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Efficacy of novel 6-phytase from *Buttiauxella* sp. on ileal and total tract nutrient digestibility in growing pigs fed a corn-soy based diet

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ABSTRACT

The current study evaluated the effect of different levels of a 6-phytase from *Buttiauxella* sp. expressed in *Trichoderma reesei* on ileal and total tract nutrient digestibility in growing pigs. Twelve ileal cannulated pigs (initial BW = 25 kg) were randomly assigned to 1 of 6 treatments in a replicated 6 × 6 Latin square design to give 12 observations per treatment. The experimental diets consisted of a corn soybean meal-based control diet (NC), NC supplemented with 4 levels of phytase (i.e. 250, 500, 1000 and 2000 FTU/kg) and a low-protein diet (5% casein) used to quantify endogenous amino acid (AA) losses. All diets contained titanium dioxide as an indigestible marker. Pigs were given their daily feed allowance at a rate of 45 g/kg BW determined at the beginning of each experimental period. Each period lasted for 9 d with 5 d of adaptation to experimental diets followed by 2 d of faecal and 2 d of ileal digesta collection. Data were analyzed using the mixed model procedures of SAS. The final model had treatment as the main effect as pen and period effects were non-significant. Increasing levels of 6-phytase supplementation linearly increased ($P < 0.05$) apparent ileal digestibility (AID) of dry matter (DM), crude protein (CP) and gross energy GE. Compared with NC, AID of phosphorus (P) increased ($P < 0.05$) by 12.7, 46.6, 49.1 and 77.4% with 250, 500, 1000 and 2000 FTU/kg of phytase, respectively along with AID of calcium (Ca) showing a tendency for improvement. Mean AID of indispensable and dispensable AA improved ($P < 0.05$) by 2.3, 2.0 and 1.1% and 2.1, 1.2 and 1.2% for diets containing 500, 1000 and 2000 FTU/kg of phytase, respectively. Mean standard ileal digestibility (SID) of indispensable and dispensable AA improved ($P < 0.05$) by 1.7, 1.2 and 2.8% and 1.0, 0.2, and 3.6% for diets with 500, 1000 and 2000 FTU/kg of phytase, respectively. Apparent total tract digestibility (ATTD) of DM, CP and GE responded linearly ($P < 0.05$) to increasing levels of phytase. The ATTD of Ca and P increased ($P < 0.05$) by 18.2, 30.4, 24.5 and 33.8% and 46.8, 98.4, 99.7 and 125.3% for diets supplemented with 250, 500, 1000 and 2000 FTU/kg of the 6-phytase, respectively. In conclusion, the supplementation of diets with a *Buttiauxella* 6-phytase significantly enhanced the AID and ATTD of Ca, P and other nutrients in pigs, in a dose dependent manner.

Abbreviations: AA, amino acids; AID, apparent ileal digestibility; ATTD, apparent total tract digestibility; Ca, calcium; CP, crude protein; DM, dry matter; GE, gross energy; NC, negative control; P, phosphorus; SID, standardized ileal digestibility.

