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PREBIOTIC POTENTIAL OF XYLANASE ENZYME SUPPLEMENTED WHEAT OFFAL IN BROILER CHICKENS

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Abstract: The recent development of antibiotics-resistant pathogens in poultry, which poses a threat to human health, has necessitated the search for an alternative to antibiotic growth promoters (AGPs) to improve the gut microflora in poultry diets. One of the alternatives to AGPs is probiotics which are beneficial organisms. The by-products of the digestion of polysaccharides for which poultry do not have enzymes to digest are called prebiotics. Prebiotics serve as food for probiotics. The application of enzymes makes this digestion possible. The prebiotic potentials of enzyme supplemented high fibre feedstuffs (HFFs) are not known. This study was conducted to assess the prebiotic potential of xylanase enzyme supplemented wheat offal on broiler chickens (*in-vivo*). The replacement of maize with wheat offal irrespective of levels supplemented with 100ppm xylanase enzyme caused a reduction in feed intake and an increase in weight gain and better feed conversion ratio. Birds fed diet with 20% wheat offal supplemented with a xylanase enzyme outperformed birds fed diets with 10 or 30% wheat offal supplemented with a xylanase enzyme and birds fed the control diet. The birds had normal weights of vital organs with good structural consistency. The identification of microbes (fungi and bacteria) showed that dietary levels of wheat offal (10, 20 or 30% inclusion) with supplementation of a xylanase enzyme enhanced the growth of beneficial microbes, which resulted in the inhibition or elimination of the opportunistic/pathogenic microbes. It was concluded that enzyme supplementation of high fibre feedstuffs could improve the growth performance, nutrient retention and increase concentration of beneficial microbes in the guts. The use of enzymes is therefore recommended when HFFs are required as a prebiotic source in the guts of broilers.

Key words: prebiotics, enzymes, intestinal microflora, broiler nutrition, digestibility.

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Introduction

Wheat offal is one of the high fibre feedstuffs that were screened and that met the first criteria for classification of any feedstuff as a potential prebiotic. Currently, there is a lot of interest in improving gut health by managing the colonic microbial population. This is traditionally done by the consumption of probiotics which are live microbial food supplements (Atteh, 2000). Probiotics will multiply faster in an environment that is rich in their food. Prebiotics are food for probiotics. Prebiotics are mostly oligosaccharides, some of which are by-products of fibre breakdown. They are mostly non-digestible by enzymes in the gut (Bailey et al., 1991). Fructans and arabinoxylans are naturally occurring non-digestible oligosaccharides in wheat that exhibit prebiotic properties. Wheat offal is made up of the coarse outer covering of wheat kernel, the seed coat, the major part of the germ, as separated from clean and scoured wheat in the process of wheat milling. Wheat bran is low in energy and high in fibre and does not allow for large inclusions in the poultry diet. This is because arabinoxylans (pentosans) being a major component of the non-starch polysaccharide fraction in cereals are not easily digested by monogastrics, and are abundantly present in wheat bran and wheat (Montagne et al., 2003). The anti-nutritive activity of arabinoxylans in wheat and wheat bran has been widely studied and found to depress the growth rate, feed digestibility and feed conversion efficiency when used in high quantity in broiler diets (Montagne et al., 2003).

Supplementation of wheat-based diets with xylanase enzymes such as arabinoxylanases and pentosanases has been reported to be capable of breaking down the arabinoxylans, as well as to reduce the adverse effects of some anti-nutritional compounds (Cowieson and Acamovic, 2003). Enzyme supplementation of wheat offal causes the breakdown of its cell wall with the release of oligosaccharides which can improve the probiotic population, thus improving digestibility and gut health. There is, however, little or no information on the prebiotic potentials of enzyme supplemented wheat offal in chickens. This study is therefore designed to assess the prebiotic potential of xylanase enzyme supplemented wheat offal on broiler chickens.

Materials and Methods

Sourcing and management of birds – A total of one thousand, nine hundred and twenty (1,920) day-old broiler chicks of the 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 1. A xylanase enzyme used is a bacteria xylanase feed enzyme (nutrase, a pure endo-1, 4-beta-xylanase) produced by *Bacillus subtilis* to break down the arabinoxylan fraction into shorter polysaccharides

(xylose monomers) with a decrease of viscosity, liberation of nutrients and improved zootechnical performances. It was supplied by Nutrex, Belgium.

Experimental design – Birds were fed a control diet (50% maize) or diets in which wheat offal 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 five (5) weeks. Live weight was recorded weekly while feed intake was recorded daily in grams and excreta samples were 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).

Table 1. Composition of experimental diet (%).

Ingredients	1	2	3	4	5	6	7	8
Maize (%)	50	50	40	40	30	30	20	20
Wheat offal (%)	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: Ground nut 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. B_2, 0.3g, Vit.B_6, 8.0mg, Vit.B_{12}, 8.0g, Nicotinic acid, 3.0g, Ca-pantothenate, 50mg, Fe, 10.00g, Al, 0.2g, Cu, 3.5mg, Zn, 0.15mg, I, 0.02g, CO_2, 0.01g, Se.

