



ORIGINAL ARTICLE

Ileal digestibility of amino acids, phosphorus, phytate and energy in pigs fed sorghum-based diets supplemented with phytase and Pancreatin[®]

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Summary

The effects of phytase supplementation on the apparent ileal digestibility (AID) of amino acids (AA) have been inconsistent. Two experiments evaluated the effect of providing a mixture of pancreatic enzymes (Pancreatin[®]) to growing pigs fed sorghum–soybean meal diets supplemented with phytase on the AID of AA, energy, and phosphorus (P), as well as the ileal digestibility (ID) of phytate; there were four periods per experiment. In Experiment 1, eight pigs (BW 22.1 ± 1.3 kg) were fitted with a T-cannula at the distal ileum. Each period consisted of 9 days; 7 days for diet adaptation, and 2 days for digesta collection. Treatments (T) were: (i) basal sorghum–soybean meal diet, (ii) basal diet plus Pancreatin[®], (iii) basal diet plus phytase and (iv) basal diet plus phytase and Pancreatin[®]. Phytase increased the digestibilities of phytate and P ($p < 0.001$), but did not affect the AID of AA and energy ($p > 0.10$). Except for methionine ($p = 0.07$), Pancreatin[®] did not affect the AID of AA. Phytase and Pancreatin[®] did not interact ($p > 0.10$). Experiment 2 was similar to Experiment 1, but Pancreatin[®] was infused into duodenum. Pancreatin[®] infusion did not affect the AID of AA ($p > 0.10$); and tended to reduce ($p = 0.09$) the AID of lysine. Phytase × Pancreatin[®] interactions were not observed ($p > 0.10$). In conclusion, phytase and Pancreatin[®] did not improve the AID of AA in growing pigs fed sorghum–soybean meal diets indicating that phytates did not affect AA digestibility.

Introduction

Cereal grains especially sorghum and corn as well as soybean meal, contain significant amounts of phytates (Maga, 1982). Phytic acid or *myo*-inositol hexaphosphoric acid is an ester of phosphoric acid and inositol (Reddy et al., 1982), and is the most common form of phytates. These also interact with other minerals such as calcium, zinc, iron and magnesium to form phytate–mineral complexes. Thus, phytates reduce the digestibility of phosphorus (P) and

calcium (Ca) in pigs (Zimmermann et al., 2002; Liao et al., 2007). Also, phytate–mineral complexes interact with proteins forming phytate–mineral–protein complexes (Reddy et al., 1982), which are stabilized by electrostatic interactions (Lázúty and Lázúty, 1998).

Several studies have demonstrated that the addition of microbial phytase releases P from the phosphorus–phytate complex, increasing its digestibility in pigs (Yi et al., 1996; O’Quinn et al., 1997). As phytates also interact with proteins, an improvement

in the digestibility of protein upon phytase supplementation would be expected as well. However, previous studies failed to show any improvement in the digestibility of protein and amino acids (AA) in pigs fed phytase supplemented diets, based on corn–(Traylor *et al.*, 2001), sorghum–soybean meal (Cervantes *et al.*, 2004), or barley–peas–canola meal (Liao, 2005). Phytates may inhibit the activity of pancreatic enzymes (Singh and Krikorian, 1989) via direct binding or by chelation of cations that are necessary for enzyme activation (Greiner and Konietzny, 2006) or the *de novo* formation of phytate:protein complexes in the stomach (Maenz, 2001). Thus, the lack of response regarding protein digestibility to phytase supplementation may be due to reduced trypsin activity caused by its interaction with phytates. Pancreatin[®] is a lyophilized extract of porcine pancreatic tissue containing high trypsin activity. The hypothesis of the present study was that phytase supplementation in combination with Pancreatin[®] might improve nutrient digestibility. The objective of this study was to determine the effect of adding Pancreatin[®] to a sorghum–soybean meal diet supplemented with phytase on the apparent ileal digestibility (AID) of AA, energy and P in pigs.