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1. Introduction

Phytic acid (myo-inositol hexakisphosphate) also known as phytate when in salt form, is the primary storage form of phosphorus (P) in various cereal grains and oil seeds. In swine, the phytate P availability is limited (Hegeman and Grabau, 2001) as the endogenous mucosal phytase activity is often not meaningful. Consequently, diets need to be supplement with inorganic P to meet the pig's P requirement. In addition, the inefficient degradability of phytate results in excessive faecal excretion of P. In non-ruminants, phytate has shown to bind with nutrients in the gut thus limiting nutrient digestibility and utilization (Woyengo et al., 2009). Phytic acid being negatively charged, can potentially bind with positively charged molecules such as minerals, particularly Ca, Zn, Fe and Mg and basic amino acids (Cromwell, 1992; Maenz, 2001). Phyate has been shown to increase endogenous amino acid losses in broilers by either interacting with endogenous enzymes or increasing secretion of gastrointestinal mucin (Cowieson et al., 2004) which may alter the maintenance requirements (Cowieson et al., 2008). Several studies have been published wherein supplementation of cereal based diets with phytase have shown to be beneficial in counteracting the negative effects of phytate (Bedford, 2000; Cowieson et al., 2006; Ravindran et al., 2006). Apart from enhancing the absorption and retention of nutrients, phytase supplementation has been shown to reduce endogenous losses, probably through the dephosphorylation reaction in which phytic acid molecule is broken down to lower molecular weight inositol phosphate esters with a reduced capacity to stimulate endogenous secretions (Cowieson et al., 2008). The possible sources of phytase enzyme with respect to non-ruminants include endogenous phytase from the small intestinal mucosa, microbial phytase from the gut microbes in the large intestine, plant phytase present in particular feedstuff and exogenous microbial phytase (Selle and Ravindran, 2008). In non-ruminants, the endogenous mucosal phytase has reported to be incapable of hydrolyzing adequate amounts of phytate-bound P (Humer et al., 2014). Moreover, during hind-gut fermentation in pigs, calcium (Ca) has shown to depress phytate hydrolysis mediated by gut microbial phytase (Sandberg et al., 1993). Hence, supplementation of exogenous microbial phytase in diets is a common method to increase nutrient absorption. Though phytase supplementation in non-ruminants has been effective in improving P digestibility (Bedford, 2000), the effect of this enzyme on other nutrients has not always been consistent (Adeola and Sands, 2003; Peter and Baker, 2001). The factors that contribute to this inconsistency could be the variation in feed ingredients that make up the diet (Liao et al., 2005a), differences in protein source (Traylor et al., 2001 and Selle and Ravindran, 2008), the level of enzyme added and the concentration of the phytate to the enzyme (Ravindran et al., 2006). The objective of the present study was to determine the effects of increasing levels of 6-phytase from *Buttiauxella* sp. expressed in *Trichoderma reesei* on ileal and total tract nutrient digestibility in growing pigs fed a corn-soybean meal based diet.

2. Materials and methods

The use of animals in this study was reviewed and approved by the University of Manitoba Animal Care Committee, and pigs were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

2.1. Animals, housing, diets, and surgical procedure

Twelve barrows (Yorkshire × Landrace × Duroc) weighing approximately 18 kg were obtained from the University of Manitoba's Glenlea Swine Research Unit and housed individually in pens with raised plastic-covered expanded metal floors in an environmentally controlled room with ambient temperature set at 22 °C in the T.K. Cheung Centre for Animal Science Research. Pigs had free access to water from drinking nipples throughout the study.

The experimental diets (Table 1) consisted of a corn soybean meal-based control diet (NC), NC supplemented with 4 levels of phytase (i.e. 250, 500, 1000 and 2000 FTU/kg) and a low-protein diet (5% casein) used to quantify endogenous amino acid (AA) losses. Diets were formulated to meet or exceed NRC (1998) nutrient specifications for growing pigs. All the diets contained titanium dioxide (TiO_2) as an indigestible marker.

Each pig upon arrival was weighed and placed in individual pens that were appropriately labelled. After a 5-d adaptation period, pigs were surgically fitted with a T-cannula at the distal ileum as described by Nyachoti et al. (2002) (Tables 2–5).

2.2. Feeding and digesta collection procedure

After a 14-d recovery period, pigs were assigned at random to the dietary treatments in a replicated 6 × 6 Latin square design to give 12 observations per treatment. All pigs were about 25 kg BW at the start of the trial period. Pigs were given their daily feed allowance at a rate of 45 g/kg BW determined at the beginning of each experimental period. Daily rations were offered in 2 equal meals at 0800 and 1700 h as a mash form. Each experimental period lasted 9 days. In each period, after 5-d acclimation to the experimental diets, faecal samples were collected continuously for a total of 12 h each on d 6 and 7. Ileal digesta samples were collected continuously for a total of 12 h each on d 8 and 9. Digesta were collected at hourly intervals into transparent plastic bags [containing 10% (v/v) formic acid to minimize bacterial activity] attached to the barrel of the T-cannulas with hose clamps. Digesta and faecal samples were immediately frozen at −20 °C until further processing.

Table 1
Composition of experimental diets (as-fed basis).

