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Research Article

Effect of a multi-carbohydrase and phytase complex on the ileal and total tract digestibility of nutrients in cannulated growing pigs

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Abstract

The current study evaluated the effect of a multi-carbohydrase and phytase complex (MCPC) on ileal and total tract nutrient digestibility in growing pigs. A total of 8 male pigs (initial BW = 30.7±1.1 kg) have been surgically fitted with a T-cannula at the distal ileum and randomly allotted to 4 groups. The experiment has been conducted according to a 4×4 Latin square design, each period lasting 10 days. Pigs were fed 4 experimental diets, which consisted of 2 basal diets (BD1, low phytate; BD2, high phytate) with or without MCPC supplying at least 1800 U xylanase, 6600 U α-arabinofuranosidase, 1244 U β-glucanase, and 1000 U phytase per/kg corn-soybean based diet. High phytate diet reduced ($P < 0.05$) the apparent ileal digestibility (AID) of crude protein by 1.4% and the apparent total tract digestibility (ATTD) of organic matter, crude protein and gross energy by 1.7, 2.3 and 1.9%, respectively, and tended to decrease ($P = 0.10$) the ATTD of Ca by 17.3%, relative to low phytate diet. Dietary supplemental MCPC increased ($P < 0.05$) AID of P and Ca by 34.2% and 31.1% for BD1 and 26.7% and 41.3% for BD2, respectively, as well as increased ($P < 0.05$) ATTD of crude fat, P, and Ca by 1.4%, 45.6% and 9.6% for BD1 and 3.1%, 66.0% and 52.7% for BD2, respectively. MCPC addition did not significantly increase AID and (or) ATTD of crude protein, organic matter and starch. A trend to enhanced protein digestibility was only observed in low phytate BD1 diet. In conclusion, dietary supplemental enzymes rich in MCPC is effective in improving the AID of P and Ca and ATTD of crude fat, P, and Ca.

Keywords: Enzyme, Fecal digestibility, Ileal digestibility, Nutrient, Phytate, Pig

INTRODUCTION

The major storage form of phosphorus (P) in many plant feeds is phytate (myo-inositol hexaphosphate, IP_6) (Lindberg et al., 2007). Since the monogastric livestock lack phytase in their gastrointestinal tracts, IP_6 can barely be used as P source for animals. In addition, IP_6 is capable of chelating minerals including Ca, Fe, Zn, Mn, and Cu and forming insoluble complexes, and any method enhancing P availability may increase the availability of these elements in feed as well (Pagano et al., 2007). In addition, IP_6 can also bind to protein and reduce the utilization of the proteins and amino acids (Kiarie et al., 2010). Microbial phytase has been widely used as a feed additive for domestic animals to improve utilization of IP_6 from plant feeds (Lei et al., 2013). Most of the IP_6 is in the indigestible fibrous part of cereal grains (Reddy et al., 1982) which also contains non-starch polysaccharides (NSP) that can reduce the nutrient digestion (Passos et al., 2015). Dietary supplementation of NSP degrading carbohydrases might also be beneficial for the availability of P, proteins and amino acid (Lindberg et al., 2007; Passos et al., 2015). However, results for phytase and carbohydrase combinations on nutrient utilization have been inconsistent (Selle et al., 2003; Kim et al., 2005; Woyengo et al., 2008).

Therefore, the aim of the current study was designed to evaluate the effects of the newly developed multi-carbohydrase

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and phytase complex (MCPC) supplementation of a corn-soybean based diet on the ileal and total tract digestibility of nutrients in growing pigs fed low and high levels of phytate.

