



Performance, nutrient utilization and serum biochemical characteristics of weanling pig with dietary supplementation of pancreatic enzymes

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ABSTRACT

The objective of the study was to determine the effect of pancreatic enzymes on the growth, nutrient utilization and blood biochemical parameters. Growth performance and biochemical parameters of pigs (144), 29±1day-old and 10±0.5 kg with dietary supplementation of pancreatic enzymes were evaluated in a 21-day feeding trial. And nutrient digestibility of eighteen 10±0.5 kg pigs with dietary addition of pancreatic enzymes were studied in a 11-day metabolic trial. They were blocked by bodyweight and sex (1:1) and allocated to 3 dietary treatments. The treatments were a negative control (NC) diet without pancreatic enzymes, NC with trypsin at 90U/kg, lipase at 150U/kg and amylase at 210U/kg (NCT1), and NC with trypsin at 180U/kg, lipase at 300U/kg and amylase 420U/kg (NCT2). The results showed the dietary supplementation with high level pancreatic enzymes could remarkably increase average daily gain (ADG) and tend to increase average daily feed intake (ADFI). In comparison with NC, significance was observed in triglyceride in both NCT1 and NCT2. And there was significant decrease in urea nitrogen in NCT2 in total protein in NCT2 compared with NC. In addition, the digestibilities of crude protein, crude fat and organic matter were enhanced with pancreatic enzymes addition.

Key words: Biochemical indices, Digestibility, Growth, Pig

The after-weaning pigs suffer the trauma of separation from the sows, a change in housing and especially piglets feed changed from sow's milk to solid feed (Giang *et al.* 2010), which often reduced the daily energy and nutrient intake (Pluske *et al.* 1996) and caused serious stress. Serious stress produced a general depression in pancreatic enzymatic activities (Lindemann *et al.* 1986) during the first week after weaning; and early weaning resulted in decrease in some digestive enzyme level was not offset in enzyme production until the pigs had reached 6 weeks of age (Hartma *et al.* 1961). Moreover both jejunal villus height and uniform microvilli on the jejunal villi were remarkably reduced for weaning pigs and increased by 14 d post-weaning (Cera *et al.* 1988), which are thought to decrease the digestive and absorptive capacity of the small intestine (Hampson 1986, Li *et al.* 2001). In addition, the gastrointestinal microbial balance will lose for the change of diet, which increases the opportunities for pathogens to colonize the gut (Fuller 1989,

Jensen 1998). Many of these factors that are interrelated and connected with growth lag are related with changes in the small intestinal morphology and digestive enzymes production. (Kitt *et al.* 2001)

Therefore, aim of this research was to study the effect of supplementing a piglet diet with different levels of pancreatic enzymes on growth performance, nutritive digestibility and blood biochemical indices.

MATERIALS AND METHODS

Crossbred (Duroc×Yorkshire×Landrace) pigs (72 barrows and 72 gilts), 29±1day-old and weighing 10±0.5 kg were used for the 21-day feeding and growth trials. The pigs were blocked by BW and sex (female: male=1:1) and allocated to dietary treatments in randomized complete block design, such that the average BW of the pigs was similar cross treatments. The diet (Table 1) was maize-soybean-based. The dietary treatments were (i) NC diet, which met or exceed NRC (1998) requirements for DE and CP; (ii) NC + trypsin (NCT1) added at 90U/kg, lipase added at 150U/kg and amylase added at 210U/kg; (iii) NC + trypsin (NCT2) added at 180U/kg, lipase added at 300U/kg and amylase added at 420U/kg. Each

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Table 1. Composition of the experimental diet

Components	Diet
Corn	550.0
Soybean meal	300.0
Fish meal	20.5
Wheat bran	50.0
Soybean oil	38.1
Dicalcium phosphate	15.0
Limestone	13.4
Vitamin-mineral premix (1%) ^a	10.0
Salt	2.2
Lysine	0.5
Met	0.3
Nutrient metabolic energy (MJ/kg) ^b	14.21
Crude protein ^b	209.0
Crude protein ^c	205.0
Lysine ^c	11.4
Calcium ^c	9.8
Phosphorus ^c	7.5

