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Aspergillus flavus Degraded Brewer Dried Grains for Broiler Chicken Diet: Performance and Nutrient Digestibility Parameters

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Improvement of agro industrial by-products via microbial biodegradation in order to subside nutritional challenges cannot be over flogged. This study was conducted to investigate the possible improvement in nutritional quality of brewer dried grains (BDG) using *Aspergillus flavus* as biodegrading agent and the consequent effect of degraded BDG on the performance and nutrient utilization by broiler chickens. Undegraded and degraded BDG was used to compound rations for broiler birds for 8 weeks. The undegraded was used at 7% inclusion level and the degraded was used at 3, 5 and 7%. 150 day old chicks were randomly allocated to 5 treatments. 30 birds were allocated to each treatment with three replicates each. *Aspergillus flavus* was cultured onto BDG using Solid State Fermentation for a period of 7 days. Chemical analysis of undegraded and degraded BDG showed that biodegradation altered the chemical composition. The crude protein improved from 27.89 to 39.12 g / 100 gDM (28.71%). The ash improved from 4.39 to 6.22 g /

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100 gDM (29.42%). The gross energy increased from 4.92 to 7.10 kcal/kg which showed 30.70% improvement. However, the crude fibre and the detergent fibre reduced after fungal biodegradation. The crude fibre reduced from 14.85 to 11.17 g / 100 gDM. At both starter and finisher phases, there were significant (P<0.05) differences in feed intake, weight gained and feed conversion ratio and birds placed on degraded BDG (DBDG) showed better nutrient utilization than ones on undegraded BDG (UBDG). The relative cost benefits (RCB) showed that the chickens on DBDG gave better economic gain than the ones on UBDG treatment.

Keywords: Aspergillus flavus; solid state fermentation; brewer dried grains; broilers.

1. INTRODUCTION

The stiff competition between humans and animals over the limited supply of conventional feed ingredients has resulted in a near collapse of the poultry industry in Nigeria [1]. This has brought about the need to improve the scientific knowledge for utilizing low cost locally available agro-industrial by-products in poultry feeds in order to reduce the feed cost. As feed constitutes 60-70% of the total cost of production, any attempt to reduce the feed cost may lead to a significant reduction in the total cost of production. Agro-industrial by products (AIBs) refers to the by-products derived in the industry due to processing of main products; they can be referred to as the secondary products or waste products after the main products have been obtained. Agro-industrial by-products cannot be added in high levels because of their low energy content, high fiber content, poor available nutrients and also because they contain non-starch polysaccharides which can account for about 70-90% of the cell wall. Agro-industrial by-products in most developing countries vary from primary processing of farm produce to wastes from agro-allied industries. Some of these wastes are left unutilized, promoting environmental pollution and hazard. According to [2] agro-industrial by-products contain considerable amount of energy and protein which are present intracellularly in the fibrillian complex. The residue represents potential valuable and renewable resources which find application in various areas that include use as animal feed. Potential agro-industrial by-products used for compounding livestock and poultry feed include cocoa husk, sorghum distillers waste, wheat offal, rice bran, cassava peels and brewer dried grains [3]. Brewer's dried grain BDG is the by-product of brewing industry, the residue containing rice grits, corn grits, corn starch, wheat starch, sorghum grits, oat and barley grits as well as husks produced as by-products in the production of beer. Brewer's dried grain (BDG) is a valuable source of CP, ME and many of the B-vitamins and rich in P, but relatively low in Ca. It is considered to be good sources of un-degradable protein and water soluble vitamins [4]. The disadvantages of using Brewer Dried Grains compared to some conventional sources of feed have been identified to include; nucleic acid toxicity, palatability and limitation of sulphur amino acid in the product. The problem of nucleic acid toxicity is said to be solved naturally due to the availability of the enzyme (uriase) in mammals which enables them overcome the problem of nucleic acid toxicity. The inclusion of agro-industrial by-products in monogastric diet is limited by its fibrousness [1,4]. The high presence of the fiber in agro-industrial by-product does not make the content to be readily digested by the monogastric animals. Although the animals innate enzyme production is enough to handle normal digestive activities, but the presence of high fiber content and lack of sufficient micro-organisms in the stomach makes digestion difficult. The advent of biotechnology, specifically, fungal biotechnology, with its inexpensive mode of application, has been used as a tool for the effective conversion of these wastes into useful products. The use of biological means in degradation of waste, especially agro-industrial by-products has greater advantages over the use of chemical because biotechnologically synthesized products are less toxic and environmentally friendly [5]. Fungi can increase the protein and soluble sugars and reduce the complex carbohydrates of these wastes Iyayi and Aderolu, 2003. *Aspergillus flavus* is a saprotrophic fungus with a cosmopolitan distribution. It is best known for its colonization of cereal grains, legumes, and tree nuts. According to [6], *Aspergillus flavus* posses the capacity to degrade the non-starch polysaccharide contents of the agro-industrial by-products converting them to simple sugars with a beneficial increase in energy and protein in these by-products. Objectives of this study include: Assessment of the effect of *Aspergillus flavus* on degrading brewer dried grain; Assessment of the effect of feeding BDG on the performance of broilers and assessment of effect of *Aspergillus flavus* degraded brewer dried grain on the nutrient utilization of broilers.

