Effects of protease on growth performance and carcass characteristics of growing-finishing pigs

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Abstract

A study was conducted to investigate the effects of dietary protease supplementation on growth performance and carcass characteristics of growing-finishing pigs that started with a lower bodyweight than pigs in the control group. A total of 96 growing pigs (initial bodyweight (BW) 31.6 ± 2.97 kg) were allotted to two dietary treatments (48 pigs/treatment, 8 pigs/pen) with different initial BW (34.37 vs. 28.78 kg). The dietary treatments were a control diet (CON), based on maize and soybean meal (grower diets) and wheat (finisher diets), and a protease diet (PRO), which was the CON diet to which 0.02% protease was added. Pigs were fed these dietary treatments (high BW-CON, low BW-PRO) for 12 weeks, comprising six weeks growing and six weeks finishing. In the growing period, PRO had higher average daily gain (ADG) and gain to feed (G:F) ratio than CON. The significant difference in bodyweight that was observed at the beginning of the experiment between the treatment groups was still observed at the end of the growing period. In the finishing period, PRO had reduced feed intake, average daily feed intake (ADFI), and increased G:F ratio compared with CON. At the end of the experiment, there was no significant difference in BW between the treatment groups as a result of lower feed intake and ADFI and higher ADG and G:F ratio. In addition, there were no differences in carcass characteristics between the groups. Therefore, standalone protease supplementation may positively affect the growth performance of growing-finishing pigs through improved feed efficiency without negative effects on carcass characteristics.

Keywords: Carcass evaluation, dietary enzyme, growth promoter, swine
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Introduction

Soybean meal is generally the primary protein source of swine diets (Kim *et al*., 2016). However, it is not an ideal dietary component owing to the presence of anti-nutritional factors and relatively lower content of certain essential amino acids (methionine and lysine) compared with proteins of animal origin (Friedman & Brandon, 2001). Anti-nutritional factors, including inhibitors of digestive enzymes and lectins, may contribute to poor digestibility and adverse nutritional and other effects (Friedman & Brandon, 2001; Jo *et al*., 2012). Hence, soybean meal must be heat treated to reduce anti-nutritional factors before inclusion in diets. But some protease inhibitors, such as Bowman-Brick inhibitors and the Kunitz inhibitors of chymotrypsin and trypsin, may remain after heat prossesing (Friedman & Brandon, 2001). Moreover, overheating may influence negatively the availability of certain amino acids (e.g. lysine and arginine) and the value of proteins (McDonald *et al*., 2011).

Dietary enzyme supplementation has been widely used as a way to improve nutrient digestibility and utilization efficacy, and may reduce feed costs in the animal industry (Adeola & Cowieson, 2011). Protease, a dietary enzyme, has been used generally in swine diets for many years as part of enzyme cocktails (Zuo *et al*., 2015). Most studies on protease supplementation in swine diet have shown positive effects on nutrient digestibility and growth performance (Ji *et al*., 2008; Jo *et al*., 2012; Omogbenigun *et al*., 2004; Yin *et al*.,

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2004, 2001; Zuo *et al*., 2015). Recently, standalone proteases have been available commercially and have shown beneficial effects on nutrient digestiblity and growth performance of pigs (Adeola & Cowieson, 2011; Guggenbuhl *et al*., 2012; Mc Alpine *et al*., 2012a; O'Doherty & Forde, 1999; Zuo *et al*., 2015). In the pig production industry, there is demand for effective ways to promote the growth of growing-finishing pigs. Antibiotics were used to prevent various diseases and to promote growth, but were reported to increase the risk of antibiotic resistance, resulting in their being banned for use in animal diets (Park *et al*., 2016). So, if an effective alternative way for promoting growth is available, it has potential to reduce costs and increase the economic efficiency of pig and pork production (Song *et al*., 2015; Mun *et al*., 2017;). Therefore, this research investigated the practical effects of dietary protease supplementation on growth performance and carcass characteristics of growing-finishing pigs with different initial BW.

Materials and Methods

The experimental protocol for this research was reviewed and approved by the Institutional Animal Care and Use Committee of Chungnam National University, Daejeon, Republic of Korea (approval #CNU-0611). This experiment was conducted at the facility of Chungnam National University Farm.

