EFFECT OF *ASPERGILLUS NIGER* TREATED FIBRE FEEDSTUFFS ON GROWTH PERFORMANCE AND CARCASS CHOLESTEROL OF GROWING PIGS

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ABSTRACT

The study evaluated copper and zinc availability from Aspergillus niger fermented fibre feedstuffs for effects on growth and carcass cholesterol of growing pigs. Three fibre feedstuffs (Wheat Bran (WB), Brewer Spent Grain (BSG) and Palm Kernel Cake (PKC) were prepared by solid-state fermentation with A. niger to determine bioavailability of copper(Cu) and zinc (Zn). Growth trial was conducted using thirty-six growing pigs with average initial weight of 25.0 ± 0.52 kg, randomly assigned to six dietary treatments in a 3 x 2 factorial experiment of three fibre sources (WB, BSG and PKC) with or without A. niger treatment in a 63 d experiment. The diets were formulated to contain treated and untreated fibre feedstuffs at 30% inclusion level.
The results showed that treatment of the fibre feedstuffs with A. niger increased Zn availability in WB, BSG and PKC by 53.35%, 7.90% and 4.54% respectively while copper availability increased by 60.94% and 16.40% in WB and PKC but depressed availability (-1.70%) in BSG treated feedstuff. The growth response showed no significant difference (P >0.05) but the average total cholesterol in pigs fed the diets appeared to decrease significantly (p<0.05) across treatment groups. These values varied between 32.00 mg/100g and 45.56 mg/100g for pigs fed A. niger treated wheat bran and untreated PKC diets respectively. The findings of this study showed that A. niger improved the composition of Cu and Zn, and pigs fed treated BSG diet had the fastest growth while those fed treated PKC diet produced better carcass cholesterol content.

Keywords*: fibre feedstuffs, A. Niger, copper, zinc, performance, growing pigs*

INTRODUCTION

Copper (Cu) and Zinc (Zn) are essential micro minerals performing multifarious functions in pigs. They are involved in a number of growth promoting processes, improving nutrient digestibility, feed intake and gut morphology, and form important units in metabolic processes (Jondreville *et al.*, 2003; Debski, 2016). Diets are important sources of these micronutrients and most conventional feed ingredients especially cereal grains are limited in Cu and Zn (Uddin *et al.*, 2014). In addition, the high phytate content of these grains further reduce availability in pigs fed cereal-based diets (Lombi *et al.*, 2011). The deficiency of these micronutrients are poor growth and development, compromised immune status and sometimes death (McDonald *et al.*, 2011).

Recently, there is increasing production of agro-industrial by-products from wheat, palm kernel oil and brewery processing, and their utilization as suitable and least cost alternative to cereal grains. However, these by-products contain high fibre levels capable of encapsulating essential nutrients and further making them unavailable to pigs (Kornegay, 2001; Lisbeth *et al.*, 2008; Amjad *et al.*, 2011; Swiatkiewicz *et al.*, 2012). Efforts to avert these micronutrient challenges require nutritional manipulations mostly targeted at dietary supplementation which to a larger extent causes some levels of buildup in tissue owing to oversupply, hence contributing to the development of antimicrobial resistance and pollution of soil and groundwater (Li *et al.*, 2021).

An alternative is the use of exogenous phytase to degrade the phytic bond releasing phosphorus among other nutrients. The high cost of enzyme supplementation lends credence to the development of least-cost measures such as solid-state fermentation with microbial phytase, especially *Aspergillus niger*. Studies have shown that the fungi produced lipase using wheat bran as both substrate and inducer in solid-state fermentation to degrade lipids (Acda and Chae, 2002; Santos *et al.*, 2014; Dwini *et al.*, 2020). Similarly, Cu and Zn play crucial roles in the reduction of cholesterol content especially the low-density lipoprotein cholesterol in mammals (Gunasekara *et al* 2011). It is envisaged that Cu and Zn which form complexes with phytate could also be released by *A. niger* treatment. This study was designed to evaluate the growth performance and carcass cholesterol of growing pigs to copper and zinc availability from *Aspergillus niger* fermented fibre feedstuffs.