^aThe active ingredients added in the vitamin-mineral premix were as follows (per kg): retinylpalmitate, 1.750kIU; cholecalciferol, 0.2kIU; DL- α -tocopheryl acetate, 11 mg; menadione, 0.5 mg; thiamin, 1 mg; riboflavin, 3 mg; pyridoxine, 1.5 mg; cyanocobalamin, 0.015 mg; nicotinic acid, 12.5 mg; Ca-pantothenate, 9 mg; folic acid, 0.3 mg; choline chloride, 400 mg; Mn, 3 mg as $\text{MnSO}_4 \cdot \text{H}_2\text{O}$; Fe, 80 mg as $\text{FeSO}_4 \cdot \text{H}_2\text{O}$; Zn, 80 mg as $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$; Cu, 5.0 mg as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; iodine 0.14 mg as KI; Se, 0.25 mg as NaSeO_3 .

^bCalculation according to the Feed Composition and Nutritive Values of China (Twentieth Edition 2009).

^cThe nutrient of the diet were determined by standard AOAC (2000) methods (No 945.18).

treatment had 6 replicate pens / diet, with 8 pigs / replicate pen. The pigs were fed the diets as mashed for 29day, data on ADG and ADFI were taken after 21days.

Crossbred (Duroc $\times$ Yorkshire $\times$ Landrace) pigs (9 barrows and 9 gilts) weighing 10 ± 0.5 kg were used for the 11-day metabolic trial, including 7-day preliminary experiment and 4-day experiment. The treatments and the diet were same with feeding trial. The pigs were blocked by BW and sex (female: male=1:1) and allocated to dietary treatments in a randomized complete block design. Each treatment had 6 replicates, with 3 replicates per sex. The pigs were in individual stainless-steel pens (1.3 $\times$ 0.7 m) which were equipped with water nozzles, stainless steel feeders, and plastic-coated, expanded metal floors, as described by Adeola *et al.* (1995). The pens were located in an environmentally controlled building maintained at 27 ± 1 with a 12 h light (06:00–18:00) and 12 h dark cycle. Feed and water were provided for *ad lib*. Chromic oxide (0.20%) was added as inert indicator to calculate the apparent digestibilities of dry matter, organic matter, crude protein, crude fat, calcium and phosphorus. Fecal was collected in the last 4 days at 08:00 h after the piglets were fed the diets containing the indicator

for seven days in the last 4 days to enable comparison of the effect of the pancreatic enzymes added at different enzyme activity. The feces collected were immediately stored frozen at -20°C until analysis. For determination of the blood characteristics, 2 pigs (1 male and 1 female) were randomly selected from each pen and venous blood samples were then taken via anterior vena cava puncture in non-heparinized tubes for serum biochemical indices analysis on the final days of the experiment 1.

Chemical analyzes: Dried fecal and feed samples were ground to pass through 1-mm screen using a mill grinder. The fecal samples were dried at 50°C for 72 h. The nitrogen content was determined by Kjeldahl-method, and crude protein (CP) was calculated as $\text{N} \times 6.25$. The concentration of P was measured by phosphorus vanadium molybdate yellow colorimetric method. The Ca content was determined by permanganometric method. Crude fat was determined by soxhlet method. Albumin was measured by bromocresol green method. Total proteins were determined by biuret double reagent through two point terminal method. Urea nitrogen was measured by O-phthalaldehyde (OPA) method. Glucose was determined by glucose oxidase method. Triglyceride was measured by GK-GPO-POD method. Chromium in the fecal was determined by atomic absorption spectroscopy method.

Calculations and statistical analysis: Digestibility (%) was calculated according to the following equation:

$$\text{Digestibility} = \frac{(1 - \text{fecal nutrient (g/kg)} / \text{diet chromium (g/kg)})}{\text{nutrient (g/kg)} / \text{fecal chromium (g/kg)}} \times 100$$

All obtained data were evaluated statistically using SAS. Student-Newman-Keuls test was used to determine the significance of the main effects ($P \leq 0.5$). The data are presented as means, standard error of means (SEM) and *p*-value.

