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Apparent and Standardized Total Tract Digestibility of Phosphorus in *Aspergillus niger* Treated Wheat Offal, Brewers' Dried Grain and Palm Kernel Cake Fed to Growing Pigs

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Abstract:
The apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) of phosphorus (P) in a *Aspergillus niger* treated wheat offal (WO), Brewers Dried Grain (BDG) and palm kernel cake (PKC), were measured using growing pigs (Large White X Hampshire) of average weight 21.02 ± 0.63 kg in a 2 x 3 factorial arrangement. Six diets based on WO, BDG and PKC with or without *Aspergillus niger* treatment were formulated. A P-free diet was also formulated to measure endogenous losses of P. The results of the experiment showed that the ATTD and STTD of P in treated WO (72.22 and 83.26 %), BDG (84.61 and 88.21 %) and PKC (82.36 and 85.68 %) were greater ($p < 0.05$) than in untreated WO (53.54 and 58.08 %), BDG (67.83 and 73.92 %) and PKC (78.47 and 82.37 %) respectively. The microbial treatment of the diets also reduced ($p< 0.05$) the excretion of P in the faeces from (1.13 to 0.71 g), (0.53 to 0.43 g) and (0.56 to 0.55 g) in WO, BDG and PKC respectively. Findings from this study showed that *Aspergillus niger* treatment of the WO, BDG and PKC significantly ($p<0.05$) improved the ATTD and STTD of phosphorous. Also, *Aspergillus niger treatment* of WO, BDG and PKC could reduce P-excretion in growing pigs fed these feedstuffs.

Keywords: Phosphorus, *Aspergillus niger,* diets, growing pigs, digestibility

Introduction

Phosphorus (P) is an essential mineral required by growing pigs for their metabolic process. Currently, diets fed to pigs contain phytate phosphorus which is not readily available for utilization. Consequently, when excreted it forms an environmentally harmful component of swine manure. Phosphorus as a nutrient has posed challenges for non-ruminant nutritionists due to the inefficiency of its use in cereal-based diets, expense of P supplementation, and potential environment pollution effects of P in animal waste (Adeola *et al*., 2004). The application of biotechnological tool such as Solid State fermentation (SSF) has been effective in improving P digestibility as well as reducing the excretion of this mineral in manure (Guggenbuhl *et al*., 2007 and Lawal *et al*., 2013). The use of microbial treatment to enhance digestibility and assimilation of nutrients may alleviate the problem of manure management in swine production, at the same time reducing environmental pollution (Sharpley *et al*., 1994) in humid tropics of Nigeria.

Digestibility of P is usually expressed as the apparent total tract digestibility (ATTD) and standardized total tract digestibility (STTD) values. Relative bioavailability and apparent total tract digestibility (ATTD) of P have been measured in many feed ingredients, which is the difference between P intake and P excretion in faeces (Jongloed *et al*., 1992; Bruce and Sandston, 1995) as reported by Ferdinando (2010). Basal EPL (Endogenous Phosphorus Loss) can be directly measured by feeding pigs a P- free diet and this value can be used to correct ATTD for EPL to calculate STTD (Standardized total tract digestibility) of P.

Treatment of these test ingredients with *Aspergillus niger* due to its phytase activity could make the phosphorus in them more available for pigs in their diets. This needs to be well investigated, hence this study.

Materials and Methods

The study was carried out at the Swine Unit of the Obafemi Awolowo University (OAU) Teaching and Research Farm, Ile-Ife (latitude 7° 28' N and longitude 4° 33' E and altitude of about 244 m above the sea level) and at laboratories of the Departments of Animal Sciences and Microbiology, OAU Ile-Ife and Central Research Laboratory, the Federal University of Technology, Akure. The test ingredients were sourced for in Ile-Ife while the experimental pigs were sourced from T and B farm Opa area, Ile Ife.

The apparent and standardized total tract digestibility was conducted on the three test ingredients. Seven diets were formulated (Table 1), Two diets each (treated and untreated) were based on PKC, BDG, and WO. The test ingredients served as the source of P in the diets and a P- free diet was formulated as the seventh diet. Each of the diets was fed to the pigs at daily rate of 4 % of live weight in line with the procedure of Onyimonyi *et al.* (2010). Water was provided *ad libitum* throughout the period of the study. A total of 21 growing pigs, Large White x Hampshire (21.02 ± 0.63 kg) was used for the seven treatments with three animals per treatment and each served as a replicate. The animals were adjusted for a period of 5 days in the metabolic cage followed by 4 days of faecal and urine collection. The animals were starved for 12 hours prior to experimental

period to clear the gut of previous meal. The pigs were also starved for another 12 hours at the end of the feeding period to ensure total faecal collection of the faeces arising from the diet offered.