Materials and methods

Experiment 1

Eight crossbred (Landrace × Hampshire × Duroc) pigs obtained from the Swine Experimental Unit of the Universidad Autónoma de Baja California, with an average initial BW of 22.1 ± 1.3 kg, were fitted with a simple T-cannula at the distal ileum. The surgical procedure was adapted from the procedure described by Sauer *et al.* (1983). A detailed description of pre- and postoperative care was previously reported by Li *et al.* (1993). The cannulas were prepared from Tygon tubing (Norton Performance Plastics, Wayne, NJ, USA). Pancreatin[®] was purchased from Sigma-Aldrich Co., St Louis, MO, USA; it contains trypsin, amylase, lipase and ribonuclease activity. According to the supplier, Pancreatin[®] had a protease activity equivalent to 214 USP units; 1 USP unit is defined as the amount of pancreatin that releases 15 nmol of tyrosine per minute from casein at pH 7.5 and 40 °C. The phytase used was of microbial origin (*Aspergillus niger*; Natuphos[®]; DSM Food Specialties, Delft, the Netherlands) and had a phytase activity equivalent to 10 000 FTU/g. One FTU is defined as the amount of phytase that liberates 1 μ mol of

Table 1 Composition (%) of the basal diet

Ingredient	%
Sorghum	72.9
Soybean meal, 48% CP	24.2
Calcium carbonate	0.93
Dicalcium phosphate	1.19
Iodized salt	0.35
Vitamin and mineral premix*	0.20
Chromic oxide	0.20

*Supplied per kilogram of diet: vitamin A, 4800 IU; vitamin D₃, 800 IU; vitamin E, 4.8 IU; vitamin K₃, 1.6 mg; riboflavin, 4 mg; D-pantothenic acid, 7.2 mg; niacin, 16 mg; vitamin B₁₂, 12.8 μ g; Zn, 64 mg; Fe, 64 mg; Cu, 4 mg; Mn, 4 mg; I, 0.36 mg and Se, 0.13 mg.

ortho-phosphate per minute from Na-phytate 5.1 mmol/l at pH 5.5 and 37 °C.

Pigs were fed one of four experimental diets (Table 1): Diet 1, basal sorghum–soybean meal diet formulated to contain 18.2% CP, 0.89% apparent ileal digestible lysine, and to meet the requirements of the other essential AA, P, Ca and ME for pigs (NRC, 1988); Diet 2, basal diet supplemented with 1050 FTU/kg diet; Diet 3, basal diet supplemented with 591 mg Pancreatin[®]/kg diet and Diet 4, basal diet supplemented with 1050 FTU and 591 mg Pancreatin[®]/kg diet. Based on the reported trypsin activity contained in Pancreatin[®], the addition of 591 mg of Pancreatin[®] to Diets 3 and 4, would digest at least 14.8 g protein/kg feed. This amount is equivalent to 40% the undigested protein in the basal diet assuming it contains 80% digestible protein. A vitamin and mineral premix was supplemented to the basal diet. Also, 0.20% chromic oxide was added to the diets as a digestibility marker in order to calculate digestibility rates.

The experiment was carried out as a repeated 4 × 4 Latin square design (Steel and Torrie, 1980); there were four experimental periods. Each experimental period comprised 9 days, 7 days of diet adaptation followed by 2 days of collection of ileal digesta. The barrows were fed equal amounts twice daily, at 07:00 and 19:00 hours. Feed intake was limited to 3.0 times the DE requirement for maintenance (NRC, 1998) based on the average BW of the pigs at the start of each experimental period. The feed was mixed with water at a ratio of 1 to 1. Phytase and Pancreatin[®] were added and mixed with the feed at the same time water was added. The pigs consumed their ration in 15 min or less. Ileal digesta were continuously collected during 12 h, every collection day, from 07:00 to 19:00 hours, in plastic bags tied to the barrel of the cannula. The bags were removed and replaced as soon as they were filled

with digesta; no bag remained attached to the cannula longer than 15 min. Ileal digesta were pooled within pig and collection period, and stored immediately after collection at -20°C .

Experiment 2

This experiment was similar to Experiment 1, except that Pancreatin[®] was infused into the small intestine, via a duodenal cannula placed at proximal duodenum (approximately 15 cm from the pylorus), rather than mixed with the diet. The purpose of the infusion was to avoid the acidic environment of the stomach (Manners, 1976), thereby preventing the denaturalization of the enzymes contained in Pancreatin[®]. Eight barrows, average initial BW 38.3 kg (± 2.8), were fitted with two simple T-cannulas: one at proximal duodenum (approximately 15 cm from the pylorus) and one at the distal ileum (approximately 15 cm from the ileo-caecal valve). The experimental diets, periods and digesta collection were the same as those described for Experiment 1. Prior to infusion, Pancreatin[®] was diluted in 120 ml distilled water at 36.5°C . Then, 20 ml were infused directly into the duodenum via the duodenal cannula using a 50 cm³ syringe, at each of 15, 30, 45, 60, 90 and 120 min post-feeding.

