Effects of different dietary RelePro protease supplementation dosage on performance and ammonia emissions of broilers

Abstract: The purpose of this experiment was to study the effects of dietary protease (RelePro) supplementation dosages on broiler performance and excreta ammonia emissions. A single-factor experiment design was adopted and a total of 960 1-day-old Arbor Acres (AA) commercial broilers were randomly allotted to 6 dietary treatments (0, 50, 75, 100, 150, and 200 mg/Kg RelePro protease supplementation, respectively) with 8 replicate pens and 20 birds per pen in a 42-day experiment. The results showed that dietary RelePro protease supplementation significantly affected average daily gain ($P < 0.01$) and reduced the feed-to-gain ratio ($P = 0.071$) of broilers, but there was no significant difference in the average daily feed intake during all groups ($P > 0.05$). Average daily gain in PC+$E_{75\text{-}150}$ groups especially in PC+E_{100} group significantly increased compared with the PC group ($P < 0.05$). Dietary RelePro protease supplementation significantly decreased excreta NH_3 emissions ($P < 0.01$), excreta NH_3 emissions in PC+$E_{75\text{-}200}$ groups were higher than those in PC group and PC+E_{50} group ($P < 0.05$). In conclusion, broliers have better performance and less ammonia emissions compared with the PC group when dietary RelePro protease (75-150 mg/Kg RelePro protease supplementation) was supplemented in the diets. the production performance was the best, and the NH_3 release is lowest in the 100 mg/Kg RelePro protease supplementation group.

Keywords: broiler; RelePro protease; growth performance; ammonia emissions

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. The application of protease is one of the effective measures to improve protein utilization and reduce ammonia emissions.

1 Materials and analysis

1.1 Experimental design

A single-factor experiment design (Table 1) was adopted, and a total of 960 1-day-old Arbor Acres (AA) commercial broilers with a body weight of 42.4±0.5g were used in a 42-day experiment. The broilers were randomly allotted to 6 dietary treatments (0, 50, 75, 100, 150, and 200 mg/Kg RelePro protease supplementation, respectively) with 8 replicate pens and 20 birds per pen.

Table 1. Experimental design

Treatments	RelePro Protease/(mg/Kg)
PC	0
PC+E_{50}	50
PC+E_{75}	75
PC +E_{100}	100
PC +E_{150}	150
PC+E_{200}	200

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. Protease (RelePro 1×10^5U/g) is produced by ADDiCAN. One unit of 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)

Items / %	1-21d	22-42d
Ingredients		
Corn	54.24	56.03
Soybean oil	1.32	2.84
Soybean meal	27.38	14.51
Cotton Seed meal	3.00	6.00
Rapeseed meal	3.00	6.00
Corn DDGS	4.00	8.00
Corn Gluten Meal	2.50	2.50
NaCl	0.30	0.30
$CaHPO_4$	1.38	0.88
Limestone	1.60	1.64
L-Lys·HCl	0.28	0.42
DL-Met	0.11	0.11

Cys	0.07	0.02
Choline Chloride (60%)	0.20	0.15
Premix [a]	0.50	0.50
Zeolite	0.11	0.10
Total	100.00	100.00
Nutritional Level		
ME/（MJ/kg）	2.95	3.05
CP	21.49	19.50
Lys	1.15	1.05
Met	0.45	0.42
Met-Cys	0.89	0.80
Thr	0.85	0.72
Try	0.29	0.23
Ca	1.00	0.90
Total P	0.67	0.61
AP	0.45	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, 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 (30ml formalin, 15g potassium permanganate per cubic meter) for 48 hours. After the house was ventilated for five days, the feeding was started.

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 should 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 Excreta ammonia emissions analysis

On d 42, the fresh excreta samples of mixtures of feces and urine were collected from the bottom tray of each cage to determine the ammonia emission. Ammonia emission of each excreta sample was determined as described by Moore, Daniel, Edwards, & Miller, (1996). In brief, the total sampled manure from each cage was thawed and then homogenized, after which 100 g subsamples in triplicate were placed in 2-L plastic containers equipped with air inflows and outflows. The samples were incubated at 22 °C and ammonia-free air (the air passed through two consecutive 1 *M* HCl traps and one trap containing deionized water) was continuously passed through each chamber. The ammonia volatilized from the excreta was trapped in two consecutive boric acid traps containing 100 mL of 0.32 *N* H_3BO_4 solution each. At 24-h post-incubation, the traps were titrated with 0.10 *N* HCl to determine the ammonia content. The ammonia emission (NH_3 release) was expressed as milligrams per 100 g of excreta.

1.6 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 are 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 and ammonia emission

Table 3 Effects of different dietary RelePro protease supplementation dosages on performance

and ammonia emission of excreta in broilers

Treatment	ADG(g)	ADFI(g)	F/G	NH_3 release (mL/100 g fecal)
PC	70.98[c]	121.81	1.72	3.5 [a]
PC+E_{50}	71.54[bc]	122.69	1.72	3.0 [a]
PC+E_{75}	72.07[ab]	123.45	1.71	2.0[b]
PC+E_{100}	73.40[a]	123.74	1.69	0.9 [c]
PC+E_{150}	73.38[a]	124.62	1.70	1.2 [c]
PC+E_{200}	71.33[bc]	121.52	1.70	1.1 [c]
SEM	0.30	1.06	0.01	0.02
P Value	<0.01	0.130	0.071	<0.01

It can be seen from Table 3 that dietary RelePro protease supplementation significantly affected average daily gain ($P < 0.01$) and had a tendency to reduce the feed-to-gain ratio ($P = 0.071$) of broilers, but the average daily feed intake was no significant difference during all groups ($P > 0.05$). Average daily gain in PC+$E_{75\text{-}150}$ groups significantly increased compared with the PC group ($P < 0.05$), especially PC+E_{100} group. Dietary RelePro protease supplementation significantly decreased excreta NH_3 emissions ($P < 0.01$), excreta NH_3 emissions in PC+$E_{75\text{-}200}$ groups were higher than those in PC group and PC+E_{50} group ($P < 0.05$).

3 Summary

Dietary RelePro protease supplementation (75-150 mg/Kg RelePro protease supplementation) can obtain better performance and less ammonia emissions compared with the PC group, especially 100 mg/Kg RelePro protease supplementation group has the best production performance and lowest NH_3 release.