



Original article

Effects of the inclusion of black soldier fly larvae (*Hermetia illucens*) meal on growth performance and blood plasma constituents in broiler chicken (*Gallus gallus domesticus*) production



Khairiyah Mat^{a,b,*}, Zulhisyam Abdul Kari^a, Nor Dini Rusli^{a,b}, Mohammad Mijanur Rahman^{a,b}, Hasnita Che Harun^{a,b}, Syed Muhammad Al-Amsyar^{a,b}, Mohamad Faiz Mohd Nor^a, Mahmoud A.O. Dawood^{c,d}, Aziza M. Hassan^e

^a Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia

^b Institute of Food Security and Sustainable Agriculture, Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia

^c Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, 33516 Kafrelsheikh, Egypt

^d The Center for Applied Research on the Environment and Sustainability, The American University in Cairo, 11835 Cairo, Egypt

^e Department of Biotechnology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

ARTICLE INFO

Article history:

Received 6 September 2021

Revised 2 October 2021

Accepted 14 October 2021

Available online 22 October 2021

Keywords:

Animal feed

Black soldier fly larvae

Birds

Fish meal

Poultry

Soybean meal

Protein replacement

ABSTRACT

The aim of this study was to identify the effect of inclusion of defatted black soldier fly larvae (Def-BSFL) meal as a protein source on the performance and blood plasma constituents of broiler chickens. A total of 360-day-old chicks were assigned into four dietary groups, which included four different levels of Def-BSFL meal namely control (0% BSFL), T1 (4% BSFL), T2 (8% BSFL) and T3 (12% BSFL) for six weeks experimental feeding period. At the end of the experiment, the blood samples of three birds from each treatment were collected to measure plasma constituents. Birds fed control and T1 diets demonstrated higher feed intake during the finisher stage compared with T2 and T3 diets. The heaviest weight for the 6-week feeding trial was recorded at T1 (1043.8 ± 65.9 g). Birds fed T1 (1.1 ± 0.0) and T3 (0.9 ± 0.1) diets displayed lower feed conversion ratio during the finisher stage than those fed control (1.7 ± 0.1) and T2 (1.8 ± 0.3) diets. Birds fed the control diet demonstrated the highest red blood cell with mean and standard deviation of 7.5 ± 0.34, whereas those fed the T2 diet showed the highest haemoglobin levels with mean and standard deviation of 15.8 ± 0.24. Birds fed T1, T2, and T3 diets exhibited a higher number ($P < 0.05$) of monocytes than those fed a control diet. There were no differences in white blood cell count across all the groups. In addition, birds fed the T2 diet showed higher ($P < 0.05$) blood urea nitrogen followed by the T3, control, and T1 diets. As a conclusion, the 4% Def-BSFL in the broiler chicken diet could be used to replace fish meal (FM) and soybean meal (SBM) without compromising bird performance and blood traits.

© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

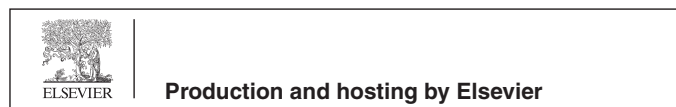
1. Introduction

Malaysia has experienced high self-sufficiency for poultry production; it was 128% and decreased to 103% in 2019

* Corresponding author at: Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia.

E-mail address: khairiyah@umk.edu.my (K. Mat).

Peer review under responsibility of King Saud University.



(Department Veterinary Service, 2020). In general, Malaysia's broiler production cycle occurs 5.33 times a year. Moreover, its average feed conversion ratio (FCR) is 1.67, which indicates the effectiveness of broilers turning feed into meat. Due to the expansion of the poultry industry in Malaysia, the cost of feed has always been a burden to producers since some of the raw materials need to be imported. Among the six essential components in formulating feed, the most expensive are the protein elements. Nowadays, there are a lot of application of replacement from plant and animal protein sources to animal feed (Pati et al., 2016; Rahman et al., 2021; Zulhisyam et al., 2021; Zulhisyam et al., 2020). To reduce the cost of import, developed countries have expended significant effort in

<https://doi.org/10.1016/j.sjbs.2021.10.027>

1319-562X/© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

locating and utilising diverse local sources for feed ingredients, particularly protein material.

