IN-VITRO ASSESSMENT OF SOME HIGH FIBRE FEEDSTUFFS AS PROBIOTICS IN BROILER DIETS

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ABSTRACT
Recent development of antibiotics-resistant 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 are probiotics which are beneficial organisms. Prebiotics are by-products of digestion of polysaccharides which poultry do not have enzymes to digest but are food for probiotics. Advent of enzymes makes this digestion possible. The prebiotic potentials of enzyme supplemented high fibre feedstuffs (HFFs) are not known. This study was conducted to screen some HFFs such as wheat bran (WB), maize bran (MB), palm kernel cake (PKC), rice husk (RH) and brewers dried grain (BDG) for production of oligosaccharides through *xylanase* digestion. Five high fibre feedstuffs (HFFs) (WB, BDG, PKC, RH and MB) screened for oligosaccharides production using the Benedict's test showed that all the feedstuffs screened except maize bran tested positive for the presence of oligosaccharides. The increase in dietary levels of HFFs generally could increase feed intake, reduced weight gain and increased feed/gain ratio ($P<0.05$). Enzyme Supplementation generally reduced feed intake while improving crude protein retention ($P<0.05$). There was no significantly different ($P>0.05$) interaction between the nutrients and enzyme studied. It was concluded that enzyme supplementation of high fibre feedstuffs could improve growth performance, nutrient retention and increased concentration of beneficial microbes in gut. Use of enzymes is therefore recommended when HFFs are required as prebiotic source in the gut of broilers.
Keywords: Prebiotics, enzymes, fermentation, nutrient, retention.

INTRODUCTION
The use of indigestible fermentable carbohydrates as prebiotics is founded on "colonization resistance" according to Van der Waaij (1989), where short chain fatty acids (SCFAs) are produced through saccharolytic fermentation in the hindgut, with a low gut pH while improving mineral absorption and inhibition of acid sensitive pathogens. This causes the proliferation of indigenous microbiota in the hindgut at the same time (Apajalahti, 2005). The fermentation of poorly digestible proteins present in the hindgut on the other hand (putrefactive fermentation) results in branched chain fatty acids (BCFAs), amines, phenols, indoles and ammonia which have toxic effects.

The *in vitro* digestibility technique offers a potential for evaluation of nutritive value of feed in the absence of live animals. *In vitro* studies in monogastric animals involve treatment of feed samples with pepsin at low pH and the product is then neutralized and treated with a solution containing pancreatic enzyme with the rate of disappearance of the different nutrients determined (Atteh, 2002).

The objective of this work is to screen high fibre feedstuff such as Wheat bran, Maize bran, Palm kernel cake (PKC), Brewer's Dried Grain (BDG) and Rice Husk for their prebiotics properties through Benedict's test and to later determine the *in-vitro* digestibility of the test ingredients.

MATERIALS AND METHODS
Preparation and screening of five high fibre feedstuffs using Benedict's Test
Five feedstuffs (Wheat-bran (WB), Maize bran (MB), Palm-kernel cake (PKC), Brewer-dried grain (BDG) and rice-husk (RH)) were selected on the basis of their high fibre / low protein content and use in animal feed manufacturing. They were purchased from a livestock feed manufacturer in Ilorin, Kwara State. The samples were air dried and ground and their proximate composition and fibre assay were

determined according to methods of AOAC (2005); Van Soest (1973) and Van Soest *et al.* (1991). Aliquot samples of the enzyme digested feeds and controls were put into test tubes. The aliquot samples in test tubes were then mixed with 3ml Benedict's reagent, boiled for 3-5 minutes and allowed to cool. A yellow precipitate was observed which was then filtered off. This was done to eliminate the monosaccharide leaving behind the oligosaccharides. The aliquot samples were then further treated with dilute 2ml of sulphuric acid (H_2SO_4), boiled for 3-5 minutes so as to hydrolyze the oligosaccharides and then 3ml of Benedict's reagent was added. A deep red/brown precipitate was formed which indicated the presence of oligosaccharides.

Experimental Design and Chemical Analysis

The trial consisted of a 5 x 2 factorial combination of feed ingredients and supplementation with enzyme. Five grams of each ingredient (wheat bran, rice husk, maize bran, Palm Kernel cake and Brewers dried grain) were weighed into a test tube with or without enzyme at the recommended rate (100ppm). Thus there were total of 10 treatments each with 4 replicates. 10mls of pepsin in 0.1M hydrochloric acid was added to each sample and the content incubated at 40^{0}C for 30minutes on a vibrator. Samples were then neutralized with 0.2M sodium hydroxide followed by addition of 5ml of pancreatin (0.2% w/v) in a buffer solution, such that pH of 6-7 was maintained. The mixture was incubated for 2 hours with shaking at a temperature of 40°C. The content of each test tube was thereafter filtered using filter paper and a vacuum pump. The digested material was discarded while the residue was oven-dried at 70°C for 24hours.

In vitro Nutrient Digestibility was calculated using the formula:

$$ND = \frac{NS - NR}{NS} \times 100$$

Where ND = Nutrient Digestibility

NS = Nutrient in feed (5g x coefficient of nutrient content)

NR = wt of residue x coefficient of nutrient content of residue

Proximate Analysis of five feedstuffs

The samples of oven-dried residue were weighed and subjected to proximate analysis using method of the AOAC (2005). Crude protein was determined using the Kjeldahl procedure. Ether extract was determined by subjecting the samples to petroleum ether extract (b.p. 60-80^{0}C) in a Soxhlet apparatus. Crude fibre of the samples was determined by the method described by Cummings and Macfarlane (2002).

