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Research Paper

Effect of Dietary Protease Supplementation on Growth Performance, Carcass Traits, and Blood Biochemical Characteristics of Native Chickens

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ABSTRACT

Introduction: Native chicken farming plays an important role in agriculture, and protease enzyme supplementation has been shown to enhance digestion and nutrient absorption. **Materials & Methods:** The experiment was conducted on 128 native chickens (aged 5–12 weeks), arranged in a completely randomized design with four treatments and four replications to assess the effect of protease enzyme supplementation in the diet on growth performance, carcass traits, and hematological characteristics of native chickens. The experimental chickens were fed and given water ad libitum, and the same basal diet was provided to the experimental birds, consisting of a control diet (the diet without protease) and three different levels of protease enzyme supplementation (0.1, 0.3, and 0.5%).

Results: The results showed that the 0.3% protease enzyme supplementation group had significantly ($P<0.05$) increased final body weight (1.854 g/bird) and daily weight gain (30.02 g/bird/day) compared with the control group; meanwhile, the feed conversion ratio (FCR) was also significantly improved ($P<0.05$). Moreover, carcass weight (1.273 g/bird), breast weight (215.7 g), and thigh weight (155.5 g) exhibited a substantial increase in the 0.3% protease group ($P<0.05$), while there was no significant effect on other slaughter characteristics ($P>0.05$). However, protease enzyme supplementation had no effect on immune organ indices, hematological parameters, or liver function, including gamma-glutamyl transferase (GGT), alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) ($P<0.05$), apart from a significant difference in liver weight (43.5 g) among treatments.

Conclusion: This suggests that the addition of 0.3% protease enzyme can improve the growth performance and carcass quality of indigenous chickens, all without any negative effect on health status.

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1. Introduction

In several countries, indigenous chickens play a crucial role in the agricultural industry, especially in rural areas where they are traditionally reared using age-old methods. According to Linh and Qui [1, 2], local chickens are highly adaptable to the environment, exhibit strong disease resistance, and produce high-quality meat that meets consumer preferences. Native chickens are considered to have lower feed conversion efficiency and slow growth rates as compared to industrial chickens, thus resulting in much higher production costs [3]. Based on these analyses, the most significant research avenues involve enhancing the well-being and performance of local chickens through the implementation of nutritional measures.

Over the past few years, antibiotic usage in the poultry sector has been reduced to mitigate the risk of antibiotic resistance [4, 5] and to provide food safety. This encourages the innovation of alternative nutritional solutions, such as exogenous enzyme application to facilitate digestion and nutrient absorption [6, 7]. One such solution is protease enzymes, which facilitate the breakdown of harder-to-digest proteins into more absorbable peptides and amino acids [8, 9]. As a result, protease helps improve the feed conversion ratio (FCR), increase growth performance, and enhance protein digestion efficiency [10]. Additionally, this enzyme has a positive impact on gut health [6], supports the immune system [4], and does not negatively affect blood biochemical indices such as liver enzymes, cholesterol, and glucose [11].

Protease has been shown to improve poultry productivity and meat quality in earlier research. According to [7], protease can aid in the breakdown of protein from plant sources, such as corn and soybeans, enhancing growth performance and reducing feed costs. Additionally, the protease enzyme improves the quality of meat by reducing abdominal fat, and increasing the yield of breast meat [12]. Additionally, protease regulates blood biochemical markers that reflect protein metabolism and general poultry health, including serum protein, blood urea, and liver enzymes [13]. However, most current studies focus on industrial chickens, while data on the impact of this enzyme on native chickens remains limited.

Based on the above context, this study was conducted to evaluate the impact of adding protease enzymes to the diet on the growth, meat characteristics, and hematological indices of indigenous chickens. This was one of the few studies focusing on indigenous chicken breeds, which exhibit different growth and digestion characteristics compared to industrial chickens. The research results provide important scientific data to optimize nutrition, enhance the efficiency of indigenous chicken farming in a sustainable manner, reduce dependence on antibiotics, and limit negative environmental impacts.

2. Materials and Methods

2.1. Location and time

The experiment was conducted at the Animal Experimental Farm, Tra Vinh University, from September to December 2024. All research activities complied with the regulations of the Science and Technology Council regarding animal experiments.

