



Preliminary evaluation of papain: its enzymatic characteristics and effects on growth performance and nutrient digestibility in weaned piglets

BAOMING LING¹, DINGYUAN FENG², JIANJUN ZUO³ and ZHONGYUE ZHANG⁴

South China Agricultural University, Guangzhou City, Guangdong Province, P.R. China

Received: 27 December 2011; Accepted: 7 June 2012

ABSTRACT

The present study was conducted to investigate the influence of papain (*Carica papaya* L.) on performance and nutrient digestibility of weaned piglets and to evaluate whether soy protein ingredients supplemented with papain could replace part of high grade animal protein ingredients in weaned piglet feed. The results showed that Km, optimal pH and temperature of papain were 6.6875 mg/mL, 7.0° and 55°C respectively. It had a good stability when either pH ranged from 5.0 to 9.0 or temperature ranged from 4 to 65°C. The addition of papain at 75 mg/kg level significantly increased *in vitro* crude protein digestibility of soybean meal and piglet feed. Piglets (300; (Pietrain×Duroc)×(Landrace×Yorkshire)) weaned at 21±1 days with an initial body weight (BW) of 5.92±0.47 kg were assigned by BW, sex, and litter according to a randomized complete block design. The animal trial consisted of 5 dietary treatments with 6 replicates/treatment and 0 piglets/replicate. The dietary treatments were positive control (PC), negative control (NC), NC + 50 mg/kg papain, NC + 75 mg/kg papain, and NC +00 mg/kg papain. The results showed that papain significantly decreased diarrhea incidence of piglets fed the diets containing high level of soy-source protein material. There was a linearly improved apparent crude protein digestibility with the increasing dosage of protease. In conclusion, papain supplementation could be considered as an effective solution to break the inclusion limit of soybean source protein ingredients in weaned piglet diet.

Key words: Enzymatic characteristics, Growth performance, Nutrient digestibility, Papain, Piglets, Weaned piglets

The feed ingredients used in newly weaned piglet diet should have high digestibility because of the weak digestion function of piglets and weaning stress. Concentrate whey protein, high quality fish meal, spray-dried plasma protein, spray dried egg meal, and skim milk powder (Pluske 2003, Li 2003) are used in their diets but these are expensive and usually in short supply. Soy-source protein ingredients are available in abundance at low price, but are used limitedly in their feed for their low digestibility. Rooke *et al.* (1998) found that piglet performance at weaning could be improved by protease-treated soybean meal and thought this may be a potential method to increase the inclusion rate of soybean meal in weaned piglet feed. Similarly, a more healthy mucosal status and better growth performance of weaned piglets could be attributed to the addition of exogenous protease in soybean meal based diets (Dierick *et al.* 2004). Microbial proteases, mainly from *Bacillus subtilis*, *Aspergillus niger* and yeast, are used comprehensively in feed industry (Ling *et al.* 2009).

However, literature is very limited in using plant proteases in animal nutrition. Papain (EC 3.4.22.2), a thiol protease extracted from unripened fruits of *Carica papaya* L. which is now widely cultivated in South China. Papain has been widely used in food and other industries for its good stability, proteolytic activity and lower price (Ashie *et al.* 2002, Xiong and Xu 2008). The present study was conducted to evaluate whether the addition of papain could increase inclusion rate of soy source protein materials in weaned piglet diet and decrease the dependency on high grade animal protein ingredients by increasing the digestibility of soy-source protein ingredients.

MATERIALS AND METHODS

In vitro trials

Enzyme activity analysis for papain: Enzyme activity assay method is the analysis methods of Chen and Ji (2005), which is similar to that of Weetall and Mason (1973). The filtrate was read spectrophotometrically at 275 nm. The buffer for papain solution, activator solution, and casein solution was 0.1 mol/litre sodium phosphate, pH 7.0. 0.1 spectrophotometrical absorbance unit was equivalent to one enzyme unit according to the above enzyme assay method.

