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Effects of dietary protease supplementation on behaviour, slaughter performance, meat quality and immune organ indices of broilers

Xixi Li[1], *Xinxin Wang*[1], *Yi Lv*[1], *Wenfeng Ma*[1,2], *Xiaohong Wu*[1], *Wenrui Zhen*[1], *Furong Zhao*[1]*

[1]*College of Animal Science and Technology, Henan University of Science and Technology, Luoyang, P.R. China*

[2]*Anhui Province Key Laboratory of Animal Nutrition Regulation and Health, Anhui, P.R. China*

**Corresponding author: zhaofur@haust.edu.cn*

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Abstract: This study was conducted to investigate the effects of protease supplementation on the behaviour, slaughter performance, meat quality, and immune organ indices of broilers. A total of 240 one-day-old Arbor Acres broiler chickens were randomly allocated to four treatments, with six replicates per treatment and 10 broilers per replicate. Chickens were fed corn-soybean meal basal diets supplemented with 0, 4, 8, and 12 IU/g of protease. Compared with 0 IU/g of protease supplementation, 12 IU/g of protease increased the average and minimum time of feeding and drinking behaviour ($P < 0.05$); increased the average, maximum, and minimum time of preening behaviour ($P < 0.05$); and decreased feeding, walking, and stretching frequency ($P < 0.05$). pH_{24h} of breast and thigh muscles had improved ($P < 0.05$). The shear force of the breast muscle and the cooking loss of the thigh muscle had decreased ($P < 0.05$). Compared with 0 IU/g of protease, 4 IU/g of protease increased the average and minimum time of lying behaviour ($P < 0.05$) and decreased stretching, scratching, and aggressive frequency ($P < 0.05$). The shear force of the thigh muscle and spleen index had enhanced ($P < 0.05$). Protease can be used as an additive in a broiler diet for production.

Keywords: enzyme; normal and abnormal behaviour; carcass traits; meat characteristics; immunity; poultry

Attacking, scratching, and feather pecking lead to injury, feather damage, reduced meat or egg production efficiency, and even death in severe cases. On the one hand, people want to acquire high-quantity and high-quality meat from livestock and poultry; the yield and quality of meat obtained after slaughter are often less than expected (Duarte and Gionbelli 2021). Although the breeding of broilers has greatly increased growth rate and meat production, muscle structure, and metabolism-related defects or myopathy caused by anaerobic metabolism, such as pale, soft, and exudative, deep, pectoral, and myopathy, spaghetti meat, white striping (WS), and wooden breast (WB), often occur in production,

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which affects the meat flavour, juiciness, taste, and tenderness. On the other hand, the natural resistance and immunity of poultry are limited; the poultry cannot resist the proliferation and invasion of pathogenic microorganisms (Ghosh et al. 2011). By interfering with the antigen present, reducing or blocking the development of immune antibodies, and affecting the function of immune cells, immunosuppressive disorders could harm immune organs and reduce the normal immunological response (Yang et al. 2020). The ban of antibiotic growth promoters (AGPs) might cause more diseases related to the early immune system. By changing the immunological status of broiler chickens, antibiotics can be utilised at subtherapeutic levels in poultry production to enhance growth and safeguard birds' health (Lee et al. 2012). Therefore, finding a green and efficient feed additive for the healthy production of broilers is necessary.

Protease, also known as peptidase and proteinase, is a general term for enzymes that hydrolyse protein substrates into peptides and amino acids (Matkawala et al. 2021). Numerous studies have shown that proteases, including alkaline protease, acid protease, and neutral protease, play an active role by improving energy and amino acid utilisation in the diet, stimulating protein digestion, enhancing the integrity of the intestinal tract, promoting the activity of digestive enzymes, and increasing the total tract retention (Kamel et al. 2015; Zuo et al. 2015; Stefanello et al. 2016; Cowieson et al. 2018). In recent years, proteases have been widely used in poultry, and they have presented a series of positive effects on improving body weight, feed intake, and feed conversion rate of broilers (Jabbar et al. 2020). Under coccidial infection, the dietary supplementation of protease greatly improves the body weight of broilers, alleviating the adverse effects of the disease on growth (Peek et al. 2009). Broilers fed with protease show an improvement in carcass yield without alteration in fat deposition rates (Law et al. 2017), and the crude protein content of meat increases (Sarica et al. 2020). Xu et al. (2017) reported that diets supplemented with protease had increased the breast meat yield of broilers.

At present, no study has focused on the effect of protease supplementation on broiler behaviour. Thus, the present study aims to perform a preliminary exploration of the effect of protease on broiler behaviour and to evaluate the effects of protease on the slaughter performance, meat quality, and immune organ index of broilers, thereby providing a reference for the scientific improvement of broiler health and production.

MATERIAL AND METHODS

The experiment was conducted in Luoyang, Henan Province, China. The experimental animal protocol was approved by the Animal Care Committee (case no. 2018009).