MATERIALS AND METHODS

Experimental location and sources of feed ingredients

The study was conducted at the Swine Unit of the Teaching and Research Farm, Obafemi Awolowo University, Ile–Ife, Nigeria. The feed ingredients were purchased from the Adom feed mill in Ibadan, Nigeria. *Aspergillus niger* was obtained from the culture bank of the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

Isolation of fungi and biodegradation

Fungi isolates were obtained from previously cultured plates. The fungi were subcultured on malt agar slants for further use. The inoculums of isolated *A. niger* were prepared by pouring 10 ml of sterile distilled water into the spores of each agar slant. The filtrate of this isolate was diluted with more sterilized water until a spore count of approximately 7.44 x 10^5 per ml was obtained using the Haemocytometer (Onilude and Oso, 1999). 300 grams of each ground fibrous feedstuff was placed in each of three 500 ml conical flasks corked with cotton wool and sterilized at 160^{o}C for 2 hours.

Sterile water was added at the rate of 75 ml per 300 grams of each fibrous feed to raise the moisture of the feeds as described by Lawal *et al.* (2012). About 10 ml of inoculums (*A. niger*) was used to inoculate each of the containing flasks under aseptic conditions. The inoculated feed samples were incubated in the oven for 5 days at 30 ^{o}C. Each of these 300 grams samples was then poured into 30 kg sterilized and moistened test ingredients and mixed thoroughly. They were allowed to ferment in the bigger containers for seven (7) days and were stirred daily.

The samples were oven dried after seven (7) days at 70 ^{o}C while dried samples were kept for the preparation of the diets and laboratory analysis.

Copper and zinc availability in the fibrous feedstuffs

Following the treatment of palm kernel cake (PKC), brewer spent grain (BSG) and wheat bran (WB) with *A. niger,* the concentrations of Cu and Zn in the treated diets were determined following the procedure of AOAC (2000) using the Atomic Absorption Spectrophotometric method. Samples were prepared by weighing 2g of each of the test ingredients in crucibles and allowed to ash in a muffle furnace for 3h. The ash residues were digested with 6N hydrochloric acid for 30 minutes.

The digested samples were filtered with Whatsmann filter paper. The filtrates were absorbed at 320.9nm and 213.9nm for Cu and Zn respectively using BUCK Scientific Atomic Spectrophotometer (Model 210 VGP).

Experimental diets, animals and management

There were six experimental diets formulated with three fibre feedstuffs (Table 1). Diets UWB and TWB contained untreated and treated WB, Diets UBSG and TBSG contained untreated and treated BSG while Diets UPKC and TPKC contained untreated and treated PKC. Each test ingredient was included at 30 % of the diet. A total of 36 growing crossbred (Hampshire x Large White) pigs with average initial weight of 25.0 ± 0.52 kg were used for the experiment.

Table 1: Composition of experimental diets

	DIETS					
INGREDIENTS (%)	**UWB**	**TWB**	**UBSG**	**TBSG**	**UPKC**	**TPKC**
Maize	45.00	45.00	45.00	45.00	45.00	45.00
Wheat Bran	30.00	30.00	-	-	-	-
Brewer Spent Grain	-	-	30.00	30.00	-	-
Palm Kernel Cake	-	-	-	-	30.00	30.00
Groundnut Cake	13.00	13.00	13.00	13.00	13.00	13.00
Soybean Meal	7.00	7.00	7.00	7.00	7.00	7.00
Fish Meal	1.00	1.00	1.00	1.00	1.00	1.00
Bone Meal	1.50	1.50	1.50	1.50	1.50	1.50
Limestone	2.00	2.00	2.00	2.00	2.00	2.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25
*Vit.-mineral premix	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100
Calculated Analysis						
Crude Protein (%)	16.97	17.02	18.27	18.37	16.03	18.70
Crude Fibre (%)	4.90	3.80	8.11	5.00	6.62	4.00
ME (Kcal/kg)	2837.6	2837.6	2920.40	2920.4	2891.16	2891.6

