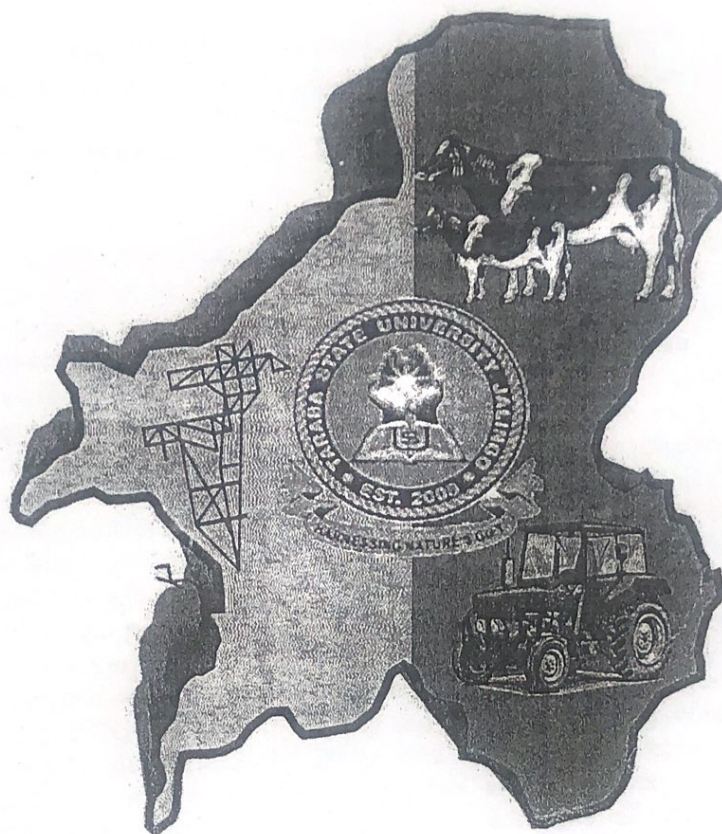


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EFFECTS OF ENZYME SUPPLEMENTATION ON THE PREBIOTIC POTENTIAL OF BREWERS DRIED GRAINS IN BROILER CHICKENS.

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Abstract

The development of antibiotics-resistance pathogens in poultry which poses threat to human health has necessitated the search for alternative to antibiotic growth promoters (AGPs) to improve gut microflora in poultry diets. One of the alternatives to AGPs is probiotics which are beneficial organisms. Prebiotics, are by-products of digestion of polysaccharides which poultry do not have enzymes to digest and serve as food for probiotics. Advent of enzymes makes this digestion possible. The prebiotic potentials of enzyme supplemented high fibre feedstuffs (HFFs) are not known. A total of 1920 day old broiler chicks of *Arbor Acre* strain randomly assigned to 8 treatments each with 8 replicate cages of 30 birds were used in a 4 x 2 factorial combination for a period of 5 weeks. This study was conducted to assess the prebiotic potential of xylanase enzyme supplemented Brewers dried grains (BDG) on broiler chickens probiotics population. The replacement of maize with Brewers Dried Grain irrespective of levels supplemented with 100ppm *xylanase* enzyme caused a reduction in feed intake and an increase in weight gain and better feed conversion rate (FCR). Birds fed diet with 10% BDG supplemented with *xylanase* enzyme out-performed birds fed diets with 10 or 30% BDG supplemented with *xylanase* enzyme and closer to the birds fed the control diet which had better FCR. The enzyme supplementation of BDG helped in increasing and improving protein, ether extract and fibre digestibilities. The dietary levels of BDG (10, 20 or 30% inclusion) with supplementation of enzyme *xylanase* enhanced the growth of beneficial microbes which resulted in inhibition or elimination of the opportunistic/pathogenic microbes. The 10% inclusion level of BDG supplemented with *xylanase* enzyme gave the best result of a beneficiary reduction in the cost of production with the best improved broiler performance. Enzyme supplementation of high fibre feedstuffs could improve growth performance, nutrient retention and fibre digestibility. Use of enzymes is therefore recommended when HFFs are required as feedstuffs for broilers chicken.

Key words: Prebiotics, enzymes, fermentation, nutrient, retention

Introduction

The cost of feed and feeding has been identified as the biggest factor militating against livestock industry in Nigeria. It has been observed that poultry production in Nigeria was not economically profitable due to the prevailing high cost of imported feed (Bolu *et al.*, 2015). The cereal component of feed is always cheaper than the protein ingredients in countries where cereal production is not a problem (Iyayi *et al.*, 2005). The increasing cost of feed ingredients has resulted in concerted research efforts by various bodies directed towards alternative sources of feed ingredients to reduce the dependence on the

expensive conventional ingredients. Maize has been reported with a high metabolisable energy of 14.37MJ/kg compared to brewer's dried grains (8.29MJ/kg), but the proximate chemical composition of the latter reveals its higher crude protein - CP (18%), lysine (0.90%), methionine (0.40%), ether extract (6.0%), calcium (0.20%) and available phosphorus (0.16%) values than maize (Atteh, 2004).