At the end of the experiment, 10 birds per replicate from each treatment were randomly selected and euthanized by bleeding through the carotid artery for collection of 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 the population and profile of microorganisms present in the broiler gut. Five grams of each sample were put into a sterile universal bottle containing 100ml of sterile water and shaken thoroughly. The mixture was allowed to settle and the supernatant was decanted into separate test tubes to be used as a stock solution. Serial dilutions of the stock were made and a 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^{0}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 4^0C. The isolates were identified according to the scheme of Chio et al. (1994). In isolation of bacteria, identification was based on 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. The pour plate technique was used with the NA (nutrient agar) plates incubated at 35^0C for 2–3 days. Bacteria were identified in their unique colonies. They were subcultured by streaking out on nutrient agar. Bacterial cells on NA were left for 2–3 days at 35^0C before their pure cultures were isolated and stored on slants containing nutrient agar at 4^0C. These slants served as the 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 temperatures, NaCl concentration, anaerobic growth, citrate utilization test, hydrogen sulphide production, urease test, acid production, sporulation test, growth in liquid medium at varying temperatures, NaCl solution, and deamination of amino acids.

Cost-benefit analysis – Cost-benefit analysis was carried out, taking into consideration the cost of maize, wheat offal and the enzyme (xylanase) as they are 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 the 5% level of significance. All significantly different means were separated using the Duncan's multiple range test of the same software package.

Results and Discussion

Table 2 shows the effects of dietary levels of wheat offal with or without enzyme supplementation on the performance of broilers. Feed intakes by birds fed the control diet and those fed diet with 10% wheat offal were comparable and they were significantly lower than those of birds fed diets with 20% wheat offal ($p<0.05$). Feed intakes of birds fed 20% and 30% wheat offal were not significantly different. Birds fed diets with wheat offal gained more weight than those fed the control diet ($p<0.05$). Weight gains of birds fed diets with 20% or 30% wheat offal were comparable and significantly higher than those of birds fed diet with 10% wheat offal ($p<0.05$). Feed gain ratios of birds fed the control diet to those fed diets with 10% or 30% were comparable and significantly higher than those fed diet with 20% wheat offal ($p>0.05$). Thus, birds fed diet with 20% wheat offal had a better feed gain ratio compared to other levels of wheat offal ($p<0.05$). Enzyme supplementation increased feed intake and weight gain ($p<0.05$) but had a

numerical decrease effect on feed/gain ratio, though not significant ($p>0.05$). There was no significant effect of the interaction between dietary levels of wheat offal and enzyme supplementation on the performance parameters ($p>0.05$).

Table 2. Effects of dietary levels of wheat offal with or without xylanase supplementation on performance of broilers (0–5wks) and nutrient digestibility.

BDG (%)	Feed consumed (g/bird/day)	Weight gain (g/bird/day)	FCR	Crude protein (%)	Crude fibre (%)	Ether extract (%)
0	75.40^{b}	40.60^{c}	1.90^{a}	62.90^{a}	75.10^{a}	63.90^{a}
10	78.90^{b}	44.00^{b}	1.80^{a}	61.20^{ab}	71.10^{a}	61.50^{ab}
20	86.80^{a}	52.80^{a}	1.60^{b}	61.00^{ab}	65.30^{b}	60.90^{b}
30	88.40^{a}	49.70^{a}	1.70^{a}	58.00^{b}	59.50^{c}	57.80^{b}
SE	75.40^{b}	40.60^{c}	1.90^{a}	62.90^{a}	75.10^{a}	63.90^{a}
Enzyme supplementation (ES) (ppm)						
0	85.50^{a}	45.40^{b}	1.80	60.10	67.20	61.70^{b}
100	79.30^{b}	48.20^{a}	1.60	61.40	68.60	65.40^{a}
SEM±	1.59	1.10	0.24	1.59	1.62	1.09
WO x ES	NS	NS	NS	NS	NS	NS

Column means with different superscripts are significantly different ($p<0.05$), NS: Not significant, S: Significant; FCR – Feed conversion ratio (kg of feed intake/kg of weight gain).

Cost-benefit analysis for replacing maize with wheat offal with or without enzyme supplementation is shown in Table 3. Cost-benefit analysis showed that the 20% inclusion level of wheat offal supplemented with xylanase enzyme gave the best numerical result of a beneficiary reduction in the cost of production with the best improved broiler performance in terms of weight gain.

Table 3. Cost-benefit analysis for replacing maize with wheat offal with or without enzyme supplementation.

Sources of variation	Cost of producing/kg (N)	Percentage reduction in price of feed (%)	Cost of raising 1kg of broilers (N)	Percentage reduction in raising 1kg of broilers (%)
Dietary levels of WO without xylanase				
0	130.00	0.00	260.00	0.00
10	120.00	8.33	240.00	8.33
20	110.00	18.18	242.00	7.44
30	100.00	30.00	230.00	13.04
Dietary levels of WO with xylanase				
0	133.00	-2.26	239.00	8.79
10	123.00	5.69	209.00	24.40
20	113.00	15.04	180.80	43.81
30	103.00	26.21	185.40	40.24