At the conclusion of both experiments, ileal digesta were thawed, pooled within pig and period for the same diet, and homogenized. A sub-sample of each homogenate was freeze-dried, and ground through a 1-mm mesh screen. Samples of diets and ileal digesta were analysed for DM and CP (method 984.13; AOAC, 2006). In Experiment 1, the content of P (method 946.06; AOAC, 2006), phytate (method 986; AOAC, 2006) and gross energy (adiabatic bomb calorimeter Model C 2006 basic, IKA-Werke GMBH & Co. KG, Staufen, Germany; benzoic acid was used as a standard) in Diets 1 and 2, as well as ileal digesta from pigs fed these two diets, were analysed. AA analyses (method 982.30; AOAC, 2006) for both experiments, and phytate content (method 986.11; AOAC, 2006) for Experiment 1 were performed at the University of Missouri Experiment Station Chemical Laboratories in Columbia, MO. Cysteine and tryptophan were not determined. Chromic oxide was analysed according to Hill and Anderson (1958). Chemical analysis of the basal diet is shown in Table 2.

The data of each experiment were analysed according to a repeated 4×4 Latin Square, with a 2×2 factorial arrangement, using the GLM procedure of SAS (SAS, 1988). The main effect of phytase and

Table 2 Chemical analysis and energy content of the basal diet (as-fed basis)

Item	%
Analysed	
Dry matter	88.0
Crude protein	18.3
Total phosphorus	0.59
Calcium	0.60
Phytate	0.68
Indispensable amino acids	
Arginine	1.13
Histidine	0.47
Isoleucine	0.80
Leucine	1.82
Lysine	0.92
Methionine	0.27
Phenylalanine	0.95
Threonine	0.64
Valine	0.93
Gross energy, kJ/kg	17.100

Pancreatin[®], and their interaction were tested. Probability levels of $p \leq 0.05$ and $0.05 < p \leq 0.10$ were defined as significant differences and tendencies respectively. The effect of phytase supplementation on P, phytate, and energy digestibility (Diet 1 vs. Diet 2) was determined by means of a *t*-student comparison. The pigs used in these experiments were cared for in accordance with the guidelines established by Canadian Council on Animal Care (1993).

Results

Pigs remained healthy and consumed their daily ration during both experiments. Also, leakage of intestinal content through the fistulas (duodenum or ileum) of the pigs did not occur in any of the two experiments. The analysed phytate content of the basal diet was 6.83 mg/g feed (Table 2). The digestibilities of phytate and P were determined in pigs consuming the basal (Diet 1) and the phytase added (Diet 2) diets of Experiment 1, to ensure that the phytase was active. The supplementation of phytase increased the ileal digestibility (ID) of phytate ($p = 0.001$; Table 3) and the AID of P ($p = 0.002$). Phytase supplementation did not affect ($p > 0.10$) the AID digestibility of energy.

In Experiment 1, phytase supplementation to the sorghum-soybean meal diet did not affect the AID of CP ($p = 0.66$; Table 4) or AA ($p > 0.10$). Pancreatin[®] supplementation did not affect either the AID of CP ($p = 0.28$) or AA ($p > 0.05$), although tended to reduce the AID of methionine ($p = 0.07$). There was

Table 3 Ileal digestibility (%) of phosphorus, phytates and energy in pigs fed a basal sorghum-soybean meal diet with or without supplemental phytase (Experiment 1)

Item	Diet		SEM	p-Value
	Basal	Phytase		
Phosphorus	39.9	51.9	2.40	0.002
Phytate	0.50	36.4	5.20	0.001
Energy	75.1	75.0	0.74	0.876

a trend for Pancreatin® to reduce the AID of methionine compared to the basal diet by 4.4 percentage units (pu). Except for methionine ($p = 0.10$), a phytase \times Pancreatin® interaction was not observed ($p > 0.10$).

The AID values of crude protein and essential AA in pigs fed the phytase supplemented sorghum-soybean meal diets and infused into duodenum with Pancreatin® (Experiment 2) are presented in Table 5. Phytase supplementation did not affect the AID of CP ($p = 0.71$) or AA ($p > 0.10$). Pancreatin® infusion did not affect the AID of AA ($p > 0.10$); and the infusion of Pancreatin® tended to reduce ($p = 0.09$) the AID of lysine. Also, a phytase \times Pancreatin® interaction was not observed ($p > 0.10$). In both experiments, the AID value of arginine was the highest whereas that of threonine was the lowest.