Brewers dried grains is the material remaining after grains have been fermented during the beer producing process. It has a high nutritional value and stable price. Its nutrient content varies in a certain range from plant to plant and has been reported to depend on the type of grain used in the initial brewing

process (barley, wheat, corn etc.), the proportion being fermented and fermentative process being used (Dibner and Richards, 2005; Olajide *et al.*, 2013). BDG is being used in both ruminant and monogastric animal feed as it is considered to be a good source of undegradable protein (having one of the highest values of bypass protein in commonly available feed stock) and water-soluble vitamins (Olajide *et al.*, 2013). According to Atteh (2004) its dietary inclusion should be limited to 5-10% in swine and poultry diets for efficient production. It has a high crude fibre of 20% with the non-starch polysaccharide (NSP) limiting its desirability in poultry diets.

It's been reported that dietary enzymes can be used to aid the digestive process in the gastrointestinal tract of the animal (Choct, 2006; Ohimain *et al.*, 2013). Its use in poultry feeds has predominantly been related to the hydrolysis of fibre or NSP fractions in cereal grains which cannot be digested by the endogenous enzyme secretions of poultry (Pylkas *et al.*, 2005). Exogenous enzymes could be added to the feed or used during feedstuff's processing and has the potential to improve feed efficiency, reduce pollution associated with poultry manure and increase the use of low cost feed ingredients (Choct, 2006). The increasing knowledge of the structure of cell walls of grain has made it easy to produce specific enzyme tailored towards the substrates of interest in commercial supplements (Ohimain *et al.*, 2013). The performance of microwave-assisted

Table 1 Composition of Experimental Diet (%)

Ingredients	1	2	3	4	5	6	7	8
Maize (%)	50	50	40	40	30	30	20	20
Brewer's Dried Grain (%)	0	0	10	10	20	20	30	30
Xylanase (ppm)	0	100	0	100	0	100	0	100
Basal ingredients (%)	50	50	50	50	50	50	50	50
Total	100	100	100	100	100	100	100	100

Basal diets : Groundnut cake(GNC) - 26%, corn bran- 1%, soybean meal- 12%, fishmeal(72%)- 4%, palm oil- 2%, oyster shell- 2%, bone meal- 2%, salt- 0.25%, methionine- 0.25%,lysine- 0.25% and vitamin premix- 0.25%.(*)Vitamin/ mineral premix contained the following: (Univit. 15 Roche) 1500 I.U, Vit A, 1500 I.U, Vit D, 3000 I.U, Vit E, 3.0g, Vit K, Vit. B₁, 0.3g, Vit.B₆, 8.0mg, Vit.B₁₂, 8.0g, Nicotinic Acid, 3.0g, Ca-Pantothenate, 50mg, Fe, 10.00g, Al, 0.2g, Cu, 3.5mg, Zn, 0.15mg, I, 0.02g, CO₂, 0.01g, Se).

At the end of the experiment, 10 birds per replicate from each treatment was randomly selected and euthanized by bleeding through the carotid artery for collection of

extractions on BDG has revealed that it can be exploited as a source of arabinoxylo-oligosaccharides with prebiotic activity (Binns, 2013).

This present study was conducted to investigate the prebiotics potential in the gut content of broiler chickens fed Brewers Dried Grain (BDG) treated with Xylanase.

Materials and Methods

Sourcing and Management of birds

A total of 1920 day old broiler chicks of *Arbor Acre* strain purchased from Yammfy Farm hatchery at Ilemona, Kwara State were used for this experiment. The birds were housed in an electrically heated battery cage and fed the experimental diet shown in Table

Experimental design

Birds were fed a control diet (50% maize) or diets in which brewers dried grain (BDG) was added at 10, 20, or 30% replacing maize in the control diet. Each of these diets was given with or without 100 PPM xylanase enzyme in a 4 x 2 factorial combination. Thus, there were 8 treatments each with 8 replicate cages of 30 birds. The experiment was conducted for a period of 5 weeks. Live weight was recorded weekly while feed intake was recorded daily in grams and excreta samples collected over a 72 hour period. The experimental diets and excreta samples were analyzed for their chemical constituents using the procedures outlined by AOAC (2008).

digesta from the gastro-intestinal tract (GIT) to determine the microbial profile. The carcasses were subsequently opened and the entire GIT removed using aseptic techniques. It was

excised and the luminal contents of the crop, jejunum, duodenum, ileum and caecum were collected and pooled together according to the procedure described by Kalantar *et al.* (2014). This was done to determine population and profile of microorganisms present in the broiler gut. Five grams of each sample was put into sterile universal bottle containing 100ml of sterile water and shaken thoroughly. The mixture was allowed to settle and the supernatant decanted into separate test tubes; this serves as stock solution. Serial dilutions of the stock were made and 0.1ml aliquot was spread on Potato Dextrose Agar (PDA) plate containing 1% streptomycin (to inhibit bacterial growth). The plates were incubated at room temperature (27 – 31°C) for 48 hours (Bengmark, 2001). After incubation, representative fungal colonies were picked from each plate and purified on fresh PDA (for other fungi) and YPDA (Yeast Peptone Dextrose Agar) plates for yeast. The purified isolates were transferred to PDA and YPDA slants incubated at room temperature for 48 hours and stored at 4°C. The isolates were identified according to the scheme of Chio *et al.* (1994). In isolation of bacteria, identification was made on the basis of their Gram's reaction and biochemical tests with reference to Bergey's manual of determinative bacteriology. The direct culturing of the samples was carried out by pipetting 1ml of the dissolved sample in sterile distilled water aseptically on sterile Petri-dishes. Pour plate technique was used with the NA (Nutrient Agar) plates incubated at 35°C for 2-3 days. Bacteria were identified in their unique colonies. They were subcultured by streaking out on Nutrient Agar. Bacterial cells on NA took 2-3 days at 35°C before their pure cultures were stored on slants containing Nutrient agar for bacteria and later stored at 4°C. These slants served as stock culture for bacteria. Other tests were carried out to identify the morphological and biochemical characteristics of the microbes such as Gram's staining reaction, spore staining, motility test, catalase test, Voges-Proskauer test, methyl-red