A total of 96 pigs (Duroc × (Landrace × Yorkshire)) with average initial BW of 31.6 ± 2.97 kg were used for 12 weeks in this study. Pigs were assigned to two dietary treatments (48 pigs/treatment, 8 pigs/pen) with different initial BW (34.37 vs. 28.78 kg, P <0.05). The dietary treatments consisted of i) a control diet (CON) based on maize and soybean meal (grower diets) or wheat (finisher diets), which were assigned to pigs weighing 34.37 ± 1.43 kg; and ii) a protease diet (PRO), which was the CON diets to which 0.02% protease enzymes had been added and which were assigned to pigs weighing 28.78 ± 2.96 kg. The protease was a commercial product (Ronozyme® ProAct, DSM Nutritional Products, Kaiseraugst, Switzerland), which contained 75,000 protease units/g and was derived from *Bacillus licheniformis*. Dietary treatments were formulated to meet or exceed the NRC (2012) estimates of nutrient requirements of growing and finishing pigs (Table 1). Pigs were fed their grower diets for six weeks and then their finisher diets for six weeks. No antibiotic growth promoters were used, and all diets were provided in meal form. All pigs were housed in an environmentally controlled room and allowed free access to diets and water throughout the experimental period.

The BW of pigs was recorded at the initiation of the experiment and at the end of each period. The amount of dietary treatments provided per pen was recorded for each period and feed remains were weighed at the end. ADG was determined by dividing the difference between final BW and initial BW by the experimental periods. ADFI was obtained by dividing the feed intake per pig by the period of each experiment. ADG was divided by ADFI to calculate the ratio between ADG and ADFI G:F(G:F) for each pig.

The pigs were transferred from the farm to a local commercial slaughterhouse after the finishing experimental period. Approximately four hours before transport, feed was withdrawn. To reduce stress, the pigs were showered with water, and water was freely available for drinking during lairage. The pigs were rested for about eight hours. The final live BW of pigs was recorded, and then they were slaughtered through electrical stunning and scalding-singeing. The hot carcass weight was recorded, and dressing percentage was calculated by comparing final live BW and hot carcass weight.

Back fat depth at the P2 position (65 mm down the left side from the midline at the level of the last rib) was measured at 9 weeks and 12 weeks of the experimental period using an ultrasound scanner (Anyscan BF, SongKang GLC Co., Gyeonggi-do, Republic of Korea). Fat-free lean (%) was calculated with the National Pork Producer Council equation (NPPC, 1999).

Data were analysed with the PROC GLM procedure of SAS (SAS Inst. Inc., Cary, NC, USA). The experimental unit was the pen. The statistical model for growth performance and carcass characteristics included dietary treatments as the fixed effect and initial BW as the covariate. Results are presented as mean ± standard error of mean. Statistical significance and tendency were considered at P <0.05 and $0.05 \leq P$ <0.10, respectively.

Results and Discussion

The PRO diets were fed to the pigs with lower initial BW, and the CON diets were fed to the pigs with higher initial BW (28.78 vs. 34.37 kg, P <0.05) (Tables 1 and 2). During the growing period, the PRO pigs had higher (P <0.05) ADG (0.944 vs. 0.877 kg/d) and G:F ratio (0.450 vs. 0.401 kg/kg) than the CON pigs, but there were no differences in feed intake and ADFI. At the end of the growing period, however, the differences in BW between the groups were still observed (68.45 vs. 71.19 kg, P <0.05). These differences were not found at the end of the finishing period, as a result of lower (P <0.05) feed intake (122.63 vs. 138.13 kg) and ADFI (2.920 vs. 3.290 kg/d), and higher (P <0.001) G:F ratio (0.319 vs. 0.271 kg/kg) in the PRO pigs (Table 3). For the entire period, pigs in the PRO consumed less feed (feed intake and ADFI, P

<0.05) and gained more weight (ADG, P <0.05) compared with those in the CON (Table 4). This result implies that protease supplement in a diet increased growth via improvement of feed efficiency (G:F ratio, P <0.001).

Table 1 Composition of basal diets for growing and finishing pigs (as-fed basis)

Item	Growing diet	Finishing diet
Ingredient (%)		
Maize	53.25	44.20
Soybean meal, 44%	23.57	13.30
Wheat	-	27.00
Dried distiller's grains with solubles	4.00	5.59
Copra meal	5.00	-
Palm kernel meal	1.40	-
Sugar beet pulp	4.55	-
Molasses, sugar beet	2.50	4.00
Soybean oil	2.42	2.56
Monocalcium phosphate	1.03	0.79
Limestone	0.79	0.98
Salt	0.50	0.50
Vitamin-mineral premix[1]	0.50	0.50
L-Lysine-HCl	0.33	0.43
DL-methionine	0.07	0.03
L-Threonine	0.08	0.11
L-Tryptophan	0.01	0.01
Total	100.00	100.00
Calculated energy and nutrient contents		
Metabolizable energy (Mcal/kg)	3.30	3.30
Crude protein (%)	18.00	15.86
Crude fat (%)	8.27	4.94
Crude fibre (%)	2.62	2.79
Neutral detergent fibre (%)	16.04	10.08
Acid detergent fibre (%)	9.50	4.07
Calcium (%)	0.66	0.59
Phosphorus (%)	0.56	0.52
Lysine (%)	1.14	0.96
Methionine (%)	0.35	0.28
Threonine (%)	0.76	0.63
Tryptophan (%)	0.21	0.17