Present address: ¹Ph.D. Scholar (lingbm863@yahoo.com.cn)
²Professor (fengdy@scau.edu.cn), ³Associate Professor (zuojs@scau.edu.cn), ⁴Ph.D. Scholar (dfzhongyue@qq.com), College of Animal Science.

Relative enzyme units: We assume the highest enzyme activity treatment among several treatments was 00%, and the enzyme activity of other treatments was expressed as the percentage of the highest enzyme activity.

Measure of Michaelis constant (Km) for papain: Five 0.1 mL mg/mL papain solutions were taken. After the addition of activator to papain solution, 2, 3, 4, and 5 mg/mL casein solutions were added to 5 different papain solutions to determine enzyme activity, respectively. Michaelis constant were obtained by Lineweaver-Burk method.

Measure of optimal pH of papain: Seven 0.1 mL mg/mL papain solution were measured. Then activator solution and casein solution at pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 were added to papain solution to determine the enzyme activity, respectively. Citrate buffer solution, phosphate buffer solution, and barbitone sodium-hydrochloric acid buffer solution were used to adjust pH values of papain solutions to be less than 5.0, from 6.0–7.0, and above 8.0, respectively.

Measure of stability of papain at different pH values: 1 mg/ml papain were prepared at pH values ranging from 3.0–9.0 and stored at 20 °C for 3h. Then, pH values of papain solutions were adjusted to 7.2 to determine the enzyme activity at different pH values.

Measure of optimal temperature of papain: Eight 0.1 mL mg/ml papain solutions were taken and activator solutions were added to papain solutions. After incubation at 25°, 35°, 45°, 55°, 65°, 75°, 85°, and 95°C for 0 min, 5 mg/mL casein solutions were added to determine the optimal enzyme activity.

Measure of stability of papain at different temperature: Eight 0.1 mL mg/mL papain solutions were incubated at 4°, 25°, 35°, 45°, 55°, 65°, 75°, and 85°C for 30 min, and were cooled to determine enzyme activity.

In vitro protein digestibility analysis of soy-source ingredients and piglet feed: Protein soybean meal (43%), full fat soya, and piglet feed samples were collected, and ground to pass 0.25 mm screen. A series of dosage of papain (0 25, 50, 75,00 mg/kg) were added to g sample of soybean meal, full fat soya, and piglet feed, respectively. Pepsin was added to sample. The sample was incubated at 37°C, pH 2 for 1 h, and neutralized by 0.6 mol/litre NaOH. Then phosphate buffer solution containing 0.1% pancreatic enzymes at pH 7.6 was added to samples. After incubation at 37°C for 6h, the reaction was stopped by 5 mlitre 5%TCA. The residues were filtered by #3 Whatman filter paper. The protein levels in residues were measured to calculate protein digestibility described by Cone and van der Poel (1993).

Feeding trial

Animals, feeding management, and experimental design: A total of 300 [(Pietrain×Duroc)× (Landrace×Yorkshire)] piglets weaned at 21±1 days with an initial body weight (BW) of 5.92±0.47 kg were assigned by BW, sex, and litter according to a randomized complete block design. The trial

consisted of 5 dietary treatments with 6 replicates/treatment and 10 piglets/replicate (pen), and sex was balanced in each pen. All pigs were housed in an environmentally-controlled house with temperature maintained at approximately 25 °C. Every pen was equipped with an automatic feeder and a nipple drinker and all piglets were supplied with feed and water *ad lib*. The study was approved by Animal Welfare and Protocol Committee of South China University of Technology. Five dietary treatments were as follows: Trt1, positive control (PC) containing 22.21% soy-source protein ingredients; Trt 2, negative control (NC) containing 30% soy source protein ingredients; Trt 3, NC + 50 mg/kg papain; Trt 4, NC + 75 mg/kg papain; Trt 5, NC +00 mg/kg papain. Ingredients and nutrient composition of experimental diets were shown in Table 1. The nutrient levels fulfilled or

Table 1. Composition and nutrient level of the basal diet (g/kg, as-fed basis)