test, starch hydrolysis, casein hydrolysis, gelatin hydrolysis, oxidase test, indole test, nitrate reduction, sugar fermentation test, growth at different temperature, NaCl concentration, anaerobic growth, citrate utilization test, hydrogen sulphide production, urease test, acid production, sporulation test, growth in liquid medium at varying temperature, NaCl solution, and deamination of amino acid test.

Cost-benefit Analysis - Cost-benefit analysis was carried out, taking into consideration the cost of maize, wheat offal and the enzyme (*xylanase*) as they were related to the performance of birds.

Statistical analysis

All Data collected were subjected to two way analysis of variance (ANOVA) using the PRO GLM (General Linear Model) of SAS (2008) at 5% level of significance. All significantly different means were separated using the Duncan's Multiple Range Test of the same software package (SAS, 2008).

Results and Discussions

Table 2a shows the effects of dietary levels of BDG with or without enzyme supplementation on the performance of broilers. Feed intake by birds fed the control diet was significantly lower than those fed diets with BDG irrespective of levels ($p < 0.05$). Increase in dietary levels of BDG had significantly increased feed intake ($p < 0.05$). Birds fed the control diet gained more weight than those fed diets with dietary levels of BDG ($p > 0.05$). Weight gain by birds fed diets with 20% or 30% BDG were comparable and significantly lower than those fed diet with 10% BDG ($p < 0.05$). Feed gain ratio of birds fed diets with dietary levels of BDG had significant increased effects and were significantly higher than those fed the control diet ($p < 0.05$). Thus birds fed the control diet had a better feed gain ratio compared to other levels of BDG ($p < 0.05$). Enzyme supplementation significantly increased feed intake and weight gain ($p < 0.05$). There was no

significant interaction between dietary levels of Brewers Dried Grain and enzyme supplementation on Feed Intake and Weight Gain parameters ($p>0.05$). There was a significant interaction ($p<0.05$) between dietary levels of BDG and enzyme supplementation on feed/gain ratio, details of

which is shown in Table 2b. Thus, enzyme supplementation reduced feed/gain ratio for birds fed the control diet and diets with 10 or 20% BDG. However there was no significant effect of enzyme supplementation in birds fed diet with 30% BDG ($P>0.05$).

Table 2a Effects of Dietary Levels of Brewers Dried Grain with or without Xylanase Supplementation on Performance of Broilers (0-5wks).

	Feed Consumed (g/bird/day)	Weight Gain (g/bird/day)	FCR
BDG (%)			
0	55.90 ^d	31.90 ^a	1.80 ^d
10	60.30 ^e	29.00 ^b	2.10 ^e
20	63.80 ^b	26.70 ^c	2.40 ^b
30	66.00 ^a	26.00 ^c	2.50 ^a
SE	0.55	0.32	0.02
ES (100ppm)			
0	62.90 ^a	27.10 ^b	2.30 ^a
100	60.10 ^b	29.70 ^a	2.10 ^b
SE	0.15	0.22	0.02
BDG*ES	NS	NS	S

Column means with different superscripts are significantly different, NS: not significant, S: Significant, FCR: Feed conversion ratio

Table 2b Details of interaction on Feed Conversion Ratio

Dietary Supplementation with Brewers Dried Grain (BDG) (%)

ES (ppm)	0	10	20	30	SE
0	1.90 ^e	2.40 ^{cd}	2.50 ^{bc}	2.60 ^a	
100	1.60 ^f	1.80 ^e	2.30 ^d	2.50 ^b	0.03

Table 3 shows cost benefit analysis of replacing maize with or without enzyme supplemented Brewers Dried Grain. Enzyme supplementation at 0 and 10% BDG inclusion level resulted to a percentage decrease (11.44

and 5.01 % respectively) while other treatments resulted to a percentage increase in cost of raising a broiler to 1kg as compared to the control diet.

Table 3 Cost benefit analysis of replacing maize with or without enzyme supplemented BDG

Source of Variation	Cost of Producing/kg (N)	Percentage reduction in price of feed (%)	Cost of Raising 1Kg of Broilers (N)	Percentage reduction to Raise 1Kg of Broilers (%)
Dietary levels of BDG without xylanase				
0	111.50	0.00	210.70	0.00
10	107.00	4.21	254.70	-20.80
20	102.50	8.78	253.20	-20.10
30	96.50	15.00	250.90	-19.10
Dietary levels of BDG with xylanase				
0	114.50	-2.62	186.60	11.40
10	110.00	1.36	200.20	5.00
20	105.50	5.69	242.70	-15.20
	99.50	12.06	247.70	-17.60

The effect of dietary level of BDG with or without enzyme supplementation on nutrient retention is shown in Table 4. Increased in dietary levels of BDG had significant effects on Crude protein and Crude fibre but no effect on Ether extract. Crude protein of birds fed the control diet was significantly higher than those of birds fed diets with BDG irrespective of level. ($p < 0.05$). Dietary levels of BDG had significant decreased effects on the protein retention by the birds. Crude fibre retention by birds fed the

control diet was significantly higher than those of birds fed diets with BDG irrespective of level ($p < 0.05$). Dietary levels of BDG had a significant decreased effects on the crude fibre by the birds ($p < 0.05$). Enzyme supplementation had significant increased effects on Crude Protein, Ether Extract and Crude Fiber ($p < 0.05$). There was no significant interaction between enzyme supplementation and dietary levels of BDG on Crude Protein, Ether Extract and Crude Fiber retentions ($P > 0.05$).