2.2. Feed formulation

All the chickens in the experiment were raised under uniform conditions regarding diet and feed management. The feed used in the study was a mixed diet that met the nutritional standards recommended by NRC [14] for broilers, with the ingredient composition and nutritional value analyzed before use. The experimental treatments were designed with different levels of protease enzyme supplementation (0%, 0.1%, 0.3%, and 0.5%) in the diet, and the feed was provided ad libitum throughout the experiment. The nutritional composition of the feed is recorded in Table 1.

2.3. Bird management

With four treatments that corresponded to four levels of protease enzyme supplementation, the experimental model was set up using a completely randomized design. The control group received mixed feed without any protease enzyme supplementation; E1 received mixed feed supplemented with 0.1% protease; E2 received mixed feed supplemented with 0.3% protease; and E3 received mixed feed supplemented with 0.5% protease. There were 128 experimental chickens in total, with each replication corresponding to an experimental unit made up of eight chickens of the same weight. To ensure consistent experimental conditions, each chicken was raised independently in a different cage. During the rearing process, the chickens received vaccinations on a predetermined schedule, which included oral, ocular, wing web, and intramuscular vaccinations against flu, smallpox, and Gumboro (first and second doses) as well as ND-IB vaccines (first and second doses). The chickens were fed twice a day at 6:30 AM and 5:00 PM as part of a consistent care and feeding schedule. To ascertain the actual

Table 1. Feed ingredient and chemical composition of the diet

Ingredients	DM	OM	CP	EE	CF	ME (Kcal/kgDM)	%Diet
Corn	89.2	98.4	7.43	1.46	1.55	3.669	42
Rice bran	87.5	92.6	11.2	8.64	7.52	2.71	29.3
Soybean meal	90.2	94.2	45.6	1.88	3.8	2.671	15.8
Fish meal	90.5	86.5	53.4	9.56	0.63	3.189	10.7
DCP	100	14.8	-	-	-	-	0.3
Stone	100	-	-	-	-	-	1.3
Vitamin and mineral premix	100	-	-	-	-	-	0.3
Salt	-	-	-	-	-	-	0.3

Chemical analysis of the diet

Chemical composition	CP	Ca	Lys	Met	ME	P
%	19.5	1.1	1.1	0.4	3.102	0.7

Noted: The premix composition per kilogram includes 2,500,000 UI of vitamin A, 600,000 UI of vitamin D3, and 4,000 mg of vitamin E. Additionally, it contains 400 mg of vitamin K3, 80 mg of folic acid, and 100,000 mg of choline. The mineral content consists of 14 g of manganese, 40 g of zinc, 32 g of iron, 48 g of copper, 0.5 g of iodine, 0.28 g of cobalt, and 0.04 g of selenium

consumption, the leftover feed was weighed the following morning at 7:00 AM. The farm's water system freely supplied clean drinking water, so the chickens never experience a water shortage. To maintain biosecurity and reduce the chance of disease transmission, feeders and drinkers were cleaned every day, and the coop was routinely inspected and sanitized. A schematic diagram of the experimental design is shown in Figure 1.

2.4. Growth performance

Body weight, feed intake, and FCR were among the metrics used to assess the growth performance the experimental chickens. To track their growth, the chickens were weighed at the start of the experiment (at 5 weeks old) and every two weeks until the end (at 12 weeks old). The difference between the initial and final average weights divided by the number of feeding days was used to calculate the average daily gain (ADG). By keeping track of the quantity of feed given and the quantity of leftover feed each day, the amount of feed consumed (FI) was calculated. Based on that, the FCR, which represents the effectiveness of feed utilization, was calculated by dividing the total amount of feed consumed by the total weight gain of the chickens during the experiment.

2.5. Carcass traits

After the experiment ended at the 12th week, all chickens in each treatment were weighed before proceeding with the slaughter. The slaughter process was carried out according to standard methods, ensuring compliance with ethical regulations in animal research in Vietnam. After bleeding and plucking, the chickens were eviscerated to determine the meat quality parameters. The evaluation criteria included carcass weight, carcass percentage, breast weight, breast percentage, thigh weight, and thigh percentage. In addition, some important internal organs such as the liver, heart, gizzard, and intestines were also collected and weighed to determine the influence of protease enzymes on the development of these organs.