Ingredients	Positive control	Negative control
Corn (CP8%)	415.1	412.1
Full fat soybean	142.1	160.6
Whey powder (CP12%)	120	120
Soybean meal (CP43%)	50	100
Flour	50	50
Whey protein concentrate (CP 34%)	50	0
Soybean protein concentrate (CP 64%)	30	40
Spray dried plasma protein (CP 78%)	30	30
Fish meal	30	0
Glucose	25	25
Sucrose	20	20
Dicalcium phosphate	7	11.5
Premix ¹	10	10
Soybean oil	7.6	5.2
L-lysine ·HCl	3.1	4
Limestone	3.6	4
Zinc oxide	2.5	2.5
L-threonine	1.2	1.8
DL-methionine	1.3	1.7
Choline chloride(50%)	1	1
L-tryptophan	0.5	0.6
<i>Nutrient level²</i>		
Digestible energy (MJ/kg)	14.52	14.52
Crude protein (%)	21	21
Calcium (%)	0.6	0.6
Available phosphorus (%)	0.45	0.45
Lysine (%)	1.53	1.54
Methionine + cysteine (%)	0.86	0.86
Threonine (%)	1.05	1.05

¹ Provided per kilogram of complete diet: Fe, 305 mg; Cu 250 mg; Mn, 51 mg; Zn,1910 mg; I, 0.5 mg; Se, 0.5 mg; Co, 0.5 mg; vitamin A,19,200 IU; vitamin D₃, 4,800 IU; vitamin E, 60 IU; vitamin K₃, 6 mg; vitamin B₁, 6 mg; vitamin, B₂ 12 mg; vitamin, B₆ 7.2 mg; vitamin B₁₂, 0.05 mg; nicotinamide, 60 mg; D-calcium pantothenate, 30 mg; folic acid, 3.6 mg; biotin, 0.6 mg.²Calculated values.

exceeded nutrient requirements recommended by NRC (1998) for 5–10 kg weaned piglets. These diets are typical commercial weaned piglet feed in Chinese market.

Data recording and sample collecting: The trial lasted 4 days. Feed intake and number of a pigs with diarrhea were recorded every day. Piglet body weight was recorded at 8 h after feeding in the beginning and at the end of trial. Average daily gain, average daily feed intake, and feed conversion were calculated from body weight and feed intake. Diarrhea incidence (%) = (total number of pigs with diarrhea) / (number of total pigs × trial days). From the eighth day after the beginning of the trial, the piglet feed was supplemented Cr_2O_3 at 2 g/kg levels as inert indicator. The fecal samples from at least 2 piglets each pen were collected during third and fourth day. The fecal samples and feed samples were stored at -20° for analysis of apparent digestibility of dry mater, crude protein, and gross energy (AOAC 1990).

Data analysis: Data were analyzed by one-way ANOVA of SPSS (Ver.3.0 for Windows). If differences in treatment means were detected by ANOVA, Duncan's multiple range test was applied to separate means. Contrast statements were utilized to test for linear or quadratic effect. A significance level of $P < 0.05$ was used during analysis.

RESULTS AND DISCUSSION

Characteristics of enzymes: The enzyme activity of papain was determined to be 41,170 U/g by using enzyme activity assay method (Chen and Ji 2005). Michaelis constant (K_m) means that the initial rate of reaction V , produced by an enzyme when the substrate concentration is high enough to saturate the enzyme, is related to the rate of reaction v at a lower substrate concentration c by the formula $V = v(1 + K_m/c)$. Equation of Michaelis constant (K_m) was showed as $y = 0.0642x + 0.0096$ by Lineweaver-Burk method. Based on this equation, K_m of papain to casein substrate was 6.6875 mg/mL. Fig. 1 showed that papain enzyme activity was high from 4.0 to 9.0 pH and the optimal pH value for papain enzyme was 7.0. Papain was stable after 3 h storage at pH from 5.0 to 9.0 (Fig. 2). Enzyme activity of papain was high during 45° – 75°C and optimal temperature was 55°C (Fig. 3). Thermal stability of papain was good after incubation during 4 – 65°C for 30 min (Fig. 4). As temperature increased to be more than 65°C , the enzyme activity of papain decreased significantly.

