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## Effects of $\alpha$ -galactosidase supplementation on nutrient digestibility, growth performance, intestinal morphology and digestive enzyme activities in weaned piglets



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### ABSTRACT

A total of 90 weaned piglets (initial body weight 7.86  $\pm$  0.98 kg) were used in a 28-d trial to evaluate effects of  $\alpha$ -galactosidase supplementation on nutrient digestibility, growth performance, intestinal morphology and digestive enzyme activities. Piglets were randomly allotted to 1 of 3 dietary treatments with 5 pens per treatment and 6 piglets per pen in a randomized complete block design. The three dietary treatments were corn-soybean basal diets supplemented with 0 (control), 100 or 200 FTU/kg  $\alpha$ -galactosidase. Piglets fed diets supplemented with  $\alpha$ galactosidase had greater (P < 0.05) apparent total tract digestibility (ATTD) of dry matter, gross energy and crude protein, and apparent ileal digestibility (AID) of isoleucine, leucine, threenine, alanine and serine than the control piglets. Supplementation of  $\alpha$ -galactosidase improved (P < 0.01) average daily gain (ADG) and reduced (P < 0.01) diarrhea rate compared with the control. The villus height to crypt depth ratio in the duodenum and jejunum of piglets fed diets containing  $\alpha$ -galactosidase were greater (P < 0.05) than those in the control piglets. Piglets offered 100 FTU/kg  $\alpha$ -galactosidase had greater (P < 0.05) villus height in the duodenum than those offered the control diet. Compared with the control, supplementation of  $\alpha$ galactosidase significantly enhanced (P < 0.05) trypsin activity in the duodenum and jejunum and lipase activity in the jejunum. The propionate concentration in the cecum decreased (P < 0.05) by  $\alpha$ -galactosidase supplementation compared with the control. However, there was no difference in these indexes between  $\alpha$ -galactosidase supplemented treatments. In conclusion, supplementation of  $\alpha$ -galactosidase at 100 or 200 FTU/kg enhanced nutrient digestibility and ADG, and reduced diarrhea rate of weaned piglets by improving intestinal morphology and digestive enzyme activities.

#### 1. Introduction

Soybean meal is the most commonly used protein supplement of plant origin with a high and consistent product quality in pig diets worldwide (Jezierny et al., 2010). However, it also contains significant amounts of raffinose family oligosaccharides (RFOs), in particular raffinose and stachyose, which are  $\alpha$ -1,6-galactosides linked to the C-6 of the glucose moiety of sucrose (Martínez-

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Abbreviations: AA, amino acids; ADFI, average daily feed intake; ADG, average daily gain; AID, apparent ileal digestibility; ATTD, apparent total tract digestibility; BW, body weight; CP, crude protein; DM, dry matter; EE, ether extract; FCR, feed conversion ratio; GE, gross energy; OM, organic matter; RFOs, raffinose family oligosaccharides; VFA, volatile fatty acids

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Villaluenga et al., 2008; Kim et al., 2015). The RFOs cannot be digested in the small intestine of monogastric animals due to the absence of endogenous  $\alpha$ -galactosidase necessary for the hydrolysis of the  $\alpha$ -1,6-linkages (Karr-Lilienthal et al., 2005). These indigestible RFOs can increase digesta viscosity and alter intraluminal osmotic pressure in the intestine (Freire et al., 1991), impairing the interaction between endogenous enzymes and nutrients, as well as nutrient absorption by the intestinal mucosa, thus negatively affecting nutrient digestibility and growth performance (Choct et al., 2010). Moreover, the microflora in the gut metabolize the RFOs and produce large amounts of carbon dioxide, hydrogen, and small quantities of methane and short chain fatty acids (VFA), resulting in flatulence and diarrhea (Fleming, 1981; Naczk et al., 1997). As a result, it is crucial to develop an effective strategy to eliminate anti-nutritional effects of RFOs and improve the utilization of sovbean meal.