Item	Diets[a]					
	NC	NC + 250	NC + 500	NC + 1000	NC + 2000	5% Casein
Ingredient (g/kg)						
Corn	627.5	627.5	627.5	627.5	627.5	–
Soybean meal	330.0	330.0	330.0	330.0	330.0	–
Casein	–	–	–	–	–	50.0
Cellulose	–	–	–	–	–	50.0
Sucrose	–	–	–	–	–	200.0
Corn starch	–	–	–	–	–	636.0
Limestone	11.5	11.5	11.5	11.5	11.5	11.0
Monocalcium phosphate	2.0	2.0	2.0	2.0	2.0	10.0
Iodized Salt	4.0	4.0	4.0	4.0	4.0	3.0
Vitamin-mineral premix[b]	10.0	10.0	10.0	10.0	10.0	10.0
Vegetable oil	10.0	10.0	10.0	10.0	10.0	25.0
Titanium dioxide	5.0	5.0	5.0	5.0	5.0	5.0
Analyzed composition						
Gross energy (MJ/kg)	16.5	16.5	16.5	16.5	16.5	16.4
Crude protein (g/kg)	213.1	215.3	213.9	218.7	211.6	50.5
Ca (g/kg)	5.9	6.3	6.3	5.7	5.7	5.7
Total P (g/kg)	3.9	3.9	4.1	3.9	3.9	2.9
Indispensable AA (g/kg)						
Arginine	12.9	13.6	13.2	13.3	12.5	1.9
Histidine	6.1	6.4	6.2	6.3	5.6	1.8
Isoleucine	7.1	7.4	7.1	7.4	6.8	2.2
Leucine	16.0	16.6	16.1	16.7	15.6	4.4
Lysine	10.8	11.4	11.0	11.4	10.5	3.5
Methionine	2.3	1.7	2.2	1.9	2.1	0.7
Phenylalanine	9.1	9.5	9.2	9.5	8.9	2.2
Threonine	7.7	8.1	7.9	8.1	7.6	2.1
Valine	8.4	8.6	8.3	8.8	8.2	3.1
Dispensable AA (g/kg)						
Alanine	9.0	9.3	9.1	9.6	8.7	1.4
Aspartic acid	20.0	20.9	20.7	21.0	19.5	3.5
Cysteine	2.3	2.2	2.6	1.9	2.2	0.3
Glutamic acid	35.3	36.1	36.2	36.3	34.3	10.5
Glycine	8.0	8.3	8.1	8.4	7.6	0.3
Proline	12.2	12.8	12.4	12.7	11.3	5.5
Serine	10.4	10.9	10.7	10.9	10.2	2.7
Tyrosine	6.1	6.3	6.1	6.3	6.0	1.8

[a] The NC was supplemented with 0, 250, 500, 1000 and 2000 FTU/kg of 6-phytase from *Buttiauxella* sp. expressed in *T. reesei* (*Axtra® PHY, Danisco Animal Nutrition/Dupont*). The analyzed in-feed phytase activities were 275, 482, 1170 and 2152 FTU/kg for diets containing 250, 500, 1000 and 2000 FTU/kg of phytase, respectively. One FTU of enzyme activity is defined as the amount of enzyme that liberates 1 μmol of inorganic P per min at 37 °C and pH 5.5 (Engelen et al., 2001).

[b] Supplied the following per kg of finished feed: vitamin A, 2000 IU; vitamin D, 200 IU; vitamin E, 40 IU; vitamin K, 2 mg; choline, 350 mg; pantothenic acid, 14 mg; riboflavin, 7 mg; folic acid, 1 mg; niacin, 21 mg; thiamin, 1.5 mg; vitamin B6, 2.5 mg; biotin, 70 mg; vitamin B12, 20 mg, Cu, 25 mg; Zn, 150 mg; Fe, 100 mg; Mn, 50 mg; I, 0.4 mg; Se, 0.3 mg.

2.3. Sample preparation and chemical analyses

Ileal digesta samples were thawed and pooled for each pig and period, homogenized in a blender (Waring Commercial, Torrington, CT), sub-sampled, and freeze-dried. Faecal samples were dried in an oven at 50 °C for 5 d, pooled per pig within a period, and sub-sampled. Dried digesta and faeces along with experimental diets were ground to pass through a 1 mm screen before chemical analysis. Ileal digesta, faecal, and feed samples were analyzed for dry matter (DM), AA (digesta and feed only), crude protein (CP), gross energy (GE), Ca, P and TiO_2. Feed and faecal samples were also analyzed for sodium (Na), potassium (K), magnesium (Mg), chlorine (Cl), zinc (Zn), iron (Fe), and copper (Cu).