Discussion

The supplementation of phytase to the basal diet increased the digestibility of phytate from 0.5% to

36.4%, and that of P by 30.1%. Similar increment in the AID of P was obtained by Liao *et al.* (2007) in pigs fed a low phytate barley-corn-soybean meal diet supplemented with 2000 FTU/kg diet. Also, Liao *et al.* (2006) reported that the supplementation of 1000 FTU/kg diet to wheat-soybean meal, wheat-soybean meal-canola meal, or wheat-peas-canola meal diets, increased the apparent total tract digestibility of P by 22.4%, 27.2% and 20.5% respectively. These results demonstrate that the phytase used in the present experiments was active.

The AA digestibility results obtained in this study are in agreement with those reported by several authors. Traylor *et al.* (2001) found that supplemental phytase had very little effect, if any, on the AID of CP and AA in growing pigs fed cornstarch-based dehulled soybean meal diets. In addition, Liao *et al.* (2005) did not find any effect of supplementing phytase to corn-soybean meal, or wheat-soybean meal diets on the AID of AA; however, they found improvements in the AID of isoleucine, leucine and lysine in weanling pigs fed wheat-soybean meal-canola meal diets. These authors suggested that the effect of supplementing microbial phytase on the AID of AA depends on the composition of the diet.

In contrast, Mroz *et al.* (1994) and Kemme *et al.* (1999) reported small increases in the AID of AA upon phytase supplementation to different diets for growing pigs. Mroz *et al.* (1994) reported significant increases in the AID of arginine (2.5 pu) and methionine (3.9 pu) when phytase was supplemented to a corn-tapioca-barley-peas-soybean meal diet at a rate of 800 FTU/kg diet. On average, the AID of the indis-

Table 4 Apparent ileal digestibilities (%) of crude protein and amino acids in pigs fed a sorghum-soybean meal diet supplemented with phytase (Phy) and Pancreatin® (Pan) (Experiment 1)

Diet					SEM	p-Value		
	1	2	3	4		Phy*	Pan†	Phy \times Pan
Phytase, FTU/kg	0	0	1050	1050				
Pancreatin, mg/kg	0	591	0	591				
Crude protein	72.7	71.5	72.2	70.9	0.56	0.66	0.28	0.97
Essential amino acids								
Arginine	83.0	83.0	82.6	82.2	0.35	0.36	0.81	0.81
Histidine	75.6	75.1	75.7	73.9	0.51	0.62	0.26	0.52
Isoleucine	73.5	72.8	73.0	72.2	0.57	0.65	0.51	0.99
Leucine	73.2	72.3	73.3	71.5	0.59	0.81	0.26	0.72
Lysine	77.8	77.0	77.7	76.3	0.48	0.71	0.27	0.74
Methionine	67.5	62.9	66.3	66.0	0.64	0.47	0.07	0.10
Phenylalanine	74.6	74.0	74.5	73.3	0.53	0.74	0.38	0.78
Threonine	64.0	62.2	63.6	61.8	0.78	0.79	0.27	0.97
Valine	70.0	69.5	70.0	68.4	0.64	0.67	0.42	0.64

*The phytase activity in Natuphos® was 10 000 FTU/g.

†Pancreatin® digests 25 times its weight of casein in 60 minutes at pH 7.5 at 40 °C and converts 25 times its weight of potato starch into soluble carbohydrates in 5 min in water at 40 °C (Sigma Co., St Louis, MO, USA).

Table 5 Apparent ileal digestibility (%) of crude protein and amino acids in pigs fed a sorghum-soybean meal diet supplemented with phytase (Phy) and infused in duodenum with Pancreatin® (Pan) (Experiment 2)

Diet	1				2				3				4				p-Value		
	0	0	1050	1050	0	591	591	1050	1050	0	591	591	1050	1050	SEM	Phy*	Pan†	Phy × Pan	
Phytase, FTU/kg	0	0	1050	1050															
Pancreatin, mg/kg	0	591	0	591															
Crude protein	71.0	69.6	70.6	70.9	1.16	0.71	0.63	0.48											
Essential amino acids																			
Arginine	77.8	77.3	78.1	79.0	0.82	0.25	0.82	0.40											
Histidine	72.7	70.3	72.1	71.1	1.26	0.54	0.60	0.26											
Isoleucine	77.5	75.3	76.8	76.9	1.16	0.94	0.16	0.54											
Leucine	63.4	61.8	64.0	60.7	1.31	0.73	0.42	0.39											
Lysine	69.6	66.0	68.2	67.6	1.41	0.84	0.09	0.56											
Methionine	75.7	73.5	75.0	74.7	1.51	0.94	0.18	0.32											
Phenylalanine	72.1	69.9	71.4	72.2	1.16	0.82	0.27	0.42											
Threonine	57.0	54.9	55.2	57.7	1.47	0.73	0.86	0.13											
Valine	68.3	66.3	67.5	67.4	1.19	0.90	0.40	0.42											

*The phytase activity in Natuphos® was 10 000 FTU/g.

