Research Article

Hermetia illucens Larva Meal as Fish Meal Alternative to Improve Growth, Hematological and Hepatic Enzyme Parameters of Broilers

Nguyen Thuy Linh, Nguyen Hoang Qui*

Department of Animal Science and Veterinary Medicine, School of Agriculture and Aquaculture, Tra Vinh University, Tra Vinh City, Vietnam.

Abstract | Insect is a potential protein source for poultry production with lower cost and better growth. By this importance, a total of seventy-two broilers from six to twelve weeks old were allotted to three treatments with 24 chickens per treatment using a completely randomized design to determine the effect of replacing fish meal (FM) by *Hermetia illucens* larva meal (HIM) in the diet on growth and health of broilers with three treatments of a 15% replacement of FM by HIM, a 30% of FM replacement by HIM and control treatment without replacement of HIM for FM. The results showed that 30% of HIM replacement in diets improved the growth performance; better body weight gain (P<0.05), lower feed intake and improved feed conversion ratio (P<0.05) during 6-12 weeks of age. Additionally, the study showed that replacing FM by HIM affected the carcass, thigh and breast weight (P<0.05); but did not show any effects on heart, liver and gizzard. The study also recorded longer small intestine in treatment of 30% HIM replacement. Furthermore, cooking loss and pH of breast and thigh meat was not different (P>0.05). A significant effect of HI in replacing FM recorded in immune organ weights, especially, improving bursa of Fabricius and thymus weight (P<0.05). The inclusion of 15% and even 30% HI in diets to replace FM did not record changes in hematological and hepatic enzyme indicators. It can be concluded that FM replacement by HIM could improve growth and health of broilers without detrimental effect.

Keywords | Blood parameter, Broiler, Growth, *Hermetia illucens*, Hepatic enzyme

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***Correspondence** | Nguyen Hoang Qui, Department of Animal Science and Veterinary Medicine, School of Agriculture and Aquaculture, Tra Vinh University, Tra Vinh City, Vietnam; **Email:** nhqui@tvu.edu.vn
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INTRODUCTION

Poultry production is one of fast-growing industry that brings good economic value to farmers. Poultry farming has not only grown in quantity, but also made many advances in farming methods, especially the application of new and low-cost feed to the production. In the context of Vietnam, it has been observed that small-scale poultry farms often demonstrate a predilection for indigenous breeds due to their higher level of desirability among the local consumers (Delabouglise *et al*., 2019). Moreover, it has been shown that these local breeds exhibit a high level of adaptability to their surrounding environment (Linh *et al*., 2022).

The poultry industry has experienced significant growth, resulting in producers facing a persistent challenge in the form of high feed costs, primarily due to the necessity of importing certain raw ingredients. The costliest constituents in feed formulation, as identified by (Mat *et al*., 2022), are the protein components, which encompass fish meal (FM). Currently, there exist a multitude of applications pertaining to the substitution of plant and animal protein sources in animal feed (Qui and Linh, 2023; Rahman *et al.*, 2021). Besides, there have been numerous global researches conducted on the utilization of insects as a viable protein source (Auza *et al.*, 2023; Bellezza *et al.*, 2021; Gasco *et al.*, 2021; Kierończyk *et al.*, 2022; Kim *et al.*, 2021). The black soldier fly- *Hermetia illucens* (HI) has the potential to serve as a viable protein alternative in chicken diets. Over the course of the previous decade, a considerable body of research has been dedicated to investigating the utilization of the black soldier fly in animal feed, as evidenced by studies undertaken by (Mat *et al.*, 2022; Auza *et al.*, 2023; Chobanova *et al.*, 2023; Mat *et al.*, 2021). Indeed, insects can be utilized as a substitute for protein sources at varying inclusion percentages in order to reduce feed production expenses and achieve the most economically efficient formulation. According to a study conducted by (Mat *et al.*, 2022), HI has been identified as a potentially beneficial nutritional source for various livestock and poultry species, including layer hens, broiler chickens, fish, quail, and pigs. The presence of high levels of important amino acids, such as lysine, in HIM has been found to contribute to the promotion of animal growth and increased protein content (Shumo *et al.*, 2019). As indicated in a prior investigation, the larvae of flyers possess the potential to serve as valuable feed sources due to their high protein content, ranging from 37% to 63% (Barragan-Fonseca *et al.*, 2017; Qosimah *et al.*, 2023). A study conducted by Dabbou *et al.* (2021) revealed that the nutritional composition of HIM comprises 58.08% crude protein and 19.05% crude fat. In contrast, FM was discovered to possess 58.68% crude protein and 6.14% crude fat, indicating a disparity in their respective nutrient profiles.

