Effects of RelePro protease supplementation in low-protein diets on performance of broilers

Abstract: The purpose of this experiment was to study the effects of protease (RelePro Protease) supplementation in low-protein diets on broiler performance. A single-factor experiment design was adopted and a total of 800 1-day-old Arbor Acres (AA) commercial broilers were randomly allotted to 5 dietary treatments with 8 replicate pens and 20 birds per pen in a 42-day experiment. Treatments included a positive basal control diet (PC group) and a negative control diet (NC group, the dietary crude protein level was reduced by 0.5% on the basis of PC group), and 3 NC diets supplemrnted with 100, 200 and 300 mg/kg of RelePro protease (NC+E_{100}, NC+E_{200}, NC+E_{300}).

The results showed that the average daily gain was significantly decreased in the NC group compared with the PC group and three RelePro protease supplementation groups ($P < 0.05$), but there was no significant difference between the PC group and three RelePro protease supplementation groups ($P > 0.05$); dietary RelePro protease supplementation (200-300 mg/Kg) in low-protein diet reduced feed-to-gain ratio compared with the PC/NC group ($P < 0.05$). In conclusion, production performance significantly decreased when dietary crude protein level reduced, dietary RelePro protease supplementation in low-protein diet can improve production performance of broilers, especially 200 mg/Kg RelePro protease was supplied in low-protein.

Keywords: broiler; low-protein diet; RelePro protease; growth performance

Protein is the main component of the feed formula which accounting for 50-55% of the total cost, and has become a key factor affecting the profit of feed and farming. At present, the high price of protein feed has significantly increased cost of farming, and reduced the profitability of farming. Excessive dietary protein will limit the digestion of protein feed by the animal itself, which not only result waste of raw materials, but also cause diarrhea and increase nitrogen emissions in the feces, increasing environmental pollution when undigested protein accumulates and ferments in the hind intestines of animals. Various breeding companies and feed companies vigorously promote and apply low-protein diet technology under the national call for "energy saving, emission reduction, ecological breeding", and improving the digestibility and utilization of protein feed in animals is one of the core

technologies in the application of low-protein diet. The application of RelePro protease is one of the effective measures to improve protein utilization.

1 Materials and analysis

1.1 Experimental design

A single-factor experiment design (Table 1) was adopted and a total of 800 1-day-old Arbor Acres (AA) commercial broilers with a body weight of 37.7 ± 0.3 g were used in a 42-day experiment. The broilers were randomly allotted to 5 dietary treatments with 8 replicate pens and 20 birds per pen. Five treatments were consisted of: Positive Control (PC, a basal diet), Negative Control (NC, the dietary crude protein level was reduced by 0.5% on the basis of the positive control group), NC+E_{100} (NC group diets with 100 mg/Kg protease supplementation), NC+E_{200} (NC group diets with 200 mg/Kg RelePro protease supplementation), NC+E_{300} (NC group diets with 300 mg/Kg protease supplementation).

Table 1. Experimental design

Treatments	CP level/%		RelePro Protease/(mg/Kg)
	1-21d	22-42d	
PC	21	19	0
NC	20.5	18.5	0
NC+E_{100}	20.5	18.5	100
NC+E_{200}	20.5	18.5	200
NC+E_{300}	20.5	18.5	300

1.2 Diet design.

The basal maize-soybean meal diet (Table 2) was formulated according to the nutrients requirements for the broilers recommended by the Feeding Standard of Chicken, except for crude protein (CP) level. RelePro Protease (RelePro Protease, 1×10^5U/g) is produced by ADDiCAN INC. One unit of RelePro protease activity is defined as the amount of enzyme which release 1 μg tyrosine Folin-positive amino acids and peptides per minute from hydrolyzing casein to at Ph 10.5 and temperature of 40°C.

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

Ingredients	1-21d		22-42d	
	Negative Control	Positive Control	Negative Control	Positive Control
Ingredients				
Corn	57.93	59.53	62.39	64.01
Soybean meal	35.16	33.76	30.2	28.81
Soybean oil	2.77	2.555	3.72	3.50
Limestone	1.16	1.175	1.13	1.13
$CaHPO_4$	1.84	1.84	1.6	1.60
DL-Met	0.21	0.2	0.13	0.12
L-Lys·HCl	0.1	0.11	0.07	0.09
Thr	0.04	0.04	0.03	0.02
NaCl	0.4	0.4	0.4	0.40
Compound vitamin [a]	0.03	0.03	0.03	0.03
Choline Chloride	0.16	0.16	0.1	0.10
Premix [b]	0.20	0.20	0.20	0.20
Total	100.00	100.00	100.00	100.00
Nutritional Level				
ME/（Mcal/kg）	12.34	12.34	12.76	12.76
CP	21.00	20.50	19.00	18.50
Lys	1.15	1.12	1.01	0.97
Met	0.50	0.49	0.40	0.39
Thr	0.81	0.79	0.73	0.71
Ca	1.00	1.00	0.90	0.90
AP	0.45	0.45	0.40	0.40