Table 4: The effect of dietary replacement of maize with or without enzyme supplemented BDG on Nutrient Retention.

	Crude Protein (%)	Crude Fiber (%)	Ether Extract (%)
BDG(%)			
0	83.10 ^a	74.30 ^a	63.80
10	73.60 ^b	66.20 ^b	63.70
20	70.00 ^c	60.80 ^c	65.60
30	65.20 ^d	58.50 ^d	64.90
SE	0.78	0.76	1.12
ES (100ppm)			
0	69.20 ^b	59.60 ^b	63.20 ^b
100	76.70 ^a	69.40 ^a	66.80 ^a
SE	0.55	0.79	0.54
BDG*ES	NS	NS	NS

Column means with different superscripts are significantly different, NS: not significant, S: Significant

Table 5a shows the effects of dietary levels of BDG with or without enzyme supplementation on the microbial gut profile of broilers. Increase in dietary levels of BDG from 0% to 30% had significant effects on the TVC, TCC, LBC, FC and pH ($p < 0.05$), but no effect on FCC. TVC for those birds fed diets with dietary levels of BDG were significantly higher than birds fed the control diet ($p < 0.05$). TVC of birds fed diets with 10% and 20% BDG were comparable but significantly lower

than birds fed diet with 30%BDG. TCC of birds fed the control diet was significantly lower than those fed diets with 10% or 30%BDG ($p < 0.05$), but comparable with birds fed diet with 20%BDG ($p > 0.05$). TCC of birds fed diets with BDG were comparable irrespective of levels ($p > 0.05$). LBC of birds fed the control diet was significantly lower than those fed diets with 20% and 30%BDG ($p < 0.05$), but comparable with birds fed diet with 10%BDG ($p > 0.05$). LBC of birds fed

diets with 30% BDG was significantly higher than those of birds fed diets with 10% or 20%BDG. FC of birds fed the control diet was significantly lower than those fed diets with dietary levels of BDG ($p<0.05$). FC of birds fed diets with BDG were comparable irrespective of levels ($p>0.05$). pH of birds fed the control diet was significantly lower than those fed diet with 20%BDG ($p<0.05$), but comparable with those of birds fed diets with 10% or 30%BDG ($p>0.05$). pH of birds fed

diets with BDG were comparable irrespective of levels($p>0.05$). Enzyme supplementation had significant effects on TCC, LBC and pH but no significant effects on TVC, FCC and FC. There was significant increased effects in TCC and pH but decreased effect in LBC by enzyme supplementation ($p<0.05$). There was no significant interaction between enzyme supplementation and dietary levels of Brewers Dried Grain on the microbial gut profile parameters ($p>0.05$).

Table 5a Effects of dietary levels of Brewers Dried Grain with or without enzyme supplementations on the microbial gut profile of broilers.

Treatment	Ph	TVC	TCC	FCC	LBC	FC
BDG (%)						
0	5.50 ^b	5.80 ^c	4.70 ^b	2.40	1.30 ^c	3.50 ^b
10	5.70 ^{ab}	6.60 ^b	5.60 ^a	1.60	3.70 ^{bc}	3.60 ^a
20	5.70 ^a	6.80 ^b	5.50 ^{ab}	1.80	3.10 ^b	3.20 ^a
30	5.60 ^{ab}	7.70 ^a	6.20 ^a	1.70	2.70 ^a	3.40 ^a
SE	0.04	0.16	0.26	0.44	0.17	0.16
ES (100ppm)						
0	5.70 ^a	6.50	5.20 ^b	1.50	1.80 ^a	2.10
100	5.60 ^b	6.90	6.00 ^a	0.70	2.60 ^b	2.50
SE	0.03	0.11	0.18	0.31	0.12	0.11
BDG*ES	0.01	0.61	0.56	0.71	0.079	0.29

Total Viable Counts (TVC), Total Coliform Counts (TCC), Faecal Coliform Counts (FCC), Lactobacillus Counts (LBC) and Fungi Counts (FC).