The inclusion of HI in chicken diets has been shown to enhance growth performance owing to its high protein content (Mat *et al.*, 2022; Auza *et al.*, 2023; Dabbou *et al.*, 2021). The study conducted by Schiavone *et al.* (2017) provided evidence that defatted HIMs have the potential to serve as a valuable source of apparent metabolizable energy and digestible amino acids for broiler chickens. This suggests that incorporating defatted HIMs into broiler diets may lead to improved efficiency in nutrient digestion. According to the findings of Marono *et al.* (2017), the utilization of defatted HI larva meal in the diet of laying hens resulted in improved feed efficiency. However, it should be noted that there were detected detrimental impacts on feed consumption. Furthermore, the addition of HIMs in the diet has been documented to have a beneficial impact on the blood profiles of laying hens, as described by (Loponte, 2017; Cullere *et al.*, 2016). In addition, the inclusion of HI larva meal in the diet of growing quails, specifically during the period from 10 to 28 days of age, resulted in similar productive performances and carcass features when compared to quails that were fed standard soybean meal and oil-based diets, as reported by (Loponte *et al.*, 2017). According to a study conducted by Schiavone *et al.* (2017), the substitution of soybean oil with HI larva fat in broiler chickens' diets, whether at a rate of 50% or 100%, does not result in any detrimental impacts on growth performance or blood parameters. In a study conducted by (Chobanova *et al.*, 2023), it was demonstrated that the substitution of dietary soybean meal with HIM should be approached cautiously, taking into account various factors such as insect processing technology, supplementation of feed additives, accounting for non-protein nitrogen, maintaining mineral balance, optimizing fatty acid profile, and ensuring adequate amino acid supplementation.

As previously mentioned, there exists a multitude of functions attributed to HIM in animal production. However, there is a dearth of research pertaining to the substitution of HIM with FM in order to enhance the growth and health of local chickens. The aim of this present investigation is to assess the impact of HIM replacement in the diet on the growth performance, carcass features, hematological and hepatic enzyme markers of broilers.

MATERIALS AND METHODS

Location and time

The study was implemented in Tra Vinh University, Tra Vinh Province, Vietnam from August 2023 to October 2023. All procedures for animal health and care were complied with Vietnamese regulations. The approval number of this research was 59/KH-NNTS from the Tra Vinh University.

Experimental design

A total of 72 chickens were allocated among three treatment groups. One treatment involves the use of eight birds, ensuring a balanced representation of both male and female individuals. The research employed local poultry species, specifically referred to as Noi chicken, ranging from the sixth to twelfth week of their growth period. The chickens were provided with unrestricted access to feed for all experimental conditions. The experimental treatments consist of three groups: (a) the control treatment (CON) with FM but does not have inclusion levels of HIM, (b) treatment 1 (RP1) which involves a 15% FM replacement by HIM, and (c) treatment 2 (RP2) which involves a 30% replacement of FM by HIM. Table 1 presents the specific information regarding replacement and nutritional compositions. Each treatment group of birds was housed in individual cages constructed with plastic nets. The floor was adorned with a layer of husk infused with Balasa bio-yeast. The study employed a 12:12 light-dark cycle using natural light as the treatment condition. Every treatment group was equipped with automated feeders and drinkers. The avian specimens underwent a period of rearing, ranging from one to six weeks to acclimate to the experimental environment. During this stage, the birds were not yet fed with an HIM. All birds were administered vaccinations to protect against highly pathogenic avian influenza, Newcastle disease, and Gumboro disease. At the end of the study, birds were slaughtered in a humane manner by the method of cervical dislocation.

