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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.