Table 5b Colonial and Morphological Characteristics of fungi in experimental broiler gut

Isolates	Colonial Characteristics	Morphological Characteristic Under Microscope	Identification
1	Loose white mycelia changing to yellowish black on sporulation under 72 hours of incubation	Conidiophore is smooth wall, non-septate hyaline near the vesicle. Residue was globose and conidia were in chains, rough with no spine. Phialide is uniform. Hyphae were septate and conidia in chains more or less globose and rough.	<i>Aspergillus niger</i>
2	Loose white mycelial turning yellowish green with colorless reverse side on petri dish	Heavily walled conidiophore, uncolored, non-septate, coarsely roughened. Vesicle is globose at maturity and spinnate. A matured vesicle was globose and spinnate although some were elliptical.	<i>Aspergillus flavus</i>
3	Fluffy white mycelia becoming dirty brown later covering about 10cm width on PDA plate.	Sporangia are supported by a large apophysate columella atop a long stalk, the sporangiophore. Sporangiophores arise among distinctive, root-like rhizoids. Rhizoids and stolons were light brown while the collumelae were light brown. The sporangiophores were irregular, round, oval and elongated.	<i>Rhizopus stolonifer</i>
4	Colonies are white with purple brown patches. Colour of the under side was yellow at the early stage with cells having single cell microconidia.	Production of both macro- and microconidia from slender phialides. Macroconidia are fusiform to sickle-shaped, mostly with an elongated apical cell and pedicellate basal cell. Microconidia are one or two-celled, hyaline, fusiform to ovoid, straight or curved, (banana shaped with blunt hook ends with foot cells)	<i>Fusarium solani</i>
5	Colonies are flat, smooth, moist, glistening or dull, and cream in color.	Cell buds were observed under the microscope. They are unicellular, globose, and ellipsoid to elongate in shape. Multilateral (multipolar) budding is typical. Pseudohyphae, if present, are rudimentary. Hyphae are absent. <i>Saccharomyces</i> produces ascospores which are globose and located in asci. Each ascus contains 1-4 ascospores. Ascospores are stained with Kinyoun stain and ascospore stain. When stained with Gram stain, ascospores appear Gram-negative, while vegetative cells appear Gram-positive.	<i>Saccharomyces cerevisiae</i>
6	Pale green blue velvety colony which later turned darker green shades. Surface is covered by a thin overgrowth of hyaline, mycelium bearing some conidiophores appearing flocculent.	Numerous erect single conidiophores are seen under the microscope forming colony with radial pattern, margin and lobed. Conidiophores are smooth walled while the metulae is cylindrical, smooth and walled bearing 3 - 6 phialides which are flask shaped and thick walled and the conidia subglobose.	<i>Penicillium chrysogenum</i>

Table 5c Results of Biochemical tests on bacteria in the gut of experimental broiler chickens

Tests	Salmonella	Lactobacillus	Streptococci	Campylobacter	E.coli	Isolates	Isolates	Clostridium	Staphylococcus
						Bifidobacterium	Bacillus		
						m			st
Gram Stain	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve
Catalase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Oxide	-ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve
Nitrate reduction	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Arch Hydrolysis	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve
Methyl Red	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve
oges proskauer	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve
asein	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
ydrolysis									
ielatin	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve
ydrolysis									
adole Test	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
I2S production	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve
Nitrate reduction	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve
lrea Hydrolysis	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	Variable
deamination test	-ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve
oxidase test	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve
Motility	Motile	Non Motile	Non motile	Non motile	Motile	Non = motile	Non motile	Non motile	Non motile
Shape	Rod	Rod	Cocci	Rod	Rods	Cocci	Rod	Rod	Cocci Rod
Capsule	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve

+ = Positive, - = negative

Table 5d Results of Biochemical tests on *Saccharomyces cerevisiae*

Test	Result
Acid Production	-ve
Urease	+ve
Glucose	+ve
Raffinose	+ve
Fructose	-ve
Galactose	+ve
Sucrose	-ve
Mannitol	+ve
Lactose	+ve
Maltose	+ve
Inositol	+ve

Table 5e shows the effects of dietary levels of Brewers Dried Grain with or without enzyme supplementation on the number of fungi identified in the crop, ileum and caecum pooled together. Dietary levels of wheat offal from 0 to 30% without xylanase supplementation had no effect on the number of beneficial fungi identified in the gut of the birds. *Penicillium chrysogenum* was the only beneficial fungus identified in the gut of bird fed the control diet and those birds fed diets with dietary levels of BDG. Two species of opportunistic/pathogenic fungi namely; *Rhizopus*

stolonifer and *Aspergillus niger* were identified in the gut of birds fed the control diet and four species (*Fusarium solani*, *Rhizopus stolonifer* *Aspergillus niger* and *Aspergillus flavus*) were identified in the gut of those birds fed diets with dietary levels of BDG. Enzyme supplementation of the dietary levels of BDG increase the number of beneficial fungi identified and a decrease effect on the number of the opportunistic/pathogenic fungi identified in the gut of the birds. With enzyme supplementation: the number of beneficial fungi identified in the gut of birds fed the control diet was still *Penicillium chrysogenum* while beneficial fungi identified in the gut of those birds fed diets with dietary levels of BDG had increased to two species namely; *Penicillium chrysogenum* and *Saccharomyces cerevisiae*. In the gut of birds fed the control diet with enzyme supplementation the number of pathogenic fungi identified had reduced to three species (*Fusarium solani*, *Rhizopus stolonifer* and *Aspergillus flavus*) while only *Aspergillus flavus* was identified in the gut of birds fed diet with 10% BDG without xylanase supplementation and *Aspergillus flavus* and *Rhizopus stolonifer* were identified in those birds fed diets with 20 or 30% BDG without xylanase supplementation

Table 5e Identification of Microbes (Fungi)