Table 1: Ingredients and chemical compositions of the diets.

Ingredients	Treatments		
	CON	RP1	RP2
Corn	13	13	13
Broken rice	25	25	25
Rice bran	46.3	46.3	46.5
Fish meal	13.6	11.6	9.4
HI meal	0	2	4
Lysine	0.5	0.5	0.5
Methionine	0.5	0.5	0.5
Salt	0.3	0.3	0.3
Dicalcium phosphate	0.5	0.5	0.5
Vitamin mineral premix	0.3	0.3	0.3
Total	100	100	100
CP	17	17	17
ME (Kcal/kg)	3022	3020	3016

Growth and carcass performance

On the initial day of treatment, specifically during the sixth week, the birds' weights were measured and recorded. Throughout the whole experimental period, which spanned from week 6 to week 12, the body weight (BW), weight gain (WG) and feed intake (FI) of all treatment birds were gathered and assessed on a weekly basis in order to determine the daily FI, daily WG, and feed conversion ratio (FCR). The final BW measurement was obtained during the twelfth week before proceeding to the slaughtering process. At the end of the treatment period, the birds were euthanized, with a total of six birds per experimental group. The birds were plucked and eviscerated, and subsequent removal of the feet, head, neck, and abdominal fat resulted in the acquisition of the chilled carcass. Subsequently, the weights of heart, liver, bursa of Fabricius, spleen, thymus and breast and thigh were promptly noted. The weights of the breast and thigh were quantified as a percentage of the total live weight.

Blood sample analysis

Birds were taken blood via vein at end of the study. The concentrations of total protein, globulin, total cholesterol, albumin, density lipoprotein cholesterol (low and high), glucose and triglycerides were determined and documented in milligrams per decilitre (mg/dL). A total of six experimental birds with an age of 12 days, were selected from each experimental unit randomly. At the conclusion of the investigation, about 2 millilitres of blood were collected from each avian specimen with 5 ml disposable syringes that were equipped with 23-gauge needles. The samples were expeditiously transferred into haematological tubes, EDTA, and subsequently placed in a refrigerated container within 48 hours of sample collection. The samples were later transferred to a medical center where they underwent biochemical and haematological analysis. Additionally, the levels of aspartate-aminotransferase (AST) and alkaline phosphatase (ALP) were determined using enzymatic methods on a Cobas 6000 Analyzer (Agilent Technologies, Santa Clara, CA, USA).

Data analysis

The data of this study was calculated using the Minitab software tool (version 17 for Windows, Minitab Inc., UK). In order to mitigate the impact of inadequate replication, the experimental unit for growth performance was defined as the pen, while for all other characteristics, it was the individual bird. The data underwent statistical analysis using a one-way analysis of variance (ANOVA), followed by Tukey's test, to assess growth performance, carcass attributes, and blood parameters. The graphs were generated using the Microsoft 365- Excel software. The significance level was determined to be $P < 0.05$.

Table 2: The effect of fish meal replacement by HI meal on BW and BWG.

Treatment	BG, g/bird		BWG, g/bird/day		
	W6	W12	W7-9	W10-12	W7-12
CON	436.4	1197^{b}	22.49	13.76^{b}	18.13^{b}
RP1	441	1234^{b}	23.24	14.55^{b}	18.90^{b}
RP2	443.7	1300^{a}	23.99	16.82^{a}	20.40^{a}
SEM	7.93	14.87	0.52	0.33	0.29
P	0.81	<0.05	0.21	<0.05	<0.05

Noted: $^{a, b, c}$: different superscripts differ significantly at P<0.05. CON: control treatment without replacement; RP1: involved a 15% replacement of HI meal (2% in diet) for fish meal; RP2: involved a 30% replacement of HI meal (4% in diet) for fish meal.