The proteolytic enzyme product used in this study was *Bacillus subtilis*-derived alkaline protease (250 000 IU/g) produced by Henan New Yangshao Biological Science and Technology Co., Ltd, China. The Chinese national standard GB/T 23527-2009 stipulates that the activity of microbial protease is determined by the Folin method or ultraviolet spectrophotometry. Solid enzyme powder (1 g or 1 ml of liquid enzyme) hydrolyses casein to produce 1 μg of tyrosine in 1 min under specific temperature and pH conditions; this enzymatic activity unit is expressed as IU/g or IU/ml.

Experimental design, animals, and diets

A total of 240 one-day-old Arbor Acres broiler chicks with similar body conditions were purchased from a local hatchery. They were weighed on arrival and randomly allocated to four treatments, with six replicates per treatment and 10 broilers per replicate. Broilers were fed corn-soybean meal basal diets as the control. In other treatment groups, broilers were fed basal diets supplemented with protease at concentrations of 4, 8, and 12 IU/g. The experiment lasted for 42 days, and it was divided into two periods, namely, starter (from 1 to 21 days of age) and finisher (from 22 to 42 days of age).

All broilers were raised in 24 wire cages with dimensions of 950 mm × 900 mm × 400 mm (10 broilers each). Diets were formulated to meet NRC (1994) nutrient recommendation (Table 1). Adequate cleaned and sterilised feeders and drinkers were prepared, and broilers were allowed food and water ad libitum. The room temperature was set to 33–35 °C in the first week. This temperature was gradually reduced by 2 °C to 3 °C weekly until the final temperature of 25 °C was reached,

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Table 1. Composition and nutrient levels of the basal diets (air-dry basis)

Ingredients (%)	Starter (1–21 days)	Grower (22–42 days)	Nutrient levels[3]	Starter (1–21 days)	Grower (22–42 days)
Corn	60.8	62.6	ME (MJ/kg)	12.6	13.1
Soybean meal	30.0	27.5	crude protein (%)	21.0	19.0
Corn gluten meal	3.00	2.00	ether extract (%)	4.50	6.95
Soybean oil	2.00	4.00	crude fibre (%)	2.20	2.20
Limestone	1.60	1.60	crude ash (%)	5.60	4.49
$CaHPO_4$	1.30	1.30	calcium (%)	1.02	1.00
NaCl	0.30	0.30	total phosphorus (%)	0.55	0.53
Lysine	0.35	0.20	digestible tryptophan (%)	0.24	0.22
Methionine	0.20	0.10	digestible methionine (%)	0.52	0.39
Mildew preventive[1]	0.08	0.08	digestible lysine (%)	1.27	1.08
Antioxidant	0.06	0.06	digestible threonine (%)	0.78	0.72
Premix[2]	0.23	0.23			
Total	100	100			

[1]Sodium propionate

[2]Premix provided the following per kg of the diet: vitamin A, 6 500 IU; vitamin D_3, 3 000 IU; vitamin E, 80 IU; vitamin K, 5 mg; vitamin B_1, 4 mg; vitamin B_2, 5.5 mg; vitamin B_6, 5 mg; niacin, 30 mg; pantothenic acid, 12 mg; folic acid, 1 mg; Mn, 80 mg; Fe, 110 mg; Cu, 12 mg; Zn, 70mg; I, 0.4 mg; Se, 0.2 mg

[3]Calculated values

after which the temperature was kept constant until the end of the trial. Good ventilation was maintained in the house, and a photoperiod system of 16 h light and 8 h dark was used during breeding. All broilers received routine vaccination, and no antibiotic or *Coccidioides* was used.

Behaviour

Behavioural indicators were determined by target sampling (Eklund and Jensen 2011). Four target broilers per replicate (24 broilers per treatment) were randomly labelled with biological dyes. One camera was set up in each replicate to record and observe the behaviour of broilers from 21 days of age to the end, and the target broilers were photographed continuously for 24 h on the first three days of every week. The behavioural analysis system (The Observer XT 12; Noldus, Wageningen, Netherlands) was used to analyse the behaviour after scan samplings were converted by a computer. The behavioural observation was performed for 24 h/replicate/day. The frequency, average time, maximum time, and minimum time of each target broiler behaviour were calculated. The frequency refers to the number of times that a certain behaviour occurred per unit time (1 h); the average time (s) refers to the average duration of certain behaviour per unit time (1 h), and the maximum time and minimum time (s) indicate that certain behaviour could continue to occur in a unit of time (1 h). The behavioural category and description of broilers were in accordance with those mentioned in Riber et al. (2017) and Senaratna et al. (2015) (Table 2).