Without Enzyme	Beneficial Fungi	Opportunistic/Pathogenic Fungi
Control	<i>Penicillium chrysogenum</i>	<i>Rhizopus stolonifer</i> , <i>Aspergillus niger</i> , <i>Fusarium solani</i> , <i>Rhizopus stolonifer</i> <i>Aspergillus niger</i> , <i>Aspergillus flavus</i>
10%	<i>Penicillium chrysogenum</i>	
20%	<i>Penicillium chrysogenum</i>	<i>Fusarium solani</i> , <i>Rhizopus stolonifer</i> <i>Aspergillus niger</i> , <i>Aspergillus flavus</i>
30%	<i>Penicillium chrysogenum</i>	<i>Fusarium solani</i> , <i>Rhizopus stolonifer</i> <i>Aspergillus niger</i> , <i>Aspergillus flavus</i>
With Enzyme	Beneficial Fungi	Opportunistic/Pathogenic Fungi
Control	<i>Penicillium chrysogenum</i>	<i>Fusarium solani</i> , <i>Rhizopus stolonifer</i> , <i>Aspergillus flavus</i> <i>Aspergillus flavus</i>
10%	<i>Penicillium chrysogenum</i> , <i>Saccharomyces cerevisiae</i>	
20%	<i>Penicillium chrysogenum</i> , <i>Saccharomyces cerevisiae</i>	<i>Rhizopus stolonifer</i> , <i>Aspergillus flavus</i> <i>Rhizopus stolonifer</i> , <i>Aspergillus flavus</i>
30%	<i>Penicillium chrysogenum</i> , <i>Saccharomyces cerevisiae</i>	

Table 5f shows the summary of the identified bacteria in the crop, ileum and caecum pooled together. Replacement of Maize with BDG with or without xylanase supplementation had an effect on the number of the microbes (bacteria) in the crop, ileum and caecum. In the gut of birds fed diets with dietary levels of wheat offal from 0 to 30% without xylanase enzyme supplementation; *Lactobacillus* sp was the only one beneficial bacterium identified while the pathogenic bacteria identified were six genera namely; *E. coli*, *Streptococcus*, *Staphylococcus*, *Clostridium*, *Campylobacter* and *Salmonella* species. Enzyme supplementation of the dietary levels of BDG had increase effects on the number of beneficial bacterial and reduce effects on the number of the

pathogenic bacteria identified in the gut of the broiler chickens. With enzyme xylanase supplementation: birds fed diet with 0% WO (control) though the number of beneficial bacteria was still one (*Lactobacillus* sp) but the pathogenic bacteria. Identified had reduced to five genera namely: *E. coli*, *Streptococcus* sp, *Staphylococcus* sp, *Clostridium* sp, *Campylobacter* sp. compared. Birds fed dietary levels of BDG with xylanase supplementation had three genera of beneficial bacteria identified in their gut namely: *Lactobacillus* sp, *Bacillus subtilis* and *Bifidobacterium* sp while the number of pathogenic bacteria had reduced to two genera (*Campylobacter* sp and *Staphylococcus* sp.).

Table 5f Identification of Microbes (Bacteria)

Without Enzyme	Beneficial Bacteria	Pathogenic Bacteria
Control	<i>Lactobacillus plantarum</i>	<i>E. coli</i> , <i>Streptococcus</i> sp, <i>Staphylococcus</i> sp, <i>Clostridium</i> sp, <i>Campylobacter</i> spp, <i>Salmonella</i> sp.
10%	<i>Lactobacillus plantarum</i>	<i>E. coli</i> , <i>Streptococcus</i> sp, <i>Staphylococcus</i> sp, <i>Clostridium</i> sp, <i>Campylobacter</i> sp, <i>Salmonella</i> sp.
20%	<i>Lactobacillus plantarum</i>	<i>E. coli</i> , <i>Streptococcus</i> sp, <i>Staphylococcus</i> sp, <i>Clostridium</i> sp, <i>Campylobacter</i> sp, <i>Salmonella</i> sp.
30%	<i>Lactobacillus plantarum</i>	<i>E. coli</i> , <i>Streptococcus</i> sp, <i>Staphylococcus</i> sp, <i>Clostridium</i> sp, <i>Campylobacter</i> sp, <i>Salmonella</i> sp.
With Enzyme	Beneficial Bacteria	Pathogenic Bacteria
Control	<i>Lactobacillus plantarum</i>	<i>E. coli</i> , <i>Streptococcus</i> sp, <i>Staphylococcus</i> sp, <i>Clostridium</i> sp, <i>Campylobacter</i> sp.
10%	<i>Bacillus subtilis</i> , <i>Lactobacillus plantarum</i> , <i>Bifidobacterium</i>	<i>Campylobacter</i> sp, <i>Staphylococcus</i> sp.
20%	<i>Lactobacillus plantarum</i> , <i>Bacillus subtilis</i> , <i>Bifidobacterium</i>	<i>Campylobacter</i> sp, <i>Staphylococcus</i> sp.
30%	<i>Lactobacillus plantarum</i> , <i>Bacillus subtilis</i> , <i>Bifidobacterium</i>	<i>Campylobacter</i> sp, <i>Staphylococcus</i> sp.