For the past decade, numerous studies on the black soldier fly (*Hermetia illucens*) larvae (BSFL) have been conducted on animal feed (Dabbou et al., 2018; Mat et al., 2021; Nekrasov et al., 2018). Apart from the fact that BSFL can be reared easily, it is a sustainable and efficient resource for alternative protein sources (Widjastuti et al., 2014). The BSFL has been established as one of the primary insects being commercialized for use as a protein source, biofuel production, and waste management (Kooienga et al., 2020). Indeed, BSFL can be used to substitute protein sources at different inclusion rates to cut feed production costs and to acquire the most cost-effective formulation. BSFL can be a good nutritional source for layer hens (Jansen, 2018), broiler chickens (Cockcroft, 2018), fish (Xiao et al., 2018), quail (Mat et al., 2021) and pigs (Nekrasov et al., 2018). According to Schiavone et al. (2018), defatted BSFL meal serves as a rich source of apparent metabolized energy and digestible amino acid for broilers with highly efficient nutrient digestion. BSFL contains high levels of essential amino acids such as lysine, which helps boost animal growth and other protein content (Shumo et al., 2019). Nevertheless, the chicken will be eventually consumed by humans. Thus, the health status and condition of the bird must be prioritized in broiler production. Haematological and biochemical analyses are required to verify any abnormalities in blood cells due to feeding birds a diet containing BSFL meal.

Consequently, based on the previously described background, this research has assessed the impact of defatted BSFL meal as a protein source on the growth performance and blood parameters of broiler chickens. The objectives of the current study are to (1) evaluate the effect of defatted BSFL meal utilisation on growth performance, (2) investigate the impact of defatted BSFL meal utilisation on blood plasma constituents, and (3) determine the most appropriate inclusion level of defatted BSFL meal for broiler chickens.

2. Materials and methods

2.1. Ethics statements

This study was carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals and was approved by the Animal Ethical Committee of Universiti Malaysia Kelantan (Malaysia).

2.2. Experimental birds and husbandry condition

The total of 360 (Average weight of 48 g) male Cobb broiler chicks (1 day-old) were procured from Prima Mekar Enterprise, a farm in Jeli, Kelantan, Malaysia. Birds were reared in poultry house, Agro Techno Park, Universiti Malaysia Kelantan, Jeli Campus (5.75019963763, 101.873947466) since day one to day 42 (6 weeks) and separated into four treatment groups (three pens for treatment and 30 birds each pen). In the first two weeks, the birds were kept warm by bulb lights to sustain the temperature that is required for regular breeding procedures. The experimental diets were prepared with 4 different levels of Def-BSFL meal inclusion namely control (0% BSFL), T1(4% BSFL), T2 (8% BSFL) and T3 (12% BSFL). The feeding trial lasted for 6 weeks, and the birds were fed with a BSFL-formulated starter diet for the first 2 weeks, grower diet for another 2 weeks, and finisher diet for the last 2 weeks with free access to water. The experimental birds were vaccinated against Newcastle disease and infectious bronchitis at 7 and 21 days old, as well as against infectious Bursal disease at 13 days old. Anticoccidial compounds were used at a preventive

dose from 22 to 29 days old. The birds were housed in floor pens with appropriate size (0.75 sqft per bird) and covered with sawdust litter.

2.3. Preparation of defatted BSFL meal

The defatted Black Soldier Fly meal was provided by Nutrition Technologies Sdn. Bhd., from a commercial facility located in the state of Johor. The larvae were fed a diet of pre-consumer food waste composed entirely of plant ingredients. After feeding for 7 days the fresh larvae were separated from the frass and feed residue and were thoroughly washed in tap water. The larvae were then dried at a maximum temperature of 80 °C. The dried larvae were introduced to an expeller press to generate an oil fraction and a press cake fraction. The press-cake was milled in a hammer mill before packaging. No additives or additional ingredients were introduced at any stage of the production process for the insect meal.