Slaughter performance

At 42 days of age, one broiler per replicate (six broilers per treatment) was randomly selected and dissected after killing by carotid artery bloodletting. Feeding and drinking were prohibited for 12 h before slaughter. Slaughter performance, which includes the percentage of dressing yield, eviscerated yield, half-eviscerated yield, breast muscle, thigh muscle, and abdominal fat, was determined. The percentage of dressing, eviscerated, and half-eviscerated yields was expressed as percentages of live body weight. The breast muscle, thigh muscle, and abdominal fat, including the abdomen and outside of the stomach, were separated from the slaughtered body and expressed as percentages of eviscerated carcass weight.

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Table 2. Categories and description of broilers behaviour

Behaviour	Definition
Feeding	Having the head in (s)triking with the beak at the feed in the feeder.
Drinking	Having the beak in touch with the drinker.
Walking	Moving forward by taking one or more steps.
Standing	The bird is upright; both legs stretched, maintaining the body elevated from the floor while not engaged in other activities.
Lying	The bird's body is resting on the floor with at least one leg stretched to the side.
Preening	Beak related behaviour that beak touches the plumage of the bird itself while lying or standing.
Stretching	Extending one wing and one leg at the same side of the body.
Scratching	The bird is scratching floor with its feet.
Pecking	Pecking in the litter and ingesting litter materials.
Aggressive	Hopping toward another bird, frontal threatening, leaping toward another bird, may involve kicking, wing-flapping, and aggressive pecking.

Meat quality

The indicators of meat quality included pH_{45min}, pH_{24h}, shear force, drip loss, and cooking loss of breast and thigh muscles after the slaughter at 42 days of age.

The samples from the left side of the breast and thigh muscles from each replicate were cut into pieces with dimensions of 5 cm × 1 cm × 1 cm. A portable pH meter (PHBF-260; Cany Precision Instruments Co., Ltd, P.R. China) was used to measure pH_{45min} and pH_{24h} of breast and thigh muscles three times, and the measurement site of each sample was kept consistent. A meat tenderness tester (RH-N50; Guangzhou Runhu Instruments Co., Ltd, P.R. China) was used to determine shear force. The cutting knife was perpendicular to the direction of the muscle fibre, and the average value of the three points was calculated.

The right side of the breast and thigh muscle samples from each replicate was cut into pieces with dimensions of 5 cm × 1 cm × 1 cm. After weighing, the samples were hung in a preservation box with a thin string. They were refrigerated at 4 °C for 24 h. The juices on the surface were gently wiped off with filter paper, and then the sample was reweighed to calculate the drip loss percentage. Samples from the right side of the breast and thigh muscles (approximately 30 g) were placed in a water bath at 80 °C for approximately 15 minutes. The surface moisture was wiped off with filter paper when the room temperature was reached. Finally, the samples were reweighed to calculate the percentage of cooking loss.

Immune organ indices

The thymus, spleen, and bursa of Fabricius were excised from the slaughtered body and weighed individually. Immune organ indices were calculated as the percentage of immune organs to live body weight of animals.

Statistical analysis

The present data were processed by using Excel 2016. SPSS v20.0 (IBM Inc., NY, USA) was used for statistical analysis. Behavioural data were analysed for variance using the Kruskal-Wallis H-test. The differences in slaughter performance, meat quality, and immune organ indices were detected by one-way ANOVA. Duncan's test was used for significant difference analysis among treatments. The analysis results were expressed as mean values and standard errors, and the significance level was set at $P < 0.05$.

RESULTS

Behaviour

The effects of dietary protease supplementation on normal behaviour are presented in Table 3. Compared with 0 IU/g of protease supplementation, 12 IU/g of protease had the lowest feeding frequency ($P < 0.05$). The average and minimum time were up-regulated by 26.72 and 17.89 s ($P < 0.05$), respec-

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Table 3. Effects of dietary protease supplementation on the normal behaviour of broilers