MATERIALS AND METHODS

Pigs, diets, and sample collection

Our animal protocol was approved by the Institutional Animal Care and Use Committee of Huazhong Agricultural University, China. A total of 8 male pigs (Duroc × Large White × Landrace; body weight at 30.7±1.1 kg) have been surgically fitted with a T-cannula at the distal ileum and randomly allotted to 4 groups. Pigs have been housed in individual metabolic crate that allowed freedom of movement and allowed a 14-d recovery period. After the recovery, pigs were fed one of the 4 experimental diets, which consisted of 2 basal diets (BD1, low phytate; BD2, high phytate; Table 1), with or without MCPC (Rovabio Advance Phy, Adisseo France S.A.S., France) supplying at least 1800 U xylanase, 6600 α-arabinofuranosidase, 1244 U β-glucanase, and 1000 U phytase per/kg diet. All diets contain 0.5% titanium dioxide (TiO_2) as an indigestible marker and were fed as mash. The experiment has been conducted according to a double 4×4 Latin square design. The pigs have been fed with a daily feed allowance at 4% body weight in 2 equal meals at 0800 and 1600 and adjusted every week. Pigs were allowed free access to water. Each period lasted 10 days, including 5 days of adaptation, followed by 3 days of feces collection and 2 days of ileal digesta collection. Feces have been collected using plastic bags attached to the skin around the anus. Digesta samples have been collected for 2 d using bags containing diluted formic acid attached to the opened cannula barrel from 08:00 to 20:00 h (Yáñez et al., 2011). Collected feces and digesta samples have been pooled for each pig within experimental period and frozen at -20°C.

Chemical analyses

The organic matter, gross energy, crude protein, crude fat, starch, P, Ca, and ash in the BD were analyzed according to previous studies (Noblet et al., 1994; Varley et al., 2011; Conde-Aguilera et al., 2016). Briefly, the gross energy of samples was determined using an adiabatic oxygen bomb calorimeter according to procedures outlined by the Association of Official Analytical Chemists (AOAC, 1980), and starch content was measured using the Ewers polarimetric method. In the AOAC (1990) frame, the content of Ca and P is determined by spectrophotometry; the content of crude protein is measured by Kjeldahl method; the content of crude fat is determined by diethyl ether extraction; and determined the content of ash and organic matter after completely burned. The AID and ATTD of components in the diet were calculated using the equation as described before (Yáñez et al., 2010).

Statistical analysis

Data has been subjected to ANOVA using the General Linear Model using SAS (SAS Inst. Inc., Cary, NC). The statistical model included the period, animal number, phytate level, MCPC and phytate × MCPC interaction. Tukey test was used in order to compare the means. Differences were considered significant at $P < 0.05$.

Table 1: Composition of basal diets and nutritional contents[1].

Ingredients	Percentage (%)		Calculated nutrients	Content	
	BD1	BD2		BD1	BD2
Corn	58.00	50.62	NE, MJ/kg	9.80	9.80
Corn distillers	15.00	15.00	Crude protein	17.62	15.80
Rice bran	2.53	15.00	Crude fat	5.30	7.12
Soybean meal	13.25	6.00	Dig. Lysine	0.95	0.95
Rapeseed meal	7.00	7.00	Dig. Methionine	0.28	0.31
Soybean oil	1.09	2.94	Dig. Methionine+cysteine	0.52	0.52
DL-methionine	0.00	0.05	Ca	0.66	0.66
L-lysine	0.42	0.59	Digestible P	0.31	0.31
L-threonine	0.03	0.11	Phytic P[2]	0.31	0.46
Tryptophan	0.02	0.04	Starch	39.03	38.06
Calcium carbonate	1.09	1.10	Soluble arabinoxylan[2]	0.77	0.66
$CaPO_3$	0.20	0.19	Insoluble arabinoxylan[2]	5.60	5.01
Salt	0.37	0.37	Total arabinoxylan[2]	6.37	5.67
Titanium oxide	0.50	0.50			
Premix[2]	0.50	0.50			

[1]BD = based diet; BD1= low phytate; BD2 = high phytate.
[2]Premix provided/kg diet: retinyl acetate, 10000 IU; cholecalciferol 2500 IU; dl-α-tocopheryl acetate, 50 IU; menadione, 5.0 mg; thiamin, 2.0 mg; riboflavin, 5.0 mg; pantothenic acid, 12.0 mg; pyridoxine, 10.0 mg; niacin, 30.0 mg; biotin, 0.2 mg; folic acid, 1.5 mg; cyanocobalamin 0.05 mg; choline chloride 1500 mg; iron, 100 mg; copper, 20 mg; manganese, 25 mg; zinc, 100 mg;selenium, 0.3 mg;iodine, 0.3 mg.
[2]Calculated values.