[1] Provided per kilogram of diet: vitamin A, 10,000 IU; vitamin D_3, 2,000 IU; vitamin E, 48 IU; vitamin K_3, 1.5 mg; riboflavin, 6 mg; niacin, 40 mg; D-pantothenic acid, 17 mg; biotin, 0.2 mg; folic acid, 2 mg; choline, 166 mg; vitamin B_6, 2 mg; and vitamin B_{12}, 28 µg; Fe, 90 mg from iron sulfate; Cu, 15 mg from copper sulfate; Zn, 50 mg from zinc oxide; Mn, 54 mg from manganese oxide; I, 0.99 mg from potassium iodide; Se, 0.25 mg from sodium selenite

Supplementation of exogenous enzymes in a diet, especially protease as a part of an enzyme mixture, has been widely used to improve the efficiency of nutrient utilization and minimize anti-nutritive effects, and thereby was expected to improve growth performance (Adeola & Cowieson, 2011). In the results of in vitro tests, the addition of enzyme complexes (xylanase, protease and phytase) tended to increase the ileal digestibility of dry matter, even though it depended on test ingredients (Kong *et al*., 2015). Other studies also showed that enzyme mixtures including protease had a positive impact on nutrient digestibility, bacterial populations in the large intestine, and growth performance of weaned pigs (Yi *et al*., 2013), faecal nutrient digestibility of growing pigs (Ji *et al*., 2008), and the performance of growing pigs without improving apparent total tract digestibility (Jo *et al*., 2012).

Table 2 Growth performance of growing pigs fed diets with and without proteases[1]

Item	**Treatments[2]**		**SEM**	***P* value**
	CON	**PRO**		
Initial bodyweight (kg)	34.37	28.78	0.25	< 0.05
Final bodyweight (kg)	71.19	68.45	0.77	< 0.05
Feed intake (kg)	90.63	88.25	2.00	0.424
ADG (kg/d)	0.877	0.944	0.017	< 0.05
ADFI (kg/d)	2.159	2.103	0.047	0.424
G:F ratio (kg/kg)	0.401	0.450	0.008	< 0.01

[1] Values are presented as the least squares mean of six replicates (8 pigs/replicate). The growing period was six weeks
[2] CON: control diet; PRO: CON + 0.02% protease (Ronozyme®)
ADG: average daily gain, ADFI: average daily feed intake, G:F ratio: ratio between ADG and ADFI

Recently, standalone proteases of swine diets have been used via several commercially available products (Guggenbuhl *et al*., 2012; Mc Alpine *et al*., 2012a, 2012b; Zuo *et al*., 2015). Supplementation of protease alone in diets of young pigs has shown similar advantages. For example, inclusion of proteases in maize and soybean meal-based diet increased apparent ileal digestibility (AID) of amino acids in piglets (Guggenbuhl *et al*., 2012). Coated proteases in diets of weaned pigs showed improved apparent total tract digestibility of nitrogen (N) and energy. It also resulted in improved AID of some indispensable amino acids, nutrients (Pan *et al*., 2016), crude protein and standardized ileal digestibility (Yu *et al*., 2016). Other studies confirmed that weaned pigs fed protease supplemented diets improved nutrient digestibility and growth performance (Zuo *et al*., 2015; Tactacan *et al*., 2016).

Some studies of growing or finishing pigs did not confirm positive effects of protease on growth performance, although its inclusion in diets improved AID of amino acids, gross energy (O'Shea *et al*., 2014) and faecal nitrogen digestibility (Mc Alpine *et al*., 2012a) when diets were based on wheat distillers and rapeseed meal. On the other hand, another study showed the positive possibility of neutral protease on the growth performance of growing pigs, resulting from a better feed conversion ratio without improved digestible energy and nutrient digestibility (O'Doherty & Forde, 1999). Similar results were found in the current study.. Low BW pigs fed diets with added protease during the growing-finishing period showed improved growth performance through high feed efficiency compared with high BW pigs fed diets without protease.

Supplementation of exogenous enzymes in diets may complement the digestive system by degrading certain nutrients that are resistant to endogenous digestive enzymes (O'Doherty & Forde, 1999; O'Shea *et al*., 2014) or neutralize anti-nutritional factors such as enzyme inhibitors and improve nutrient digestibility (Zuo *et al*., 2015). Moreover, it has been reported that the activity of digestive enzymes in the stomach and pancreatic tissue is decreased dramatically after weaning (Hedemann & Jensen, 2004; Zuo *et al*., 2015). Thus, the addition of protease in diets of weaned pigs could be helpful in the digestion of certain types of protein that are resistant to pig digestive enzymes and neutralize protease inhibitors, resulting in improved nutrient digestibility and growth performance. On the other hand, protease supplementation may have less effect on growth performance because the digestive system is more developed in pigs at the growing-finishing stage compared with weaned pigs, and improved digestibility of crude protein and amino acids may not always lead to increase growth. Based on the results of this study and previous ones, the supplementation of protease alone in swine diet has an effect on nutrient and energy digestibility, regardless of basal diet ingredients, protease type, pig growth stage, intestinal development status and so on. On the other hand, the effects of protease on growth performance, especially growing-finishing pigs, may be affected by those factors.