2.4. Preparation of experimental diets

Defatted BSFLM (approximately 11% crude fat as fed) was procured from a commercial manufacturer (Nutrition Technologies Sdn. Bhd., Malaysia) and used as a protein source to partially replace FM and SBM in this study. Feed was formulated according to broiler chicken (Cobb strain) requirements in relation to growth stages, namely, starter, grower, and finisher phases, according to National Research Council (1994) using the Win Feed 2.8 software package (WinFeed UK Limited, Cambridge, UK). The formulation is isocaloric which mean that the dietary treatments have the same caloric density for each phase as described in de Souza Vilela et al. (2021), the diets were designed on the basis of chemical compositions and the ME value for defatted BSFL meal, while other ingredients were chosen in accordance with the INRAE-CIRAD-AFZ (2021) composition and nutritive values of feed. Feed was formulated with four different level of inclusion rates of Def-BSFL meal in the experimental diets at control (0%), T1 (4%), T2 (8%) and T3 (12%). Due to Dabbou et al. (2018) lower level of BSFL meal that 15% of inclusion rate is more suitable as the findings suggest that it may have a deleterious impact on the FCR during the growing and finishing periods, as well as intestinal morphology, particularly in animals fed with high levels of inclusion in their diet. In addition, Onsongo et al. (2018), concluded that BSFL can replace up to 15% of the conventional feed ingredients in broiler diets for better economic return. The fine powder of Def-BSFL were finely grounded and mixed with other ingredients; maize, soybean meal, fish meal, dicalcium phosphate, limestone, methionine, antioxidant, premix and vegetable oil. In the present study, vegetable oil as a binder to produce feed pellets. The mixture was then pelleted by passing it through a mincer of 3 mm die to produce 3 mm diameter size of pellets (Cerrate et al., 2009). These were sundried to about 10% moisture content, packed in polythene bags and kept safe dry for use. Table 1 presents the gross composition of all experimental diets.

2.5. Chemical composition of experimental feeds

As indicated in Table 2, the proximate composition of the experimental diets is comparable to the control diets. The formulated feed samples were pulverised and tested to analysed the proximate components in accordance with AOAC method (AOAC, 2005) to determine content for dry matter (DM) (method 934.01), crude protein (CP) (method 2001.11), ether extract (EE) (method 920.39), and ash (method 954.02).

Table 1

Gross composition (g/100 g dry weight) of four experimental diets.

Ingredients (%)	Control (0% BSFL)			T1 (4% BSFL)			T2 (8% BSFL)			T3 (12% BSFL)		
	S	G	F	S	G	F	S	G	F	S	G	F
Maize	60.4	61.2	65.3	68.2	64.2	67.6	61.8	58.1	61.1	59.7	57	59.6
SBM	20	14	15	17.9	12.8	10	15.4	10.8	10	13.5	10	12
FM ¹	11.2	11.9	8.2	4.3	8.4	8.5	5.6	9	7.2	5.9	7.7	4.16
Def-BSFL ²	0	0	0	4	4	4	8	8	8	12	12	12
DCP	0.4	0.4	0.3	0.2	0.35	0.4	0.3	0.3	0.5	0.3	0.2	0.5
LMS	0.6	0.8	0.4	0.7	0.6	0.5	0.6	0.55	0.6	0.5	0.8	0.3
Lysine	0	0.1	0.2	0.1	0.2	0.27	0.2	0.3	0.3	0.32	0.42	0.38
MET	0.3	0.33	0.26	0.1	0.15	0.13	0.1	0.3	0.16	0.15	0.26	0.2
Antioxidant	1.1	1.27	1.3	0.4	1.5	0.6	1	2.45	1.64	1	2.32	1.26
Premix ³	5	5	4.04	3.1	2.8	3	6	5.2	5.5	5.63	4.3	4.6
Vegetable oil	1	5	5	1	5	5	1	5	5	1	5	5
Total	100	100	100	100	100	100	100	100	100	100	100	100

Abbreviation: S, Starter; G, Grower; F, Finisher; DCP, Dicalcium Phosphate; LMS, Limestone; MET, Methionine; SBM, Soybean meal; FM, Fish meal; CP, crude protein; EE, ether extract; CF, crude fibre.

¹Fishmeal 550.

²Hi. Protein; Crude fat 11%, crude fibre 8%, crude protein 47%, ash 21% & moisture 5%.

³Vitamin premix (/kg in premix): vitamin A 800,000 IU, vitamin D3 160,000 IU, vitamin E 15 g, vitamin K3 325 mg, vitamin B1 1500 mg, vitamin B2 1250 mg, vitamin B6 1100 mg, vitamin B12 4 mg, vitamin C 2.5 g, creatine 5.5 g, folic acid 70 mg, Biotin 125 mg, Nicotinic acid 4 g, D-pantothenic acid 4.5.

⁴Three replicates of each feed sample were subjected to chemical analysis in this study.