Dry matter content was determined according to the AOAC (1990; method 925.09) by oven drying 5 g of sample at 102 °C overnight. Gross energy was measured using an adiabatic bomb calorimeter (model 6400, Parr Instrument, Moline, IL) which had been calibrated using benzoic acid as a standard. Nitrogen content was determined using the combustion method (method 990.03; AOAC, 1990) using the LECO N analyser (model CNS-2000; LECO Corp., St. Joseph, MI) and CP was calculated as nitrogen × 6.25. Samples for analysis of Ca, P, Na, K, Mg, Zn, Fe, and Cu were ashed for 12 h and digested according to AOAC (2005; method 985.01) and read on a Varian inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA). Chloride levels in the samples were determined at Central Testing Laboratory Ltd. Manitoba, Canada according to AOAC (2006; method 943.01). Titanium contents were determined according to the procedures described by Lomer et al. (2000) and read on an inductively coupled plasma mass spectrometer (Varian Inc., Palo Alto, CA). Amino acid contents were

Table 2
Coefficients of apparent ileal amino acid digestibility in growing pigs fed a corn-soybean meal based diets supplemented with enzyme.

Item	Diets[1]					SEM	*P*-value		
	NC	NC + 250	NC + 500	NC + 1000	NC + 2000		T[2]	L[3]	Q[4]
Indispensable AA									
Arginine	0.865	0.875	0.877	0.879	0.876	0.008	0.208	0.642	0.154
Histidine	0.483	0.505	0.522	0.532	0.478	0.024	0.239	0.901	0.063
Isoleucine	0.816^{b}	0.831^{ab}	0.832^{a}	0.841^{a}	0.836^{a}	0.009	0.037	0.255	0.135
Leucine	0.820	0.833	0.837	0.838	0.833	0.010	0.317	0.502	0.103
Lysine	0.812	0.825	0.826	0.833	0.826	0.010	0.284	0.498	0.105
Methionine	0.845^{a}	0.740^{c}	0.850^{a}	0.777^{d}	0.826^{b}	0.011	<0.0001	<0.0001	<0.0001
Phenylalanine	0.823	0.840	0.843	0.846	0.842	0.009	0.153	0.443	0.155
Threonine	0.701^{b}	0.725^{a}	0.725^{a}	0.736^{a}	0.711^{b}	0.013	0.050	0.983	0.049
Valine	0.773	0.784	0.784	0.797	0.789	0.011	0.336	0.252	0.177
Dispensable AA									
Alanine	0.758	0.778	0.777	0.791	0.776	0.012	0.100	0.409	0.084
Aspartic acid	0.774^{b}	0.790^{a}	0.798^{a}	0.804^{a}	0.788^{ab}	0.010	0.029	0.325	0.027
Cysteine	0.721^{a}	0.641^{b}	0.723^{a}	0.603^{c}	0.699^{a}	0.021	<0.0001	0.277	0.606
Glutamic acid	0.841	0.841	0.847	0.860	0.849	0.013	0.128	0.166	0.053
Glycine	0.667	0.685	0.682	0.703	0.677	0.022	0.194	0.433	0.159
Proline	0.760	0.792	0.774	0.775	0.771	0.021	0.498	0.791	0.776
Serine	0.766^{b}	0.786^{a}	0.791^{a}	0.795^{a}	0.782^{a}	0.011	0.046	0.724	0.037
Tyrosine	0.813^{b}	0.835^{a}	0.837^{a}	0.843^{a}	0.832^{a}	0.009	0.026	0.436	0.092

[abc] Means in the same row with different superscripts differ ($P < 0.05$).
[1] NC supplemented with 0, 250, 500, 1000 and 2000 FTU/kg of 6-phytase from *Buttiauxella* sp. expressed in *Trichoderma reesei*.
[2] T, treatment effect.
[3] L, linear effect for treatments with 250, 500, 1000 and 2000 FTU/kg of enzyme.
[4] Q, quadratic effect for treatments with 250, 500, 1000 and 2000 FTU/kg of enzyme.

Table 3
Coefficients of standardized ileal amino acid digestibility in growing pigs fed a corn-soybean meal based diets supplemented with enzyme.