Table 3 Growth performance of finishing pigs fed diets with and without proteases[1]

Item	Treatments[2]		SEM	*P* value
	CON	PRO		
Initial bodyweight (kg)	71.19	68.45	0.77	< 0.05
Final bodyweight (kg)	108.60	107.45	1.30	0.544
Feed intake (kg)	138.13	122.63	4.63	< 0.05
ADG (kg/d)	0.891	0.929	0.031	0.411
ADFI (kg/d)	3.290	2.920	0.110	< 0.05
G:F ratio (kg/kg)	0.271	0.319	0.007	< 0.001

[1] Values are presented as the least squares mean of six replicates (8 pigs/replicate). The finishing period was for six weeks
[2] CON: control diet; PRO: CON + 0.02% protease (Ronozyme®)
ADG: average daily gain, ADFI: average daily feed intake, G:F ratio: ratio between ADG and ADFI

Table 4 Growth performance of growing-finishing pigs fed diets with and without proteases[1]

Item	Treatments[2]		SEM	*P* value
	CON	PRO		
Initial bodyweight (kg)	34.37	28.78	0.25	< 0.05
Final bodyweight (kg)	108.60	107.45	1.30	0.544
Feed intake (kg)	228.75	210.88	5.50	< 0.05
ADG (kg/d)	0.884	0.937	0.016	< 0.05
ADFI (kg/d)	2.724	2.512	0.066	< 0.05
G:F ratio (kg/kg)	0.325	0.374	0.007	< 0.001

[1] Values are presented as the least squares mean of six replicates (8 pigs/replicate). The growing-finishing period was for 12 weeks
[2] CON: control diet; PRO: CON + 0.02% protease (Ronozyme®)
ADG: average daily gain, ADFI: average daily feed intake, G:F ratio: ratio between ADG and ADFI

No significant differences in carcass characteristics were found between the PRO and the CON groups (Table 5). This result was consistent with those of a previous study, which showed that the inclusion of protease in growing-finishing diets had no significant effect on carcass weight, kill out, back fatback fat depth, and lean meat (O'Shea *et al*., 2014). However, in the same study (O'Shea *et al*., 2014), positive effects of proteases on growth performance were not found. Meanwhile, keratinase supplementation in the diet of nursery pigs increased loin muscle area, showing potential for improving carcass quality in growing-finishing pigs (Wang *et al*., 2011). So, further investigation is needed to clarify positive effects of proteases on carcass characteristics.

Table 5 Carcass characteristics of finishing pigs fed diets with and without proteases[1]

Item	Treatments[2]		SEM	*P* value
	CON	PRO		
Final live bodyweight (kg)	108.60	107.45	1.30	0.544
Hot carcass weight (kg)	83.55	82.81	0.98	0.606
Dressing percentage (%)	76.94	77.07	0.05	0.068
Initial back fat depth (week 9, mm)	14.58	14.11	0.50	0.519
Finial back fat depth (week 12, mm)	17.20	16.73	0.53	0.545
Back fat depth change (mm)	2.62	2.62	0.20	0.994
Fat-free lean (%)[3]	58.13	57.99	0.20	0.655

[1] Values are presented as the least squares mean of six replicates (8 pigs/replicate). The growing-finishing period was 12 weeks
[2] CON: control diet; PRO: CON + 0.02% protease (Ronozyme®)
[3] Fat-free lean (%) was calculated with the equation of NPPC (1999)

Conclusions

The results in this study showed that single protease supplementation during the growing-finishing period could practically increase the growth of pigs with relatively lower bodyweight by improving feed efficiency, but had no disadvantages in carcass characteristics. However, further studies may be needed to clarify the underlying mechanisms of improving feed efficiency in growing-finishing pigs and the subsequent effect on carcass characteristics by dietary protease supplementation.

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Authors' contributions

JC, KSK and HBK designed this experiment. JC, KSK, HBK, SP, JK, SK, BK, SHC, JYC and IHP performed the animal experiment, measured and acquired the data, and evaluated the carcass characteristics. JC, KSK and HBK analysed the data and wrote the manuscript. JHC and MS supervised all processes through performing the experiment to writing the manuscript. All authors read and approved the final manuscript.

Conflict of interest declaration

The authors have declared that there are no competing interests.

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