The increased feed intake of the birds on the diets containing Brewers Dried Grain (BDG) is understandable, BDG contain high fibre which tends to increase the total fibre content of the diets and dilute other nutrients. Birds must therefore eat to meet their energy requirement to sustain rapid growth and development hence the increased feed intake. This is in agreement with the report of Chong *et al.* (2008) that birds' attempt, as a priority, to consume a certain quantity of feed necessary to meet their energy requirements. Observation in this study also agreed with the views of Onifade and Babatunde (1998) and Aderolu *et al.*, (2007) who reported increased feed intake with increasing levels of dietary brewers dried grain (BDG). Decreased in weight gain with increased in dietary level of BDG was recorded in this study. This may be due to higher dietary fibre resulting from increasing levels of BDG which might have decreased nutrient availability and consequently, weight gain. This observation was in agreement with Aderolu *et al.*, (2007) and Bolu *et al.*, (2015) who reported lower weight gained as the dietary level of BDG and DDG increased respectively. Feed conversion ratio was significantly higher (poorer) with corresponding increase in BDG inclusion level. This may be as a result of increased dietary fibre with increased level of dietary BDG inclusion which could have altered the texture of the feed coupled with change in colour, taste and possibly odour of the diets. Jorgensen *et al.*, (1996) and Aderolu *et al.*, (2007) reported that these factors can independently or combine together to affect feed consumption and utilization. Birds do not have the innate ability to utilize fibre efficiently. This observation also agreed with the works of Ander (1992) and Onifade and Babatunde (1998) who reported that efficiency of food utilization decreased as the concentration of BDG in diets increased.

There were significant ($P < 0.05$) effect of *xylanase* enzyme supplementation on feed intake, weight gain and feed conversion ratio

of broiler birds. This result was in agreement with the findings of Atteh (2004) and Ohimain *et al.*, (2013) who reported that enzyme supplementation improved some of the performance characteristics of broilers. Enzyme supplementation improves energy content of feeds resulting in animals reaching their energy requirement faster in enzyme supplemented diets than diets without enzyme supplementation and as such consumes less. In this study enzyme supplementation reduced feed consumption and improved feed conversion ratio. Birds fed diets with supplemented BDG with *xylanase* consumed lesser feed, gained more weight and as such had a lesser (better) feed conversion ratio as compared to those without enzyme. The supplementation of enzyme caused a better performance of birds fed with high fibre diets, as exogenous enzyme supplementation have been reported to render fibrous polysaccharides and other anti-nutritional factors utilizable by non-ruminants (May *et al.*, 1994; Jorgensen *et al.*, 1996; Choct, 2006).

BDG inclusion in the diets brought about non significant ($P > 0.05$) decline in digestibility coefficients of ether extract and ash. This agreed with the findings of Babatunde *et al.* (1975) corroborated by Panda (2005) who also observed insignificant decreases in dry matter, energy, nitrogen and ether extract digestibilities when BDG based diets were fed to growing pigs. According to these authors, the decline in dry matter digestibility may be attributed to increases in the dietary levels of crude fibre as BDG levels increased in the diets. However, there was significant reduction in digestibility coefficients of crude protein and crude fibre with increased BDG in the diets. This was supported by the report of Yaakugh *et al.* (1988) who observed significant ($P < 0.05$) depressions in the digestibility of dry matter, energy, protein, ether extract, crude fibre and ash as the levels of BDG replacing maize increased in the diets of pigs.

Enzyme supplementation has a significant effect on the treatments as those supplemented with enzyme has a higher (better) nutrient retention (CP, CF and EE). This may be as a result of the enzyme effect which degraded the non-starch polysaccharides as enzyme supplementation was reported to improve fibre, ether extract and protein digestibility (Bedford, 1992 and Ohimain *et al.*, 2013). Iyayi and Davis (2005), Iyayi *et al.* (2005) and Li *et al.* (2008) observed increased in crude fibre digestibility and concluded that the enzymes must have acted on cellulose, glucuronoxylans, arabinoxylans and mannan thereby reducing the crude fibre content and subsequently increased the energy contents and NFE digestibility.

Dietary BDG inclusion level had a significant effect on the weight of drumsticks. This might have resulted from the prolonged time of eating in the 30% BDG group due to higher level of dietary fibre. From observation, birds stand most of the time they eat and they eat to meet their energy requirements and as such the feed intake, eating time will be increased. This might subsequently affect the drumsticks. This prolonged eating time is in consonance with Hertland *et al.*, (2004) reported that birds fed high fibre diet spend more time eating and appear quieter than those fed low fibre diets.

The percentage weight of the proventriculus increased with increased level of BDG without enzyme supplementation while enzyme supplementation at all inclusion level had similar effect. This may be attributed to the fibre content and hence bulkiness of diets without enzyme supplementation. The proventriculus is known to produce pepsin and Hcl for gastric digestion. Increase in proventriculus size suggests an increase in enzyme production for digestive purposes with increase in dietary level of BDG (Boisen and Fernandez, 1995). Enzyme supplementation led to a decrease in the weight of the empty gizzard as enzyme supplementation is believed to breakdown fibre thereby reducing the bulkiness of feed that was being digested in the gizzard. The reduction in bulkiness of enzyme supplemented diets can be said to be

the reason for a lower percentage weight of the empty gizzard. Gizzard worked less in the presence of enzyme (Madrigal *et al.*, 1993).