2.6. Immune organ indices

After the experiment concluded at the 12th week, some chickens from each treatment group were randomly selected to evaluate the mass and ratio of the immune organs. The organs, including the Fabricius pouch, thymus, and spleen, were collected immediately after slaughter and cleaned. The immune organ index was calculated using the Equation 1 [1]:

1. *Immune organ index=(Immune organ weight/Body weight)×1,000*

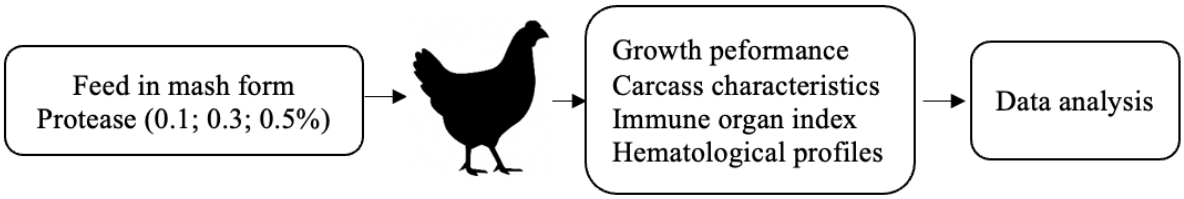

Figure 1. The experimental design

This measurement helps assess the development of immune organs and the response of chickens to the addition of protease enzyme in their diet.

2.7. Blood biochemical parameters and liver enzymes

After the experiment concluded at the 12th week, blood samples were collected from the wing veins of chickens in each treatment group. Each blood sample was collected into a test tube containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) and then centrifuged to separate the serum for biochemical analysis. The biochemical parameters determined included total serum protein, total cholesterol, triglycerides, glucose, globulin, creatinine, albumin, and the albumin/globulin ratio. Liver damage indices were assessed through liver enzymes, including gamma-glutamyl transferase (GGT), alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP). Serum samples were analyzed using the SMT-120VP analyzer (Chengdu Seamaty Technology Co., Ltd., China) with standard kits according to the manufacturer's instructions. The obtained data were statistically processed to evaluate the impact of protease enzyme on blood parameters, thereby reflecting the nutritional status, metabolism, and overall health of the experimental chickens.

2.8. Data analysis

The data obtained from the experiment were statistically processed using Minitab software, version 16. Growth indicators, meat quality characteristics, immune organ indices, and biochemical parameters were analyzed using one-way analysis of variance (ANOVA) in a completely randomized design. When there was a statistically significant difference ($P<0.05$), the Tukey test was used to compare the differences between the treatments. The results are presented as Mean±SEM. $P<0.05$ were considered statistically significant, while $P>0.05$ were regarded as not showing a significant difference between the treatments.

3. Results

3.1. Growth performance

The addition of protease enzyme to the diet significantly affected several growth parameters of the experimental chickens (Table 2). Body weight at week 12, ADG, and FCR showed significant differences between the treatments ($P<0.05$). The group supplemented with 0.3% protease achieved the highest final body weight and had the most improved FCR, while the control group had significantly lower values. The average daily feed intake did not differ between the treatments ($P>0.05$), indicating that the enzyme did not affect the feed consumption level but positively impacted the nutritional efficiency.

Table 2. Growth performance of native chickens from 5-12-week-old

Criteria	Treatments				SEM	P
	Control	E1	E2	E3		
5-week-old (g/bird)	360.8	371.7	382.5	343.5	12.05	0.210
12-week-old (g/bird)	1684^{b}	1774^{ab}	1854^{a}	1728^{b}	25.31	<0.01
Daily weight gain (g/bird/day)	27^{b}	28.62^{ab}	30.02^{a}	28.27^{ab}	0.601	<0.01
Daily feed intake (g/bird/day)	66.5	66.44	66.44	66.57	1.177	0.990
FCR	2.46^{a}	2.32^{ab}	2.21^{b}	2.36^{ab}	0.04	0.020

Note: Different letters in the same row indicate statistically significant differences.

Table 3. Carcass characteristics of native chickens from 5-12-week-old

Criteria	Treatments				SEM	P
	Control	E1	E2	E3		
Live weight (g/bird)	1713^{b}	1783^{ab}	1843^{a}	1722^{b}	15.83	<0.01
Carcass weight (g/bird)	1153^{b}	1202^{ab}	1273^{a}	1167^{ab}	24.49	0.030
Carcass percentage (%)	67.3	67.4	69.02	67.8	1.138	0.700
Breast weight (g/bird)	209.3^{b}	216.3^{ab}	237.8^{a}	215.7^{ab}	6.052	0.040
Breast percentage (%)	18.19	18	18.75	18.48	0.723	0.880
Thigh weight (g/bird)	151.6^{b}	158.3^{ab}	181^{a}	155.5^{b}	5.597	0.020
Thigh percentage (%)	13.16	13.19	14.22	13.32	0.434	0.320
Liver weight (g)	30^{b}	33.25^{ab}	43.5^{a}	36.5^{ab}	2.534	0.020
Heart weight (g)	9	9	10	9	0.288	0.090
Gizzard (g)	48.67	44.17	46.17	47.58	2.519	0.630
Small intestine weight (g)	91.67	92	95.5	90.83	1.572	0.240
Large intestine weight (g)	35.33	47.33	49.5	38.67	4.225	0.120