Item	Diets[1]					SEM	*P*-value		
	NC	NC + 250	NC + 500	NC + 1000	NC + 2000		T[2]	L[3]	Q[4]
Indispensable AA									
Arginine	0.923^{b}	0.928^{b}	0.933^{ab}	0.932^{ab}	0.943^{a}	0.008	0.048	0.069	0.532
Histidine	0.824	0.817	0.849	0.845	0.873	0.024	0.313	0.014	0.897
Isoleucine	0.876^{c}	0.886^{bc}	0.891^{ac}	0.896^{ab}	0.904^{a}	0.009	0.015	0.031	0.384
Leucine	0.866	0.875	0.881	0.880	0.887	0.010	0.239	0.133	0.291
Lysine	0.863	0.871	0.875	0.880	0.885	0.010	0.246	0.121	0.283
Methionine	0.891^{a}	0.814^{c}	0.895^{a}	0.844^{bc}	0.887^{a}	0.011	<0.0001	<0.0001	0.003
Phenylalanine	0.864	0.878	0.883	0.883	0.889	0.009	0.129	0.144	0.340
Threonine	0.820	0.833	0.839	0.844	0.849	0.013	0.175	0.088	0.398
Valine	0.849	0.854	0.859	0.865	0.874	0.011	0.215	0.045	0.449
Dispensable AA									
Alanine	0.837	0.850	0.852	0.860	0.865	0.012	0.149	0.074	0.341
Aspartic acid	0.830	0.841	0.850	0.855	0.853	0.010	0.058	0.051	0.119
Cysteine	0.813^{a}	0.748^{b}	0.809^{a}	0.724^{b}	0.810^{a}	0.021	<0.0001	0.102	0.809
Glutamic acid	0.889	0.885	0.893	0.903	0.904	0.013	0.064	0.052	0.136
Glycine	0.895^{b}	0.894^{b}	0.902^{b}	0.910^{b}	0.942^{a}	0.022	0.017	0.011	0.874
Proline	1.179^{b}	1.168^{b}	1.172^{b}	1.156^{b}	1.265^{a}	0.021	<0.0001	0.002	0.024
Serine	0.875^{c}	0.884^{bc}	0.893^{ab}	0.893^{ab}	0.908^{a}	0.011	0.024	0.028	0.409
Tyrosine	0.862^{b}	0.879^{ab}	0.883^{a}	0.887^{a}	0.888^{a}	0.009	0.040	0.088	0.308

[abc] Means in the same row with different superscripts differ ($P < 0.05$).
[1] NC supplemented with 0, 250, 500, 1000 and 2000 FTU/kg of 6-phytase from *Buttiauxella* sp. expressed in *Trichoderma reesei*.
[2] T, treatment effect.
[3] L, linear effect for treatments with 250, 500, 1000 and 2000 FTU/kg of enzyme.
[4] Q, quadratic effect for treatments with 250, 500, 1000 and 2000 FTU/kg of enzyme.

determined according to AOAC (1990; method 982.30). Briefly, about 100 mg of each sample was digested in 4 mL of 6 M HCl for 24 h at 110 °C followed by neutralization with 4 mL of 25% (wt./vol.) NaOH and cooled to room temperature. The mixture was then equalized to 50 mL volume with sodium citrate buffer (pH 2.2) and analyzed using an AA analyzer (Sykam GmbH, Fürstenfeldbruck, Germany). Samples for analysis of sulfur-containing AA (Methionine and Cysteine) were subjected to performic acid oxidation before acid hydrolysis. Tryptophan was not analyzed. The in-feed phytase activity was analyzed according to the method of Engelen et al. (2001).

Table 4
Apparent ileal digestibility (AID) coefficients of nutrients and DE content of corn-soybean meal based diets supplemented with enzyme fed to growing pigs.