Abdominal fat was significantly lower in BDG based diets as compared to diet with 0% BDG inclusion level. This might be as a result of high level of fibre which acts as diluent thereby preventing excess fat deposition in the body of the animals. This is in agreement with Babatunde, *et al.*, (1975) who reported that fibrous feeds, because of their lower content and availability of energy tend to promote better carcasses with higher lean meat and lower fat content. Enzyme supplementation caused a numerical increase in abdominal fat, this result agree with Cafe *et al.* (2002) who reported that broilers fed enzyme supplemented diet had significantly higher proportion of abdominal fat.

The pH of all treatments apart from enzyme supplemented 0% BDG inclusion level were similarly affected but significantly higher than that of enzyme supplemented 0% BDG inclusion level. This could be the reason why the birds fed 0% BDG enzyme supplemented diets had better performance in terms of feed consumption, weight gained and feed conversion ratio. Low pH (acidic condition) has been reported by Mabelebele *et al.*, (2014) to aid digestion better in broiler chicken although he reported a higher pH value (4.90) in crop of broiler chickens.

It was observed that TVC of treatments supplemented with enzyme was significantly (enzyme supplementation of diets with BDG at 20%) or numerically (enzyme supplementation of diets with BDG at 0%, 10%, and 30%) higher than their respective counterparts without enzyme supplementation. This is due to the prebiotic potential of BDG as BDG has been reported to have prebiotic potential most especially when supplemented with appropriate enzyme. The LBC of the latter was numerically (0%, 10% and 20%) or significantly (30%) lower than the former while the FCC was numerically higher. The LBC population is observed to increase the TVC while reducing the non-beneficial bacteria population. This proves the superiority of beneficial bacteria (LBC) over

non-beneficial ones (TCC and FCC) as the beneficial bacteria were suppressed by the beneficial ones; hence, the prebiotic potential of BDG. Enzyme supplementation at 0% was significantly higher than other treatments which appeared similar. Enzyme supplementation was seen to reduce the pH of the treatment.

The TCC and pH of enzyme supplemented diets were significantly lower to those without enzyme supplementation while the LBC was significantly higher. The lower TCC and higher LBC population can be as a result of the prebiotic properties of BDG that was enhanced by enzyme supplementation. The enzyme action might have resulted to the lower pH in birds fed enzyme supplemented diets as lower pH was reported by Mabelebele *et al.*, (2014) to aid digestibility of dietary fibre.

Dietary BDG inclusion level had significant effect on the TCC, LBC and pH values but other parameters measured were not significantly affected. The total coliform count and pH value of enzyme supplemented diets was significantly lower while the *Lactobacillus* population was higher than diets without enzyme supplementation. The significant lower pH value may be as a result of enzymatic reaction on the enzyme supplemented diets thereby causing a lower pH (more acidic pH) to aid more digestion (Fuller, 1977 and Panda *et al.*, 2005). This is also in accordance to what Mabelebele *et al.*, (2014) reported that a lower pH could be the reason for better digestion in birds. The significantly lower TCC and higher LBC in enzyme supplemented diets may be as a result of BDG having prebiotic properties (supporting the growth of *Lactobacillus* in order to suppress the growth of total coliform bacteria). Thus, findings in this study showed that inclusion of BDG without enzyme supplementation increased the concentration of pathogenic/opportunistic microbes (fungi and bacteria) against the beneficial microbes while enzyme supplementation increased the concentration of beneficial microbes

(Bezkorovainy, 2001). This finding is in accordance with Bradley *et al* (1994), Binns (2013) and Kalantar *et al.*, (2014) who indicated that Barley diets increased the total number of bacteria and the population of gram negative, coliform and clostridium bacteria (pathogenic microbes), conversely decreased the population of lactic acid bacteria (beneficial microbes) in the intestinal digesta compared to other diets. Inversely, birds fed on barley + enzyme diet had higher number of lactic acid bacteria (Crittenden and Playne, 1996; Cowan, 1999; Collins and Gibson, 1999).

The results obtained from this study showed that replacement of maize with Brewers Dried Grain irrespective of levels supplemented with 100ppm *xylanase* enzyme caused a reduction in feed intake and an increase in weight gain and better FCR. In all these parameters, it is observed that birds fed diet with 10% BDG supplemented with *xylanase* enzyme out-performed birds fed diets with 10 or 30% BDG supplemented with *xylanase* enzyme and closer to the birds fed the control diet which was with better FCR.

It can be deduced that enzyme supplementation of BDG helped in increasing and improving protein, ether extract and fibre digestibilities corroborating the report of Bedford (1992). The weights of vital organs in this trial showed that the birds were in good health conditions during the trial period corroborating the report of Bounous and Stedman (2000) on normal avian haematology and body condition.

The microbes identified (Fungi and Bacteria) showed that dietary levels of BDG (10, 20 or 30% inclusion) with supplementation of enzyme *xylanase* enhanced the growth of beneficial microbes which resulted in inhibition or elimination of the opportunistic/pathogenic microbes.

The cost benefit analysis revealed that 10% inclusion level of BDG supplemented with *xylanase* enzyme gave the best result of a beneficiary reduction in the cost of production with the best improved broiler performance.

Conclusion

Enzyme supplementation of high fibre feedstuffs improved growth performance, nutrient retention and fibre digestibility. Use of enzymes is therefore recommended when HFFs are required as feedstuffs for broilers chicken.

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