UWB; Untreated Wheat Bran; TWB: Treated Wheat Bran; UBSG: Untreated Brewer' Spent Grain; TBSG: Treated Brewers' Spent Grain; UPKC: Untreated Palm Kernel Cake; TPKC: Treated Palm Kernel Cake; ME: Metabolizable energy;
**Grower premix supplied the following per kg diet: vitamin A 10,000,000 IU; vitamin D 32,000,000 IU; vitamin E 8,000 IU; vitamin K 2,000 mg; vitamin B 1 2,000 mg; vitamin B 2 5,500 mg; vitamin B 61,200 mg; vitamin B 12 12 mg; biotin 30 mg; folic acid 600 mg; niacin 10,000 mg; pantothenic acid 7,000 mg; choline chloride 500,000mg; vitamin C 10,000 mg; iron 60,000 mg; Mn 80,000 mg; Cu 800 mg; Zn 50,000 mg; iodine 2,000 mg; cobalt 450 mg; selenium 100 mg; Mg 100,000 mg; anti-oxidant 6,000 mg.*

The experimental pigs were fed 4% of their body weight while water was supplied *ad libitum* throughout the experimental duration. Data were taken weekly on feed intake and weight gain while feed-to-gain ratio was evaluated on treatment basis. The experimental design was 3 x 2 factorial arrangement of three fibre (WB, BSG and PKC) sources with or without *A. niger* treatment. The experimental duration was 63 days.

Carcass cholesterol determination

At the end of the experimental trial, three pigs per treatment were sacrificed for cholesterol determination. Meat samples were collected at the *longissimus dorsi* on treatment basis. Carcass cholesterol was determined on treatment basis using the normal phase of High Performance Liquid Chromatography (HPLC).

Chemical and statistical analyses

The proximate composition of the test ingredients was carried out following the procedure of AOAC (2000). Data were analysed using the General Linear Model procedure of the SAS (2002) package.

RESULTS AND DISCUSSION

Effect of A. niger treatment on copper and zinc composition in test fibrous feedstuffs

The results of *A. niger* treatment on fibre feedstuffs are shown in Table 2. Upon treatment of the fibre feedstuffs with *A. niger*, the availability of copper (Cu) and zinc (Zn) in the treated feedstuffs improved significantly. For the treated feedstuffs, WB produced the largest (60.94%) increase in Cu availability, PKC had the least (16.40%), and BSG gave a depressed effect (-1.70%). However, for the per cent improvement in Zn availability, there was a downward decreasing trend from WB (53.35%) through BSG (7.90%) to PKC (4.54%). Generally, treated wheat bran (TWB) had the highest per cent differences in both Cu and Zn availability, while treated palm kernel cake (TPKC) had better Cu availability compared with treated brewer spent grain (TBSG). The negative value (-1.70%) obtained for TBSG implied a decrease in Cu availability upon treatment with *A. niger*.

The increased Cu and Zn availability observed in the treated fibre feedstuffs could be attributable to the fibre degrading activity of *A. niger* to unwrap the minerals from the protective covering of the fibre, thus improving availability. Similar findings were observed by Selle and Ravindran (2007); Espinosa and Stein (2021) on fibre feedstuffs using microbial phytase and found that *A. niger* could break the phytic-bound minerals, hydrolyzing between 35 and 50% Zn and Cu in diets of pigs.

Table 2: Copper and zinc composition in treated and untreated fibrous feedstuffs

Treatments	Copper (mg/kg)	% Difference	Zn (mg/kg)	% Difference
UWB	16.72		53.40	
TWB	26.91	60.94	81.89	53.35
UBSG	30.72		78.33	
TBSG	30.20	-1.70	84.55	7.90
UPKC	19.86		89.32	
TPKC	23.12	16.40	93.38	4.54

UWB; Untreated Wheat Bran; TWB: Treated Wheat Bran; UBSG: Untreated Brewer' Spent Grain; TBSG: Treated Brewers' Spent Grain; UPKC: Untreated Palm Kernel Cake; TPKC: Treated Palm Kernel Cake; ME: Metabolizable energy;

Proximate composition of A. niger treated and untreated fibrous feedstuffs

After *A. niger* treatment of fibre feedstuffs, the crude protein (CP) content of TWB, TBSG and TPKC increased significantly by 25.60 %, 23.43 % and 0.50 % than in the untreated fibre feedstuffs respectively. The fibre content in TWB, TBSG and TPKC decreased significantly in a downward trend by 42.03%, 30.88% and 4.36% than in the untreated feedstuffs respectively (Table 3). Importantly too, the ether extract increased almost proportionately from TWB through TBSG and TPKC by a factor of 3.1%, 13.92% and 21.38% higher than the untreated fibre feedstuffs respectively.

