



## Effects of RelePro protease supplementation in low-protein diets on growth performance and diarrhea rate of weaned piglets

**Abstract:** The objective of this study was to evaluate the effects of RelePro protease (RelePro protease) supplementation in low-protein diets on growth performance of weaned piglets. A total of 150 weaned piglets ( $6.99 \pm 0.21$  Kg) were randomly divided into six groups. Treatments were as follows: Positive Control (PC, a basal diet, CP 19.0%), Negative Control (NC, the dietary crude protein level was reduced by 0.5% on the basis of the positive control group, CP 18.5%), and four RelePro protease supplementation groups (NC+E<sub>150</sub>, NC+E<sub>200</sub>, NC+E<sub>250</sub>, NC+E<sub>300</sub>, 150, 200, 250 and 300 mg/Kg RelePro protease supplementation in the low-protein diet, respectively). The experimental period is 28 days. The results showed that the NC group significantly reduced average daily gain ( $P < 0.05$ ) compared with PC group and NC+E<sub>200-300</sub> groups, and significantly increased feed-to-gain ratio ( $P < 0.05$ ) compared with NC+E<sub>250</sub>. Dietary RelePro protease supplementation in the low-protein diet could alleviate this problem, increase average daily gain and decrease feed-to-gain ratio. In conclusion, dietary RelePro protease supplementation in the low-protein diet improved the growth performance and diarrhea rate of weaned piglets, reduce the feed-to-gain ratio, especially 250 mg/Kg RelePro protease supplementation group.

**Keywords:** low protein; RelePro protease; weaned piglets; growth performance; diarrhea rate;

The world is affected by environmental pollution by rapid development of industries including the swine industry and the two most harmful contaminants are nitrogen and phosphorus. Some people reported that total nitrogen excretion was decreased by 8% for nitrogen intake decreasing by 1%. From an economic and environmental point of view, decreasing crude protein (CP) and supplementing an enzyme cocktail in a diet could be an effective strategy for the pig industry to reduce production cost and pollution. However, some studies have reported that low-protein diets could decrease growth performance of weaned piglets. Exogenous enzymes were expected to solve these problems. RelePro protease is a generic term for an enzyme that breaks down proteins. Supplementation of RelePro protease in diets can improve growth performance in animals. Therefore, the objective of this study was to evaluate the effects of RelePro protease supplementation in low-protein diets on growth performance and nutrient digestibility of weaned piglets.

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## 1 Materials and analysis

### 1.1 Experimental design

In the experiment, a total of 150 ternary hybrid weaned piglets (Duroc×Landrace×Large Yorkshire,  $6.99 \pm 0.21$  Kg) were randomly divided into 6 treatments, each treatment had 5 replicates, and each replicate had 5 piglets. Six treatments were as follows: Positive Control (PC, a basal diet, CP 19%), Negative Control (NC, the dietary crude protein level was reduced by 0.5% on the basis of the positive control group, CP 18.5%), and four RelePro protease supplementation groups (NC+E<sub>150</sub>, NC+E<sub>200</sub>, NC+E<sub>250</sub>, NC+E<sub>300</sub>, 150, 200, 250 and 300 mg/Kg RelePro protease supplementation in the low-protein diet, respectively), and the experimental period is 28 day

Table 1. Experimental design

Treatments	Diets	Dosages of RelePro RelePro protease (mg/Kg)
PC	PC	0
NC	NC	0
NC+E <sub>150</sub>	NC + RelePro RelePro protease	150
NC+E <sub>200</sub>	NC + RelePro RelePro protease	200
NC+E <sub>250</sub>	NC + RelePro RelePro protease	250
NC+E <sub>300</sub>	NC + RelePro RelePro protease	300

### 1.2 Diet design

The basal maize-soybean meal diet (Table 2) was formulated according to the nutrient requirements for the weaned piglets recommended by the Feeding Standard. RelePro protease (RelePro RelePro protease,  $1 \times 10^5$ U/g) is produced by ADDiCAN. The enzyme activity is defined as the enzyme amount requirement for hydrolyzing casein equivalent to release 1 µg tyrosine Folin-positive amino acids and peptides per minute under the conditions of temperature 37°C and pH value of 8.5. The amount of enzyme is a unit of enzyme activity U.