Items		Treatment			
		1	2	3	4
Feeding	frequency/time	7.61 ± 3.28^{a}	6.73 ± 2.17^{ab}	5.96 ± 1.97^{ab}	4.79 ± 2.34^{b}
	the average time (s)	69.1 ± 32.0^{b}	75.8 ± 21.2^{ab}	75.7 ± 27.4^{ab}	95.8 ± 30.6^{a}
	maximum time (s)	165 ± 56.8	174 ± 48.7	162 ± 45.2	189 ± 55.0
	minimum time (s)	21.8 ± 15.5^{b}	20.9 ± 13.2^{b}	29.5 ± 19.5^{ab}	39.7 ± 24.4^{a}
Drinking	frequency/time	3.79 ± 1.28	3.61 ± 1.51	3.69 ± 1.54	2.88 ± 1.93
	the average time (s)	20.5 ± 7.64^{b}	20.1 ± 10.61^{b}	16.8 ± 3.49^{b}	28.8 ± 14.0^{a}
	maximum time (s)	39.2 ± 13.3^{ab}	37.1 ± 18.6^{ab}	31.1 ± 12.4^{b}	42.4 ± 14.8^{a}
	minimum time (s)	9.44 ± 4.53^{b}	9.37 ± 5.28^{b}	7.87 ± 1.86^{b}	19.8 ± 13.8^{a}
Walking	frequency/time	33.2 ± 11.7^{a}	29.9 ± 14.8^{a}	28.8 ± 9.58^{ab}	20.3 ± 11.7^{b}
	the average time (s)	7.38 ± 2.51	8.33 ± 3.87	7.42 ± 1.89	7.27 ± 2.24
	maximum time (s)	26.5 ± 11.1	29.0 ± 15.9	23.8 ± 9.85	24.2 ± 9.98
	minimum time (s)	2.15 ± 0.88	2.31 ± 1.13	2.27 ± 0.89	2.24 ± 0.81
Standing	frequency/time	21.5 ± 11.3	19.0 ± 9.87	21.2 ± 8.96	18.9 ± 12.6
	the average time (s)	11.5 ± 5.32	11.9 ± 5.23	13.8 ± 7.78	12.6 ± 5.28
	maximum time (s)	42.3 ± 19.5	34.3 ± 11.9	48.6 ± 29.6	44.4 ± 16.9
	minimum time (s)	2.69 ± 1.41	2.88 ± 1.54	2.90 ± 1.13	2.47 ± 1.24
Lying	frequency/time	19.8 ± 4.97^{ab}	21.5 ± 9.77^{a}	18.1 ± 3.98^{ab}	14.8 ± 5.48^{b}
	the average time (s)	227 ± 91.2^{b}	330 ± 102.8^{a}	290 ± 91.7^{ab}	252 ± 116.2^{b}
	maximum time (s)	784 ± 182.5	877 ± 149.6	830 ± 171.3	869 ± 234.4
	minimum time (s)	27.8 ± 51.5^{b}	116 ± 119.3^{a}	64.9 ± 65.5^{ab}	29.8 ± 54.4^{b}
Preening	frequency/time	9.68 ± 3.53	11.1 ± 4.48	11.3 ± 4.07	9.37 ± 3.12
	the average time (s)	18.7 ± 5.35^{b}	22.0 ± 12.5^{b}	19.0 ± 5.53^{b}	30.6 ± 11.1^{a}
	maximum time (s)	55.9 ± 18.3^{b}	69.6 ± 33.7^{ab}	56.5 ± 24.1^{b}	87.8 ± 33.6^{a}
	minimum time (s)	4.52 ± 1.83^{b}	5.75 ± 3.52^{ab}	5.44 ± 1.12^{ab}	7.53 ± 5.14^{a}
Stretching	frequency/time	4.23 ± 2.14^{a}	2.52 ± 0.93^{b}	3.19 ± 1.49^{ab}	2.71 ± 1.82^{b}
	the average time (s)	3.49 ± 1.27	5.06 ± 2.34	4.35 ± 2.17	3.93 ± 1.38
	maximum time (s)	6.29 ± 3.48	8.95 ± 5.53	7.11 ± 5.08	6.07 ± 2.79
	minimum time (s)	2.19 ± 0.75	2.65 ± 1.45	2.49 ± 1.21	2.37 ± 0.96

Treatment 1 = control, supplemented with 0 IU/g of protease; Treatment 2 = supplemented with 4 IU/g of protease; Treatment 3 = supplemented with 8 IU/g of protease; Treatment 4 = supplemented with 12 IU/g of protease

[a,b]The same row with different superscripts means significant difference ($P < 0.05$)

tively, whereas no significant difference was found for the maximum time ($P > 0.05$). The frequency of drinking behaviour was not significantly different among the treatments ($P > 0.05$), whereas the average time with the addition of 12 IU/g of protease significantly increased by 8.27, 8.66, and 11.96 s compared with the other treatments ($P < 0.05$). In addition, the minimum time increased by 10.33, 10.40, and 11.90 s ($P < 0.05$). The frequency of walking behaviour declined linearly with the increase of protease concentration in the basal diet, and 12 IU/g of protease significantly decreased the frequency of walking behaviour by 12.84 and 9.53 times compared with 0 and 4 IU/g ($P < 0.05$), respectively. Protease had no effect on the average time, maximum time, and minimum time of walking behaviour ($P > 0.05$). No significant difference in average time, maximum time, minimum time, and frequency of standing behaviour was found among the treatments ($P > 0.05$). The lying frequency of the treatment fed with 4 IU/g of protease was higher than that of the treatment fed with 12 IU/g ($P < 0.05$). The average time was significantly improved by 103.89 and 78.12 s ($P < 0.05$), and the minimum time was significantly improved by 88.05 and 86.09 s in the 4 IU/g compared with 0 and 12 IU/g treatment ($P < 0.05$),

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respectively. The maximum time of lying behaviour was not significantly different among the treatments ($P > 0.05$). The frequency of preening behaviour was not significantly different among the treatments ($P > 0.05$), whereas the average time at 12 IU/g of protease supplementation was significantly increased by 11.93, 8.61, and 11.63 s compared with the other treatments ($P < 0.05$). The maximum time at 0 and 8 IU/g of protease was significantly lower than that at 12 IU/g ($P < 0.05$), and the minimum time at 0 IU/g was significantly lower than that at 12 IU/g ($P < 0.05$). The frequency of stretching behaviour at 0 IU/g was significantly higher than that at 4 and 8 IU/g ($P < 0.05$), but the average, maximum, and minimum times were not significantly different ($P > 0.05$).