†Pancreatin® digests 25 times its weight of casein in 60 min at pH 7.5 at 40 °C and converts 25 times its weight of potato starch into soluble carbohydrates in 5 minutes in water at 40 °C (Sigma Co., St Louis, MO, USA).

pensable AA increased by 0.7 pu. Kemme *et al.* (1999) reported significant increases in the AID of isoleucine (2.1 pu), lysine (2.4 pu), threonine (2.9 pu) and tryptophan (4.4 pu) upon supplementation of phytase to a corn-soybean meal diet at a rate of 900 FTU/kg diet. The average of the AID of the indispensable AA increased by 2.2% pu. Although the majority of the studies shows that phytase supplementation increases or tends to increase the AID of AA, the magnitude of the overall improvement was only about 1 to 2 pu; sometimes, these increases were significant, albeit of a small magnitude.

In theory, the magnitude in response to phytase supplementation depends on the phytate content and the activity of intrinsic phytase in the diet. Therefore, the largest response would be expected when the diet is high in phytate and has a low activity of intrinsic phytase. However, Liao *et al.* (2005) reported no improvements in the AID of AA of growing pigs when phytase was supplemented to either high- or low-phytate P diets. Based on these results, Liao *et al.* (2005) suggested that the content of phytate-P *per se* is not the primary determinant for a response to phytase. Rather, it may be the amount of protein (AA) that is complexed with phytate-P; this may include the interaction of phytate with the pancreatic proteases and intestinal peptidases.

According to Honig and Wolf (1991), phytic acid and phytate may form complexes with protein and free AA at pH values normally occurring in the small intestine of the pig. The interaction between phytate and protein may provoke changes in protein struc-

ture that can decrease enzymatic activity, and protein solubility and digestibility (Greiner and Konietzny, 2006). Also, it is possible that insoluble complexes of phytate-protein or phytate-AA are formed inside the small intestine, which could be less susceptible to hydrolysis by pancreatic and intestinal enzymes. *In vitro* studies conducted with rats show that phytates reduce the digestion of casein and bovine serum albumin by pepsin (Knuckles *et al.*, 1989) and may inhibit the activity of digestive enzymes (Reddy *et al.*, 1982; Singh and Krikorian, 1982). Based on those findings, phytase supplementation to the diet might result in the release of protein and AA and, as a consequence, an increase in the digestibility of dietary protein.

However, the results from the present and other *in vivo* studies (e.g. Traylor *et al.*, 2001) are not consistent with these *in vitro* results. Supplementing phytase did not affect the AID of AA in pigs fed sorghum-soybean meal diets. This discrepancy may be partially explained by the fact that protein- and AA-phytate complexes are stabilized by weak electrostatic interactions (Láztity and Láztity, 1998), which can be disrupted by pH alterations such as those occurring in the gastrointestinal tract of pigs, without the need of any specific enzyme. Also, Maenz (2001) suggested that there is *de novo* formation of phytate-protein complexes in the gastrointestinal tract when optimal pH conditions exist. At acidic pH (2.8), phytate is negatively charged and can form strong electrostatic linkages with the basic lysine, arginine and histidine residues (Desphande and

Damodaran, 1989). These authors indicate that such interactions may cause a modification in structure brought about by close packing of protein molecules around the relatively small and highly charged phytate anion, leading to the formation of an insoluble complex. This is expected to occur in the stomach where pH gets as low as 2.5. Indeed, Okubo *et al.* (1976), who studied the pH range at which the glycinin component of soy proteins binds to phytates, found that the maximal binding of phytate per mole of glycinin was at pH 2.5. However, these authors did not observe any binding above the isoelectric point (pH 4.9). Thus, since digesta moves back to the small intestine where pH is higher (duodenum, 6.2; ileum, 7.4; Jongbloed *et al.*, 1992), a minimal interaction between proteins and phytates in the small intestine would be expected; this might explain the lack of effect of phytase on protein digestibility. Noteworthy, most of the phytates are degraded in the gastroduodenum section and the released phosphates are absorbed in the small intestine (Jongbloed *et al.*, 1992).