Faeces and urine were collected separately on daily basis every morning (7.30 – 8.30 am). Samples of faeces collected were oven dried at 60 ^{0}C, weighed and were put in a plastic bag and stored in deep freezer at (-10 to -20 ^{0}C) for phosphorus analysis. Urine was collected, the record of weight and volume was taken, about 10 % of each day's collection was stored in 10 ml of 10 % sulphuric acid to prevent nitrogen losses by evaporation of ammonia and keep the urine pH below 4.

Table 1: Gross composition of experimental diets

	WO		BDG		PKC		
Ingredients (%)	UT	T	UT	T	UT	T	P-free
WO	50.00	50.00	-	-	-	-	-
BDG	-	-	50.00	50.00	-	-	-
PKC	-	-	-	-	50.00	50.00	-
Sucrose	20.00	20.00	20.00	20.00	20.00	20.00	22.00
Soy-bean Oil	-	-	-	-	-	-	6.00
Limestone	1.20	1.20	1.20	1.20	1.20	1.20	0.80
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamins/Premix*	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Cassava flour	28.10	28.10	28.10	28.10	28.10	28.1	70.00
Potassium Carbonate	-	-	-	-	-	-	0.40
Magnesium Oxide	-	-	-	-	-	-	0.10
Total	100	100	100	100	100	100	100

WO = Wheat Offal, BDG = Brewer Dried Grain, PKC = Palm Kernel Cake, P – Free: Phosphorus free, TRT =Treatment, T= Treated, UT=Untreated,*Grower premix supplied per kg diet: vitamin A 10,000,000 IU; vitamin D 32,000,000 IU; vitamin E 8,000 IU; vitamin K 2,000mg; vitamin B1 2,000 mg; vitamin B2 5,500 mg; vitamin B61,200 mg; vitamin B12 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.

Results and Discussion

The phosphorus digestibility and retention in the growing pigs fed treated agro industrial by-products are shown in Table 2. The Phosphorus intake, fecal output, urine phosphorus output, Phosphorus retention and the apparent total tract digestibility (ATTD) were significantly ($p<0.05$) affected by the treatments. P intake increased significantly ($p<0.05$) from 2.46 to 3.65 g, 1.66 to 2.80 g , 2.62 to 3.10 g in pigs fed WO, BDG and PKC respectively when treated with *A. niger*. Phosphorus output in faeces was also reduced significantly ($p<0.05$) from 1.13 to 0.71 g for WO, 0.56 to 0.43 g for BDG and 0.77 to 0.56 g for PKC when treated with *A. niger.* The total P intake in pigs fed untreated WO, BDG and PKC was less than that in pigs fed treated ones. When the P intake was small, the proportion of the endogenous phosphorus loss (EPL) of P in the total quantity of excreted P increases (Kim *et al.,* 2012). P retention also increased ($p < 0.05$) from 53.93 to 80.44 % for WO, 67.81 to 84.57 % for BDG and 78.46 to 82.34 % for PKC when subjected to microbial treatment. The ATTD of P increased ($p<0.05$) from 53.94 to 72.22 % for WO, 67.83 to 84.61 % for BDG and 78.47 to 82.36 % for PKC when treated with *A. niger.* The basal EPL observed was not significantly ($p> 0.05$) influenced by the microbial treatment of the test ingredients. In this experiment, the basal EPL was measured by feeding a P-free diet and a value of 0.103 g/kg DMI was obtained.