The enzyme  $\alpha$ -galactosidases (EC 3.2.1.22) are a group of exo-type carbohydrases which catalyze the hydrolysis of the terminal  $\alpha$ -1,6-linked-galactose residues from galactose oligosaccharides like raffinose and stachyose (§en, Eryılmaz, Bayraktar, & Önal, 2011). *In vitro* study demonstrated the addition of  $\alpha$ -galactosidase led to decrease in sucrose, raffinose and stachyose, and increase in fructose, glucose, and galactose at 37 °C in buffer pH 5.5 (Frias et al., 2003). Consequently, supplementation of exogenous  $\alpha$ -galactosidase in feed may be an effective approach to break down the RFOs in the intestine, resulting in improved digestibility (Smiricky et al., 2002). At present, researches on the efficiency of  $\alpha$ -galactosidase in pig diet are few, and provided inconsistent results (Baucells et al., 2000; Pan et al., 2002). Moreover, the few researches have mainly involved growing pigs. When pigs get older, their fermentative capacity probably increase and thus they get more from RFOs, which may reduce the impact of enzyme (Longland et al., 1994). As a consequence, the response of weaned piglets to the  $\alpha$ -galactosidase supplementation may be stronger than growing pigs due to their immature fermentative capability. Therefore, the objective of the present study was to evaluate the effects of supplementing corn-soybean based diets with  $\alpha$ -galactosidase on nutrient digestibility and growth performance (average daily gain, average daily feed intake and feed conversion rate) in weaned piglets. Special attention was also focused on the digestive mechanisms (intestinal morphology, digestive enzyme activities and volatile fatty acids concentrations in the cecum) likely to be involved in these changes.

#### 2. Materials and methods

All experimental procedures used in this study were reviewed and approved by the China Agricultural University Animal Care and Use Committee (Beijing, China). Soybean meal and extruded soybean were obtained from the China Agriculture University Animal Experimental Base. The analyzed chemical composition of soybean meal or extruded soybean is presented in Table 1.

#### 2.1. Preparation of $\alpha$ -galactosidase

The  $\alpha$ -galactosidase used in this experiment was provided by VTR Bio-tech Co. LTD (Guangdong, China). The  $\alpha$ -galactosidase was produced by *Aspergillus Niger* and the optimal pH range was from 3.5 to 6. Activity of  $\alpha$ -galactosidase was determined based on the method described by Rezessy-Szabó et al. (2007). One FTU of  $\alpha$ -galactosidase activity was defined as the amount of enzyme that liberates 1 µmol of  $\rho$ -nitrophenol per min at 37 °C and pH 5.5. The analyzed activity of  $\alpha$ -galactosidase used in this study was 1094 FTU/g.

#### 2.2. Animals, diets, and housing

A total of 90 piglets (weaned at 28 d; Duroc × [Landrace × Large White]) with an initial body weight (BW) of 7.86  $\pm$  0.98 kg were randomly allocated to one of three dietary treatments in a 28 d study. Each treatment was replicated with five pens of six piglets (3 barrows and 3 gilts) per pen. The three dietary treatments were corn-soybean basal diets supplemented with 0 (control), 100 (0.01%) or 200 FTU/kg (0.02%)  $\alpha$ -galactosidase (Table 2). All diets were formulated to meet or exceed the nutrient requirements for weaned piglets recommended by NRC (2012) and fed as mash. Chromium oxide (2.5 g/kg) was added to all diets as an indigestible marker.

| Item                    | Soybean meal | Extruded soybean |
|-------------------------|--------------|------------------|
| Dry matter              | 889          | 877              |
| Crude protein           | 446          | 358              |
| Ether extract           | 19.8         | 189              |
| Ash                     | 60.3         | 41.3             |
| Organic matter          | 828          | 836              |
| Neutral detergent fiber | 157          | 120              |
| Acid detergent fiber    | 90.3         | 60.6             |
| Calcium                 | 3.2          | 3.0              |
| Total phosphors         | 5.9          | 4.2              |
| Sucrose                 | 59.5         | 44.3             |
| Raffinose               | 13.5         | 9.6              |
| Stachyose               | 56.2         | 31.4             |

Table 1

Analyzed chemical composition of soybean meal and extruded soybean (g/kg, as-fed).

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