Statistical analysis

All data collected were subjected to analysis of variance using the model for factorial design (General Linear Model procedure SAS Institute (2000)). Significant differences between treatments means were separated using the Duncan multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

The result of Oligosaccharides test for the five (5) feedstuffs in Table 1 was positive for all the test feedstuffs except maize bran. Table 2 shows the *in vitro* nutrient retention of five ingredients supplemented with or without *xylanase.* The protein digestibility of all the five ingredients were significantly different ($P<0.05$) except for PKC and BDG that were comparable ($P>0.05$). This reveals that it contribute a percentage of the required CP of the body but the concentration/ type of amino-acid present also influence its availability as well as when the amino acid is in free or bound forms in the feedstuff. The fibre digestibility of RH, PKC and BDG were comparable ($P>0.05$) but significantly different from that of wheat bran and maize bran ($P<0.05$). The CF present are either in the digestible or non-digestible forms enhance the need for exogenous enzymes to help in the pre-digestion of the non-digestible or bound nutrients. Fat digestibility of all the five ingredients were significantly different from each other with BDG having the highest value (58.2%) and rice husk with the lowest value (21.1%)($P< 0.05$). This might have influence the percentage of energy contribution by the feedstuff. The effect of exogenous enzyme inclusion in the feedstuff significantly affected only the CP with a significant increase in CP when exogenous enzyme was included in the feedstuff preparation. *Xylanase* supplementation caused a significant increase in

protein digestibility of wheat bran, maize bran, rice husk, palm kernel cake and brewers dried grain ($P<0.05$) but there was just a numerical increase in fibre digestibility and fat digestibility of all the five ingredients ($P>0.05$). There was no significant interaction between inclusion of enzyme and the five ingredients on the nutrient retention parameters ($P>0.05$).

The concept of prebiotics was initially defined as "non-digestible feed ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (Gisson and Roberfroid, 1995). There are many reasons for exclusion of high fibrous feed ingredient in poultry diets, among which is decreased digestibility of nutrient (Bach, 1997; Dusterhorft *et al.*, 1993) as a result of arabinoxylans which are the main Non Starch Polysaccharide (NSP) contained in cereal based feed ingredients. Arabinoxylans are known to increase viscosity of digestive content, hence interfered with digestion which can be detrimental to nutrient utilization (Montagne *et al.*, 2003; Gallinger *et al.*, 2004). Enzymes help to decrease viscosity of digestive content resulting in improvement in nutrient digestibility. Mod *et al.* (1983) opined that the release of nutrient from fibrous feedstuff with addition of exogenous enzyme is possible *in vitro*. In this study, the use of fibrous diet in the absence of supplementary enzyme caused a decrease in nutrient digestibility while the addition of supplementary enzyme improved nutrient digestibility. This observation agrees with the results of Cowieson and Acamovic (2003) when peanut was supplemented with enzymes. It was observed that the enzyme (*xylanase*) supplementation of all the feed ingredients except rice husk caused an increase in crude protein digestibility. This agrees with Boisen and Fermandez (1995) who reported that ileal protein digestibility of the *in vitro* was higher than those of *in vivo*. This guarantees that the carbohydrates investigated will resist gastric activity and enzymatic hydrolysis up to 45-83% *in-vivo*. All feedstuffs tested positive for the presence of oligosaccharides, except maize bran, and therefore met the first criteria for classification of any feedstuff as a potential prebiotic (Manning and Gibson, 2004; Ruberfroid, 2007).

In conclusion therefore, among the five high fibre feedstuffs (WB, MB, PKC, BDG and RH) investigated for the production of oligosaccharides only four feedstuffs (WB, PKC, BDG and RH) shows the presence of Oligosaccharides. Therefore, it can be concluded that all the four feedstuffs met the first criteria for classification of any feedstuff as a prebiotic potentials and can be used as alternatives to Antibiotics Growth Promoters.

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Table 1 Effects of Benedict's test on five test feedstuffs for oligosaccharides

Feedstuff	Result
Rice husk	Positive
Palm Kernel Cake	Positive
Brewer's Dried Grain	Positive
Maize bran	Negative
Wheat bran	Positive

Note: Positive means feed shows the presence of Oligosaccharides
Negative means feed shows the absence of Oligosaccharides

Table 2 Effects of ingredients and enzyme supplementation on *in-vitro* nutrient retention.

Ingredients (I)	Crude Protein (%)	Crude Fibre (%)	Ether Extract (%)
Wheat bran (WB)	69.50^{b}	53.50^{b}	56.10^{b}
Maize bran (MB)	59.80^{c}	84.60^{a}	51.10^{c}
Rice husk (RH)	73.30^{a}	49.20^{c}	21.10^{d}
Palm Kernel Cake (PKC)	53.10^{d}	47.30^{c}	38.90^{e}
Brewer's Dried Grain (BDG)	53.70^{d}	48.00^{c}	58.20^{a}
Enzyme (ppm)(E)			
0	59.80^{b}	55.70	45.00
100	63.40^{a}	56.80	45.30
I x E	**NS**	**NS**	**NS**
± SEM	1.75	1.76	1.72

$^{a, b, c, d}$ – means within column with different superscripts are significantly different ($P<0.05$)