Table 2

Proximate analysis (g/100 g dry weight) of four experimental diets.

Nutrient composition ¹ (%)	Control (0% BSFL)			T1 (4% BSFL)			T2 (8% BSFL)			T3 (12% BSFL)		
	S	G	F	S	G	F	S	G	F	S	G	F
CP (%)	22.2	19.7	18.1	21.6	19.7	19.7	22.3	20.5	19.5	23.3	21.0	20.0
EE (%)	2.0	13.6	12.9	2.2	14.2	14.2	5.9	14.8	13.1	6.0	13.1	15.4
Ash (%)	5.2	8.1	8.0	7.8	7.2	7.2	10.6	9.3	9.6	10.2	9.1	8.6
DM (%)	71.8	97.2	94.3	85.7	94.0	94.0	88.8	94.3	95.4	85.9	95.3	94.1
ME (kcal/kg)	2773.4	3502.57	3450.4	2754.6	3392.5	3392.5	2928.1	3421.0	3426.1	2827.2	3396.5	3524.1

Abbreviation: CP, Crude protein; EE, Ether extract; DM, Dry matter; ME = Metabolisable energy.

¹A total of three replicates of each meal sample were subjected to chemical analysis

2.6. Measurement of birds growth parameters

Throughout the study, the animals were given access to unlimited amounts of food and water. The clinical indicators of disease or mortality were observed on a daily basis. The body weights of all chicks were measured and recorded at an individual level at the beginning of the trial and on day 14, 28 and 42. The feed intake was determined by subtracting the remaining feed every morning from the initial feed provided to them. Each trial consisted of three biological replicates of broiler house. The average daily gain (ADG) and feed intake (FI) were measured at the level of the individual and the level of the pen, at the completion of each growth phase. ADG was measured by dividing the total body weight gain by number of days. The feed conversion ratio (FCR) was calculated for every growth phase as well as for the entire experimental period. FCR was calculated by dividing FI by ADG.

2.7. Haematological parameters

The blood samples were taken from each bird at day 42 of the study for analysis of serum and haematological components. Two ml blood samples were obtained by puncture of the left-wing vein using a vacutainer blood tube, needles, and syringes as described by Hong et al. (2012). The blood samples were analysed to measure white blood cells (WBCs), monocytes (MONs), lymphocytes (LYMs), granulocytes (GRAs), mean platelet volume (MPV), haematocrit (HCT), mean corpuscular volume (MCV), haemoglobin (HGB), red blood cells (RBCs), mean cell haemoglobin (MCH), mean cor-

puscular haemoglobin concentration (MCHC), and red cell distribution width (RDW) by using Mythic 18 Vet haematology analyser (Lancashire, UK). In addition, blood samples were also analysed for biochemical constituents, including alanine aminotransferase (ALT), blood urea nitrogen (BUN), calcium (Ca), creatine kinase (CK), gamma-glutamyl transferase (GGT), magnesium (Mg), phosphorus (P) and total protein (TP) using the IDEXX VetTest[®] Chemistry Analyzer (Maine, USA).

2.8. Statistical analysis

All data were subjected to analysis of variance (ANOVA) using SPSS software (version 23). A cage for growth rate was the experimental unit, whereas the individual bird was used for blood parameters. ANOVA was used to analyse the collected data for chemical composition of the diets, growth performance, and blood parameters. Linear regression was used to test the response for increases of Def-BSFL inclusion rate. The differences between treatments were compared using the Duncan multiple range test at 5% probability levels. The data were expressed as the mean and standard deviation.

3. Results

3.1. Broiler growth performances

Throughout the whole study time, the birds displayed their full range of vitality as no signs of any disorder were observed in all the