The study was approved by the College of Animal Science and Technology Animal Protocol Review Committee. Housing and care of the animals conformed to Chinese Department of Agriculture Guidelines.

RESULTS AND DISCUSSION

Growth performance: Effect of pancreatic enzymes on growth performance was shown in Table 2. Pigs fed the high level pancreatic enzymes had higher average daily gain (ADG) ($P < 0.05$) and tended to have higher ADFI ($P = 0.0636$) compared with pigs fed the negative control diet. No difference was observed ($P > 0.05$) in ADFI and ADG in comparison NCT1 with NC. Feed conversion was not significantly ($P > 0.05$) increased with pancreatic enzymes supplementation.

Pancreatic enzymes which contain trypsin, lipase and amylase are natural chemicals that help break down fats, proteins and carbohydrates. However, the stress of weaning usually caused disordered digestive enzymes secretion (Hartma *et al.* 1961, Lindemann *et al.* 1986, Owsley *et al.*

Table 2. Effects of pancreatic enzymes on growth performance of weaned pigs

Item	NC	NCT1	NCT2	SEM	p-Value
BW	10.29	10.45	10.17	0.1	0.5901
ADFI	513.0	510.2	543.6	7.0	0.0636
ADG	348.9 ^b	354.3 ^b	377.5 ^a	5.5	0.0498
FCR	1.47	1.44	1.44	0.03	0.8903

1986, Jensen *et al.* 1997) and low antibody concentration (Levast *et al.* 2010). Therefore it is necessary to supply digestive enzymes for weaned pigs. The positive effect on post-weaning piglets was observed by Liu *et al.* (2010), the bodyweight, feed intake and feed efficiency of the test groups were superior to those of the control group ($P < 0.05$) with lipase supplementation. In addition, digestive enzymes could be supplemented in diets of aquatic livestock except for young animals. The performance of fishing black carp was increased (Chen *et al.* 2009) with neutral protease addition. For the synergism of different kinds of enzymes, the digestive enzymes were generally combined with non-starch polysaccharide enzymes, which showed better performance in animals (Lin *et al.* 2007) and increased corn AMEn values. (Dourado *et al.* 2009). Overall, the results of the study indicated the diets as a result of a digestive enzymes mixture supplementation can improve the performance of the piglets when compared with NC group.

Blood biochemical indices: Blood biochemical indices were affected with pancreatic enzymes supplementation. (Table 3) Although albumin and glucose were affected by dietary treatment, no difference was observed ($P > 0.05$). Supplementation of pancreatic enzymes in the NC diet significantly lowered ($P < 0.05$) urea nitrogen in NCT2. Triglyceride in blood were enhanced ($P < 0.5$) by different levels of pancreatic enzymes addition.

Albumin is important for maintaining oncotic pressure, and for transporting many substances in the blood, which was measured to assess liver function. In this study it was lowered, therefore we presume the secretion of liver was probably inhibited and more research should be launched about the effect on liver with pancreatic enzymes addition in diet. Blood glucose is an important mark of energy metabolism and body health, which provides the brain and body with main source of energy. In this research, blood

glucose was improved with pancreatic enzymes supplementation, which showed more blood glucose was used for body and tissue growth. Total protein response the protein level of diet and digestion and absorption of animals. Urea excreted in urine is the primary nitrogenous end product produced by amino acid catabolism in pigs, and its concentration is directly related to the rate of urea synthesis (Zervas and Zijlstra 2002) and has both a strong and inverse relationship with lean growth and a positive relationship with fat deposition (Coma *et al.* 1995), which responses the status of protein metabolism and the situations of amino acid balance (Borg *et al.* 1987). In this study, the lower level of blood urea nitrogen and the higher level of total protein in blood indicated protein deposition was enhanced by digestive enzyme supplementation. Blood triglyceride can response the situation of lipid metabolism and is affected by nutrient level of diet (Song *et al.* 2004) and is decreased with indigestive lipid (Chiang *et al.* 1983). In this study, blood triglyceride was increased with addition of pancreatic enzymes, which demonstrated crude fat digestibility was increased. In the study of Liu *et al.* (2010), the group fed the diet blended with 10 000U/kg of recombinant lipase showed significant ($P < 0.05$) increases in blood triglyceride concentration on the seventh day postweaning.