Item	Diets[1]					SEM	*P*-value		
	NC	NC + 250	NC + 500	NC + 1000	NC + 2000		T[2]	L[3]	Q[4]
AID									
Dry matter	0.664[c]	0.660[c]	0.677[bc]	0.673[bc]	0.704[a]	0.014	0.002	0.003	0.915
Crude protein	0.771[d]	0.787[c]	0.797[bc]	0.801[bc]	0.824[a]	0.010	<0.0001	0.020	0.485
Gross energy	0.693[c]	0.688[c]	0.704[bc]	0.702[bc]	0.730[a]	0.012	0.001	0.003	0.849
Ca	0.708	0.750	0.791	0.772	0.795	0.030	0.069	0.246	0.761
P	0.393[d]	0.443[c]	0.576[b]	0.586[b]	0.697[a]	0.020	<0.0001	<0.0001	0.106
DE content (MJ/kg)	11.43[b]	11.35[b]	11.63[b]	11.59[b]	12.05[a]	0.200	0.001	0.003	0.842

[abcd] Means in the same row with different superscripts differ ($P < 0.05$).
[1] NC supplemented with 0, 250, 500, 1000 and 2000 FTU/kg of 6-phytase from *Buttiauxella* sp. expressed in *Trichoderma reesei*.
[2] T, treatment effect.
[3] L, linear effect for treatments with 250, 500, 1000 and 2000 FTU/kg of enzyme.
[4] Q, quadratic effect for treatments with 250, 500, 1000 and 2000 FTU/kg of enzyme.

Table 5
Apparent total tract digestibility (ATTD) coefficients of nutrients and DE content of corn-soybean meal based diets supplemented with enzyme fed to growing pigs.

Item	Diets[1]					SEM	*P*-value		
	NC	NC + 250	NC + 500	NC + 1000	NC + 2000		T[2]	L[3]	Q[4]
ATTD									
Dry matter	0.843[bc]	0.839[b]	0.855[ac]	0.844[bc]	0.858[a]	0.006	0.024	0.004	0.561
Crude protein	0.853[c]	0.869[b]	0.882[ad]	0.873[bd]	0.886[a]	0.007	<0.0001	0.003	0.373
Gross energy	0.835[b]	0.834[b]	0.848[a]	0.835[b]	0.852[a]	0.006	0.011	0.002	0.836
Ca	0.539[b]	0.637[a]	0.703[a]	0.671[a]	0.721[a]	0.033	0.002	0.069	0.713
P	0.312[d]	0.458[c]	0.619[b]	0.623[b]	0.703[a]	0.028	<0.0001	<0.0001	0.164
Na	0.879	0.852	0.916	0.900	0.953	0.023	0.165	0.115	0.904
K	0.734[d]	0.743[cd]	0.801[a]	0.770[bc]	0.790[ab]	0.016	0.001	0.029	0.075
Mg	0.237[d]	0.233[cd]	0.387[ab]	0.336[b]	0.411[a]	0.023	<0.0001	0.001	0.186
Cl	0.985[a]	0.981[a]	0.978[ab]	0.967[b]	0.966[b]	0.007	0.026	0.100	0.999
Zn	−0.503	−0.428	−0.269	−0.364	−0.201	0.117	0.067	0.335	0.051
Fe	0.393[a]	0.498[b]	0.257[c]	−0.113[d]	−0.056[d]	0.032	<0.0001	<0.0001	<0.0001
Cu	−0.034[b]	0.324[a]	−0.446[c]	−0.084[b]	−0.001[b]	0.046	<0.0001	<0.0001	<0.0001
DE content (MJ/kg)	13.78[b]	13.76[b]	13.99[a]	13.79[b]	14.06[a]	0.110	0.011	0.002	0.826

[abcd] Means in the same row with different superscripts differ ($P < 0.05$).
[1] NC supplemented with 0, 250, 500, 1000 and 2000 FTU/kg of 6-phytase from *Buttiauxella* sp. expressed in *Trichoderma reesei*.
[2] T, treatment effect.
[3] L, linear effect for treatments with 250, 500, 1000 and 2000 FTU/kg of enzyme.
[4] Q, quadratic effect for treatments with 250, 500, 1000 and 2000 FTU/kg of enzyme.

2.4. Calculations and statistical analysis

Apparent ileal (AID) and total tract digestibility (ATTD) coefficients were calculated using the following equation:

$$\%\,\text{Apparent nutrient digestibility} = 100 - \left\{ \left[\frac{N_d}{N_f} \times \frac{T_{\text{if}}}{T_{\text{id}}} \right] \times 100 \right\}$$

where N_d, nutrient concentration in ileal digesta or faeces (mg/kg DM); N_f, nutrient concentration in feed (mg/kg DM); Ti_f, TiO_2 concentration in feed (mg/kg DM); Ti_d, TiO_2 concentration in ileal digesta or faeces (mg/kg DM).