The effects of dietary protease supplementation on the frequency of abnormal behaviour are presented in Figure 1. The treatment fed with protease showed decreased frequency of scratching behaviour. The frequency of scratching behaviour in each treatment was 2.79, 1.35, 1.59, and 1.93 times. Notably, treatment with 4 and 8 IU/g of protease decreased the scratching frequency compared with 0 IU/g ($P < 0.05$). A downward trend in pecking behaviour was observed, although the frequency of pecking behaviour had no significant difference among the treatments ($P > 0.05$). The frequency of aggressive behaviour was 0.66, 0.10, 0.42, and 0.34 times. Supplemented with 4 IU/g of protease resulted in the lowest aggressive frequency ($P < 0.05$).

Slaughter performance

The slaughter performance parameters at 42 days of age are presented in Table 4. Dietary supplementation with protease had no effect on the percentage of dressing yield, eviscerated carcass, half-eviscerated carcass, breast muscle, thigh muscle, and abdominal fat on day 42 ($P > 0.05$).

Meat quality

The meat quality characteristics of breast and thigh muscles of broilers at 42 days of age are presented in Table 5. The value of pH_{45min} and drip loss of breast and thigh muscles were not significantly different among the treatments ($P > 0.05$). Compared

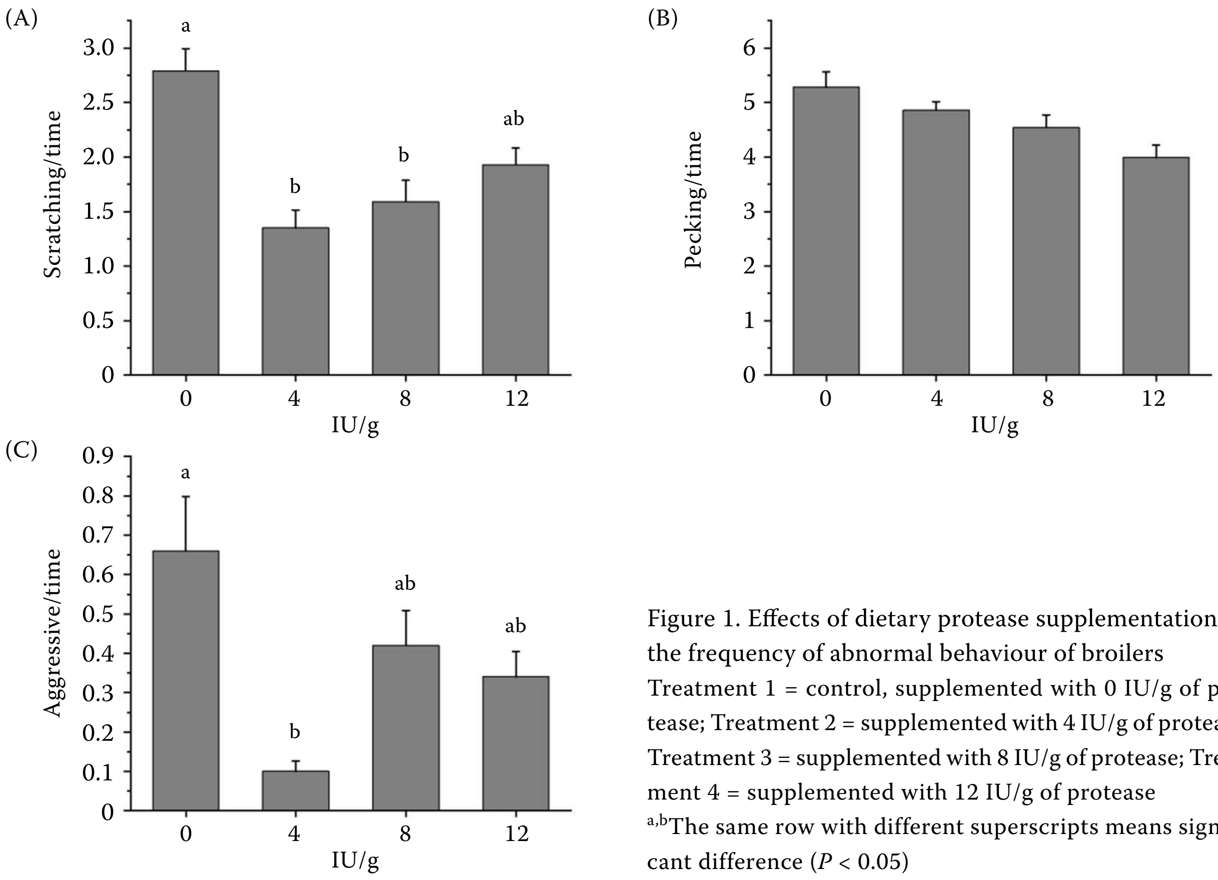

Figure 1. Effects of dietary protease supplementation on the frequency of abnormal behaviour of broilers
Treatment 1 = control, supplemented with 0 IU/g of protease; Treatment 2 = supplemented with 4 IU/g of protease; Treatment 3 = supplemented with 8 IU/g of protease; Treatment 4 = supplemented with 12 IU/g of protease
[a,b]The same row with different superscripts means significant difference ($P < 0.05$)

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Table 4. Effects of dietary protease supplementation on the slaughter performance (%) of broilers at 42 days

Items	Treatment			
	1	2	3	4
Live body weight (g)	1 564 ± 154	1 560 ± 187	1 539 ± 91.5	1 549 ± 240
Dressing yield	89.8 ± 1.24	89.8 ± 0.98	90.5 ± 1.90	89.8 ± 0.70
Eviscerated carcass	69.5 ± 1.69	68.6 ± 1.45	68.5 ± 1.63	69.2 ± 0.87
Half-eviscerated carcass	83.4 ± 1.26	82.5 ± 1.76	82.2 ± 1.74	82.5 ± 0.53
Breast muscle	27.3 ± 2.88	27.0 ± 1.72	27.1 ± 1.89	26.2 ± 2.60
Thigh muscle	24.9 ± 5.31^{ab}	22.1 ± 2.60^{b}	26.4 ± 1.40^{a}	25.6 ± 2.10^{ab}