Table 2: The analyzed nutrient composition of the based diets[1].		
Nutrients	**BD1**	**BD2**
Gross energy, MJ/kg	16.70	17.40
Crude protein, %	18.48	16.85
Crude fat, %	5.44	7.73
Ash, %	5.14	5.23
Starch, %	44.75	45.61
P, %	0.52	0.61
Ca, %	0.70	0.68

[1]BD = based diet; BD1= low phytate; BD2 = high phytate.

RESULTS

The analyzed nutrient composition of BD is presented in Table 2, including gross energy, crude protein, crude fat, starch, P, and Ca and are in agreement with their calculated values in Table 1. The AID of crude protein was significantly affected by dietary phytate content (Table 3). High phytate diet decreased ($P < 0.05$) the AID of crude protein by 1.4% units relative to low phytate diet in -MCPC groups. Dietary supplemental enzymes significantly affected AID of P, and Ca (Table 3). Specifically, dietary added MCPC increased the AID of P and Ca by 34.2% and 26.7% and 31.1% and 41.3% at low and high phytate level, respectively. However, no significant interaction between phytate level and MCPC has been observed on AID of these nutrients.

The ATTD of organic matter, crude protein, crude fat, Ca and gross energy were significantly affected by dietary phytate content (Table 3). High phytate diet decreased ($P < 0.05$) organic matter, crude protein, gross energy and Ca by 1.7, 2.3, 1.9 and 17.3%, respectively, while it increased ($P < 0.05$) crude fat by 2.8% relative to the low phytate diet in the -MCPC groups. Dietary supplemental enzymes significantly affected (Table 3) ATTD of crude fat, P, and Ca. Specifically, dietary added enzymes increased ($P < 0.05$) the ATTD of crude fat, P, and Ca by 1.4 and 3.1%, 45.6% and 66.0%, 9.6 and 52.7% at low and high phytate levels, respectively. However, no significant interaction between phytate level and MCPC has been observed on these nutrients and no treatment effect has been observed on starch ATTD.

DISCUSSION

Current study showed that the ATTD of organic matter, crude protein, gross energy, and Ca and the AID of crude protein were significantly reduced by dietary phytate content in growing pigs, as observed by Woyengo et al. (2012) and Kahindi et al. (2015). Phytate may bind to glucose, starch and amino acids and may decrease the amylase and protease enzyme activities in the small intestine reducing thereby the digestibility and utilization of nutrients (Selle et al., 2000; Pagano et al., 2007; Kiarie et al., 2010; Selle et al., 2012; Woyengo and Nyachoti, 2013). It is generally accepted that by breaking down phytate, phytase limit the insoluble complex formation between phytate and nutrients, thus improving nutrient digestibility (Lei et al., 2013; Cowieson et al., 2017; Zouaoui et al., 2018). Interestingly, the current study showed that dietary supplemental enzymes rich in phytase and multi-carbohydrase increased the AID of P and Ca, as well as the ATTD of crude fat, P, and Ca in both low and high phytate diets. This could be explained by the enzyme degradation of IP_6 and polysaccharides in the cell walls in cereals and storage cell contents in protein meals and reduction of digesta viscosity (Lei et al., 2013; Passos et al., 2015). These outcomes were similar with previous studies, which have shown that dietary supplemental either phytase, NSP degrading enzymes, or both improved the AID and ATTD of crude fat, P, and/or Ca in swine (Radcliffe et al., 2006; Woyengo et al., 2008; Selle et al., 2009; Guggenbuhl et al., 2012; Torres-Pitarch et al., 2017). Moreover, the present

Table 3: Effect of phytate level and Enzymes on the apparent total tract digestibility of nutrients.