The effects of dietary levels of wheat offal with or without enzyme supplementation on nutrient retention are shown in Table 4. Enzyme supplementation had no significant effect ($p>0.05$) on crude protein and ether extract retention. However, enzyme supplementation caused an increase in crude fibre retention ($p<0.05$). Crude protein retention in birds fed the control diet was significantly higher than in those fed diet with 30% wheat offal ($p<0.05$), but comparable with those of birds fed diet with 10% or 20% wheat offal ($p>0.05$). Protein retention in birds fed diets with wheat offal irrespective of levels was comparable ($p>0.05$). There was a decrease in the crude fat retention with an increase in the dietary level of wheat offal in birds fed diets with 20% or 30% wheat offal being significantly lower ($p<0.05$). The birds fed the control and those fed diet with 10% wheat offal were comparable ($p>0.05$) in crude fat retention. The crude fibre retention of birds fed the control diet was significantly higher than of those of birds fed diets with 20% or 30% wheat offal ($p<0.05$), but comparable with the crude fibre retention of birds fed diet with 10% wheat offal ($p>0.05$). Fibre retention of birds fed diets with wheat offal irrespective of levels was comparable ($p>0.05$). There was no significant effect of the interaction between the inclusion of enzymes and the dietary level of wheat offal on crude protein, ether extract and crude fibre ($P>0.05$).

Table 4. Effects of dietary levels of wheat offal with or without enzyme supplementation on nutrient retention of broiler chickens.

	Crude protein (%)	Ether extract (%)	Crude fibre (%)
Dietary levels of wheat offal (WO) (%)			
0	62.90^{a}	75.10^{a}	63.90^{a}
10	61.20^{ab}	71.10^{a}	61.50^{ab}
20	61.00^{ab}	65.30^{b}	60.90^{b}
30	58.00^{b}	59.50^{c}	57.80^{b}
Enzyme supplementation (ES) (ppm)			
0	60.10	67.20	61.70^{b}
100	61.40	68.60	65.40^{a}
WO x ES	NS	NS	NS
SEM±	1.59	1.62	1.09

Column means with different superscripts are significantly different; NS: Not significant, S: Significant.

Table 5a shows the effects of dietary levels of wheat offal with or without enzyme supplementation on organs and body part weights. An increase in dietary levels of wheat offal from zero to 30% had no significant effect on the heart, proventriculus, abdominal fat, liver, back, head, drumstick, shank and thigh weights ($p>0.05$). However, there were significant effects on the bursa of Fabricius, crop, full and empty gizzard, spleen, neck, wing and breast weights ($p<0.05$).

Table 5a. Effects of dietary levels of wheat offal with or without enzyme supplementation on organs and body parts expressed in percentage of live body weight of broilers.

	Heart	Bursa	Crop	Proven-triculus	Full gizzard	Empty gizzard	Abdominal fat	Liver	Spleen	Back	Neck	Head	Drumstick	Wings	Shank	Thigh	Breast
WO (%)																	
0	0.50	0.10^{b}	0.50^{b}	0.50	2.70^{b}	1.90^{b}	2.10	2.20	1.40^{a}	12.00	5.40^{a}	3.50	9.90	7.90^{b}	4.70	10.30	14.50^{b}
10	0.50	0.20^{b}	0.60^{b}	0.50	3.10^{ab}	1.90^{b}	1.50	1.90	0.20^{c}	15.80	4.80^{b}	3.40	10.60	9.00^{a}	4.00	9.60	15.30^{b}
20	0.50	0.40^{a}	0.90^{a}	0.60	3.90^{a}	2.50^{a}	2.00	1.90	0.90^{b}	14.00	5.00^{ab}	3.20	10.80	7.90^{b}	5.40	9.60	15.70^{b}
30	0.40	0.10^{b}	0.70^{b}	0.60	3.70^{a}	2.40^{a}	0.30	1.90	0.10^{c}	12.00	4.30^{c}	3.60	10.10	7.90^{b}	5.30	16.80	17.10^{a}
ES (ppm)																	
0	0.40	0.20^{a}	0.20	0.60	3.40	2.20	1.80	2.10	0.10	14.20	4.60^{b}	3.30	10.40	7.80^{b}	4.90	12.70	15.00^{b}
100	0.50	0.01^{b}	0.70	05.	3.20	2.10	1.10	1.90	0.10	13.00	5.20^{a}	3.50	10.60	8.50^{a}	4.80	10.50	16.30^{a}
WO x ES	NS	NS	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	S
SEM	0.22	0.32	0.45	0.22	0.17	0.75	0.41	0.11	0.12	0.69	0.13	0.86	0.25	0.18	0.24	1.72	0.39