2. MATERIALS AND METHODS

Brewer dried grains were obtained from a brewery located in Ibadan, Nigeria. It was dried to constant weight at 60℃. 25 kg of the dried BDG was autoclaved at 121℃ for 15 minutes. The autoclaved BDG was then inoculated with *A. flavus* under aseptic condition after adjusting its moisture level to 25%. After 7 days, the biodegradation reaction was stopped and the material dried [7]. Samples were then withdrawn for proximate analysis using the method of [8]. The *A. flavus* used was obtained from Microbiology Unit of Department of Biological Sciences of Bowen University, Iwo, Nigeria. The characterization of the obtained A*. flavus* was known by the use of manual of [9]. A total of 150 day old broiler chicks (Anak strain) were used for the experiment. There were five dietary treatments of 30 birds each and three replicate of 10 birds each. Diet 1 contained neither degraded nor undegraded BDG. Diet 2 contained 7% undegraded BDG while diets 3, 4 and 5 contained 3, 5 and 7% levels of *A. flavus* degraded BDG. The experimental design used was Completely Randomized Design. Data were analyzed statistically using the Analysis of Variance (ANOVA) technique of [10]. Where statistical significant differences were observed, the treatment means were compared according to Statistical Analysis System [11]. At the end of the starter and finisher phases, three birds were taken from each treatment (one from each replicate) and placed in cages for faecal collection. Weights of feaces were taken before and after drying. Nutrient digestibility was calculated as follows:

Nutrient Digestibility (%) = (Nutrient intake-Nutrient output x 100 / Nutrient Intake)

3. RESULTS AND DISCUSSION

Chemical analysis of undegraded and degraded BDG is presented in Table 1 and Tables 2 and 3 show the gross composition of experimental diets at starter and finisher phases respectively. From the results, biodegradation of BDG altered the chemical composition. The crude protein improved from 27.89 to 39.12 g / 100 gDM (28.71%). Enhancement was also observed in ash and gross energy. The ash improved from 4.39 to 6.22 g / 100 gDM (29.42%). The gross energy increased from 4.92 to 7.10 kcal/kg which showed 30.70% improvement. Notably, the crude fibre and the detergent fibre reduced after fungal biodegradation. The crude fibre reduced from 14.85 to 11.17 g / 100 gDM. In the same vein, cellulose came down from 7.15 to 5.55 g / 100 gDM. In addition, the ether extract also reduced after biodegradation from 7.38 to 5.84 g / 100 gDM (20.87%). Performance of broiler chickens at the starter phase is shown in Table 4. There were significant ($P<0.05$) differences in feed intake, weight gained and feed conversion ratio. The feed intake improved from 38.21 to 46.78 g/b/d and the weight gain also improved from 13.92 to 18.21 g/b/d for birds placed on 7% degraded BDG. The worst feed conversion ratio (2.76) was obtained from birds placed on treatment with 7% UBDG. Performance of broilers at finisher phase is presented in Table 5. The same pattern was seen as there were significant ($P<0.05$) differences in the feed intake, body weight gain and feed conversion ratio. In all these parameters at the finisher phase, the birds placed on DBDG performed better than the birds on UBDG. The relative cost benefits (RCB) showed that the chickens on DBDG gave better economic gain than the ones on UBDG treatment. In addition, at both starter and finisher phases, nutrients were better utilized by birds on DBDG as shown in crude protein, ether extract, crude fibre, and ash digestibility. Improvement in the nutritive quality of fibrous feed ingredients is occasioned by secretion of enzymes from fungi through solid state fermentation medium and it varies from one fungus to another. [12] recorded variations in proximate composition of brewer dried grain when subjected to biodegradation by *Aspergillus niger, Aspergillus flavus and Penicillium spp*. *Aspergillus niger* was able to effect highest percentage in the cellulose reduction owing to its vigorous growth and hence ability to produce more hydrolytic enzymes within a short time. In addition, in the previous work of [13] wheat offal (another agro industrial by product) was degraded using *Aspergillus niger* for seven days showed that there was improvement in crude protein value from 2.43 to 4.00 g / 100 gDM (39.25% improvement) while crude fibre had 28.02% reduction after biodegradation. In sub-Saharan Africa, large quantities of agricultural and agro-industrial by-products are generated and most of them are seen as waste and con-conventional feed materials. Since BDG is high in fibre, it is minimally degraded in the gastro intestinal tract of monogastrics. No doubt, this ability of *A. flavus* to reduce the fibrousness of