RESULTS AND DISCUSSION

Effect of HIM replacement on growth performance

The effect of HIM replacement versus FM in the diets (Tables 2 and 3) shows that it helps chickens improve their growth performance, particularly body weight (BG), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) (P<0.05). At first, initial weight (W6) was not different (P>0.05) while W12 shows the significance between treatments. It proves that the replacement of HIM to FM brings a positive result. BWG at W10-12 and in whole period was highest in the treatment of 30% HIM replacement (P<0.05). Table 3 shows that FCR was lowest at treatment of 30% replacement from W10-12 and W7-12 (P<0.05), whereas W7-9 was not different between treatments. Additionally, the results from W7-9 and W7-12 show the highest FI at treatment without replacement of HIM (P<0.05).

Table 3: The effect of fish meal replacement by HI meal on FI and FCR.

Treatment	FI, g/bird			FCR		
	W7-9	W10-12	W7-12	W7-9	W10-12	W7-12
CON	48.97[a]	64.33	56.65[a]	2.26	6.11[a]	4.20[a]
RP1	48.18[ab]	64.33	56.25[a]	2.19	6.30[a]	4.24[a]
RP2	45.93[b]	62.17	54.05[b]	2.26	4.12[b]	3.25[b]
SEM	0.60	0.81	0.38	0.13	0.19	0.17
P	<0.05	0.17	<0.05	0.91	<0.05	<0.05

Noted: [a, b, c]: different superscripts differ significantly at P<0.05. CON: control treatment without replacement; RP1: involved a 15% replacement of HI meal (2% in diet) for fish meal; RP2: involved a 30% replacement of HI meal (4% in diet) for fish meal.

Effect of HIM replacement on carcass characteristics

Tables 4 and 5 shows that there are no differences between treatments in heart, liver, gizzard, large intestine and caecal (P>0.05) although HIM replacement treatments show a higher weight and length. The significance was recorded in small intestine length with the highest length in treatment of 30% HIM replacement (P<0.05). Through Table 6, there are no significant differences between treatments in cooking loss of meat from breast and thigh as well as no significance in pH of breast and thigh meat (P>0.05). Figure 1 shows that Bursa of Fabricius and thymus weight were different between treatments (P<0.05) while spleen weights were not different in replacement of HIM to FM (P>0.05). The highest weights were recorded in treatment of 30% HIM replacement in both Fabricius and thymus weight.

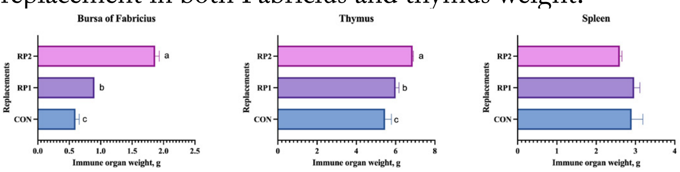

Figure 1: Immune organ weights of broilers. a, b, c: different superscripts differ significantly at P<0.05. CON: control treatment without replacement; RP1: involved a 15% replacement of HI meal (2% in diet) for fish meal; RP2: involved a 30% replacement of HI meal (4% in diet) for fish meal.

Effect of HI meal replacement on hematological and hepatic enzyme markers

Figure 2 shows that there are no detrimental effects of replacing FM with HIM in diets, on hematological parameters including protein (ranged from 4177-4573 mg/dl), albumin (1528-1592 mg/dl), globulin (ranged from 2647-2980 mg/dl), glucose (ranged from 144-145 mg/dl), total cholesterol (ranged from 69-72 mg/dl), triglyceride (ranged from 10.5-11.4 mg/dl), HDL-c and LDL-c (ranged 21.0-21.7 mg/dl) (P<0.05). Additionally, Figure 3 shows that there are no effects of HIM replacement to FM on hepatic enzyme parameters consisting of AST (ranged from 224.6-227.6 U/L) and ALP (ranged from 1421-1436 U/L).