The weights of the bursa of Fabricius of birds fed the control diet and of those fed diet with 30% wheat offal were comparable and significantly lower than those of birds fed diets with 10% and 20% wheat offal ($p<0.05$). Crop weights of birds fed the control diet and of those fed diets with 10% or 30% wheat offal were comparable and significantly lower than of those birds fed diet with 20% wheat offal ($p<0.05$). The weight of the crop of birds fed diet with 20% wheat offal was significantly higher than of those fed diets with 10% or 30% wheat offal. The weight of the full gizzard of birds fed the control diet was significantly lower than of those fed diets with 20% or 30% wheat offal ($p>0.05$), but comparable with the weight of the full gizzard of birds fed diet with 10% wheat offal ($p<0.05$). The full gizzard weights of birds fed diets with wheat offal irrespective of levels were comparable ($p<0.05$). However, the weight of the empty gizzard of birds fed the control diet and the weight of the empty gizzard of birds fed diet with 10% wheat offal were comparable ($p>0.05$), but were significantly lower than the weight of the empty gizzard of birds fed diets with 20% or 30% wheat offal ($p<0.05$). The empty gizzard weights of those birds fed diets with 20% and 30% wheat offal were not significantly different, but were significantly higher than the weight of the empty gizzard of birds fed diet with 10% wheat offal ($p>0.05$). The weight of the empty gizzard of birds fed the control diet were comparable ($p>0.05$). The spleen weight of birds fed the control diet was significantly higher than of those birds fed diets with wheat offal ($p<0.05$). The weights of spleen of birds fed diets with 10% and 30% wheat offal were comparable but significantly lower than of those fed diet with 20% wheat offal ($p>0.05$). The neck weight of birds fed the control diet was significantly higher than of those birds fed diets with 10% or 30% wheat offal ($p>0.05$), but it was comparable with the neck weight of birds fed diet with 20% wheat offal ($p>0.05$). The neck weights of birds fed diets with 10% and 20% wheat offal were comparable, but were significantly higher than those of birds fed diet with 30% wheat offal ($p<0.05$). The wing weights of birds fed the control diet and of those fed diets with 20% or 30% wheat offal were comparable ($p>0.05$), but were significantly lower than of those birds fed diet with 10% wheat offal ($p<0.05$). The wing weights of birds fed diet with 10% wheat offal were significantly higher than of those birds fed diets with 20% or 30% wheat offal ($p>0.05$). The breast weights of birds fed the control diet and of those fed diets with 10% or 20% wheat offal were comparable ($p<0.05$), and were significantly lower than of birds fed diet with 30% wheat offal. The breast weight of birds fed diet with 30% wheat offal was significantly higher than of those birds fed diets with 10% or 20% wheat offal ($p<0.05$). There was no significant effect of enzyme supplementation on the weight of the heart, crop, proventriculus, full and empty gizzard, abdominal fat, liver, spleen, back, head, drumstick, shank and thigh. However, there were

significantly increased effects on weights of neck, wings and breast but a significantly decreased effect on weight of the bursa of Fabricius. There was no significant ($p>0.05$) effect of the interaction between inclusion of enzymes and dietary levels of wheat offal on all the parameters except for the abdominal fat and breast meat ($p<0.05$), the details of which are shown in Table 5b. Thus, enzyme supplementation of the control diet and diet with 20% wheat offal increased the breast weight ($p<0.05$), while there was no significant effect of this parameter at 10% or 30% wheat offal level ($p>0.05$).

Table 5b. Effects of the interaction between dietary levels of wheat offal and enzyme supplementation on abdominal fat and breast weight ($p>0.05$).

Dietary wheat offal supplementation (%)					
Parameters	ES (100ppm)	0	10	20	30
Abdominal fat	0	2.40^{a}	1.20^{c}	0.60^{c}	0.30^{d}
	100	1.90^{b}	1.80^{b}	0.10^{d}	0.20^{d}
Breast weight	0	13.40^{c}	14.40^{bc}	15.40^{bc}	14.90^{bc}
	100	15.70^{b}	16.20^{b}	19.30^{a}	16.00^{b}

Table 6 shows the effects of dietary levels of wheat offal with or without enzyme supplementation on the microbial gut profile of broilers. An increase in dietary levels of wheat offal from 0% to 30% had a significant effect on the fungi colony count (FCC) and fecal count (FC) ($p<0.05$), but it had no significant effect on pH, total viable count (TVC), total colony count (TCC) and lactobacillus count (LBC) ($p>0.05$). The FCC for the birds fed the control diet was comparable with those of birds fed diets with wheat offal irrespective of the levels ($p<0.05$). Birds fed diet with 20% wheat offal had the significantly lower FCC compared to those of birds fed diets with 10% or 30% wheat offal ($p<0.05$). The FC for the birds fed the control diet was comparable with those of birds fed diets with wheat offal irrespective of the levels ($p<0.05$). Birds fed diet with 20% wheat offal had the significantly lower FC than birds fed diet with 30% wheat offal ($p<0.05$), but comparable with those of birds fed diet with 10% wheat offal ($p<0.05$). Enzyme supplementation had no significant effects on pH, TVC, TCC, FCC, LBC and FC ($p<0.05$), but it had a numerically increased effect on TVC, LBC and FC. There was no significant effect of the interaction between inclusion of enzymes and dietary levels of wheat offal on all the microbial gut profile parameters ($p>0.05$).

The FCC for the birds fed the control diet was comparable with those of birds fed diets with wheat offal irrespective of the levels ($p<0.05$). Birds fed

diet with 20% wheat offal had the significantly lower FCC compared to those of birds fed diets with 10% or 30% wheat offal ($p<0.05$). The FC for the birds fed the control diet was comparable with those of birds fed diets with wheat offal irrespective of the levels ($p<0.05$). Birds fed diet with 20% wheat offal had the significantly lower FC than birds fed diet with 30% wheat offal ($p<0.05$), but comparable with those of birds fed diet with 10% wheat offal ($p<0.05$). Enzyme supplementation had no significant effects on pH, TVC, TCC, FCC, LBC and FC ($p<0.05$), but it had a numerically increased effect on TVC, LBC and FC. There was no significant effect of the interaction between inclusion of enzymes and dietary levels of wheat offal on all the microbial gut profile parameters ($p>0.05$).