groups. The performance of the broiler chicks in terms of growth is summarised in Table 2. The feed intake (FI), body weight (BW), average daily weight gain (ADG), and feed conversion ratio (FCR) of birds were affected by the inclusion of def-BSFL meal in the experimental diets throughout the feeding stages (1 to 42 days) as displayed in Table 3. For starter ration, birds fed T2 diet showed higher ($P < 0.05$) ADG and FI than those fed other diets and no differences ($P > 0.05$) were observed regarding FCR among the diets. For grower ration, birds fed the T1 diet displayed significantly ($P < 0.05$) higher FI followed by T2, control and T3 diets. However, there were no differences ($P > 0.05$) in FI between the control and T1 or between the control and T2 diets. Similarly, birds fed the control diet demonstrated higher ($P < 0.05$) ADG than those fed T2 and T3 diets, and no difference ($P > 0.05$) was observed between control and T1 diets. Furthermore, there was no significant ($P > 0.05$) difference in FCR among the diets. For finisher ration, birds fed the control and T1 diets exhibited higher ($P < 0.05$) FI than those fed T2 and T3 diets. Birds fed T1 diet demonstrated higher ($P < 0.05$) ADG followed by T3, control and T2 diets, but no difference ($P > 0.05$) was observed between the control and T2 or between the control and T3 diets. Birds fed T1 and T3 diets demonstrated significantly ($P < 0.05$) lower FCR than those fed control and T2 diets.

3.2. Blood plasma constituents

Tables 4 and 5 describe the haematological parameters and serum biochemical characteristics of broiler chickens. The results revealed a significant ($P < 0.05$) effects related to the inclusion of def-BSFL were observed for all measured parameters, except for WBC, GRA, and HCT (Table 4). Birds fed the control diet demonstrated higher ($P < 0.05$) RBC and MPV than those fed other diets. However, for those fed the T1 diet, it appeared that MCH, MCHC, and LYM values were the highest ($P < 0.01$). However, birds fed the control diet demonstrated significantly ($P < 0.05$) lower MON than those fed other diets. In addition, the highest HGB was observed in birds fed the T2 diet, while MCV in the birds fed T3 diet. Birds fed the T1 diet displayed lower ($P < 0.05$) RDW than those fed other diets. Furthermore, the results of biochemistry blood parameters were significant ($P < 0.05$) for BUN, Ca, P, and TP among the diets (Table 5). Birds fed the control diet displayed significantly ($P < 0.05$) higher Ca and P than for other diets. Moreover, birds fed the T2 diet showed higher ($P < 0.05$) BUN followed by the T3, control, and T1 diets. The treatments had no significant ($P > 0.05$) effect on other parameters, including ALT, CK, GGT, and Mg.

Table 3

Performance of broiler chicken fed with different inclusion rates of Def-BSFL as protein source in the diets for six weeks. Data expressed as mean \pm SD.

Parameters	Diets					P-value
	Stage	Control (0 % BSFL)	T1(4% BSFL)	T2(8% BSFL)	T3(12% BSFL)	
Average daily feed intake (g/bird)	Starter (1–14 d)	13.3 \pm 1.2 ^a	12.7 \pm 2.1 ^a	21.0 \pm 0.9 ^b	9.0 \pm 0.2 ^a	**
	Grower (15–28 d)	34.0 \pm 1.1 ^b	37.7 \pm 2.8 ^b	35.6 \pm 3.4 ^b	28.3 \pm 2.2 ^a	***
	Finisher (29–42 d)	51.5 \pm 3.6 ^b	54.5 \pm 1.7 ^b	31.9 \pm 0.7 ^a	27.8 \pm 2.1 ^a	***
	Overall (1–42 d)	32.9 \pm 8.1	34.9 \pm 9.0	29.5 \pm 4.4	21.7 \pm 6.4	**
Body weight (g/bird)	Day-old (1 d)	45.8 \pm 1.1	45.9 \pm 0.9	46.4 \pm 0.6	42.4 \pm 4.3	ns
	Starter (14 d)	127.0 \pm 8.8 ^a	122.9 \pm 10.8 ^a	186.4 \pm 8.1 ^b	126.4 \pm 9.8 ^a	***
	Grower (28 d)	467.0 \pm 42.4 ^c	353.8 \pm 29.8 ^{ab}	311.3 \pm 19.9 ^{ab}	264.5 \pm 9.9 ^a	***
	Finisher (42 d)	891.5 \pm 47.2 ^b	1043.8 \pm 65.9 ^c	713.0 \pm 37.8 ^a	686.8 \pm 44.7 ^b	***
Average daily weight gain (g/bird)	Starter (1–14 d)	5.8 \pm 0.6 ^a	5.5 \pm 0.7 ^a	10.0 \pm 0.5 ^b	6.0 \pm 0.6 ^a	**
	Grower (15–28 d)	24.3 \pm 1.9 ^c	16.4 \pm 3.0 ^{bc}	8.9 \pm 2.0 ^{ab}	10.0 \pm 0.5 ^a	***
	Finisher (29–42 d)	30.3 \pm 0.9 ^b	49.5 \pm 1.0 ^b	17.7 \pm 2.4 ^a	30.0 \pm 2.6 ^b	***
	Overall (1–42 d)	20.13 \pm 1.3	23.8 \pm 1.6	12.2 \pm 1.7	15.3 \pm 1.2	*
Feed conversion ratio (FCR)	Starter (1–14 d)	2.3 \pm 0.2 ^a	2.3 \pm 0.3 ^a	2.1 \pm 0.3 ^{ab}	1.5 \pm 0.1 ^b	**
	Grower (15–28 d)	1.4 \pm 0.1 ^c	2.3 \pm 0.4 ^b	4.0 \pm 1.4 ^a	2.8 \pm 0.2 ^b	**
	Finisher (29–42 d)	1.7 \pm 0.1 ^b	1.1 \pm 0.0 ^a	1.8 \pm 0.3 ^b	0.9 \pm 0.1 ^a	***
	Overall (1–42 d)	1.8 \pm 0.2	1.9 \pm 0.4	2.6 \pm 0.7	1.7 \pm 0.6	**