The test ingredients, microbial treatment as well as the interaction between the test ingredients and the treatment significantly ($p<0.05$) affected the values observed for the Phytic acid contents of the test ingredients. The values obtained for WO, BDG and PKC reduced from 0.019 to 0.006, 0.001 to 0.00 and 0.022 to 0.00 g/kg respectively. *Aspergillus niger* treatment increased standardized total tract digestibility (STTD) of P ($p< 0.05$) significantly in the test ingredients due to its phytase activity (Selle and Ravindran, 2008). Standardized total tract digestibility (STTD) of P in pigs fed the test ingredients also showed significant ($p<0.05$) differences. The highest value of 88.21 % was observed in treated BDG, followed by 85.68 % in treated PKC, 58.08 % in untreated WO. The least value was observed in untreated WO. The values of STTD of P observed in this experiment disagreed with Ferdinando (2010), where the STTD of P in Distillers Dried Grain with Soluble (DDGS) without phytase (72.9 %) was not different from the STTD of P in DDGS with phytase (75.5 %). The significant ($p<0.05$) improvement in the phosphorus digestibility and retention in the growing pigs fed these treated test ingredients in this study may be due to phytase enzyme released by the *A. niger* (Selle and Ravindran, 2008).

Table 2: Phosphorus digestibility and retention of the experimental animal

Parameters	WO		BDG		PKC			P (ANOVA)			
	UT	T	UT	T	UT	T	P-Free	Pooled SEM	SRC	TRT	SRC X TRT
P Intake(g)	2.462	3.65	1.66	2.80	2.62	3.10	-		0.15	<0.0001	< 0.0001
Fecal P output (g)	1.134	0.714	0.534	0.431	0.564	0.547	-	0.06	<0.0001	<0.0001	<0.001
Urine P output (g)	0.0002	0.0001	0.0004	0.001	0.0004	0.005	-	0.00	< 0.0001	<0.0001	<0.0001
Total P output (g)	1.134	0.714	0.534	0.431	0.564	0.547		0.06	<0.0001	<0.0001	<0.0001
P retention (%)	53.93	80.44	67.81	84.57	78.46	82.34	-	2.53	< 0.0001	<0.0001	<0.0001
ATTD of P (%)	53.54	72.22	67.83	84.61	78.47	82.36	-	1.19	< 0.0001	<0.0001	<0.0001
EPL (g/kg of DMI)	0.102	0.103	0.101	0.101	0.102	0.103	0.103	0.25	0.7100	0.1820	0.3060
Fecal Phytic acid (g/kg)	0.019	0.006	0.001	0.00	0.022	0.00	0.00	2.21	< 0.0001	<0.0001	<0.0001
STTD of P (%)	58.08	83.26	73.92	88.21	82.37	85.68	-	0.45	<0.0001	<0.0001	<0.0001

WO = Wheat Offal, BDG = Brewer Dried Grain, PKC = Palm Kernel Cake, ATTD = Apparent Total Tract Digestibility, SEM = Standard Error of the Mean. SRC=Fibre source, TRT =Treatment, T= Treated, UT=Untreated, DMI =Dry Matter Intake. EPL: Endogenous Phosphorus loss, P Intake: Phosphorus intake, P- Free =Phosphorus free diet. STTD = Standardized Total Tract Digestibility.

The ability of microbial phytase to increase phosphorus availability in feed and enhance its digestibility has been reported in the findings of Pillai *et al.*, (2006) and Brana *et al.*, (2006). The total endogenous phosphorus loss (EPL) observed in this study was within the range (0.070 to 0.670 g/kg) reported by Dilger and Adeola (2006) and Pettey *et al.* (2006) where the total EPL was measured using the regression method. The values for the ATTD of P in WO, BGD and PKC in this study were in agreement with values reported by Pedersen *et al.* (2007). The ATTD of P in distillers dried grains with soluble (DDGS) was previously reported with values ranging from 50 to 68 % (Pedersen *et al.,* 2007; Stein *et al.,* 2009) and the ATTD of P in BDG measured in this experiment was similar to the greatest values in this range and higher in the treated BDG, WO and PKC (84.61, 72.22 and 82.37 %) respectively. This observation confirmed that the ATTD of P in treated BDG was much greater than in untreated WO. The result of the fecal P was similar to the results of Ferdinando (2010) who reported that Phosphorus concentration in feces reduced from 1.98 to 1.15 % ($p < 0.001$) in pigs fed corn and from 2.84 to 1.84 % ($p< 0.001$) in pigs fed SBM when phytase was used.

Conclusion

Findings from this study showed that *Aspergillus niger* treatment of the WO, BDG and PKC significantly improved the ATTD and STTD of phosphorous. Also, *Aspergillus niger* treatment of WO, BDG and PKC could reduce P-excretion in growing pigs fed these feedstuffs.

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