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Effects of an enzyme cocktail on digestible and metabolizable energy concentrations in barley, corn, and wheat fed to growing pigs

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ABSTRACT

The objective of this study was to determine the effects of an enzyme cocktail (EC), consisting of xylanase, mannanase, and protease, on digestible energy (DE) and metabolizable energy (ME) concentrations in barley, corn, and wheat fed to growing pigs. A pilot study was conducted to determine the effects of EC on the in vitro ileal dry matter digestibility (IVDMD) in 8 sources of feed ingredient including barley, corn, wheat, canola meal, copra meal, cottonseed meal, palm kernel meal, and soybean meal. The IVDMD was determined in each feed ingredients with or without the 0 or 10 g/kg of EC addition. The values for the IVDMD in barley and wheat increased ($P < 0.05$) when the EC was added. However, the EC addition did not affect IVDMD of corn, canola meal, copra meal, cottonseed meal, palm kernel meal, and soybean meal. Based on the pilot study, an animal experiment was conducted to determine the effects of the EC on DE and ME concentrations in barley, corn, and wheat. A 3×2 factorial treatment arrangement with 3 ingredients and 0 or 2 g EC/kg was used. Six barrows with 35.8 ± 3.3 kg body weight were assigned to 6 dietary treatments in a 6×6 Latin square design. There was no interaction between the ingredient and the EC addition, and the effect of the EC addition was not observed. The apparent total tract digestibility of gross energy in diets containing corn was greater ($P < 0.05$) than diets containing barley, but was less ($P < 0.05$) than diets containing wheat. The concentration of DE in barley, corn, and wheat were 3197, 3376, and 3503 kcal/kg (as-fed basis), respectively, and the respective values of ME were 3035, 3304, and 3407 kcal/kg (as-fed basis). In conclusion, the DE and ME concentrations in barley, corn, and wheat were not affected by the EC addition.

1. Introduction

Feed ingredients derived from cereal grains are the major sources of energy in swine diets and generally have non-starch polysaccharide (NSP) compounds that interfere with digestion, and, thus, decrease the efficiency of energy utilization (Bach Knudsen, 2011). The plant cell wall consists of cellulose microfibrils, which are amorphous structures of NSP such as arabinoxylans and β-glucans (Selvendran, 1984). These compounds are hardly digested or utilized in the porcine digestive tract due to a lack of enzymes, and the concentration of NSP is negatively correlated with starch in most cereal grains (Bach Knudsen, 1997) and nutrient digestibility (Yin et al., 2000). However, supplementation of exogenous enzyme can hydrolyze NSP to monosaccharides and, consequently, improve nutrient digestibility, as well as growth performance of pigs (Adeola and Cowieson, 2011). These beneficial effects of enzyme addition may be demonstrated by increased energy values in feed ingredients.

Xylan is one of the main polysaccharides in plant cell wall (Bach Knudsen, 1997). The positive linear effect of xylanase supplementation on crude protein (CP) and amino acids (AA) digestibility of growing pigs was observed previously (Barrera et al., 2004). The beneficial effects of mannan, which is the component of several NSP, on broiler chickens (Kong et al., 2011) and growing pigs (Yoon et al., 2010) were also reported.

To maximize the effect of enzyme products, many exogenous enzyme products contains 2 or more enzymes as an enzyme cocktail (EC), but the beneficial effects of EC are still debatable. Positive effects of the EC addition on apparent total tract apparent digestibility (ATTD) of gross energy (GE) were observed in previous studies (Emiola et al., 2009; Jo et al., 2012; Kiarie et al., 2012), whereas no substantial differences were observed in others (Kim et al., 2004; Owusu-Asiedu et al., 2010; Smith et al., 2010). Inconsistency of effects may be due to the variability in sources of enzymes, ingredient composition of diets,

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concentration of NSP in diets, or physiological states of the animal.

Adeola and Cowieson (2011) suggested that the effects of EC may depend on the ingredient composition of diets, and, therefore, the activation of each enzyme with their respective substrate should be considered before mixing enzymes. However, the measurement of the concentration and structure of substrates in feed ingredients is time consuming and costly. The in vitro digestibility assay (Boisen and Fernández, 1995, 1997) is a relatively simple and reliable method for the comparison of the concentration of available substrates among feed ingredients, assuming that the changes in the in vitro ileal dry matter digestibility (IVDMD) of feed ingredient by the EC addition are reproduced in in vivo energy utilization. Therefore, the objectives of the current study were to determine the IVDMD of 8 sources of commercial feed ingredient with or without the EC addition, and to determine the effects of the EC on digestible energy (DE) and metabolizable energy (ME) concentrations in barley, corn, and wheat fed to growing pigs.

