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Effects of supplemental β-mannanase on *in vitro* disappearance of dry matter in feed ingredients for swine

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Abstract

The present study was aimed to investigate the effects of supplemental β-mannanase on *in vitro* ileal disappearance (IVID) and *in vitro* total tract disappearance (IVTTD) of dry matter (DM) in barley, canola meal, copra meal, corn, corn distillers dried grains with solubles, cottonseed meal, palm kernel meal, soybean meal, and wheat for pigs. Feed ingredient samples were finely ground and divided into the control group and the treatment group. The samples of the control group were prepared to contain 990 g/kg test ingredient and 10 g/kg cornstarch, whereas the samples of the treatment group were prepared to contain 990 g/kg test ingredient and 10 g/kg β-mannanase product (8,000 units/kg in the mixed sample). A 2-step *in vitro* ileal digestion technique, which simulated the digestion and absorption processes in the stomach and small intestine, was used to determine the IVID of DM in test ingredients, whereas a 3-step *in vitro* ileal digestion technique, which additionally simulated the digestion process of the large intestine, was used to determine the IVTTD of DM in test ingredients. The *in vitro* digestion procedures were performed in triplicate for each sample. The addition of β-mannanase increased (p = 0.003) the IVID of DM in wheat and tended to increase (p = 0.063) the IVID of DM in soybean meal. The IVTTD of DM in barley, cottonseed meal, and palm kernel meal was improved (p < 0.05) by the addition of β-mannanase. In conclusion, the digestibility of nutrients for pigs may be improved when β-mannanase is added into diets containing barley, cottonseed meal, palm kernel meal, soybean meal, or wheat.

Keywords: β-Mannanase, Digestibility, Exogenous enzyme, *In vitro* disappearance, Swine

INTRODUCTION

Most commercial diets for pigs consist of corn, soybean meal, and corn distillers dried grains with solubles (DDGS) in many countries [1,2]. However, the prices of major feed ingredients have recently become unstable due to the global climate changes [3]. Thus, the demand for alternative feed ingredients has been increased. However, majority of alternative feed ingredients originating from plants are rich in non-starch polysaccharides (NSP), which are indigestible fractions by endogenous enzymes of pigs and act as antinutritional factors during the digestion and absorption processes [4,5]. For this reason, increased inclusion rates of alternative feed

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Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

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Conceptualization: Park CS, Kim BG.
Data curation: Son J.
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Methodology: Park CS.
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ingredients in diets may result in reduced digestibility of nutrients and energy in pigs. Therefore, supplementation of NSP-degrading enzymes in diets may mitigate the potential negative effects of NSP on nutrient digestibility and improve the nutrient utilization of alternative feed ingredients in swine diets.

Mannan is one of the common NSP found in many alternative feed ingredients. Monosaccharide molecules such as mannose or glucose in mannan are linked as a β-1,4-linkage [6], which cannot be hydrolyzed by the endogenous enzymes secreted by pigs [7]. Therefore, the addition of β-mannanase that can hydrolyze the mannan in alternative feed ingredients may improve the digestibility of energy and nutrients in swine diets [8,9]. The effect of β-mannanase depends on the quantity of substrates in feed ingredients. To our knowledge, however, there has been very limited research investigating the effects of supplemental β-mannanase in alternative feed ingredients. Therefore, the objective of the present work study was to investigate the effects of supplemental β-mannanase on *in vitro* disappearance of dry matter (DM) in various feed ingredients for swine.

MATERIALS AND METHODS

Enzyme product and sample preparation

The enzyme product contained 800 units/g of β-mannanase. The ingredients tested in the present study were barley, canola meal, copra meal, corn, DDGS, cottonseed meal, palm kernel meal, soybean meal, and wheat. These samples were finely ground using a coffee grinder and divided into the control group and the treatment group. The samples of the control group were prepared to contain 990 g/kg test ingredient and 10 g/kg cornstarch, whereas the samples of the treatment group were prepared to contain 990 g/kg test ingredient and 10 g/kg β-mannanase product (8,000 units/kg of mixed sample). Samples of the control and treatment groups were used for both *in vitro* ileal disappearance (IVID) and *in vitro* total tract disappearance (IVTTD) of DM. The *in vitro* digestion procedures were performed in triplicate for each sample.