Enzyme characteristics are important to evaluate the enzyme. Our results showed that the enzyme activity of papain at pH ranging from 6.5 to 8.0 was high and the optimal

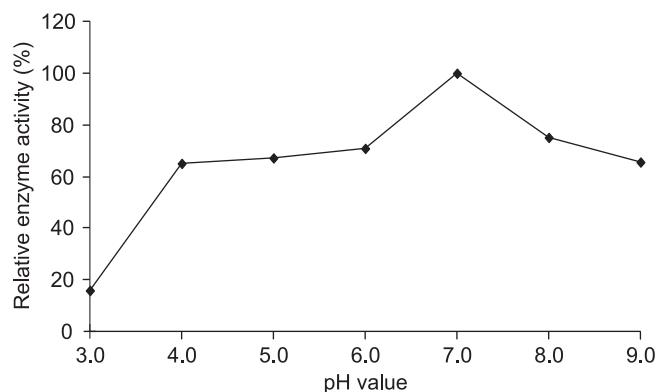


Fig. 1. Effect of pH on the activity of papain.

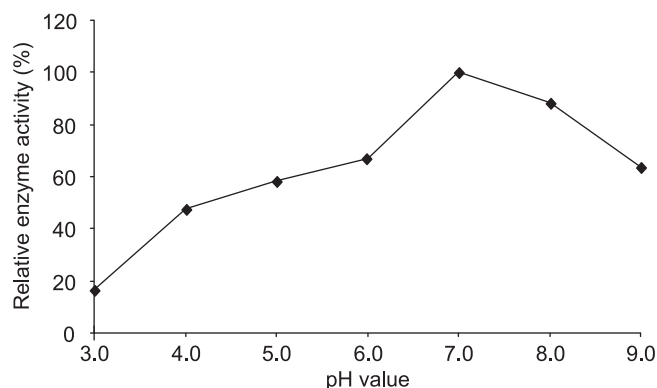


Fig. 2. Stability of papain at different pH values.

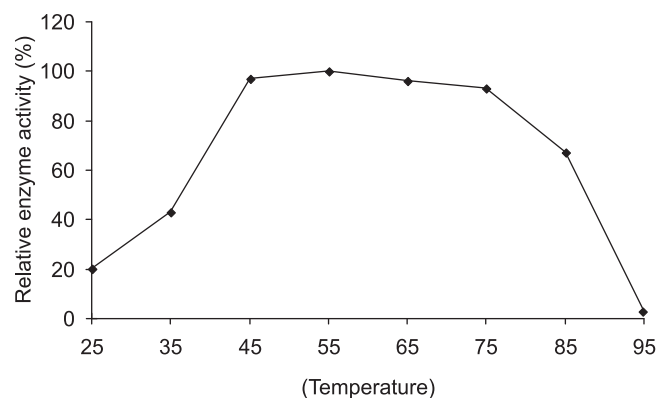


Fig. 3. Effect of temperature on the activity of papain.

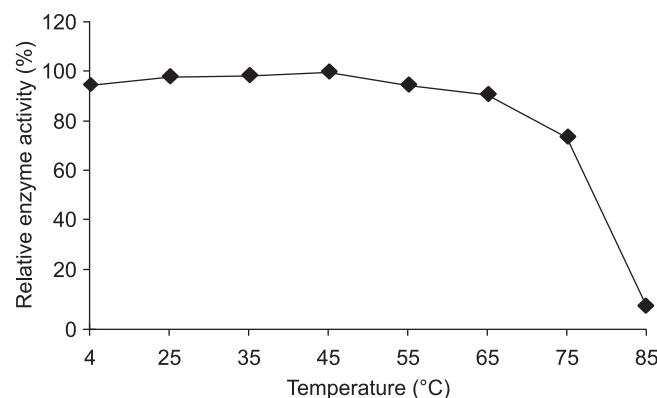


Fig. 4. Thermostability of papain.

pH value for enzyme activity of papain was 7.0. Similarly, Chen and Ji (2005) and Qu *et al.* (2008) reported that the highest enzyme activity of papain was during pH 7.0–8.0. The enzyme activity decreased significantly at temperature more than 65 °C, suggesting that the high temperature around 65–80 °C during feed processing including conditioning and pelleting might affect the recovery rate of papain enzyme activity.