Note: Different letters in the same row indicate statistically significant differences.

3.2. Carcass traits

Data on the carcass characteristics of the experimental chickens showed that the addition of protease enzyme significantly affected several parameters (Table 3). The body weight, breast weight, and thigh weight of the group supplemented with 0.3% protease were significantly higher than those of the control group ($P<0.05$), while the group supplemented with 0.5% protease showed no significant difference compared to the other groups. The relative proportions of carcass, breast, and thigh meat were not affected by enzyme supplementation ($P>0.05$). The liver weight showed significant differences among the treatments ($P<0.05$), with the group supplemented with 0.3% protease having the highest value. However, the mass of the heart, liver, small intestine, and large intestine did not show significant differences between the experimental groups ($P>0.05$).

3.3. Immune organ indices

No statistically significant difference ($P>0.05$) was observed between the treatments regarding the bursa of Fabricius, spleen, and thymus indices in all four treatments, indicating that the addition of protease enzyme did not significantly affect the development of immune organs (Figure 2). The obtained values were similar between the control group and the experimental groups, suggesting that the protease enzyme did not have a negative impact on the immune system of chickens under experimental conditions.

3.4. Blood biochemical parameters and liver enzymes

No statistically significant difference ($P>0.05$) was recorded between the treatments in terms of total protein, cholesterol, triglycerides, glucose, globulin, creatinine, albumin, and the A/G ratio (Figure 3). These results indicate that the addition of protease enzyme did not significantly affect the blood biochemical indices of the experimental chickens. The parameters in the control group and the groups supplemented with protease enzymes remained at equivalent levels, indicating stability in the nutritional metabolism and homeostasis of the chickens.

No statistically significant difference was observed between the treatments regarding liver damage indicators (Figure 4), including GGT, ALT, AST, and ALP ($P>0.05$). This indicates that the addition of protease enzymes to the diet did not negatively affect liver function of the experimental chickens, while also maintaining normal physiological activity of the liver throughout the study period.

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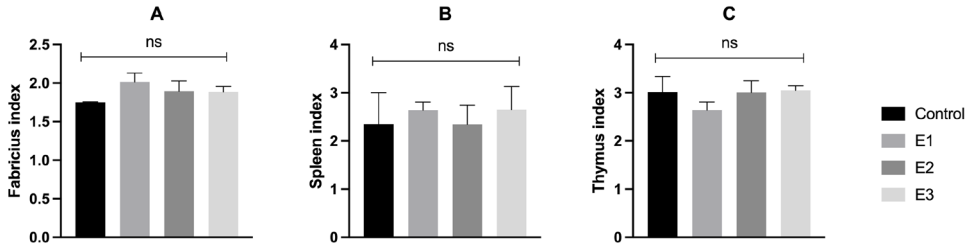

Figure 2. Immune organ indices of the native chickens

4. Discussion

The protease enzyme affects the growth ability of chickens by improving the efficiency of digestion and protein absorption in their diet, thereby optimizing nutrient metabolism. The main mechanism of protease is to cleave peptide bonds in less digestible proteins, releasing more easily absorbable peptides and amino acids, helping poultry better utilize the protein source from their feed [8, 9]. Previous studies have also shown that protease can optimize the utilization of dietary protein in poultry farming. Protease used as a feed additive can help supplement the effects of endogenous pepsin and pancreatic enzymes by enhancing the hydrolysis and solubilization of proteins [6]. This leads to improved weight gain, as the body has sufficient substrates to synthesize muscle protein and develop tissues [1]. The reduction in feed intake while maintaining good growth rates can be explained by the improvement in feed efficiency. When protein is digested more thoroughly, the amount of nutrients required to achieve the same level of growth decreases, so chickens need to consume less feed while still achieving high weight gain. Additionally, protease enzymes also help reduce anti-nutritional factors in feed [7], limiting the negative impact of undigested protein on the digestive system and gut bacteria, thereby maintaining gut health and improving feed conversion efficiency. Protease helps support the increase in intestinal villus length, thereby aiding in better nutrient absorption [15]. The research results showed that a 0.3% protease supplement yields the highest effectiveness, which may be related to the physiological limits of the chicken digestive system. When the enzyme is supplemented at the optimal level, the digestive system can operate most efficiently to break down protein without causing nutritional imbalances or metabolic disorders [16]. However, at a level of 0.5%, enzyme saturation or changes in digestive kinetics may occur, leading to a decrease in protein digestion and absorption efficiency [17]. Additionally, excessive use of enzymes can affect the gut microbiota or alter the interactions between endogenous and exogenous enzymes [18], thereby not providing the expected growth benefits.