Nutrient digestibility: Effects of pancreatic enzymes on nutrient digestibility of weaned piglets were shown in Table 4. The digestibilities of organic matter, crude protein and crude fat were significantly ($P < 0.05$) increased by dietary treatment. And there were differences ($P < 0.05$) in digestibility of calcium and a tendency ($P = 0.0519$) in digestibility of dry matter with high level pancreatic enzymes supplementation when compared with the NC group. In comparison with NC, the digestibility of phosphorus was not significantly affected by pancreatic enzymes supplementation.

The hydrolysis of dietary components occurs mainly in the stomach and in the intestinal tract due to the action of pepsin and pancreatic enzymes and intestinal enzymes. (Lee *et al.* 1984) Therefore, it is reasonable to assume that nutrient utilization improvement were responsible for pancreatic enzymes supplementation under the same dietary nutrient content. Digestibility increase of nutrient indicated an improvement of digestive capacity in the weaning pigs (Lloyd

Table 3. Effects of pancreatic enzymes on biochemical parameters of weaned pigs

Item	NC	NCT1	NCT2	SEM	p-Value
Albumin (g/L)	172.6	153.8	161.7	6.81	0.5930
Total Protein (mg/ml)	65.1	74.8	77.2	2.53	0.1022
Urea nitrogen (mg/L)	307.3 ^a	272.7 ^{ab}	248.1 ^b	10.5	0.0355
Glucose (mmol/L)	11.9	13.7	12.9	0.47	0.3421
Triglyceride (mmol/L)	21.6 ^b	27.8 ^a	27.0 ^a	1.2	0.022

Table 4. Effects of pancreatic enzymes on nutrient digestibility of weaned pigs

Item	NC	NCT1	NCT2	SEM	p-Value
Dry matter (%)	84.92	86.07	86.22	0.26	0.0519
Organic matter (%)	85.63 ^b	86.64 ^a	86.76 ^a	0.21	0.0158
Crude protein (%)	82.23 ^b	85.21 ^a	85.00 ^a	0.51	0.010
Crude fat (%)	79.08 ^b	84.65 ^a	84.30 ^a	0.91	0.0001
Calcium (%)	64.42 ^b	66.11 ^{ab}	68.14 ^a	0.67	0.043
Phosphorus (%)	65.64	66.52	68.66	0.64	0.1350

et al. 1957), therefore pigs showed better performance than negative control. A number of studies were launched in the recent years about single digestive enzymes supplementation in diets. The increase of apparent protein digestibility (Lin *et al.* 2007, Chen *et al.* 2009), the digestion improvement of starch (Richards *et al.* 2004) and digestibility increase of dry matter, organic matter and protein (Dierick and Decupere 2004) were observed by researchers in feeding trial. Generally, the anti-nutritive factors in feed are known to interfere with the digestibility of other macronutrients, thereby reducing feeding values. (Bell *et al.* 1983). Therefore non starch polysaccharide enzymes were generally combined with digestive enzymes. In the research of Li *et al.* (2010), middle and high level enzyme blends (amylase, protease and xylanase) addition both resulted in increased ($P < 0.05$) dry matter and crude protein digestibilities of non-extruded corn diets.

In conclusion, our results suggested that supplementation of pancreatic enzymes to corn-soybean diet has the ability to improve growth performance and nutrient digestibilities. Supplementation with 180U/kg trypsin, 300U/kg lipase and 420U/kg amylase cocktail allowed weaned pigs to achieve a better performance than the control group and the low level digestive enzymes group.

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