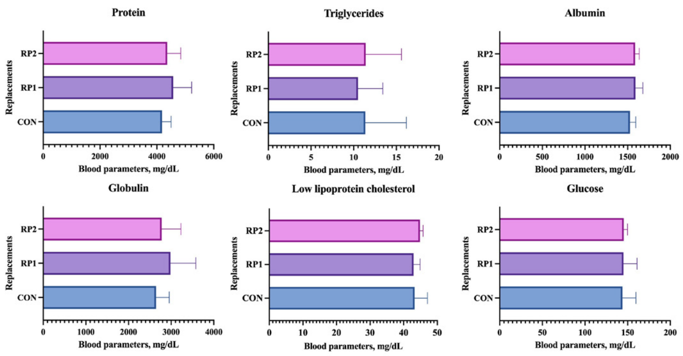

Figure 2: Haematological parameters of broiler chicken. CON: control treatment without replacement; RP1: involved a 15% replacement of HI meal (2% in diet) for fish meal; RP2: involved a 30% replacement of HI meal (4% in diet) for fish meal.

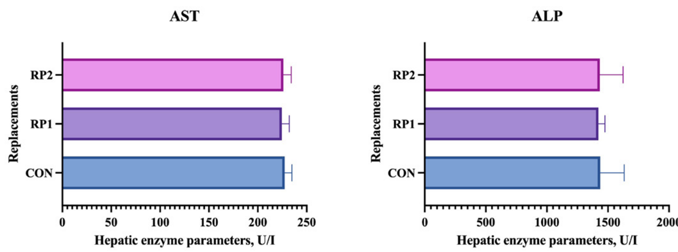

Figure 3: Hepatic enzyme parameters of broiler chickens. CON: control treatment without replacement; RP1: involved a 15% replacement of HI meal (2% in diet) for fish meal; RP2: involved a 30% replacement of HI meal (4% in diet) for fish meal.

The results indicated that substituting FM with HIM had a notable impact on the growth of broiler chickens. The growth performance may be influenced by the CP content present in the HIM. As similar to the study conducted by Auza *et al.* (2023), high CP and high CF were recorded in HIM. Insects, such as HIM, possess chitin and antimicrobial peptides (AMPs), as demonstrated in the research conducted by (Kierończyk *et al.*, 2022). The presence of chitin and AMPs in invertebrates, as highlighted by (Gasco *et al.*, 2021), can have beneficial effects on the

Table 4: The effect of HI meal replacement versus fish meal on carcass traits.

Treatment	Carcass traits						
	Live weight, g	Carcass, g	Carcass, %	Breast, g	Breast, %	Thigh, g	Thigh, %
CON	1156[b]	769.4[b]	66.52	159.0[b]	20.67	182.4[b]	23.71
RP1	1210[b]	795.6[b]	65.74	160.9[ab]	20.23	185.2[ab]	23.29
RP2	1321[a]	897.3[a]	67.99	166.8[a]	18.59	187.4[a]	20.89
SEM	22.66	6.50	0.98	1.56	0.73	0.74	0.70
P	<0.05	<0.05	0.32	<0.05	0.18	<0.05	0.59

Noted: [a, b, c]: different superscripts differ significantly at P<0.05. CON: control treatment without replacement; RP1: involved a 15% replacement of HI meal (2% in diet) for fish meal; RP2: involved a 30% replacement of HI meal (4% in diet) for fish meal.

Table 5: The effect of HI meal replacement versus fish meal on some organs.