Table 6. Effects of dietary levels of wheat offal with or without enzyme supplementation on the microbial gut profile of broilers.

	pH	TVC 1 0^7cfu/ml)	TCC (10^7cfu/ml)	FCC (10^7cfu/ml)	LBC (10^7cfu/ml)	FC (10^5cfu/ml)
WO (%)						
0	6.10	3.00	2.50	0.90^{ab}	2.00	2.50^{ab}
10	6.20	3.90	1.80	1.20^{b}	3.50	2.60^{ab}
20	6.10	6.80	1.00	0.30^{a}	4.50	3.20^{a}
30	6.10	5.90	1.60	1.40^{b}	3.80	2.90^{b}
ES (ppm)						
0	6.10	5.10	1.90	1.20	3.30	1.40
100	6.10	5.90	1.00	0.70	6.00	2.90
WO x ES	NS	NS	NS	NS	NS	NS
SEM	0.53	0.28	0.24	0.17	0.96	0.11

a, b – Means within the column having different superscripts differ significantly; Acid-base balance (pH), total viable count (TVC), total coliform count (TCC), feacal coliform count (FCC), lactobacillus count (LBC) and fungi count (FC).

Tables 7 and 9 show colonal and morphological characteristics used in identifying and classifying the various species of fungi. The fungi identified were found to belong to five genera, namely: *Penicillium*, *Aspergillus*, *Fusarium*, *Saccharomyces* and *Rhizopus.*

Table 7. Colonal and morphological characteristics of fungi in the experimental broiler gut.

Isolates	Colonal characteristics	Morphological characteristics under microscope	identification
1	Loose white mycelia changing to yellowish black on sporulation under 72 hours of incubation	Conidiophore is a smooth wall, non–septate hyaline near the vesicle. It has a globose reside and conidia were in chains, rough with no spine. Phialide is uniform. Hyphae were septate and conidia in chains were more or less globose and rough.	*Aspergillus niger*
2	Loose white mycelia turning yellowish green with colourless reverse side on the Petri dish	Heavily walled conidiophore, uncoloured, nonseptate, coarsely roughened. A vesicle is globose at maturity and spinnate. A matured vesicle was globose and spinnate although some were elliptical.	*Aspergillus flavus*
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 and the collumelae were light brown. The sporangiophores were irregular, round, oval and elongated.	*Rhizopus stolonifer*
5	Colonies are flat, smooth, moist, glistening or dull, and cream in colour.	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. *Saccharomyces* 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.	*Saccharomyces cerevisae*
6	A pale green blue velvety colony which later turned darker green shades. The 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 a colony with a radial pattern, margin and lobed. Conidiophores are smooth-walled while the metulae are cylindrical, smooth and walled bearing 3–6 phialides which are flask-shaped and thick-walled and the conidia are subglobose.	*Penicillium chrysogenum*

The results of biochemical tests on bacteria in the gut of experimental broiler chickens are shown in Table 8. It shows the various tests used in identifying and classifying the bacteria present in the broiler chicken gastrointestinal tract.

Table 8. Results of biochemical tests on bacteria in the gut of experimental broiler chickens.

Tests	Isolates								
	Salmonella	*Lactobacillus*	*Streptococci*	*Campylobacter*	*E.coli*	*Bifidobacterium*	*Bacillus*	*Clostridium*	*Staphylococcus*
Gram stain	-ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve
Catalase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Spore	-ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve
Nitrate reduction	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Starch hydrolysis	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	-ve
Methyl red	+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve
Voges proskeauer	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve
Casein hydrolysis	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Gelatin hydrolysis	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve
Indole test	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
H_2S production	+ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
Citrate reduction	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve	+ve
Urea 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 9. Results of biochemical tests on *Saccharomyces cerevisae*.

Test	Result
Acid production	-ve
Urease	+ve
Glucose	+ve
Raffmose	+ve
Fructose	-ve
Galactose	+ve
Sucrose	-ve
Mannitol	+ve
Lactose	+ve
Maltose	+ve
Inositol	+ve

Table 10 shows the effects of dietary levels of wheat offal with or without enzyme supplementation on the summary of the identified fungi and bacteria in the crop, ileum and caecum pooled together. An increase in dietary levels of wheat offal from 0 to 30% had no effect on both the number of beneficial fungi and the pathogenic/opportunistic fungi identified in the gut of the birds. *Penicilium chrysogenum* was the only beneficial fungus identified in the gut of birds fed the control diet and of those birds fed diets with dietary levels of WO. Four species of opportunistic/pathogenic fungi, namely: *Fusarium solani, Rhizopus stolonifer, Aspergillus niger* and *Aspergillus flavus* were identified in the gut of birds fed the control diet and of those birds fed diets with dietary levels of WO. Enzyme supplementation of the dietary levels of wheat offal had an increased effect on the the number of beneficial fungi identified and a decreased effect on the number of the opportunistic/pathogenic fungi identified in the gut of the birds. Two beneficial fungi species were identified in the gut of those birds fed diets with enzyme supplemented dietary levels of WO, namely: *Penicilium chrysogenum* and *Saccharomyces cerevisiae*, while birds fed the control diet with enzyme supplementation had only *Saccharomyces cerevisiae* identified in their gut. There were four species of pathogenic fungi identified in the gut of birds fed the control diet with enzyme supplementation as were observed with the control diet without enzyme supplementation, but the number of pathogenic fungi identified in the gut of birds fed diet with 10% WO with enzyme supplementation reduced to two species (*Fusarium solani* and *Rhizopus stolonifer*), while *Fusarium solani* was the only opportunuistic/pathogenic fungus identified in the gut of those birds fed diets with 20% or 30% wheat offal. 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 beneficial bacterium identified, while the pathogenic bacteria identified belonged to six genera, namely: *E. coli, Streptococcus, Staphylococcus, Clostridium, Campylobacter* and *Salmonella* species. Enzyme