Influence of papain on in vitro crude protein digestibility: The addition of papain had a linear significant effect on *in vitro* crude protein digestibility of soybean meal, full fat soya, and piglet feed (Table 2). *In vitro* crude protein digestibility of soybean meal and piglet feed supplemented with 75 mg/kg and 100 mg/kg papain was significantly higher than without papain. *In vitro* crude protein digestibility of soybean meal and pig feed linearly increased with increasing papain level from 0 to 75 mg/kg. There were no difference in *in vitro* crude protein digestibility of soybean meal and piglet feed between 75 mg/kg papain level and 100 mg/kg papain level. The addition of papain at 50 mg/kg significantly improved *in vitro* crude protein digestibility, but there was

no further increase in crude protein digestibility as papain level increased from 75 to 100 mg/kg.

Effect of papain on growth performance and diarrhea incidence of weaned piglets: There were no significant differences in daily weight gain and feed conversion between negative control diet and positive control diet. Average daily gain and feed conversion of positive control diet was numerically (10.06% and 3.07%) better than those of negative control diet (Table 3). Diarrhea incidence of negative control diet was significantly higher than that of positive control diet. The addition of papain had a significant effect on diarrhea incidence of the piglets fed negative control diets containing high percentage of soy-protein ingredients. Average daily gain and feed conversion numerically improved with increasing papain level.

Effect of papain on apparent digestibility of weaned piglet: There were no significant differences in apparent digestibility in dry matter and gross energy among treatments (Table 4). Apparent crude protein digestibility of negative control diet containing high percentage of soy protein ingredients was significantly lower than that of positive control diet. The

Table 2. Effect of papain on *in vitro* crude protein digestibility of soybean meal, full fat soya, and compound piglet feed (%)

Soy-meal	0 mg/kg papain	25 mg/kg papain	50 mg/kg papain	75 mg/kg papain	100 mg/kg papain
Soybean meal	77.36±1.53 ^b	78.83±1.66 ^{ab}	80.11±1.58 ^{ab}	82.72±1.37 ^a	82.33±1.42 ^a
Full fat soya	72.14±1.48 ^b	74.58±1.63 ^{ab}	76.39±1.41 ^a	78.14±1.59 ^a	78.55 ±1.50 ^a
Compound piglet feed	76.55±1.62 ^b	77.96±1.45 ^{ab}	79.75±1.57 ^{ab}	81.94±1.52 ^a	81.52±1.65 ^a

^{a-b}Data are mean ± SE. Means within a row not sharing the same superscript differ significantly at P<0.05.

Table 3. Effects of dietary supplementation of papain on the growth performance and diarrhea incidence of weaned piglets

Items	PC*	NC*	NC+ 50 mg/kg papain	NC+75 mg/kg papain	NC+100 mg/kg papain
Initial body weight (kg/pig)	5.92±0.28	5.92±0.27	5.92±0.27	5.92±0.25	5.92±0.29
Final body weight (kg/pig)	9.70±0.41	9.35±0.49	9.43±0.43	9.71±0.39	9.64±0.45
Average daily gain (g/d)	270.14±21.49	245.45±30.24	250.52±23.63	270.76±20.97	266.17±25.92
Average daily feed intake (g/d)	340.85±29.65	319.20±32.52	322.27±30.73	346.43±28.53	334.97±33.13
Feed conversion	1.26±0.05	1.30±0.06	1.29±0.03	1.28±0.06	1.26±0.04
Diarrhea incidence,%*	3.41±1.07 ^b	4.92±1.71 ^a	3.17±1.40 ^b	3.00±1.32 ^b	2.74±1.58 ^b

^{a-b} Data are mean ± SE. Means within a row not sharing the same superscript differ significantly at P<0.05.* PC, Positive control; NC, negative control; diarrhea incidence (%), (total number of pigs with diarrhea)/ (number of total pigs × trial days).