Treatment	Carcass traits					
	Liver, g	Heart, g	Gizzard, g	Small intestine, cm	Large intestine, cm	Caecal, cm
CON	27.00	5.47	43.63	142.3[ab]	11.07	17.50
RP1	32.67	4.80	41.80	147.6[a]	11.10	19.50
RP2	28.43	5.13	48.60	135.3[b]	12.50	18.50
SEM	1.46	0.33	1.61	2.80	0.37	0.55
P	0.07	0.43	0.06	<0.05	0.06	0.10

Noted: [a, b, c]: different superscripts differ significantly at P<0.05. CON: control treatment without replacement; RP1: involved a 15% replacement of HI meal (2% in diet) for fish meal; RP2: involved a 30% replacement of HI meal (4% in diet) for fish meal.

Table 6: The effect of HI meal replacement versus fish meal on meat quality.

Treatment	Breast				Thigh			
	Before cooking, g	After cooking, g	Cooking loss, %	pH	Before cooking, g	After cooking, g	Cooking loss, %	pH
CON	5.86	4.36	25.57	5.85	5.76	4.26	25.94	6.23
RP1	5.50	4.20	23.55	5.90	5.56	4.16	25.17	6.10
RP2	5.50	3.90	29.14	5.93	5.80	4.26	26.43	6.30
SEM	0.12	0.13	2.01	0.04	0.06	0.08	1.34	0.06
P	0.12	0.12	0.21	0.42	0.08	0.67	0.80	0.19

Noted: [a, b, c]: different superscripts differ significantly at P<0.05. CON: control treatment without replacement; RP1: involved a 15% replacement of HI meal (2% in diet) for fish meal; RP2: involved a 30% replacement of HI meal (4% in diet) for fish meal.

growth performance, gastrointestinal microbiota, and immunological response of avian species. It is commonly recognized that monogastric animals are unable to digest chitin present in their food. However, according to (Auza *et al*., 2023), the inclusion of AMP in feed which degrades chitin protein led to enhanced intestinal equilibrium, inhibition of harmful microorganisms, and promotion of the growth of good bacteria, specifically *Lactobacillus*. Consequently, this resulted in the synthesis of several easily digestible components. Additionally, it should be noted that fat is a significant nutrient found in insect biomass, occasionally in quantities that are comparable to crude protein (Benzertiha *et al*., 2020). The high fat content in HIM inclusion contributes to the presence of fatty acids in poultry meat. According to previous studies conducted by Kierończyk *et al.* (2022) and Auza *et al.* (2023), the nutritional composition of HIM exhibits similarities to soybean meal/FM and a higher fat content compared to FM. According to the research conducted by Dabbou *et al.* (2018), it has been observed that including larva meal at a concentration of 10% as a partial replacement for soybean meal is a viable option for inclusion in broiler diets during the beginning phase. The inclusion of HIM in the diet positively modulates the levels of live weight and dietary fiber intake. According to Auza *et al.* (2023), the substitution of FM with HIM larva meal at a rate of 75% in the diet was identified as the ideal feeding strategy for enhancing the utilization of feed components in village chickens, while minimizing any negative impacts on their performance.