In vitro disappearance assays

To simulate the digestion and absorption processes in the stomach and the small intestine, the IVID of DM in the test ingredients was determined using a 2-step IVID technique [10]. Briefly, 1 g of a sample was transferred into a 100-mL conical flask. A 25 mL of sodium phosphate buffer solution (0.1 *M*, pH 6.0) and 10 mL of HCl (0.2 *M*) were added. To simulate digestion conditions in the stomach, the pH was adjusted to 2.0, and 1 mL of freshly prepared pepsin solution (10 mg/mL; 250 units/mg solid, P7000, pepsin from porcine gastric mucosa; Sigma-Aldrich, St. Louis, MO, USA) was added to the sample. A 0.5 mL of chloramphenicol (C0378, Chloramphenicol; Sigma-Aldrich) solution (5 g/L of ethanol) was added to prevent the microbial growth during the incubation. Test flasks were incubated in a shaking incubator at 39℃ for 6 h. After the incubation, 10 mL of sodium phosphate buffer solution (0.2 *M*, pH 6.8) and 5 mL of NaOH (0.6 *M*) were added. Then, the acidity of the solution was adjusted to pH 6.8. Thereafter, 1 mL of freshly prepared pancreatin solution (50 mg/mL; 4 × USP,

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P1750, pancreatin from porcine pancreas; Sigma-Aldrich) was added to the solution. After incubating the solution in a shaking incubator at 39℃ for 18 h, 5 mL of 20% sulfosalicylic acid solution was added and the solution samples were left for 30 min at room temperature to precipitate the indigestible protein. The samples were then filtered through pre-dried and pre-weighed glass filter crucibles (Filter Crucibles CFE Por. 2; Robu, Hattert, Germany) containing 400 mg of celite. Test flasks were rinsed twice with 1% sulfosalicylic acid solution. Twenty mL of ethanol (95%) and acetone (99.5%) were added twice to the glass filter crucibles. Then, glass filter crucibles with undigested residues were dried at 80℃ for 24 h.

To simulate total tract digestion and absorption, the IVTTD of DM in the ingredients was determined using 3-step IVTTD technique [11]. The first and second steps were comparable to the IVID procedure except the sample weight, enzyme concentrations, and incubation time. For IVTTD, 0.5 g of sample was used, and the concentrations of pepsin solution and pancreatic solution were raised to 25 and 100 mg/mL, respectively, while the incubation periods for step 1 and step 2 were reduced to 2 and 4 h, respectively. In the third step of the IVTTD procedure, 10 mL of 0.2 *M* EDTA solution was added to the samples. The acidity was then adjusted to pH 4.8. The samples were supplemented with 0.5 mL of multi-enzyme solution (V2010, Viscozyme; Sigma-Aldrich) as a replacement for microbial enzymes, and then, the samples were incubated in a shaking incubator for 18 h at 39℃. After incubation, the samples were filtered and the undigested residues were collected and dried as described in the IVID procedure except for the drying condition of 130℃ for 6 h in this procedure.

Statistical analysis

Data were analyzed by the GLM procedure of SAS (SAS Institute, Cary, NC, USA). The model included β-mannanase addition as a fixed effect. The experimental unit was each test flask. Statistical significance and tendency of treatment effects were declared at $p < 0.05$ and $0.05 \leq p < 0.10$, respectively.

RESULTS AND DISCUSSION

The effects of supplementing β-mannanase to swine diets have been reported in previous experiments [8,12–14]. However, the results have been inconsistent. Kim et al. [12] reported that the addition of β-mannanase at 400 units/kg in corn-wheat-soybean meal-based diets increased the average daily gain, gain-to-feed ratio, and the apparent total tract digestibility of DM and gross energy in growing pigs. On the other hand, Kwon & Kim [8] reported that the digestible and metabolizable energy in copra expellers and palm kernel expellers were not affected by the addition of β-mannanase at 2,400 units/kg. This discrepancy may partially attribute to the variations in *in vivo* experiments such as differences in animal housing environments or experimental facilities. The *in vitro* techniques used in the current study may avoid the variations caused by uncontrolled factors in *in vivo* study and test the effects of dietary exogenous enzymes with many samples in a controlled environment. Additionally, the effects of supplemental enzymes are dependent upon the quantity of substrates that are not digested by endogenous

enzymes in pigs.