Protease enzymes have a significant impact on the meat yield of chickens by optimizing the digestion of protein and nutrient absorption. Meat yield is closely related to the nutritional status of broiler chickens because animals provided with adequate nutrition will promote the development of muscle tissue [15]. When the protein in the diet is more efficiently broken down, the availability of amino acids for muscle synthesis increases, helping to

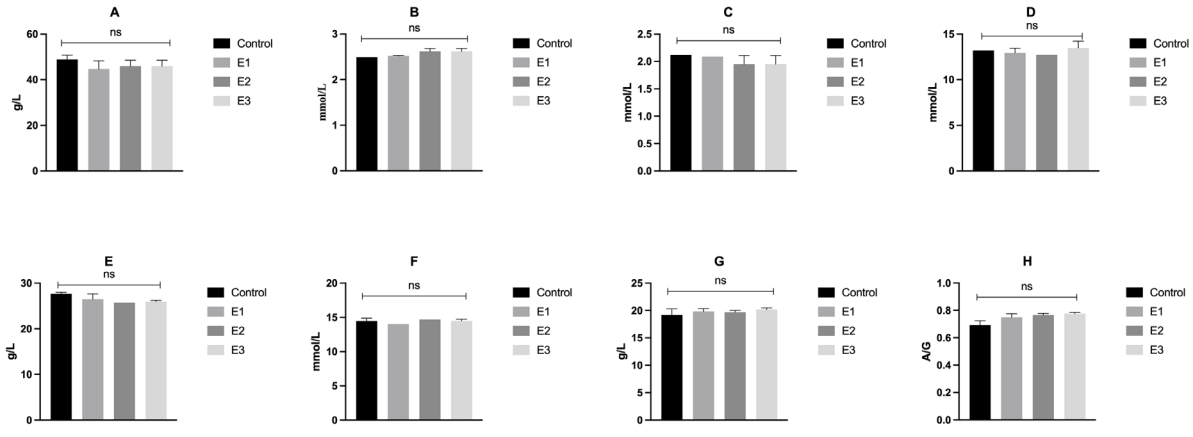

Figure 3. Blood biochemical parameters

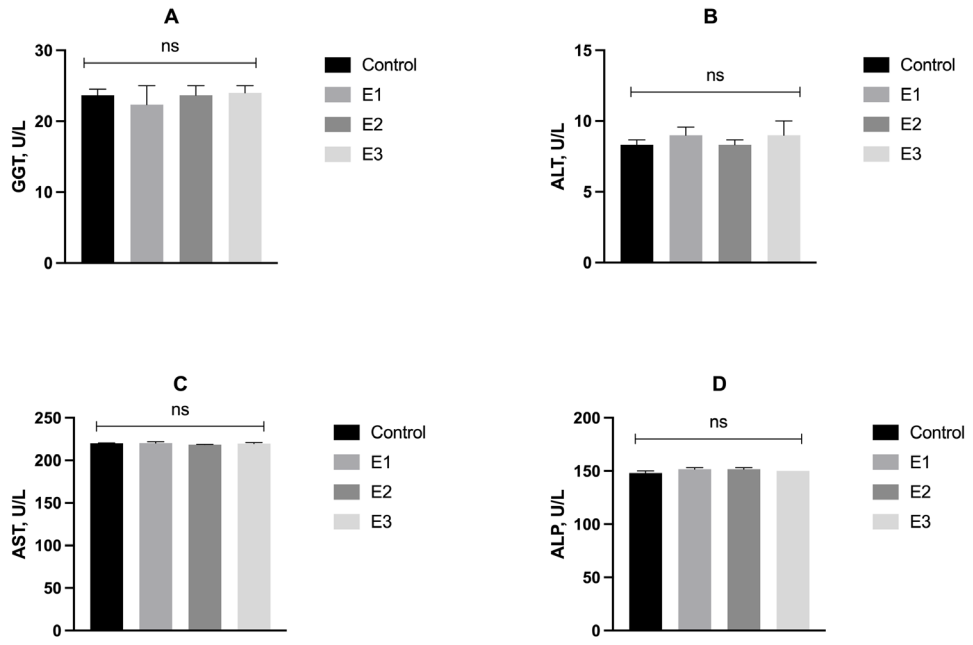

Figure 4. Liver enzyme activities

improve the mass and ratio of meat parts such as breast and thigh meat [1]. This increase reflects the enhanced protein metabolism efficiency with the support of protease enzymes. The highest efficiency was observed at 0.3% enzyme supplementation, suggesting it as the optimal level for improving meat yield without disrupting nutrient metabolism. When the enzyme supplementation level is higher, the effectiveness does not increase, possibly due to saturation in the protein breakdown capacity of the digestive system, leading to the underutilization of the supplemented amino acids [17]. This also explains why the meat yield indices do not continue to increase at higher enzyme levels. Although protease has a significant impact on carcass weight, most internal organs are unaffected, except for the liver. The liver plays a central role in protein metabolism [19], including the synthesis of plasma proteins and the regulation of nitrogen metabolism. When the ability to digest protein improves, the liver must work harder to regulate the metabolic process, leading to an increase in liver mass [20]. This increase reflects the physiological adjustment of the body to adapt to changes in protein metabolism rather than having a negative impact on the health of the chickens.

In the current study, protease enzymes did not significantly affect immune organ indices such as the bursa of Fabricius, spleen, and thymus, as well as blood parameters and liver enzyme indices in chickens. This can be explained by the mechanism of action of protease enzymes primarily on the digestive system rather than directly regulating immune activity. Previous studies have shown that the improvement of protein digestion can indirectly support the immune system by providing sufficient essential amino acids [7]. However, when the chicken's diet already ensures sufficient protein and there are no stress factors or pathogens, the addition of protease enzymes does not create a significant difference in the size and weight of the immune organs [4]. This is consistent with the studies of Park [10] and Oyeagu [15], as they did