Standardized ileal digestibilities (SID) of amino acids were calculated using the following equation:

$$\%\,\text{SID} = \text{AID} + \left[\frac{\text{EAL}}{\text{AA}_f} \times 100 \right]$$

where EAL, non specific endogenous loss of amino acids at the distal ileum (mg/kg DM intake); AA_f, dietary content of the amino acid (mg/kg DM).

EAL was measured at the distal ileum after feeding low casein protein diet and calculated according to the following equation.

$$\text{EAL} = \text{AA}_d \times \frac{T_{\text{if}}}{T_{\text{id}}}$$

where AA_d, concentration of that amino acid in ileal digesta (mg/kg DM); Ti_f, TiO_2 concentration in feed (mg/kg DM); and Ti_d, TiO_2 concentration in ileal digesta (mg/kg DM).

Data was subjected to the mixed model procedures of SAS (SAS software 9.2; SAS Institute, Cary, NC, USA). The effects of pen and period were not statistically significant and thus the final model had treatment as the main effect. Results were considered significant at $P \leq 0.05$ and tendencies were observed at $0.05 < P < 0.10$ orthogonal polynomials were used to determine linear and quadratic effects of increasing phytase.

3. Results

3.1. Apparent ileal digestibility

Most of the AA were not significantly affected by phytase supplementation except for isoleucine ($P = 0.037$), threonine ($P = 0.050$), aspartic acid ($P = 0.029$), serine ($P = 0.046$) and tyrosine ($P = 0.026$) which showed significant improvement with enzyme addition along with alanine ($P = 0.100$) showing a tendency for improvement. Increasing levels of phytase quadratically improved AID of threonine ($P = 0.049$), aspartic acid ($P = 0.027$) and serine ($P = 0.037$), along with a tendency for quadratic improvement of alanine ($P = 0.084$) and tyrosine ($P = 0.092$). Apparent ileal digestibility of DM ($P = 0.003$), CP ($P = 0.020$), GE ($P = 0.003$) and P ($P < 0.0001$) showed a linear improvement with increasing levels of enzyme. The DE content showed a linear improvement ($P = 0.003$) with enzyme addition. Also enzyme supplementation showed a tendency for improvement of AID of Ca ($P = 0.069$).

3.2. Standardized ileal digestibility

Enzyme supplementation significantly improved SID of arginine ($P = 0.048$), isoleucine ($P = 0.015$), glycine ($P = 0.017$), proline ($P < 0.0001$), serine ($P = 0.024$) and tyrosine ($P = 0.040$), with aspartic acid ($P = 0.058$) and glutamic acid ($P = 0.064$) showing a tendency for improvement. Increasing levels of phytase showed a linear improvement in SID of isoleucine ($P = 0.031$), glycine ($P = 0.011$) and serine ($P = 0.028$), with proline showing both linear ($P = 0.002$) and quadratic ($P = 0.024$) improvement. Standardized ileal digestibility of arginine ($P = 0.069$), tyrosine ($P = 0.088$), aspartic acid ($P = 0.051$) and glutamic acid ($P = 0.052$) showed a tendency for linear improvement with increasing levels of enzyme.

3.3. Apparent total tract digestibility

The ATTD of DM ($P = 0.004$), CP ($P = 0.003$), GE ($P = 0.002$), P ($P < 0.0001$), K ($P = 0.029$) and Mg ($P = 0.001$) improved linearly with increasing levels of enzyme, with Ca ($P = 0.069$) showing a tendency for linear improvement. The DE content showed a linear improvement ($P = 0.002$) with enzyme addition.