Abdomen fat	0.94 ± 0.27	0.94 ± 0.14	1.15 ± 0.47	1.19 ± 0.42

Treatment 1 = control, supplemented with 0 IU/g of protease; Treatment 2 = supplemented with 4 IU/g of protease; Treatment 3 = supplemented with 8 IU/g of protease; Treatment 4 = supplemented with 12 IU/g of protease
^{a,b}The same row with different superscripts means significant difference (P < 0.05)

with 0 IU/g of protease, 12 IU/g of protease significantly upregulated the value of pH_{24h} of breast and thigh muscles (P < 0.05). The shear force of breast muscle and the cooking loss of thigh muscle were significantly reduced (P < 0.05). Dietary inclusion of 4 IU/g of protease significantly resulted in a high shear force of thigh muscle compared with 0 IU/g (P < 0.05). The cooking loss of breast and thigh muscles was low when 8 IU/g of protease was added to the diet compared with 0 IU/g (P < 0.05).

Immune organ indices

The thymus, spleen, and bursa of Fabricius indices of broilers at 42 days of age are presented in Figure 2. No significant differences in the thymus and bursa of Fabricius indexes were found among the treatments (P > 0.05). The spleen index of broilers receiving dietary supplemented with 4 IU/g of protease was significantly higher than 0 IU/g (P < 0.05).

DISCUSSION

Behaviour

Maintaining behaviour sustains animals' physiological balance, such as feeding, drinking, resting, and comfortable behaviour, such as those associated with feather care and activity patterns (Appleby et al. 2004). In the present study, broilers

Table 5. Effects of dietary protease supplementation on the meat quality of broilers at 42 days

Items		Treatment			
		1	2	3	4
Breast muscle	pH_{45min}	6.83 ± 0.20	7.03 ± 0.14	6.99 ± 0.26	6.85 ± 0.28
	pH_{24h}	5.90 ± 0.10^{b}	5.87 ± 0.04^{b}	5.86 ± 0.14^{b}	6.25 ± 0.15^{a}
	shear force (kg/f)	27.3 ± 4.24^{a}	25.7 ± 2.31^{a}	22.2 ± 9.54^{ab}	17.6 ± 4.04^{b}
	drip loss (%)	4.24 ± 1.17	4.01 ± 0.84	3.51 ± 0.61	4.31 ± 1.22
	cooking loss (%)	22.2 ± 4.40^{a}	19.3 ± 3.67^{ab}	16.6 ± 3.76^{b}	21.2 ± 3.27^{ab}
Thigh muscle	pH_{45min}	6.76 ± 0.33	7.00 ± 0.51	7.07 ± 0.19	6.98 ± 0.27
	pH_{24h}	6.17 ± 0.21^{b}	6.37 ± 0.05^{b}	6.23 ± 0.13^{b}	6.70 ± 0.22^{a}
	shear force (kg/f)	31.4 ± 8.89^{b}	45.8 ± 13.4^{a}	33.2 ± 6.88^{ab}	36.5 ± 10.2^{ab}
	drip loss (%)	3.26 ± 1.15	3.18 ± 0.89	2.42 ± 0.26	2.68 ± 0.66
	cooking loss (%)	21.3 ± 3.39^{a}	18.3 ± 3.68^{ab}	15.4 ± 5.30^{b}	13.9 ± 3.68^{b}