Table 2 Composition and nutrient levels of experimental diets (air-dry basis)

Ingredients, %	PC	NC
Corn	36.60	41.10



Animal Nutrition

Soybean meal,43% CP	26.50	21.36
Barely	5.00	5.00
Soybean oil	3.80	3.83
Fish meal	4.00	4.00
Glucose	3.00	3.00
Sweet whey powder	12.00	12.00
Monocalcium phosphate	0.72	0.82
Sodium chloride	0.18	0.18
Lactose	5.00	5.00
Limestone	1.14	1.14
L-lysine hydrochloride	0.48	0.64
L-threonine	0.20	0.28
L-tryptophan	0.68	0.95
DL-Methionine	0.27	0.29
Premix	0.43	0.41
Total	100.00	100.00
Calculated nutrient levels		
Digestible energy, MJ/kg	14.64	14.64
Crude protein, %	19.00	18.50
Calcium, %	0.71	0.71
Total phosphorus, %	0.43	0.43
Lysine, %	1.33	1.32
Methionine+cystine, %	0.87	0.85
Tryptophan, %	0.24	0.23
Threonine, %	0.90	0.89

### 1.3 Feeding management

Pigs were housed in a completely enclosed, temperature-controlled room containing 30 pens. Each pen was equipped with a stainless-steel nipple drinking fountain and a one-sided feeding hopper. The room temperature was set to  $28 \pm 1$  °C for the first week and was gradually decreased to 25 °C by the end of the experiment water and diets were provided ad libitum throughout the experimental period. Plenty of feed was placed in the hoppers to ensure feed was always available, and hoppers were checked daily to ensure ad libitum access and to minimize feed wastage.

### 1.4 Inspection indicators and methods

#### 1.4.1 Growth performance

Record the addition and loss of feed, settle the feed intake at each replication. Record the number of each replication piglet every day, accurately record the weight of the dead piglet, and observe the anatomy of the dead piglet. On the 28rd day of the experiment, piglet per replicate were fasted for 12



h and weighted. The body weights (BW) and feed intake (FI) were recorded to calculate the average daily gain (ADG), average daily feed intake (ADFI) and feed-to-gain ratio (F/G).

$$F/G = \text{piglet feed intake} / (\text{final weight} + \text{dead piglet weight} - \text{initial weight})$$

$$\text{Average feed intake} = \text{stage F/G} \times \text{piglet weight gain}$$

### 1.4.2 Diarrhea rate

Observe and record the health of the pigs every day. Observe the diarrhea at 9:00 and 17:00 every day, record the number and score of diarrhea piglets. All data are observed and recorded by the same person. The diarrhea score is based on Table 3, and it is judged as diarrhea when score is 2 or 3.

$$\text{Diarrhea rate/\%} = [\text{Total number of diarrhea piglets} / (\text{Number of piglets} \times \text{experimental days})] \times 100$$

Table 3 Diarrhea Scoring Standard

Diarrhea conditions	Fecal appearance	Moisture content	Score
Normal	Shaped or granular	< 70	0
Mild	Soft manure, can be formed	70~75	1
Moderate	Thick, shapeless, no separation of manure and water	75~80	2
Severe	Liquid, not shaped, separation of manure and water	> 80	3

### 1.5 Statistical analysis

Data for all the treatments was preliminarily statistic using Microsoft Excel, the SPSS 19.0 software was used for one-way analysis of variance (ANOVA) followed by Duncan’s multiple comparison test. Data are shown as the means and pooled SEM,  $P < 0.05$  indicates significant difference,  $0.5 < P < 0.1$  indicates significant difference trend.

## 2 Experimental results

It can be seen from Table 4 that the NC group significantly reduced average daily gain ( $P < 0.05$ ) compared with PC group and NC+E<sub>200-300</sub> groups, and significantly increased feed-to-gain ratio ( $P < 0.05$ ) compared with NC+E<sub>250</sub>. Dietary RelePro protease supplementation in the low-protein diet could alleviate this problem, increase average daily gain and decrease feed-to-gain ratio.

Table 4 Effects of RelePro protease supplementation in low-protein diets on growth performance and diarrhea rate of weaned piglets

Treatments	ADG(g)	ADFI(g)	F/G	Diarrhea rate (%)
PC	306.15 <sup>b</sup>	430.24	1.41 <sup>a</sup>	1.35
NC	293.28 <sup>c</sup>	420.18	1.43 <sup>a</sup>	2.35
NC+E <sub>150</sub>	303.35 <sup>bc</sup>	419.88	1.39 <sup>ab</sup>	1.45
NC+E <sub>200</sub>	308.42 <sup>b</sup>	426.35	1.38 <sup>ab</sup>	1.20
NC+E <sub>250</sub>	318.88 <sup>a</sup>	435.29	1.36 <sup>b</sup>	0.65
NC+E <sub>300</sub>	308.26 <sup>b</sup>	424.77	1.38 <sup>ab</sup>	1.05
SEM	2.10	3.14	0.08	0.12
<i>P</i> value	0.011	0.148	0.045	0.358

### 3 Summary

As a whole, our findings indicated that dietary RelePro protease supplementation in the low-protein diet improved average daily gain, decreased the feed-to-gain ratio and diarrhea rate of weaned piglets. Based on the results of this experiment, 250 mg/Kg RelePro protease supplementation dosage in the low-protein diet was the most appropriate.