As shown in Table 3, the ash content increased by 64.86%, 26.30% and 49.80% for TWB, TBSG and TPKC respectively compared with the untreated feedstuffs. There were variations in the values obtained for the nitrogen-free extract (NFE) with observed variations due to the differences in other proximate parameters. *Aspergillus niger*-treated feedstuffs improved nutritional value. The increase in CP content of treated fibre feedstuffs could be due to the presence of microbial biomass in the treated diets. Shuvo *et al.* (2022) and Ahmad (2019) found that *A. niger* produced phytase that hydrolyses phytate, releasing encapsulated proteins and other nutrients. Similar observations were also reported by Ojebiyi *et al.* (2010); Bidura *et al.* (2012) and Gowanlock *et al.* (2013).

Also, the observed reduction in crude fibre content of treated feedstuffs was similarly reported by Price *et al.* (2001) and Shuvo *et al.* (2022) using fermented rice bran. Again, this observation may be due to the fibre-degrading activity of *A. niger* on fibre feedstuffs. The rise in the values obtained for ether extract of treated fibre feedstuffs could be due to the production of microbial oil by some groups of non-pathogenic saprophytes such as *A. niger*.

Similar findings were reported by Oboh *et al.* (2002) and Altop *et al.* (2019) on *A. niger* fermented cassava products and the improvement of nutritional contents of some oil seeds with *A. niger*.

Table 3: Proximate composition of fibre feedstuffs

	Fibre feedstuffs					
Parameters *(% DM basis)*	UWB	TWB	UBSG	TBSG	UPKC	TPKC
Crude Protein	15.47^{d}	19.43^{c}	25.65^{b}	31.66^{a}	18.82^{c}	18.91^{c}
Crude Fibre	8.97^{e}	5.20^{f}	13.31^{c}	9.20^{d}	15.59^{a}	14.91^{b}
Ether Extract	3.23^{f}	3.33^{e}	4.67^{c}	5.32^{a}	4.35^{d}	5.28^{b}
Ash	3.70e	6.10^{b}	5.78^{c}	12.50^{a}	2.57^{e}	3.85^{d}
Nitrogen Free Extract	67.42^{a}	54.93^{c}	44.81^{e}	30.34^{f}	56.04^{b}	47.57^{d}

Means bearing different superscripts in a row differ significantly at p<0.05

UWB; Untreated Wheat Bran; TWB: Treated Wheat Bran; UBSG: Untreated Brewer' Spent Grain; TBSG: Treated Brewers' Spent Grain; UPKC: Untreated Palm Kernel Cake; TPKC: Treated Palm Kernel Cake; ME: Metabolizable energy;

Growth performance and muscle cholesterol of pigs fed experimental diets

The performance of growing pigs fed experimental diets is shown in Table 4. The final body weight, average daily feed intake, average daily weight gain and feed conversion ratio showed no significant difference (P > 0.05) across dietary treatments. Similarly, there was no interaction effect of dietary fibre source by treatment for all growth parameters. However, there were statistical variations (p<0.05) in the final body weight, average daily weight gain and average daily feed intake of pigs by fibre sources. Pigs fed *A. niger* TWB had depressed final body weight (-1.6%) compared with pigs fed UWB based diets while pigs fed TBSG and TPKC diets had a 6.46% and 1.1% increase compared to pigs fed UBSG and UPKC diets respectively. Also, the average daily gains of pigs fed *A. niger* TWB, TBSG and TPKC increased by 0%, 10.71% and 5.6% while the average daily feed intake increased by 0.96%, 5.62% and 2.97% respectively compared with pigs on the untreated WB, BSG and PKC diets. The variations observed in this study could be attributed to the sources of fibre with differences in the nutrient composition. There was improvement in the feed to gain ratio of pigs fed *A. niger* treated diets. Pigs on TPKC diets had the better conversion ratio compared to those on TWB while pigs on TBSG had the poorest feed efficiency.