BDG and improve the crude protein content is a positive development. It will help to increase the usability of this AIB as a non-conventional feed ingredient for the monogastrics. [14] opined that the post fermentation improvement in crude protein content of the BDG may possibly be due to the secretion of some extra cellular enzymes (protein) such as amylases, xylanases and cellulases into the BDG by the fungus in order to make use of the carbohydrates in the BDG as a carbon and energy source. Besides, fungi, by nature, colonize the carbon source for utilization of available nutrients. Hence, they synthesize and produce a reasonable amount of cellulolytic extra cellular enzymes and this will catalyze the breakdown of nutrients to products that penetrate the fungal mycelia across cell membrane to increase biosynthesis and fungal metabolic activities leading to growth. The growth and multiplication of the fungal biomass in the form of single cell protein (SCP) may also be responsible for the increase in the protein content of the BDG after biodegradation. This result is consistent with the works of Iyayi and Aderolu [12] that revealed an improvement in the crude protein (CP) contents of AIBs after treatment with *Trichoderma viride.* Furthermore, the obtained result is also in line with the report of [15] who submitted that the CP content of spent straw improved from 3.42 to 6.19 after fermentation. Biodegradation by the fungus has the potential of changing the biochemical structure of the substrate and this is also expressed in changes observed in neutral detergent fibre(NDF), acid detergent fibre (ADF) and acid detergent lignin [16]. In this study, the biodegraded BDG showed a reduced cell wall component value than that of undegraded BDG as revealed in reduction of NDF, ADF and ADL contents. This development may be attributed to breakdown of lignocellulosic bonds by the *A. flavus* enzymes [17]. The decrease in the crude fibre content shows the ability of *A. flavus* to produce hemicellulases, cellulases and polygalacturinase enzymes. This furher confirms the assertion of [18] that fungal biodegradadtion of agro industrial wastes can decrease cell wall total fibre value. There was increase in gross energy probably due to degradation of polysaccharide compounds which led to increase in sugar production and availability, hence more energy. Improving the concentration of the sugars is advantageous apart from reduction of lignocelluloses in the course of improving biomass quality via fermentation [19]. Increase in ash content after fermentation suggests improvement in minerals availability. [20] opined that fermentation of agro industrial wastes will increase availability of minerals like calcium, phosphorus and potassium. This further suggest that enzymes like phytase were produced during fermentation. Phytase (myo-inositol hexakisphosphate phosphohydrolase, EC 3.1.3.8) catalyses the breaking down of phytate to myo-inositol pentakisphosphate and orthophosphate. Enhancing the digestibility of proteins and bioavailability of phosphorus and other minerals which are prone to chelation by phytic acid reduces the anti-nutritive tendencies of phytate and minimizes environmental pollution [21]. In this study, the birds placed on UBDG could not feed like the ones on degraded BDG could probably due to “gut fill effect” owing to the gritty nature of agro wastes which compelled the birds to consume more water than the feed. In addition, increase in feed consumption by birds on DBDG may be due to production of vitamin B complex and flavour compounds as a result of fermentation [22]. Besides, [23] reported that unfermented agro wastes have the tendency of increasing the viscosity of feed in the intestinal tract and this will directly reduce the transit time of the digesta in the GIT and also reduce the feed consumption by the birds. Weight gain is related to the nutrients available to the birds. It is also related to nutrient digestibility and utilization by the birds. It is vital to note that fiber in monogastric diets is primarily utilized in the hind gut (caeca, rectum and the colon) and this makes the nutrients unavailable for the animal’s use. [24] attributed poor weight gain to proliferation of bacteria in the large intestine. Feeding animals diets high in dietary fiber, particularly soluble fiber alters the rate of fecal passage, microbiota, metabolites, and efficacy of digestion [25]. Commensal bacteria in the large intestine utilize fiber as a source of energy. An increase in the energy supply increases microbial metabolism and microbial population growth. Feeding of DBDG notably minimizes the microbial load and this leads to increase in the quantity of amino acids digested in the precaecal part of the tract because the microbial population in viscous unfermented BDG diet can negatively influence digestibility of protein in ileum [26]. The best feed conversion ratio was noted in treatment with DBDG which indicates that birds were able to convert the feed to flesh better than when fed UBDG. Poor digestibility observed in the birds placed on UBDG may be due to the high level of lignifications that characterizes a typical non conventional agro waste prior to fermentation [27]. Observed that the decrease in protein digestibility might have resulted from the presence of non-starch polysaccharides present in the high fiber feed material. This reduction in digestibility is a major restriction to the use of agricultural residues. The presence of non-starch polysaccharides restricts access to protein thus restricting digestibility. The level of interference and consumption of dietary fiber to the absorption of minerals and trace elements differ widely depending on the chemical structure of the polysaccharides and its digestibility by intestinal bacteria. Increasing the dietary level of fiber caused corresponding decrease in the digestion and availability of nutrients including mineral in poultry diet [28]. Dietary fiber affects the digestion and absorption of lipids in broiler chickens as it is for other nutrients. The absorbent property of fiber affects the alimentary canal and increase in the feacal excretion of lipids, sterols, nitrogenous compounds and bile acids [29,30] recorded a reduction in the digestion and utilization of nutrients such as crude protein, ether extract and mineral (ash) when compared to fungal degraded high fiber feed material. This reduction shows that the presence of non-starch polysaccharides in dietary fiber restricts the utilization of vital nutrients present in the feed material. This reduction could be as a result of the inability of enzymes secreted by the digestive tract of poultry to completely break down complex non-starch polysaccharide bonds. This brings about the need for fungal degradation to break down complex bonds improving nutrient utilization, as fungi secrete many enzymes such as cellulase, amylase, pectinase [31] which are employed in the hydrolysis of polysaccharides.