Note: Different superscripts in each row indicate significant difference ($p < 0.05$).

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns - means within the same row with no letters are not significantly different.

4. Discussion

4.1. Chemical composition of experimental feeds

The CP content in starter, grower, and finisher rations for all treatments in the current study were in line with recommended minimum specifications for broilers (Yan et al., 2010). However, the percentage of CP was lower as compared to a previous study on starter and grower rations with 23.0% and 21.5% CP, respectively (Dabbou et al., 2018). By contrast, the CP content in the current study was higher compared to a study by Al-Qazzaz et al. (2016), as they reported that the CP percentage used ranged between 16% and 19%. The discrepancy of CP content between the current and previous studies can be explained by the usage of higher proportions of high-protein ingredients such as maize, soybean, corn gluten, and fish meals (Al-Qazzaz et al., 2016; Dabbou et al., 2018; Schiavone et al., 2018). However, CP content in the finisher stage of T2 and T3 was slightly higher as compared to Dabbou et al. (2018), at 19.47%. Yan et al. (2010) explained that feeding diets higher in crude protein tended to result in faster growth, especially in early stages, but were less efficient in conversion of CP into BWG. Nevertheless, if protein costs are high relative to energy, using lower protein diets is recommended. Furthermore, EE content in starter feed of all treatments were lower compared to the previous study of Dabbou et al. (2018) at 7.1% to 7.3%. The dissimilarities may be due to the lower amount of vegetable oil used in the current study (1%) as compared to the 4.0%–4.6% of soybean oil use in the previous study (Dabbou et al., 2018). However, EE content in grower and finisher rations of all treatments were higher compared to previous studies at 8.01% to 9.89% (Dabbou et al., 2018) and 7.1% to 10.3% (Schiavone et al., 2018). This variation may be due to the insect-rearing medium and defatting process, which influenced the EE content in both current and previous studies used slightly similar percentage of additional oil (Schiavone et al., 2018). Shumo et al. (2019) reported that the EE content in BSFL ranged between 30% and 34% and strongly relied on their feed formulation. This explained the high EE content in the feed formulation in the recent study. The common method to determine the energy available in feed is measuring the ME. The ME of the starter ration for all treatments in the current study were comparable to Al-Qazzaz et al. (2016) at 2600 to 2800 kcal/kg. By contrast, the ME for the current study was lower compared to that of Dabbou et al. (2018), as they reported that the ME used for the starter group in their study was 2999.3 kcal/kg. However, the ME

Table 4Hematological components of broiler chicken fed with different inclusion rates of Def-BSFL as protein source in the diets for six weeks. Data expressed as mean \pm SD.