Again, the low average daily feed intake observed in pigs fed BSG diets could be due to the nature of the diets which could have contributed to the poor daily weight gain of pigs fed BSG diets.
Fibre source, treatment effects and interaction between fibre and treatment had significant ($p<0.05$) effects on average total cholesterol contents of pigs fed fibre diets. There was a significant increase in the average total cholesterol contents of pigs fed UWB, UBSG and UPKC diets. The observed variations may be due to differences in fibre sources and nutrient composition, especially ether extract of the fibre feedstuffs.

Similarly, there were significant effects of fibre source, treatment and fibre x treatment interactions on pigs fed *A. niger*-enhanced diets. The pigs fed UBSG and TBSG had a rise of 3.92 % in average total cholesterol content while pigs fed UWB and TWB as well as UPKC and TPKC produced -3.03% and -14.16% reduction in cholesterol contents respectively. Again, the observed variations in cholesterol contents of pigs fed *A. niger* treated and untreated diets may be attributed to differences in the ether extract content of the diets resulting in varied fat deposition. All the values obtained for the cholesterol contents of pigs fed treated and untreated diets were within the range (30 – 81 mg/100g) obtained by Sinclair *et al.* (2010). These results agrees with the findings of Adesehinwa and Ogunmodede, (2002) that dietary fats influence the cholesterol contents. Also, the decrease in cholesterol level of *A. niger* treated WB and PKC was reported by Omojola *et al.* (2009) to be due to decrease in hepatic synthesis where Cu performs a vital role.

The reduction effect could also be due to the fermentation effect of *A. niger* to inhibit the activity of 3-hydroxy-3-methyl-glutaryl-CoA reductase, producing mevalonate, a rate-limiting enzyme which slows down the biosynthesis of cholesterol (Serruys *et al.*, 2004; Hajjaj *et al.*, 2005). Studies by Honoso and Tonoka (1995) and Anis *et al.* (2018) on the effects of *A. niger* fermented rice bran reported significant improvement in feed efficiency and reduction in carcass cholesterol content of growing pigs.

Table 4: Growth performance and cholesterol of growing pigs fed experimental diets

PARAMETERS	UWB	TWB	UBSG	TBSG	UPKC	TPKC	SEM	FIB	TRT	FIB x TRT
Initial weight (kg)	25.08	24.25	23.50	24.25	24.75	24.08	0.52	0.84	0.82	0.81
Final weight (kg)	48.92[a]	48.13[a]	41.33[c]	44.00[b]	47.50[a]	48.00[a]	0.88	0.01	0.63	0.68
ADWG (kg/day)	0.38[a]	0.38[a]	0.28[c]	0.31[b]	0.36[a]	0.38[a]	0.01	0.02	0.51	0.89
ADFI (kg/day)	1.04[a]	1.03[a]	0.89[c]	0.94[b]	1.01[a]	1.04[a]	0.02	0.01	0.47	0.76
Feed:Gain ratio	2.91	2.85	3.27	3.00	2.91	2.75	0.09	0.37	0.40	0.91
ATC (mg/100g)	33.00[d]	32.00[d]	34.22[c]	35.56[c]	45.56[a]	39.11[b]	0.85	0.03	0.02	0.03

ADWG = Average daily weight gain, ADFI = Average daily feed intake, ATC = Average Total Cholesterol
UWB; Untreated Wheat Bran; TWB: Treated Wheat Bran; UBSG: Untreated Brewer' Spent Grain; TBSG: Treated Brewers' Spent Grain; UPKC: Untreated Palm Kernel Cake; TPKC: Treated Palm Kernel Cake; ME: Metabolizable energy;

CONCLUSION

The study concluded that *Aspergillus niger* had beneficial effects as a fungal phytase on fibrous feedstuffs due to the possibility of improving Copper and Zinc availability. Also, pigs fed treated BSG and PKC diets had better growth responses while those fed *A. niger*-treated PKC and WB produced low carcass cholesterol contents.

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