2. Materials and methods

2.1. Pilot study: in vitro ileal digestibility

In vitro digestibility trial was conducted to select the feed ingredients, which had a potential to maximize the effects of EC supplementation. Three whole grain sources, including barley, corn, and wheat, and 5 oilseed meals, including canola meal, copra meal, cottonseed meal, palm kernel meal, and soybean meal, were tested. The EC consisted of xylanase (2000 unit/g, Enzyme Commission 3.2.1.8), mannanase (500 unit/g, Enzyme Commission 3.2.1.25), and protease (80 unit/g, Enzyme Commission 3.4.24.40). The xylanase was produced with a wild type strain of *Paenibacillus* sp. HY-8, the mannanase was produced with a wild type strain of *Cellulosimicrobium* sp. HY-13, and the protease was produced with a wild type strain of *Aranicola proteolyticus*. All protocols were based on 2-step in vitro ileal digestion technique suggested by Boisen and Fernández (1995) with several modifications including apparatus for incubation and the duration of drying after step 2. Prior to the analyzes, finely ground (<1 mm) ingredients were weighed to 1 ± 0.001 g. Each ingredient was analyzed in triplicate and divided into 2 groups, ingredient with or without 10 g/kg of the EC addition. The inclusion rate was determined by 5 times as much as the recommended inclusion rate of the EC in order to maximize the effects of EC. In the step 1, ground samples and EC were put into 100 mL conical flasks. A 25 mL of sodium phosphate buffer solution (0.1 M, pH 6.0) and 10 mL of 0.2 M HCl solution were added in the test flasks. Using 1 M HCl or NaOH solution, pH was adjusted to 2.0. To mimic the digestion in the stomach, 1 mL of freshly prepared pepsin solution (10 mg/mL; $\geq$250 units/mg solid, P7000, Pepsin from porcine gastric mucosa; Sigma-Aldrich, St. Louis, MO) was added in the test flasks. To prevent the contamination caused by bacteria, 0.5 mL of chloramphenicol (C0378, Chloramphenicol; Sigma-Aldrich) solution (5 g/L of ethanol) was added. Each test flask was closed with a silicon stopper and incubated in a shaking incubator at 39 °C for 6 h.

After the incubation with pepsin, step 2, which simulates the digestion in the small intestine, was performed. A 10 mL of sodium phosphate buffer solution (0.2 M, pH 6.8) and 5 mL of 0.6 M NaOH solution were added in the test flasks. Then pH was adjusted to 6.8 using 1 M HCl or NaOH solution. A 50 mg/mL of pancreatin solution (4 × USP, P1750, Pancreatin from porcine pancreas, Sigma-Aldrich, St. Louis, MO, US) was prepared and centrifuged at 2710*g* for 10 min at room temperature. A 1 mL of supernatant of fresh pancreatin solution was added into each test flask. Then, the test flasks were incubated in a shaking incubator at 39 °C for 18 h.

After the 18 h incubation, a 5 mL of 20% sulfosalicylic acid solution were added in the test flasks and incubated at a room temperature for 30 min to precipitate the indigestible protein. After the precipitation, undegraded samples were filtered in predried and -weighed glass filter crucibles (Filter Crucibles CFE Por. 2, Robu, Hattert, Germany) containing 400 mg of celite 545 using the Fibertec System (Fibertec System 1021 Cold Extractor, Tecator, HÖganäs, Sweden). The test flasks were rinsed twice by 1% sulfosalicylic acid solution and 10 mL of 95% ethanol and 99.5% acetone were added in the glass filter crucibles twice. Glass filter crucibles with undegraded samples were dried at 130 °C in a forced-air drying oven for 6 h. Dried crucibles were weighed and the dry matter (DM) weight of undegraded samples were determined. The IVDMD was calculated by the difference between DM weight of the ingredient and undegraded sample.

Data were analyzed by the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, US). The model included the EC addition as the independent variable and IVDMD of each ingredient as the dependent variable. Least squares of means for each ingredient with or without the EC addition were calculated. The experimental unit was the flask and significant differences were declared by an α-level of 0.05.

2.2. In vivo experiment for energy utilization

The experimental procedure was reviewed and approved by the Institutional Animal Care and Use Committee at the Konkuk University (Seoul, Republic of Korea). Three test ingredients were barley, corn, and wheat (Table 1). A 3×2 factorial treatment arrangement with 3 ingredients and 0 or 2 g EC/kg was used. Three ingredients including barley, corn, and wheat were used and the EC was added at 0 or 2 g/kg diet (Table 2). Diets were formulated to meet or exceed nutrient requirement estimates (NRC, 1998) except for AA. The EC was the same as that used in the pilot study and corn was used as a carrier. The inclusion rate of EC was the recommended concentration of product. Six barrows with an initial body weight (BW) of 35.8 ± 3.3 kg were randomly allotted to a 6×6 Latin square design with 6 diets and 6 periods (Kim and Kim, 2010). Pigs were individually housed in metabolism cages (0.48×1.49 m^2), which had fully slatted plastic floor and feeder. Water was freely available.

Pigs were fed at daily amounts of 3 times the estimated maintenance requirement for energy (i.e., 106 kcal ME/kg BW$^{0.75}$; NRC, 1998). The allowance was divided into 2 equal meals at 0730 and 1530 h. The ME concentration of experimental diets were calculated based on ME values described in NRC (1998). At the beginning of each period, the BW of each pig was recorded and daily feed allowance was calculated. Pigs had an ad libitum access to water filled in the feeder. After the 3-d of adaptation period, feces were collected during 4 d of collection period based on the

Table 1
Analyzed composition of barley, corn, and wheat (as-fed basis).

Item	Barley	Corn	Wheat
Dry matter (g/kg)	880	861	893
Gross energy (kcal/kg)	4046	4015	4080
Crude protein (g/kg)	126	73	117
Ether extract (g/kg)	13.9	32.7	16.1
Crude fiber (g/kg)	51.2	18.8	25.3
Ash (g/kg)	24.2	16.5	18.1
Neutral detergent fiber (g/kg)	237	112	138
Acid detergent fiber (g/kg)	78.4	24.8	27.0
Ca (g/kg)	1.40	1.60	1.40
P (g/kg)	3.05	2.60	3.00