Another possibility could be that phytates reduce the activity of pancreatic proteases or intestinal peptidases; trypsin is the most critical pancreatic protease because it activates itself and can activate the other proteases. Thus, if the activity of pancreatic proteases were reduced by phytates, the administration of exogenous trypsin to the pigs would be expected to overcome that reduction and to increase the AID of protein and AA. In Experiment 1, 591 mg Pancreatin[®] (mix of pancreatic enzymes including trypsin) were added to the basal diet, alone or in combination with phytase. Theoretically, this amount would digest at least 14.8 g dietary protein. The protein apparently digested in the basal diet was 133 g (protein intake, 183 g; protein digestibility, 72.7%). Thus, the AID of CP would be expected to increase by 14.8 g or 11.1% as a result of phytase and Pancreatin[®] supplementation. But, as shown in Table 4, no effect on the AID of protein or AA was observed. This lack of response could be attributed to the very acid pH of the stomach (approximately 2.0–2.5), which in turn may have denatured the proteolytic enzymes. Therefore, trypsin added to the diet could have lost all of its activity in stomach, before reaching the small intestine where protein digestion takes place. Thus, in Experiment 2, the same amount of Pancreatin[®] was infused directly into duodenum to prevent the loss of trypsin activity by the stomach low pH. However, no response was obtained again to phytase or Pancreatin[®] (Table 5).

The interaction between proteins and phytates may influence the enzymatic digestion of proteins in the stomach (Adeola and Sands, 2003) and small intestine of pigs (Greiner and Konietzny, 2006). Knuckles *et al.* (1989) found that phytates (hexaphosphate) inhibited from 9 to 14% pepsin digestion of casein and bovine serum albumin *in vitro*, at pH 1.5. This inhibition is not unexpected since the activity of pepsin is optimal at that low pH. However, these authors did not observe any effect of phytates on trypsin activity, and concluded that neither phytates nor its hydrolysates affected protein utilization or weight gain of rats. In contrast, Sing and Krikorian (1982) observed a substantial inhibition of trypsin activity by phytic acid in an *in vitro* study. According to Desphande and Damodaran (1989), who found no formation of insoluble complexes between phytate and trypsin or chymotrypsin at pH 7.8, the inhibition reported by Sing and Krikorian (1982) might be due to their use of high phytate:enzyme ratios. Both trypsin and chymotrypsin belong to the serine protease family in which the active site involves the catalytic triad of serine, histidine and aspartate residues (Rawlings and Barrett, 1994), and none of these AA interacts with phytates at alkaline pH in the absence of Ca²⁺.

Additionally, there is the question of how much free phytate would be available to inhibit the digestive enzymes. Considering its strong affinity to various cations and the type of interactions involved in its association with dietary protein as well as factors such as food processing and pH, little free phytate would be expected to be available to interact with enzymes in the digestive system. Also, based on data published by Maga (1982), a typical cereal–soybean meal diet for growing pigs usually contains a very high (25:1) crude protein:phytate ratio. In addition, several enzymes with different substrate specificity are involved in the protein digestion process. Thus, proteins in which some lysine or arginine side chains are complexed with phytate may not be effectively hydrolysed by trypsin, but they can be hydrolysed by other enzymes such as chymotrypsin which shows high specificity for AA with large hydrophobic side chains (e.g. phenylalanine, tyrosine, tryptophan and isoleucine). Therefore, it appears that factors other than enzyme activity are limiting the effect of phytase on protein digestibility.

The supplementation of phytase and Pancreatin[®], alone or in combination, did not affect the AID digestibility of energy. The results from Experiment 1 of this study are consistent with those obtained by Liao *et al.* (2005). They did not find any effect of

phytase supplementation (2000 FTU/kg) to low-phytate diets formulated with corn, barley, wheat, and soybean meal, on the AID of energy in growing pigs. Although high-phytate diets had significant lower digestible energy values than low phytate diets, these authors did not find any effect of phytase supplementation on the digestibility of energy.

In conclusion, under the circumstances of these studies, phytates did not interfere with the digestion process of protein and energy in growing pigs, and phytase or Pancreatin[®] did not exert any effect on the apparent digestibility of protein in pigs fed sorghum–soybean meal diets. Consequently, there is no need of supplemental trypsin and chymotrypsin in this type of diets for growing pigs.

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