supplementation of the dietary levels of wheat offal significantly increased the number of beneficial bacteria, while subduing the number of the pathogenic bacteria identified. The number of beneficial bacteria was constant (*Lactobacillus* species) with a reduction in the number of the pathogenic bacteria identified to five genera compared to 0% WO without xylanase supplementation. In the gut of those birds fed diets with 10% or 20% or 30% WO, the beneficial bacteria increased to three genera, namely: *Lactobacillus sp, Bacillus subtilis* and *Bifidobacterium* species while the number of pathogenic bacteria was reduced to four (*E. coli, Clostridium* species, *Streptococcus* species *and Staphylococcus* species), one (*Streptococcus* species) and two (*E. coli and Streptococcus* species), respectively.

Wheat offal is high in fibre and is known to affect feed intake and energy utilization of birds. The primary factor in the voluntary feed intake of chicks appears to be the need for energy (Atteh, 2000). Birds will ordinarily eat to satisfy their energy requirements. As fibre content of diets increases, the density of the diets decreases. The inclusion of fibre in feed dilutes energy concentration of diets (Sriver et al., 2003). Hence, for birds to keep a constant energy level, they have to change their feed intake as the energy density of the feed changes, hence the need for an increase in feed intake. Fibre has been included in experimental diets for monogastric animals for many years, primarily to promote constant passage of materials through the gut (Lee et al., 2003). Fibre reduces the digestibility time of feed in the gut not allowing intestinal secretions to act on the feed.

Observation from this trial showed that the feed became bulky as 30% of the maize in the control diet was replaced with wheat offal. An increase in feed intake as the dietary level of wheat offal increased without xylanase enzyme supplementation was observed. This may be a result of the birds trying to eat more to satisfy their energy requirements. This is in line with the report of Bailey et al. (1991) that birds are known to eat to satisfy their energy requirements. The reduction in feed intake in the presence of enzyme supplementation of the feed observed in this trial showed that the birds consumed less feed to meet their energy requirements. This finding is consistent with Annison and Choct (1992) and Montagne et al. (2003) who alluded that decreased feed intake in broilers fed enzyme supplemented diets could be due to the ability of the birds to fulfil their requirements with less feed. Similarly, Alander et al. (2001) reported reduced feed intake in birds fed diets supplemented with Roxaxyme G compared with the control. An increase in feed/gain ratio (FCR), when there was no enzyme in the feed, indicated that the feed was not efficiently utilized but when the feed was supplemented with xylanase, the FCR was improved. This observation was supported with published reports that the supplementation of non-starch polysaccharidases could improve the growth performance of birds fed on a wheat and/or rye diet (Annison and Choct, 1992; Bedford, 1996; Pettersson and Aman, 1989).

Table 10. Identification of fungi and bacteria.

Without enzyme	Beneficial fungi	Opportunistic/ pathogenic fungi	Beneficial bacteria	Pathogenic bacteria
Control	*Penicilium chrysogenum*	*Rhizopus stolonifer, Aspergillus niger*	*Lactobacillus plantarum*	*E. coli, Streptococcus sp, Staphylococcus sp, Clostridium sp, Campylobacter sp, Salmonella sp*
10%	*Penicilium chrysogenum*	*Fusarium solani, Rhizopus stolonifer, Aspergillus niger, Aspergillus flavus*	*Lactobacillus plantarum*	*E. coli, Streptococcus sp, Staphylococcus sp, Clostridium sp, Campylobacter sp, Salmonella sp*
20%	*Penicilium chrysogenum*	*Fusarium solani, Rhizopus stolonifer, Aspergillus niger, Aspergillus flavus*	*Lactobacillus plantarum*	*E. coli, Streptococcus sp, Staphylococcus sp, Clostridium sp, Campylobacter sp, Salmonella sp*
30%	*Penicilium chrysogenum*	*Fusarium solani, Rhizopus stolonifer, Aspergillus niger, Aspergillus flavus*	*Lactobacillus plantarum*	*E. coli, Streptococcus sp, Staphylococcus sp, Clostridium sp, Campylobacter sp, Salmonella sp*
With enzyme				
Control	*Penicilium chrysogenum*	*Fusarium solani, Rhizopus stolonifer, Aspergillus flavus*	*Lactobacillus plantarum*	*E. coli, Streptococcus sp, Staphylococcus sp, Clostridium sp, Campylobacter sp*
10%	*Penicilium chrysogenum, Saccharomyces cerevisiae*	*Aspergillus flavus*	*Bacillus subtilis, Lactobacillus plantarum, Bifidobacterium*	*Campylobacter sp, Staphylococcus sp.*
20%	*Penicilium chrysogenum, Saccharomyces cerevisiae*	*Rhizopus stolonifer, Aspergillus flavus*	*Lactobacillus plantarum, Bacillus subtilis, Bifidobacterium*	*Campylobacter sp, Staphylococcus sp.*
30%	*Penicilium chrysogenum, Saccharomyces cerevisiae*	*Rhizopus stolonifer, Aspergillus flavus*	*Lactobacillus plantarum, Bacillus subtilis, Bifidobacterium*	*Campylobacter sp, Staphylococcus sp.*