4. Discussion

4.1. Apparent ileal digestibility

The effect of supplemental phytase on AID of AA has not always been consistent (Liao et al., 2005a). The main factors to be considered are the type and solubility of protein, in situ pH, the concentrations of dietary minerals and the interactions between phytate, AA and proteolytic enzymes in the digestive tract (Liao et al., 2005a). Also the feed ingredients that make up the diet have an influence on the effect of phytase on AA digestibility. The response to phytase supplementation is not primarily dependent on the phytate-P content in the diet (Sands, 2002), but rather on the amount of AA that is bound with the phytate-P (Liao et al., 2005b), and this amount of AA that remain bound to phytate-P may vary among different ingredients. Supplemental phytase has shown to hydrolyze the phytate protein bonds and thereby enhancing their availability. In studies by Liao et al. (2005b), significant improvement or tendencies for improvement was observed for AID of CP and AA when phytase (500 and 1000 FTU/kg diet) was supplemented to a wheat-soybean meal based diet. Guggenbuhl et al. (2012a) has reported a similar improvement in AID of CP, AA, and total indispensable AA with 1000 units/kg phytase in pigs fed a corn-soybean meal based diets. Also in studies by Zeng et al. (2011), pigs fed a low-P corn-soybean meal diet with 1000 FTU/kg phytase significantly improved AID of leucine, lysine, phenylalanine, alanine and cysteine and showed a tendency for increase in AID of total AA, isoleucine, threonine, aspartic acid and serine. Moreover, Radcliffe et al. (1999) reported a linear improvement in AID of selected AA in pigs fed a corn-soybean meal based diets with 250 or 500 FTU/kg microbial phytase. The effect of phytase on AID of Ca and P in the current study was in concurrence with previous studies (Radcliffe et al., 2006; Zeng et al., 2011; Guggenbuhl et al., 2012a,b).

4.2. Standardized ileal digestibility

Comparable results were reported by Traylor et al. (2001), where a slight numerical improvement in apparent and true ileal digestibility was observed for most of the AA when semi-purified diets consisting mainly of sucrose and cornstarch with soybean meal as protein sources were supplemented with 500 units of phytase per kilogram of diet. Likewise similar results were reported by Kiarie et al. (2010), where phytase addition increased the standardized ileal AA digestibility in growing pigs fed wheat barley-based diets.

4.3. Apparent total tract digestibility

Kies et al. (2006) reported similar results where ATTD of Ca, P, Mg, K, and Cu significantly increased in a dose-dependent manner. Also O'Quinn et al. (1997) found a linear increase in Ca digestibility with increasing phytase supplementation up to 1000 FTU/kg. Furthermore Adeola et al. (1995) reported that dietary microbial phytase (1500 FTU/kg) supplementation improved the ATTD of Mg, Mn, Cu and Zn for pigs fed corn-soybean meal diet. Our observations are also in agreement with the results reported by Mroz et al. (1994) wherein supplementation of corn-soybean meal diet with microbial phytase (800 FTU/kg of diet) significantly enhanced the ATTD of DM, CP, Ca and P.

Apart from Ca and P digestibility, the results from the current study are in contradiction with Rutherfurd et al. (2014) wherein dietary phytase supplementation (1107 FTU/kg or 2215 FTU/kg) in pigs fed corn-soybean meal-based diet had no effect on digestibility of Mg, K, Fe, Mn and Zn. Similarly in studies by Jolliff and Mahan (2012), pig fed a corn-soybean meal-based diet supplemented with 1000 FTU/kg of microbial phytase did not improve ATTD of Mg and K. Apart from this, the current study also showed negative ATTD values for Zn, Fe and Cu. With less digestible micro-nutrients, negative digestibility coefficients could be observed if the variation in the nutrient contents between samples is in excess of the mean concentration of the nutrient. (Agudelo-Trujillo, 2005). McDowell (2003) had reported that while measuring digestibility of micro-minerals, larger errors result from sample contamination and unknown endogenous losses.

5. Conclusion

The study was conducted to assess the efficiency of a *Buttiauxella* 6-phytase on nutrient digestibility in growing pigs. The results showed that enzyme supplementation in corn-soybean meal basal diet significantly improved the digestibility for most of the AA. Moreover, the supplementation of diets with a *Buttiauxella* 6-phytase significantly enhanced the AID and ATTD of Ca, P and other nutrients, in a dose dependent manner. The significance of the trial mainly implies to nutrients such as phosphorus, wherein the enzyme supplementation has shown to improve the digestibility. So the dietary supplementations with inorganic forms of such nutrients can be minimized thereby reducing their excretion which poses a major environmental hazard.

Conflict of interest statement

I (Martin Nyachoti, Professor) hereby confirm that there is no conflict of interest to declare for any of the co-authors of this manuscript.

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