In the present work, the addition of β-mannanase increased (p = 0.003) the IVID of DM in wheat and tended to increase (p = 0.063) the IVID of DM in soybean meal (Table 1). However, these improvements were not observed in the IVTTD of DM in soybean meal and wheat (Table 2), likely due to the higher disappearance of DM in the last step of *in vitro* total tract digestion techniques. The IVTTD of DM in barley, cottonseed meal, and palm kernel meal was improved (p < 0.05) by the addition of β-mannanase. This observation may indicate that barley, cottonseed meal, and palm kernel meal contain mannan which are more suitable for β-mannanase used in the current study. Kwon & Kim [8] reported that the analyzed concentration of mannan in copra expellers and palm kernel expellers is 24.6% and 31.3%, respectively.

Table 1. *In vitro* ileal disappearance of dry matter (%) in test ingredients with or without β-mannanase supplementation

Item	β-Mannanase (g/kg)[1)]		SEM	*p*-value
	0	10		
Barley	76.6	76.1	0.5	0.555
Canola meal	65.2	62.4	1.1	0.219
Copra meal	51.5	51.7	0.1	0.171
Corn	75.1	75.7	0.3	0.223
Corn distillers dried grains with solubles	63.3	63.0	0.4	0.570
Cottonseed meal	63.8	63.2	0.3	0.180
Palm kernel meal	30.7	29.5	0.8	0.330
Soybean meal	75.8	77.5	0.5	0.063
Wheat	84.7	89.0	0.4	0.003

Each least squares means represents three observations.
[1)]β-mannanase was supplemented at 8,000 units/kg of mixed sample.

Table 2. *In vitro* total tract disappearance of dry matter (%) in feed ingredients with or without β-mannanase supplementation

Item	β-Mannanase (g/kg)[1)]		SEM	*p*-value
	0	10		
Barley	81.4	81.9	0.1	0.021
Canola meal	79.0	78.5	0.4	0.450
Copra meal	63.2	63.4	0.2	0.634
Corn	85.8	86.6	0.3	0.159
Corn distillers dried grains with solubles	69.5	69.6	0.4	0.886
Cottonseed meal	72.5	73.8	0.3	0.025
Palm kernel meal	42.5	43.2	0.1	0.027
Soybean meal	94.3	94.6	0.4	0.715
Wheat	90.9	90.7	0.4	0.701

Each least squares means represents three observations.
[1)]Supplemental β-mannanase was supplemented at 8,000 units/kg of mixed sample.

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Therefore, it was expected that the *in vitro* disappearance of DM in palm kernel meal increased by the addition of β-mannanase. However, it remains unclear why the IVID or IVTTD of DM in copra meal was not influence by β-mannanase. This unresponsiveness by supplemental β-mannanase is perhaps due to the variations in the structure of mannan and other polysaccharides among copra meal sources. The IVID of DM and IVTTD of DM in the control group were within the values in the literature [4,15,16].

Apparent total tract digestibility of crude protein and minerals has been reported to increase by supplementing β-mannanase to corn-soybean meal-based diets in pigs [17,18]. Beta-mannans in feed ingredients encapsulate nutrients and potentially bind to digestive enzymes [19,20]. Based on the present results, supplemental β-mannanase may have hydrolyzed β-mannan matrix and improved digestions of protein and minerals in the small intestine of pigs resulting in improved nutrient digestibility.

In conclusion, the addition of β-mannanase in feed ingredients at 8,000 units/kg increased the IVID of DM in soybean meal and wheat and the IVTTD of DM in barley, cottonseed meal, and palm kernel meal. Therefore, the digestibility of energy and nutrients for pigs may be improved when β-mannanase is added into diets containing barley, cottonseed meal, palm kernel meal, soybean meal, or wheat. Further research is warranted to investigate various doses of supplemental β-mannanase on nutrient digestibility of swine feeds.

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