [13] and least significant difference were used to separate the treatment means.

3. RESULTS AND DISCUSSION

3.1 Internal Organ Weight (% of live weight) of Birds Fed CBS-Based Diets

Results of the internal organ weights of birds fed CBS- based diets are presented in Table 4. It was observed that there was no significant difference ($P>0.05$) in the values obtained for liver and heart. However, lower weight was observed at 5%, while the higher weight was observed at 25%. There were however significant differences ($P<0.05$) for the values obtained for gizzard, spleen, pancreas and abdominal fat pad weights. Gizzard weights revealed that the lowest was observed at 10% and the highest weight at 30%, and this same trend was observed for pancreas weights also, this could be a reflection of the high fiber content of the feed, which leads to increased organ weights as a result of work which is required in the digestion. Abdominal fat pad weights were lower at 20% inclusion levels and higher on the control diet. This could be as a result of the nutrient diluting effect of fiber in the diets of monogastrics.

3.2 Gut Morphology of Broiler Birds Fed Graded Levels of CBS Based Diets

Presented in Table 5 are the results of gut morphology analysis of broiler birds fed graded levels of CBS- based diets. At both the duodenum and the ileum, significant differences were observed for villus height, crypt depth and villus: crypt ratio for birds fed the graded levels of CBS in their diets, with the results indicating that the villous height in duodenum was greater than those in the ileum for broiler birds fed varying levels of CBS–based diets. This observation is in agreement with the findings of Gholamhazan et al. [14] who observed that villi height in duodenum was greater than those in the jejunum and ileum when broiler chickens were fed diets varying in the levels of lysine. The finding above is consistent to the major role of duodenum in nutrient absorption. The villous height depends upon two factors, firstly on cellular multiplication and differentiation from the crypts and secondly on the migration of mature cells along the villous axis, accompanied by the cellular shedding from the apex. The regulation of these factors is widely considered to be the way in which the small intestine adapts to nutritional demands [15]. The duodenum villous height and crypt depth of broiler birds fed graded–levels of CBS–based diets increased significantly (P<0.05) in the CBS-diets compared to the control. However the increased in the villous height across the CBS diets did not result in increased weight gain and feed utilization. Increased villi height is expected to result in improved performance as a result of improved nutrient absorption. The increase in villi height observed in the CBS diets might indicate that the birds fed CBS diets up to 30% CBS dietary level might have had greater nutrient absorption and utilization because increase in villi height implies increased surface area for nutrient utilization [16].

Table 1. Chemical composition of cocoa bean shell

Component	Values
Dry matter (%)	84.52
Crude Protein (%)	14.98
Ether Extract (%)	2.39
Crude Fiber (%)	7.67
Ash (%)	11.66
Metabolizable Energy (kcal/kg)	2,800
Theobromine (mg/g)	50

Table 2. Composition of CBS based diets fed to broiler birds at starter phase (Kg/100kg) diets

Ingredients	0%CBS	5%CBS	10%CBS	15%CBS	20%CBS	25%CBS	30%CBS
CBS	0.00	5.00	10.00	15.00	20.00	25.00	30.00
Maize	53.00	50.00	46.00	43.00	40.00	37.00	34.00
Soya Bean Meal	38.00	36.00	35.00	33.00	31.00	29.00	26.00
Fish Meal	3.90	3.90	3.90	3.90	3.90	3.90	3.90
Bone Meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00
DCP	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Limestone	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
B-Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Proximate Analysis							
M.E(kcal/kg)	3,185.38	3,156.42	3,126.14	3,097.18	3,068.22	3,039.26	3,010.33
Crude Protein (%)	24.46	24.04	23.96	23.54	23.11	22.69	22.26
Ether Extract (%)	3.47	3.28	3.09	2.89	2.71	2.52	2.33
Crude Fiber (%)	3.55	3.76	3.80	4.03	4.84	4.95	5.21
Calcium (%)	1.20	1.55	1.79	2.04	2.29	2.53	2.46
Phosphorus (%)	0.61	0.54	0.68	0.82	0.96	1.09	1.23

M.E= Metabolisable Energy (kcal/kg)

Table 3. Composition of CBS based diets fed to broiler birds at finisher phase (kg/100kg) diets