	Low phytate		High phytate		*P*- value[1]			R^2
	-MCPC	+MCPC	-MCPC	+MCPC	Phytate	MCPC	Phytate x MCPC	
AID, % [2]								
Organic matter	82.59	82.62	82.77	83.38	0.14	0.31	0.35	0.73
Crude protein	82.35	83.69	81.23	81.87	0.04	0.14	0.60	0.62
P	47.24	63.40	50.60	64.09	0.17	< 0.01	0.36	0.88
Ca	47.60	62.40	43.67	61.69	0.33	< 0.01	0.51	0.85
ATTD, % [2]								
Organic matter	85.82	85.49	84.33	84.57	< 0.01	0.89	0.42	0.84
Starch	91.85	91.56	91.75	91.97	0.72	0.93	0.55	0.81
Crude protein	81.52	83.28	79.63	79.92	0.001	0.15	0.30	0.82
Crude fat	79.11	80.22	81.29	83.82	< 0.001	< 0.01	0.22	0.91
Gross enegy	83.19	82.56	81.64	82.05	0.03	0.81	0.24	0.84
P	32.67	47.57	27.55	45.72	0.20	< 0.01	0.54	0.78
Ca	49.59	54.34	40.99	62.60	0.10	< 0.01	0.28	0.95

[1]Data (n=32) were subject to variance analysis with fixed effect phytate (n=2), MCPC (n=2) and interaction (n=4)
[2]AID = apparent ileal digestibility; ATTD = apparent total tract digestibility; MCPC = multi-carbohydrase and phytase complex; -, diet without added MCPC; +, diet with added MCPC.

study showed that dietary supplementation of phytase plus multi-carbohydrase rich in xylanase, α-arabinofuranosidase and β-glucanase resulted in higher increased ATTD of P (66.0%) and Ca (52.7%) in higher phytate diet than dietary supplementation only of phytase at 1000 FTU/kg which resulted in increased ATTD of P (32.2%) and Ca (15.5%) (She et al., 2018). These outcomes are consistent with a previous study that showed dietary supplementation of phytase and xylanase together displayed better results of AID and ATTD of P and Ca than dietary supplementation of xylanase alone (Lindberg et al., 2007). These findings could be explained by a complementary effect between multi-carbohydrase and phytase on the digestibility of both minerals. Nevertheless, several inconsistent scenarios were observed in the present study. Dietary supplemental enzymes did not affect the AID and ATTD of crude protein as reported by Lindberg et al. (2007) and Woyengo et al. (2008). Also, the ATTD of starch was not affected neither by phytate level nor by MCPC supplementation. However, some other studies showed that dietary supplementation of either phytase or NSP-degrading carbohydrase alone or in combination can improve the digestibility of nutrients (Zeng et al., 2018; Lee et al., 2019; Sun et al., 2020). This discrepancy could also be due to different experimental conditions, including diet compositions, doses and types of enzymes, and ages of pigs. Moreover, no interaction was observed between phytic P level and MCPC on the digestibility of nutrients in this study. However, the protein digestibility tended to be increased with MCPC on low phytate diets (+1.6 and +2.2 % on AID and ATTD of protein respectively) but surprisingly not on high phytate diet. This observation might also be due to the large difference between low and high phytate diets which were not based only on phytate level difference but also on ingredients used such as high rice bran content as well as on crude fat and crude protein contents. This result may also indicate that MCPC is less impacted by level of phytic P ranging from 0.31 to 0.46%, probably due to its high active phytase at pH 3.0 present within the MCPC (Menezes-Blackburn et al., 2015), which lead to more efficient breakdown of phytate in the stomach and reduced the formation of insoluble complexes between phytate and nutrients.

CONCLUSION

In summary, the current study found that the dietary supplement of an enzymatic cocktail with phytase and multi-carbohydrase improved the ileal and total tract digestibility of P, Ca, crude fat in growing pigs and could be used as a promising enzymes product to reduce the negative effects of phytate in practice.

CONMFLICT OF INTEREST

All authors declare that there are no conflicts of interests.

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ANIMAL WELFARE S TATEMENT

The experimental design and procedures in this study were reviewed and approved by department of Animal Nutrition and Feed Science, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, China.

All animal experimental procedures were performed according to the Institutional Animal Care and Use Committee of Huazhong Agricultural University.

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