Table 1. Proximate and detergent fibre analysis of undegraded and degraded brewer dried grain (g/100 gDM)

Composition	UBDG (%)	DBDG (%)
Dry matter	90.13	87.19
Crude protein	27.89	39.12
Ether extract	7.38	5.84
Ash	4.39	6.22
Crude fibre	14.85	11.17
Gross energy (Kcal/kg)	4.92	7.10
Cellulose	7.15	5.55
Hemicellulose	4.82	3.48
Neutral detergent fibre	2.45	1.47
Acid detergent fibre	2.66	1.79
Acid detergent lignin	5.16	4.27

Table 2. Composition of experimental diet for starter phase

Ingredients	Control	7% UBDG	3% DBDG	5% DBDG	7% DBDG
Maize	54	56	53	52	51
UBDG	-	7.0	-	-	-
DBDG	-	-	3.0	5.0	7.0
Wheat offal	5.2	0.3	2.3	3.3	3.3
Groundnut cake	11.1	15	15	13	12
Soya bean meal	20	12	17	17	17
Fish meal	6	6	6	6	6
Bone meal	2	2	2	2	2
Oyster shell	1	1	1	1	1
Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10
Total	100	100	100	100	100
Metabolizable energy	2912.34	2958.71	2874.47	2906.52	2890.03
Crude protein	23.20	23.43	22.68	22.70	22.36

UBDG=Undegraded brewer dried grains; DBDG=Degraded brewer dried grains. *******Vitamin mineral premix supplied the following vitamins and trace elements per kg diet: Vit. A 12500IU, Vit. D_3 2500IU, Vit. E 40 mg, Vit. K_3 3 mg, Vit. B_1 3 mg, Vit.B_2 5.5 mg, Niacin 5.5 mg, Calcium Pantothenate 11.5 mg, Vit B_6 5 mg, Vt B_{12} 0.025 mg, Folic Acid 1 mg, Biotin 0.08 mg, Mn 120 mg, Choline Chloride 500 mg, Fe 100 mg, Zn 80 mg, Cu 8.5 mg, I 1.5 mg, Co 0.3 mg, Se 0.48 mg and Antioxidant 120 mg*

Table 3. Composition of experimental diet for finisher phase

Ingredients	Control	7% UBDG	3% DBDG	5% DBDG	7% DBDG
Maize	54	57	56	55	54
UBDG	-	7.0	-	-	-
DBDG	-	-	3.0	5.0	7.0
Wheat offal	12.4	6.5	11.0	10.1	7.5
Groundnut cake	10.2	10.2	10.2	10.2	10.2
Soya bean meal	16.7	12.6	13.1	13	14.6
Fish meal	3	3	3	3	3
Bone meal	2	2	2	2	2
Oyster shell	1	1	1	1	1
Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10
Total	100	100	100	100	100