Treatment 1 = control, supplemented with 0 IU/g of protease; Treatment 2 = supplemented with 4 IU/g of protease; Treatment 3 = supplemented with 8 IU/g of protease; Treatment 4 = supplemented with 12 IU/g of protease
^{a,b}The same row with different superscripts means significant difference (P < 0.05)

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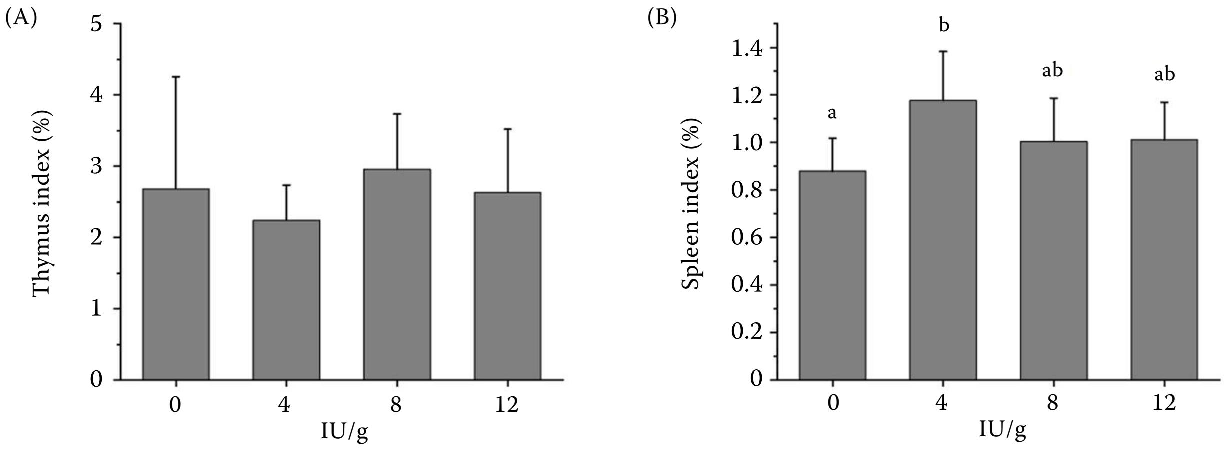

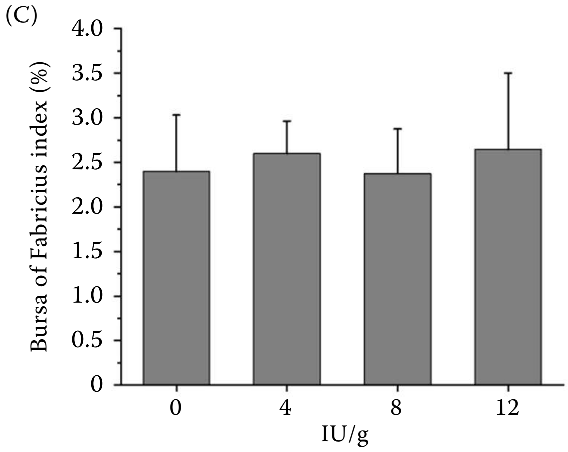

Figure 2. Effects of dietary protease supplementation on the immune organ indices (%) at 42 days

Treatment 1 = control, supplemented with 0 IU/g of protease; Treatment 2 = supplemented with 4 IU/g of protease; Treatment 3 = supplemented with 8 IU/g of protease; Treatment 4 = supplemented with 12 IU/g of protease

[a,b]The same row with different superscripts means significant difference ($P < 0.05$)

fed with a dietary inclusion of 12 IU/g of protease showed a significant increase in the average and minimum times of feeding and drinking behaviour. They showed reduced feeding frequency compared with the group that was not given supplemental treatment. Soluble non-starch polysaccharides enhance the viscosity of the intestinal content and inhibit enzymatic activity by combining with various nutritional factors and digestive enzymes. In addition, 12 IU/g of protease stimulated the expression of feeding and drinking behaviour, possibly because exogenous enzymes promote protein and energy digestibility (Fru-Nji et al. 2015) and increase the amount of nutrients in the intestine for further decomposition or absorption, thereby stimulating the development of the digestive system (Owsley et al. 1986). Moreover, this supplementation increased intestinal emptying rates. The average and minimum times of lying behaviour with protease supplemented at 4 IU/g were higher than those supplemented at 0 and 12 IU/g, and the highest lying frequency was achieved. Based on the results of this analysis, 4 IU/g of protease could promote and increase the lying time of broilers. The lying time gradually decreased with the increase of protease level, which indicated that the dosage of protease had an impact on lying behaviour. We hypothesised that 12 IU/g of protease increased the time of feeding and drinking behaviour. Preening behaviour is a comfortable behaviour. The psychological comfort of broilers improved to a certain extent at 12 IU/g of protease, and the expression of preening behaviour was evidently promoted at this rate.