Table 4. Effects of dietary supplementation of papain on apparent nutrient digestibility of weaned piglets

Items	PC*	NC*	NC+ 50 mg/kg papain	NC+75 mg/kg papain	NC+100 mg/kg papain
Dry matter (%)	88.59±1.86	86.65±2.39	88.03±2.27	87.38±2.81	88.72±1.99
Gross energy (%)	87.90±2.15	85.32±2.02	86.98±2.53	86.01±1.92	87.45±2.88
Crude protein (%)	87.52±2.32 ^a	79.84±3.41 ^b	80.71±2.09 ^b	84.63±2.34 ^{ab}	86.79 ±2.11 ^a

^{a-b} Data are mean ± SE. Means within a row not sharing the same superscript differ significantly at P<0.05.*PC, Positive control; NC, negative control.

addition of papain linearly improved apparent crude protein digestibility. Papain supplemented with 100 mg/kg level could restore apparent crude protein digestibility level of negative control diet to the level similar to that of positive control.

In vitro digestibility measure is an easy way to evaluate the effect of enzymes. Bedford and Classen (1993) and Zyla *et al.* (1995) applied this method to determine the effect of NSP enzymes and phytase. Our results showed that the addition of papain to piglet feed by adding liquid papain without feed processing significantly improved *in vitro* crude protein digestibility, and as papain level was more than 75 mg/kg, no further improvement of *in vitro* crude protein digestibility could be observed. However, the data (Tables 3, 4) indicated that negative control feed supplemented with 100 mg/kg papain had better growth performance and apparent crude protein digestibility than negative control without papain. This also suggested that the enzyme activity of papain might be decreased by feed processing temperature normally around 65–80 °C. It is necessary to protect papain by coating or thermal stable gene modification to increase thermal stability.

Dierick *et al.* (2004) reported that the addition of 0.05 and 0.1% microbial protease improved average daily gain and feed conversion, and O'Doherty and Forde (1999) reported that the addition of neutral protease in diets containing 40% pea significantly improved feed conversion. Similarly, Rooke *et al.* (1998) reported protease treated soybean meal in weaned piglet feed significantly improved growth performance. In addition, protease supplementation improved growth performance of trout (Drew *et al.* 2005) and broilers (Yu *et al.* 2007). Our results showed that the addition of papain in the diets containing high inclusion rate of soy-source protein significantly decreased diarrhea incidence and numerically improved average daily gain and feed conversion, which were in agreement with others (Rooke *et al.* 1998, O'Doherty and Forde 1999, Dierick *et al.* 2004). The application of papain in weaned piglet feed may be considered as a solution to break the inclusion limit of soy source protein.

The results showed that the addition of 100 mg/kg papain significantly improved apparent digestibility of crude protein. Similarly, Dierick *et al.* (2004) reported that the supplementation of 0.05% and 0.1% protease improved both ileal and apparent digestibility of dry matter, crude protein, fat, and energy. However, Caine *et al.* (1997) reported that pre-treatment of soybean meal by protease could not improve ileal digestibility of crude protein and amino acid. The results of O'Doherty and Forde (1999) showed that neutral protease could not improve nutrient digestibility. The difference among the present study and other research might be due to the difference in protease sources, diet compositions, or animal breeds.

In conclusion, the addition of papain significantly

increased *in vitro* crude protein digestibility of soybean meal, full fat soya and piglet feed, and the addition of papain in piglet feed significantly decreased diarrhea incidence and improved apparent crude protein digestibility. These results indicated that papain can be used to increase the inclusion rate of soy protein ingredients and decrease the usage of high quality animal protein in weaned piglet feed so as to decrease the cost of feed. Because papain enzyme activity significantly decreased as the temperature exceed 65 °C. Therefore, it is necessary for feed industry to take necessary steps such as coating or thermal stable genetic modification to improve thermal stability of enzymes to avoid the influence of high temperature of feed processing.