UBDG= Undegraded brewer dried grains; DBDG=Degraded brewer dried grains. *******Vitamin mineral premix supplied the following vitamins and trace elements per kg diet: Vit. A 12500IU, Vit. D_3 2500IU, Vit. E 40 mg, Vit. K_3 3 mg, Vit. B_1 3 mg, Vit.B_2 5.5 mg, Niacin 5.5 mg, Calcium Pantothenate 11.5 mg, Vit B_6 5 mg, Vt B_{12} 0.025 mg, Folic Acid 1 mg, Biotin 0.08 mg, Mn 120 mg, Choline Chloride 500 mg, Fe 100 mg, Zn 80 mg, Cu 8.5 mg, I 1.5 mg, Co 0.3 mg, Se 0.48 mg and Antioxidant 120 mg*

Table 4. Performance of broiler starter fed diets containing undegraded and degraded brewer dried grain

Parameter	Control	7% UBDG	3% DGDG	5% DBDG	7% DBDG	SEM
Feed intake (g/b/d)	38.21^c	37.5^d	39.29^c	42.85^b	46.78^a	1.63
Body weight gain (g/b/d)	13.92^d	13.57^d	15.00^c	16.42^b	18.21^a	0.78
Feed conversion ratio	2.74^a	2.76^a	2.61^b	2.61^b	2.57^b	0.06
Mortality (%)	1	1	0	0	1	-
Relative cost benefit (%)	0.00	3.01	3.21	3.36	4.21	-

a, b, c, Means in the same row with different superscripts differ significantly (P<0.05); UBDG=undegraded brewer dried grains; DBDG=degraded brewer dried grains

Table 5. Performance of broiler finisher fed diets containing undegraded and degraded brewer dried grain

Parameter	Control	7% UBDG	3% DGDG	5% DBDG	7% DBDG	SEM
Feed intake (g/b/d)	91.07^c	81.43^d	93.57^b	102.5^{ab}	116.79^a	2.67
Body weight gain (g/b/d)	44.28^d	38.92^e	50.00^c	56.78^b	66.79^a	1.33
Feed conversion ratio	2.05^a	2.09^a	1.87^b	1.80^b	1.75^c	0.021
Mortality (%)	0	1	1	0	0	-
Relative cost benefit (%)	0.00	2.45	3.44	3.95	4.12	-

a, b, c, Means in the same row with different superscripts differ significantly (P<0.05); UBDG=Undegraded brewer dried grains; DBDG=degraded brewer dried grains

Table 6. Nutrient digestibility of experimental broilers (starter phase)

Composition (%)	Control	7% UBDG	3% DBDG	5% DBDG	7% DBDG	SEM
Crude protein	69.70^d	68.88^d	73.20^c	75.10^b	78.67^a	2.59
Ether extract	49.30^b	46.24^c	52.34^b	55.04^a	55.92^a	2.11
Crude fibre	35.46^c	34.53^c	38.75^b	39.66^b	42.04^a	1.81
Ash	35.63^c	34.34^c	37.98^b	39.47^a	41.33^a	1.56
Dry matter	70.90^b	71.37^b	72.24^{ab}	73.03^a	73.58^a	1.88

a, b, c, Means in the same row with different superscripts differ significantly (P<0.05); UBDG=Undegraded brewer dried grains; DBDG=degraded brewer dried grains

Table 7. Nutrient digestibility of experimental broilers (finisher phase)

Composition (%)	Control	7% UBDG	3% DBDG	5% DBDG	7% DBDG	SEM
Crude protein	73.93^{c}	71.76^{d}	75.50^{c}	77.56^{b}	79.73^{a}	2.99
Ether extract	53.82^{c}	50.42^{d}	57.85^{b}	59.05^{a}	62.54^{a}	2.45
Crude fibre	50.54^{d}	49.72^{e}	52.99^{c}	55.95^{b}	57.32^{a}	2.11
Ash	46.10^{d}	45.46^{d}	48.01^{c}	49.61^{b}	51.02^{a}	1.44
Dry matter	75.05^{c}	74.16^{c}	76.60^{c}	77.44^{b}	80.04^{a}	1.58

a, b, c, Means in the same row with different superscripts differ significantly (P<0.05); UBDG=Undegraded brewer dried grains; DBDG=degraded brewer dried grains

4. CONCLUSION

The investigation has shown that solid state fermentation has beneficial effects as it can effect bioconversion of BDG into better quality feed ingredient. This can be a better option to chemical and physical treatments for improving the BDG quality for poultry's consumption. Biodegradation can also aid nutrient digestibility and utilization and this impacted well on the birds by improving their feed consumption, weight gain and feed conversion ratio. The relative cost benefit clearly shows from the results that it is economically advisable for the farmers to use *A. flavus* degraded BDG for broiler chicken production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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