[a] provided per kg of diet: vitamin A 13 500 IU, vitamin D_3 3600 IU, vitamin E 33 IU, vitamin K 3 6 mg, vitamin B_1 4.5 mg, vitamin B_2 10.5 mg, vitamin B_6 6 mg, vitamin B_{12} 0.03 mg, calcium pantothenate 18 mg, niacin 60 mg, folic acid 1.8 mg, biotin 0.165 mg, choline 1000 mg,

[b] provided per kg of diet: Zn (as $ZnSO_4$) 80mg, Fe (as $FeSO_4 \cdot 7H_2O$) 100 mg, Cu (as $CuSO_4 \cdot 5H_2O$) 8 mg, Mn (as $MnSO_4$) 120 mg, Se (as $Na_2SeO_3 \cdot 5H_2O$) 0.3 mg, I (as KI) 0.7 mg.

1.3 Animals and management

The experimental animals were raised in the replicate cage. The experimental house and cages were cleaned and disinfected with 1% sodium hydroxide solution for 24 hours before feeding, then rinsed with tap water. Experimental house, cages, feed pans, buckets and fumigation and disinfection of drinking water equipment were disinfected with Formalin and Potassium permanganate (30 ml formalin, 15 g potassium permanganate per cubic meter) for 48 hours., the feeding started after the house was ventilated for five days.

The broilers were reared according to standard routine practices. The birds were housed in stainless steel battery cages on concrete floor covered with clean rice bran and hulls. Temperature was

gradually decreased from 33°C at chick arrival to 24°C on d 22, and thereafter kept constant to trial termination on d 42. The relative humidity of the house be kept at 55%~65%. Light of 24 h/day was provided via fluorescent lights. The broilers were given *ad libitum* access to feed and water. The routine immunization program for immunization was performed.

1.4 Growth performance

Record the addition and loss of feed, settle the feed intake at each stage. Record the number of each repeated chicken every day, accurately record the weight of the dead chickens, and observe the anatomy of the dead chickens. On the 43rd day of the experiment, broiler were weighted after 12 h fasting per replicate. The average daily gain (ADG), average daily feed intake (ADFI) and feed-to-gain ratio (F/G) were calculated recorded to the body weights (BW) and feed intake (FI).

F/G = stage chicken feed intake / (stage final weight + dead chicken weight-stage initial weight)

Average feed intake = stage F/G × stage chicken weight gain

Mortality rate (%) = (number of dead chickens + number of eliminated chickens)/total number of chickens × 100%.

1.5 Statistical analysis

Data for all the treatments was preliminarily counted 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 were shown as the means and pooled SEM, $P < 0.05$ indicates significant difference, and $0.5 < P < 0.1$ indicates significant difference trend.

2 Experimental results

2.1 Growth performance

Table 3 Effects of RelePro protease supplementation in low-protein diets on performance of broilers from 1 to 42 days

Treatment	ADG(g)	ADFI(g)	F/G
PC	57.69[a]	103.59	1.80 [ab]
NC	53.72[b]	98.92	1.84 [a]
NC+E_{100}	56.76[a]	103.34	1.82 [ab]
NC+E_{200}	58.32[a]	103.99	1.78 [b]

NC+E_{300}	57.43[a]	102.98	1.79 [b]
SEM	0.21	0.98	0.03
P-Value	0.024	0.354	0.043

It can be seen from Table 3 that dietary RelePro protease supplementation significantly affected average daily gain and feed-to-gain ratio ($P < 0.05$) of broilers during the whole growth period. The average daily gain was significantly decreased in the NC group compared with the PC group and three RelePro protease supplementation groups ($P < 0.05$), but there was no significant difference between the PC group and three RelePro protease supplementation groups ($P > 0.1$). Dietary RelePro protease supplementation (200-300 mg/Kg) in low-protein diet can reduce feed-to-gain ratio compared with the PC/NC group ($P < 0.05$). It can be seen that from the performance index of the whole period, 200 mg/Kg RelePro protease supplementation in low-protein diet can obtain better production performance to other groups.

3 Summary

Reducing dietary crude protein level can significantly reduce average daily gain and increase feed-to-gain ratio, thus reduce production performance of broilers; dietary RelePro protease supplementation in low-protein diet can increase the average daily gain and decrease feed-to-gain ratio, thereby improve production performance of broilers and alleviate the adverse effects on production performance caused by the reduction of dietary crude protein level, especially 200 mg/Kg RelePro protease supplementation in low-protein diet can obtain better production performance.