ACKNOWLEDGEMENTS

The authors wish to thank Mr Guangbing Wu of Danisco Animal Nutrition and Mr Ronald Co of Canadian Jefe Nutrition Inc. for their support in the present study.

REFERENCES

- AOAC.1990. *Official Methods of Analysis*. 5th edn. Association of Official Analytical Chemists, Arlington, VA, USA.
- Ashie I N A, Sorensen T L and Nielsen P M. 2002. Effect of papain and a microbial enzyme on meat proteins and beef tenderness. *Journal of Food Science* **67**: 2138–42.
- Bedford M R and Classen H L.1993. An *in vitro* assay for predication of broiler intestinal viscosity and growth when fed rye-based diets in the presence of exogenous enzymes. *Poultry Science* **72**: 137–43.
- Caine W R, Sauer W C, Tamminga S, Verstegen M W and Schulze H.1997. Apparent ileal digestibilities of amino acids in newly weaned pigs fed diets with protease-treated soybean meal. *Journal of Animal Science* **75**: 2962–69.
- Chen F Y and Ji P X. 2005. Studies on characteristics of immobilized papain on silk fibroin. *Journal of South China Agricultural University* **26** (4): 81–83 (in Chinese with English abstract).
- Cone J W and van der Poel A F B.1993. Prediction of apparent ileal protein digestibility in pigs with a two-step *in-vitro* method. *Journal of the Science of Food and Agriculture* **62**: 393–400.
- Dierick N, Decuypere J, Molly K and Vanderbeke E. 2004. Microbial protease addition to a soybean meal diet for weaned piglets: effects on performance, digestion, gut flora and gut functions *Recent advances of research in antinutritional factors in legume seeds and oilseeds/EAAP publication* **4**: 229–33.
- Drew M D, Racz V J, Gauthier R and Thiessen D L. 2005. Effect of adding protease to coextruded flax: pea or canola: pea products on nutrient digestibility and growth performance of rainbow trout (*Oncorhynchus mykiss*). *Animal Feed Science and Technology* **19**: 117–28.
- Li D F. 2003. *Swine Nutrition*. 2nd edn. China Agricultural Press, Beijing.
- Ling B M, Feng D Y and Zuo J J. 2009. Progress in feed proteases. *Feed industry* **22**: 7–10.
- NRC.1998. *Nutrient Requirements of Swine*.10th edn. National Academic Press, Washington, DC.
- O'Doherty J V and Forde S.1999. The effect of protease and α -galactosidase supplementation on the nutritive value of peas

- for growing and finishing pigs. *Irish Journal of Agricultural and Food Research* **38**: 217–26.
- Pluske J R, Le Dividich J and Verstegen M W A. 2003. *Weaning the Pig: Concepts and Consequences*. Wageningen Academic Publishers, Netherlands.
- Qu H Z, Huang L, Zhang G H, Chai Z, Zhang L, Hao D Y and Wang X P. 2008. The comparison of enzymatic characteristics of ficin and papain. *Journal of Jilin University (Natural Science volume)* **46** (6): 1217–20 (in Chinese with English abstract).
- Rooke J A, Slessor M, Fraser H and Thomson J R. 1998. Growth performance and gut function of piglets weaned at four weeks of age and fed protease-treated soya-bean meal. *Animal Feed Science and Technology* **70**: 175–90.
- SPSS. 1995. SPSS Version 10.0 for Windows. SPSS, Inc. USA.
- Weetall H and Mason R. 1973. Studies on immobilized papain. *Biotechnology and Bioengineering* **5** (3): 455–58.
- Xiong Q C and Xu D C. 2008. Application progress of papain. *China beet and sugar*: 43–45 (in Chinese).
- Yu B, Wu S T, Liu C C, Gauthier R and Chiou P W S. 2007. Effects of enzyme inclusion in a maize-soybean diet on broiler performance. *Animal Feed Science and Technology* **34**: 283–94.
- Zyla K, Ledoux D R, Garcia A and Veum T. 1995. An *in vitro* procedure for studying enzymic dephosphorylation of phytate in maize-soybean feeds for turkey poults. *British Journal of Nutrition* **74** (1): 3–17.