not find significant changes in the immune response of healthy poultry supplemented with protease enzyme. Similarly, blood biochemical indices such as total protein, albumin, globulin, glucose, cholesterol, and triglycerides showed no differences between the treatments. This indicates that protease enzymes do not significantly alter the balance of homeostatic metabolism. According to McCafferty [9], protease enzymes primarily affect digestive efficiency without directly impacting blood biochemical parameters if the diet is not nutritionally deficient. Furthermore, the study by Sugiharto [8] also noted that the improvement in protein digestion could affect the absorption rate of amino acids but did not directly impact blood biochemical parameters if the diet was balanced. Moreover, although the liver tends to increase in weight, liver damage indicators such as AST, ALT, ALP, and GGT did not show significant differences between the experimental groups. This proves that the liver is not overloaded or damaged due to the increased protein metabolism when protease enzymes are supplemented. According to Qiu [12], some exogenous en-

zymes can promote protein metabolism without causing liver dysfunction, as long as the supplementation levels are within an appropriate range. The study by Walk [9] also noted that protease enzymes help optimize protein utilization without increasing pressure on the liver. This explains why the liver has a larger mass but shows no signs of functional damage.

5. Conclusion

The addition of protease enzyme to the diet has a positive impact on the growth performance and meat yield of local chickens. The supplementation level of 0.3% yielded the most favorable result in weight gain, feed conversion ratio, breast and thigh meat yield, and liver weight, while higher levels did not provide additional advantages. Despite these improvements, immune indices, blood biochemical parameters, and liver function markers remained unchanged, indicating no adverse effects on overall health.

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Compliance with ethical guidelines

All research activities complied with the regulations of the Science and Technology Council regarding animal experiments (Code: 137/2022/HĐ.HĐKH&ĐT-ĐHTV).

Data availability

The data that support the findings of this study are available upon request from the corresponding author.

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Authors' contributions

Conceptualization, methodology, formal analysis, investigation, and writing: All authors; Supervision: Nguyen Hoang Qui.

Conflict of interest

The authors declared no conflict of interest.

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