Ingredients	0%CBS	5%CBS	10%CBS	15%CBS	20%CBS	25%CBS	30%CBS
Cocoa Bean Shell	0.00	5.00	10.00	15.00	20.00	25.00	30.00
Maize	63.00	60.00	57.00	54.00	51.00	48.00	45.00
Soya Bean Meal	31.00	29.00	27.00	25.00	23.00	21.00	19.00
Fish Meal	0.70	0.70	0.70	0.70	0.70	0.700	0.70
Molasses	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Bone Meal	1.50	1.50	1.50	1.50	1.50	1.50	1.50
DCP	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Limestone	1.0	1.0	1.00	1.00	1.00	1.00	1.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
B-Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15	0.15	0.15	0.15
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Proximate Analysis							
M.E(kcal/kg)	3,224.93	3,195.97	3,167.01	3,138.05	3,109.09	3,080.13	3,051.71
Crude Protein(%)	20.31	19.89	19.46	19.04	18.61	18.91	18.76
Ether Extract(%)	3.62	3.42	3.24	3.05	2.86	2.67	2.48
Crude Fiber(%)	3.29	3.40	3.80	4.55	4.90	5.26	5.50
Calcium(%)	0.81	1.06	1.31	1.55	1.79	2.02	2.29
Phosphorus(%)	0.45	0.52	0.65	0.79	0.92	1.06	1.98

Table 4. Weights of internal organs (%) of broilers fed graded-levels of CBS based diets

Parameters	0%CBS	5%CBS	10%CBS	15%CBS	20%CBS	25%CBS	30%CBS
Liver	1.793	1.673	1.983	2.055	2.195	2.25	2.058
Gizzard	3.12^{b}	2.995^{b}	2.915^{b}	3.258^{ab}	3.253^{ab}	3.558^{ab}	3.728^{a}
Spleen	0.022^{bc}	0.034^{bc}	0.067^{ab}	0.094^{a}	0.179^{c}	0.088^{ab}	0.051^{abc}
Heart	0.4363	0.4875	0.4675	0.4675	0.565	0.465	0.565
Pancreas	0.162^{c}	0.258^{ab}	0.158^{c}	0.220^{b}	0.250^{ab}	0.275^{ab}	0.283^{a}
Abdominal fat	1.24^{a}	0.55^{bc}	0.99^{ab}	0.86^{ab}	0.23^{c}	0.59^{bc}	0.99^{ab}

Table 5. Gut morphology of birds fed CBS based diets

Parameters	0%CBS	5%CBS	10%CBS	15%CBS	20%CBS	25%CBS	30%CBS	SEM
Villus height								
Duodenum	1589^{bc}	1681^{b}	1740^{b}	1176^{c}	2074^{a}	1210^{c}	2402^{a}	8.10
Ileum	974.9^{c}	1806^{a}	1180^{bc}	1537^{ab}	1767^{a}	1392^{b}	135.1^{d}	3.7
Villus width								
Duodenum	165.2^{c}	212.5^{b}	219.2^{b}	192.8^{b}	148.1^{c}	153.1^{c}	253.1^{a}	5.193
Ileum	208.7^{ab}	228.5^{b}	191.3^{b}	225.7^{ab}	258.2^{a}	208.1^{ab}	233.5^{ab}	19.58
Crypt depth								
Duodenum	66.73^{cd}	149.7^{b}	45.49^{d}	98.06^{c}	189.6^{b}	301.8^{a}	152.4^{b}	3.94
Ileum	93.79^{c}	235.8^{a}	119.9^{c}	142.6^{b}	214.5^{a}	134.5^{b}	136.8^{b}	10.6

^{abc}*Means with the same super script are not significantly different (P>0.05)*

4. CONCLUSION

The findings in this study suggest that although the gut morphology of the birds fed CBS diets improved compared to the control diet to have enhanced nutrient absorption, essential nutrients such as digestive protein and energy were locked up or bonded by anti-nutritional factors such as theobromine and tannins present in the CBS diets thereby impairing the essential nutrient's bio-availability thereby impeding the growth of the birds fed diets beyond the 10% inclusion level. The results of this study showed that increasing the dietary level of cocoa bean shell in broiler chicken's diets improved their villous and crypt dimensions positively.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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