Considerable improvement in performance rates especially feed conversion ratio as a result of enzyme supplementation has also been reported in poultry (Fuller, 1997; Petterson and Aman, 1989; Ohimain and Ofongo, 2013). Similarly, Malmen et al. (2002) showed that Roxaxyme^{R} G2 supplementation significantly improved weight gain and FCR of broilers. Thus, birds fed diet with 20% wheat offal supplemented with enzymes had a relatively better feed/gain ratio compared to those fed the control diet. There were no significant effects of the interaction between levels of wheat offal and xylanase enzyme supplementation on feed intake, weight gain and feed/gain ratio.

Reduction in nutrient digestibility with an increase in dietary levels of wheat offal observed in this study is associated with the fibre that has a high level of non-starch polysaccharides. This is in line with the work of Pylkas et al. (1998) who reported that increasing the levels of wheat offal without enzyme supplementation resulted in the difficulty in feed digestibility. Enzyme xylanase supplementations efficiently broke down NSP and enhanced digestibility. It was observed that digestibility of fibre, fat and protein were higher when supplemented with enzyme. This supports the report of Zhang et al. (2005) that xylanase supplementation resulted in improvements in the degradation of NSP in the gastro-intestinal tract of broilers fed wheat-based diet relative to the control group. However, the use of exogenous enzymes in poultry results in improvement of nutrient digestibility (Malimen et al., 2002). Enzyme allows improved performance or a more efficient use of cheap low-quality carbohydrate sources without adversely affecting animal performance (Santin et al., 2001).

An increase in the weight of the bursa of Fabricius, gizzard and spleen in the birds fed diet without enzyme supplementation may be as a result of increased activity of these organs (Montague et al., 2003). Increased viscosity of the intestinal contents decreases the rate of diffusion of substrates and digestive enzymes and hinders their effective interaction leading to significant modification of the structure and function of the digestive organs (Jorgensen et al., 1996). To adapt to these changes, the activities of the intestinal secreting mechanism may be enhanced possibly leading to hypertrophy of the digestive organs. The increased weight of the crop is thought to be due to a need to store more feed as a result of the bulky nature of wheat offal, while an increased gizzard size is due to the need for more grinding activities resulting in the increased musculature consequent on the increased fibre content of the diet (Chio et al., 1994). With enzyme supplementation, a greater proportion of non-starch polysaccharide (NSP) was digested, thereby attenuating the increased function of the responding organs.

E.coli, Streptococcus species, *Staphylococcus* species, *Clostridiu* species, Campylobacter species, Salmonella species were the pathogenic bacteria and *Lactobacillus* species, *Bacillus subtilis* and Bifidobacterium species were the beneficial bacteria identified in the GIT of the broiler chickens. The concentrations of these organisms were high in this trial. This observation is in line with the work of Skorupski et al. (1997) who concluded that environmental factors, including pH, temperature, osmolarity, and certain amino acids, could increase the activity of a transcriptional activator (ToxR) and result in more virulent bacteria.

A prebiotic is a selectively fermented food ingredient that stimulates specific changes in the composition of and (or) activity in the gastrointestinal microflora, which in turn confer benefits on the host well-being and health (Roberfroid, 2007). Prebiotics, such as fructooligosaccharides (FOS), inulin, galactooligosaccharides (GOS) and glucooligosaccharides, are dietary carbohydrates that escape digestion

in the upper gastrointestinal tract and alter the bacterial composition of the gut by changing the type of the substrate provided to the existing gut microbiota (Chio et al., 1994; Gibson and Roberfroid, 1995; Alander et al., 2001; Malinen et al., 2002). Prebiotics are fermented by probiotic bacteria, producing short-chain fatty acids (SCFAs) as the end products of fermentation. The major SCFAs produced are acetate, propionate, and butyrate (Pylkas et al., 2005), and the profile of SCFAs varies among the different prebiotic sources. SCFAs have beneficial effects on human health as energy sources and by inhibiting the growth of pathogenic bacteria (Bailey et al., 1991; Blottiere et al., 1999; Pylkas et al., 2005). SCFAs also decrease colonic pH, which demonstrates the fermentability of the non-digestible carbohydrates. Beneficial microbes such *as Lactobacillus* species, *Bifidobacterium* species and *Bacillus subtilis*, which were results of fermentation of fibres, were observed in this trial. *Lactobacillus* species are capable of controlling populations of *E. coli* in the crop, and their effects are both bacteriostatic (depressing the growth of other bacteria by their secretions) and bacteriocidal (killing other bacteria by secretions) (Collins and Gibson, 1999). *Bacillus subtilis* spores in broilers exert their beneficial effects by one or more of these mechanisms. It makes use of available oxygen, thereby creating a more favourable environment for the beneficial anaerobic species to proliferate. This action enhanced the proliferation of *Lactobacillus* that helps control the growth of pathogenic bacteria especially *E. coli*. It enhances enzyme production and immune responses (Kalantar et al., 2014).