The replacement of HIM resulted to a positive impact on the weights of the carcass, breast, and thigh. It has also shown to significantly increase the size of the small intestine of broilers. No significant differences were observed in the other evaluated parameters. The variation in carcass weights could perhaps be attributed to the process of protein synthesis in avian species, mostly concentrated in the muscular tissues of the organism (Jariyahatthakij *et al*., 2018; Linh *et al*., 2021). Consequently, this resulted in an augmentation of the bird weights. The present investigation shown resemblances to the research conducted by Auza *et al.* (2023). Furthermore, it is likely that the increase in carcass weight is a result of the inclusion of lauric acid in the HIM, specifically in the form of medium chain fatty acid. This particular fatty acid also has the ability to function as an antibacterial agent within the active intestinal microbiota, specifically targeting gram-positive bacteria (Barros-Cordeiro *et al*., 2014). Moreover, the observed differences in small intestine length could perhaps be attributed to the small intestine's vital function of nutrient absorption from dietary sources. Since the process of nutritional breakdown into smaller molecules can be selectively taken up by small intestinal epithelial cells is facilitated by a cascade of enzymes (Ducatelle *et al*., 2023). Thus, it shows that the longer length of small intestine could prove high protein and nutrient content in HIM. The study demonstrated that there was responsiveness observed in the white pulp of the spleen across all groups of animals. Additionally, the treated animals exhibited a higher spleen weight compared to the control birds. This observation exhibits a resemblance to the research conducted by Bellezza *et al.* (2021) in relation to the spleen. The observed result can be attributed to the presence of chitin, which has been shown to have an immunostimulatory impact (Gasco *et al*., 2018). The study conducted by Bovera *et al.* (2016) arrived at a similar finding, as they detected an increase in spleen weight that were fed a diet including insect meal. That study attributed these results to the antibacterial and antifungal characteristics of chitin. Additionally, it has been observed that animals with higher levels of stress exhibit reduced spleen volume. This phenomenon may be an effect of corticosterone, a stress hormone, that inhibits the development of lymphoid organs (Bovera *et al*., 2016). Moreover, a higher proportion of facilities had normal or average activity levels across all groups that could select for the existence of manageable stress levels. The ideas presented in this study can be supported by the findings of Schiavone *et al.* (2018), which showed generally favourable health and welfare status in broiler chickens.

Evaluation of biochemical blood parameters, including total protein, total cholesterol, AST, ALT and other linked numbers, can be used as a predictive tool to identify specific metabolic disorders that may appear in major organs (Nunes *et al*., 2018). The data of blood profiles was similar to the study of Qui and Linh (2023). The evaluation of broiler health is reliant upon the blood levels of specific parameters, as these indicators have been linked to the presence of disease or stress (Kim *et al*., 2021). The blood values acquired in the current study indicate that the consumption of HIM does not have a detrimental effect on the health state of animals. Throughout the experiment, the hens remained in good condition without exhibiting any clinical signs of disease or irregularities, which is further supported by the analysis of their blood parameters. The findings of this investigation align with previous studies conducted by (Bellezza *et al*., 2021; Kim *et al*., 2021; Dabbou *et al*., 2021), since no significant variations in hematological markers were identified. The absence of notable distinctions in total protein and albumin levels among the treatment groups suggests that hens have the ability to regulate their blood metabolism and return to a normal metabolic state following the finisher period. This observation aligns with the findings of our recent study, which shown that a time of finishing exercise led to the normalization of plasma lipid levels through the facilitation of triglyceride transit and activation of lipolysis pathways (Jariyahatthakij *et al*., 2018).

CONCLUSIONS AND RECOMMENDATIONS

The substitution of HIM, as an alternative to FM, has been found to have advantageous impacts on the performance and health of broiler chickens. In the treatment involving 30% replacement of high HI, there is an observed linear progression in BW, BWG, as well as a linear decline in FI and FCR. The incorporation of a 30% HIM into the diet of broiler chickens has been found to enhance the overall quality of their carcasses, as seen by increased weights of the carcass, thigh, and breast, as well as an elongation of the small intestine. The research findings indicated that the consumption of HIM replacement did not result in any negative impact on meat quality as well as the hematological and hepatic enzyme parameters of broiler chickens.

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NOVELTY STATEMENT

The study provided the information of an alternative to fish meal as one of the most expensive ingredients for poultry feed. To our best knowledge, this study is the new one which recorded in local broilers in Vietnam and could widely apply for other local poultry around the word.

AUTHOR'S CONTRIBUTION

NHQ and NTL conducted the research; NTL analysed data; NHQ wrote the manuscript; All authors approved for the final manuscript

Conflict of interest

The authors have declared no conflict of interest.

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