Abnormal behaviour, such as stereotypes, self-mutilation, feather-pecking, or excessively aggressive behaviour, indicates poor welfare. Intense pecking behaviour is a serious welfare problem, and a closer look at the data showed that protease notably decreased the frequency of scratching and aggressive behaviour, which could reduce pecking frequency. The high level of welfare leads to freely expressed natural behaviour in broilers and less abnormal behaviour. These findings indicated that protease makes broilers feel comfortable. The increase of comfortable behaviour might reduce the occurrence of abnormal behaviour. Thus, the specific mechanism must be further explored.

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Slaughter performance

Slaughter performance is an important indicator of meat production traits in livestock and poultry. The current study showed that protease had no significant effect on the slaughter performance of broilers. However, conflicting conclusions on slaughter performance were found. Corn-soybean meal diets supplemented with compound enzymes, including protease, had a significant influence on breast and wing performance at 42 days of age (Dalolio et al. 2016), and supplementing poultry by-product meal with 3% protease improved the carcass yield percentage of broilers (Mahmood et al. 2017). The different effects of protease application may be due to broilers' type, level, dietary composition, breed, age, and health status.

Meat quality

Meat and meat products are protein resources; pH, shear force, drip loss, and cooking loss of muscle are important indicators of meat quality that are evaluated. Based on previous research, no difference in the meat quality of broilers fed with corn-soybean meal basal diet supplemented with 0.012 5% protease was observed; no significant changes in meat colour, pH, shear force, WHC, WB, and WS incidence were found among the treatments, although pH_{24h} showed an increasing trend. In addition, a low drip loss was observed in broilers subjected to protease treatment (Lu et al. 2020). The current study found that diets supplemented with 12 IU/g of protease improved pH_{24h} of breast and thigh muscles and decreased the shear force of breast muscle. This result was consistent with that obtained by Xu et al. (2017), who reported that coated protease in broilers fed with corn and sorghum-based diets increased the pH_{24h} value but showed decreased drip loss of the breast muscle. Plant proteases, such as bromelain and papain, resulted in a higher water retention rate in turkey meat (Doneva et al. 2015). In the present experiment, we also found that adding 8 IU/g of protease decreased the cooking loss of breast and thigh muscles. Proteolytic enzymes are used to stimulate myofibril breakage and disrupt the shape of muscle connective tissue, such as bromelain, which breaks down alanine, glycine, tyrosine, and lysine at their carbonyl terminus (Ikram et al. 2021). The decrease in shear force of breast muscle might be due to the ability of protease to effectively destroy the major muscle proteins (connective tissue/collagen and myofibrillar proteins) (Ashie et al. 2006). In broilers, muscles with larger muscle fibre diameters are more resilient than those with smaller muscle fibres (Chen et al. 2007). Animals with more moderate-sized muscle fibres produced high-quality meat (Choi and Kim 2009), which supports the current findings.

Immune organ indices

The immune organ index is an important parameter of immune status in poultry. Increasing weight and indices of immune organs are due to the growth and division of immune cells, which indicates the enhanced immunity of the body. A diet supplemented with 4 IU/g of protease significantly increased the spleen index compared with 0 IU/g. This finding was contradicted by Lu et al. (2020), who discovered that relative weights of broiler organs, including the heart, liver, spleen, and bursa of Fabricius, were not significantly different among the treatments subjected to control, protease, and AGP treatments. The relative weights of the spleen, thymus, and bursa, as well as immunocompetence (including humoral and cellular) of broilers, were not affected by various concentrations of protease supplementation in decorticated cottonseed (Sheikh et al. 2016). In the current study, broilers fed 4 IU/g of protease had an increased spleen index compared with those fed basal diets, which might be attributed to the fact that dietary supplementation of protease can improve growth performance by increasing average daily gain of broilers (Angel et al. 2011). Psychological and physiological stresses, stress duration, age, genetics, and immune status of animals are associated with the loss of immune homeostasis (Feng et al. 2012). The increased time of preening behaviour showed a comfortable state of broiler psychology, which might be related to immune status. However, the specific mechanism remains complicated and needs to be further explored.

CONCLUSION

This study showed that the dietary administration of 4 IU/g of protease can improve the immune function of broilers to a certain extent. It can reduce the frequency of abnormal behaviour. Increasing the protease level to 12 IU/g was beneficial to the expression of feeding, drinking, and preening behaviour. Thus, we recommended the addition of 4 or 12 IU/g of protease to improve the welfare and production of broilers.

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Conflict of interest

The authors declare no conflict of interest.

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