The fungi identified were both beneficial and non-beneficial. *Aspergillus niger, Aspergillus flavus, Fusarium solani* and *Rhizopus stolonifer* were the pathogenic/opportunistic fungi identified. This may be a result of contamination from the feed/diet (Dalcero et al., 1997). Replacement of maize with wheat offal supplemented with xylanase has the effect on the growth of beneficial fungi: *Saccharomyces cerevisiae* and *Penicilium chrysogenum.* It was observed in this study that inclusion of wheat offal 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. This finding corroborates the report of Ohimian and Ofongo (2013) who reported that coliforms and *E. coli* were consistently higher ($P<0.05$) in the control (maize–soybean meal diet) than in feeds supplemented with wheat offal with or without enzyme supplementation in the gut of chickens and that the population of coliform and *E. coli* were the lowest in the diet containing wheat offal and Roxazyme G2.

The beneficial microbes (both fungi and bacteria) have been shown to improve bird performance and decrease mortality (Miles and Bootwalla, 1991; Santin et al., 2001). Enzyme supplementation has a beneficial effect on animal performance (Fasuyi, 2010).

Conclusion

The replacement of maize with wheat offal irrespective of levels supplemented with 100ppm xylanase enzyme caused a reduction in feed intake and an increase in weight gain and better FCR. The birds fed diet with 20% wheat offal supplemented with xylanase enzyme outperformed birds fed diets with 10% or 30% wheat offal supplemented with xylanase enzyme and birds fed the control diet. It can be deduced that enzyme supplementation of wheat offal helped in increasing and improving protein and fibre digestibilities. The weights of vital organs showed that the birds were in good health conditions. The result of identification of microbes (fungi and bacteria) showed that dietary levels of wheat offal (10, 20 or 30% inclusion) with supplementation of xylanase enzyme enhanced the growth of beneficial microbes which resulted in inhibition or elimination of the opportunistic/pathogenic microbes. Cost-benefit analysis showed that the 20% inclusion level of wheat offal supplemented with xylanase enzyme gave the best result of a beneficiary reduction in the cost of production with the best improved broiler performance.

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PREBIOTSKI POTENCIJAL PŠENIČNOG STOČNOG BRAŠNA SA DODATKOM ENZIMA KSILANAZE KOD BROJLERSKIH PILIĆA

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R e z i m e

Nedavni razvoj patogena otpornih prema antibioticima kod živine, što predstavlja pretnju za ljudsko zdravlje, zahteva traženje alternative za antiobiotske stimulatore porasta (engl. *antibiotic growth promoters* – AGPs) kako bi se poboljšala crevna mikroflora u ishrani živine. Jedna od alternativa za antiobiotske stimulatore porasta (AGPs) jesu probiotici, koji predstavljaju korisne organizme. Nusproizvodi varenja polisahirida za koje živina ne poseduje enzime za varenje nazivaju se prebiotici. Prebiotici služe kao hrana za probiotike, pri čemu primena enzima omogućava njihovo iskorišćavanje. Prebiotski potencijali hraniva sa visokim sadržajem vlakana (engl. *high fibre feedstuffs* – HFFs) sa dodatkom enzima nisu poznati. Ovo istraživanje je sprovedeno kako bi se ocenio prebiotski potencijal pšeničnog stočnog brašna sa dodatkom enzima ksilanaze na brojlerske piliće (*in vivo*). Zamena kukuruza, pšeničnim stočnim brašnom sa dodatkom enzima ksilanaze od 100ppm, nezavisno od učešća, dovela je do smanjenja konzumiranja hrane, povećanja prirasta i bolje konverzije hrane (engl. *feed conversion ratio* – FCR). Brojleri hranjeni smešom sa 20% pšeničnog stočnog brašna sa dodatkom enzima ksilanaze, odlikovali su se boljim performansama u odnosu na brojlere koji su konzumirali smešu sa 10 odnosno 30% pšeničnog stočnog brašna uz dodatak enzima ksilanaze, kao i u odnosu na kontrolnu grupu. Pilići su imali normalnu težinu vitalnih organa sa dobrom strukturalnom konzistencijom. Identifikacija mikroorganizama (gljivica i bakterija) pokazala je da sadržaj u obroku pšeničnog stočnog brašna (10, 20 ili 30%) sa dodatkom enzima ksilanaze povećava rast korisnih mikroba, što je uticalo na inhibiciju ili eliminaciju oportunističkih/patogenih mikroorganizama. Zaključeno je da dodavanje enzima hranivima sa visokim sadržajem vlakana može poboljšati performanse porasta, iskorišćavanje hranljivih materija i povećati koncentraciju korisnih mikroorganizama u digestivnom traktu. Upotreba enzima se stoga preporučuje kada su hraniva sa visokih sadržajem vlakana potrebna, kao izvor prebiotika u digestivnom traktu brojlera.

Ključne reči: prebiotici, enzimi, crevna mikroflora, ishrana brojlera, svarljivost.

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