Blood components	Diets				P-value
	Control (0% BSFL)	T1(4% BSFL)	T2(8% BSFL)	T3(12% BSFL)	
MCV (μm^3)	60.2 \pm 1.41 ^{bc}	51.6 \pm 1.22 ^a	57.4 \pm 0.67 ^{ab}	65.1 \pm 3.69 ^c	*
MON ($10^3/\mu\text{l}$)	0.2 \pm 0.03 ^a	0.4 \pm 0.03 ^b	0.4 \pm 0.03 ^b	0.4 \pm 0.03 ^b	**
RBC ($10^6/\mu\text{l}$)	7.5 \pm 0.34 ^b	5.9 \pm 0.08 ^a	6.5 \pm 0.30 ^a	5.9 \pm 0.11 ^a	**
WBC ($10^3/\mu\text{l}$)	8.5 \pm 0.57	9.6 \pm 0.15	8.7 \pm 0.18	8.9 \pm 0.16	ns
GRA ($10^3/\mu\text{l}$)	5.2 \pm 0.52	4.9 \pm 0.39	5.0 \pm 0.27	6.3 \pm 0.20	ns
HGB (g/dl)	13.5 \pm 0.55 ^{ab}	14.6 \pm 0.37 ^{bc}	15.8 \pm 0.24 ^c	13.0 \pm 0.47 ^a	**
HCT (%)	31.5 \pm 0.68	28.9 \pm 0.30	32.6 \pm 0.72	32.2 \pm 1.55	ns
MCH (pg)	39.1 \pm 3.93 ^a	71.5 \pm 0.73 ^c	47.5 \pm 1.30 ^b	32.4 \pm 0.39 ^a	***
MCHC (g/dl)	34.0 \pm 0.61 ^a	42.3 \pm 1.10 ^b	38.2 \pm 0.88 ^{ab}	34.4 \pm 2.50 ^a	*
RDW (%)	12.9 \pm 0.20 ^b	7.5 \pm 0.27 ^a	12.2 \pm 0.15 ^b	12.6 \pm 0.43 ^b	***
MPV (μm^3)	7.9 \pm 0.37 ^b	6.4 \pm 0.23 ^a	5.9 \pm 0.37 ^a	6.4 \pm 0.24 ^a	*
LYM (%)	3.1 \pm 0.08 ^b	4.3 \pm 0.26 ^c	3.3 \pm 0.20 ^b	2.2 \pm 0.33 ^a	***

Note: Different superscripts in each row indicate significant difference ($p < 0.05$).* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns - means within the same row with no letters are not significantly different.

Abbreviations: MCV, mean corpuscular volume MON, monocytes RBCs, red blood cells WBC, white blood cell GRA, granulocytes HGB, haemoglobin HCT, haematocrit MCH, mean cell haemoglobin MCHC, mean corpuscular haemoglobin concentration RDW, red cell distribution width MPV, mean platelet volume LYM, lymphocytes.

Table 5Biochemical components of broiler chicken fed with different inclusion rates of Def-BSFL as protein source in the diets for six weeks. Data expressed as mean \pm SD.

Blood parameters	Diets				P-value
	Control (0% BSFL)	T1(4% BSFL)	T2(8% BSFL)	T3(12% BSFL)	
ALT u/l	407.3 \pm 42.2	471.0 \pm 58.0	452.0 \pm 30.3	330.7 \pm 14.5	ns
BUN mg/dl	95.5 \pm 1.8 ^b	75.6 \pm 3.5 ^a	113.3 \pm 6.2 ^c	106.9 \pm 5.4 ^{bc}	**
CAL mg/dl	8.8 \pm 0.2 ^b	7.0 \pm 0.6 ^a	7.3 \pm 0.4 ^a	7.1 \pm 0.4 ^a	*
CK u/l	212.0 \pm 3.1	217.7 \pm 5.4	215.0 \pm 19.5	175.0 \pm 9.1	ns
GGT u/l	539.7 \pm 41.3	568.7 \pm 30.4	587.0 \pm 20.2	537.0 \pm 46.4	ns
Mg mg/dl	2.5 \pm 0.1	2.6 \pm 0.2	2.9 \pm 0.1	2.7 \pm 0.3	ns
PHOS mg/dl	8.1 \pm 0.1 ^b	6.6 \pm 0.4 ^a	6.8 \pm 0.1 ^a	7.0 \pm 0.4 ^a	*
TP mg/dl	3.1 \pm 0.2 ^b	3.1 \pm 0.2 ^b	3.1 \pm 0.1 ^b	2.3 \pm 0.1 ^a	*

Note: Different superscripts in each row indicate significant difference ($p < 0.05$).* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns - means within the same row with no letters are not significantly different.

Abbreviation: T1, diets containing 4% BSFL; T2, diets containing 8% BSFL; T3, diets containing 12% BSFL. ALT, alanine aminotransferase; BUN, blood urea nitrogen; Ca, calcium; CK, creatine kinase; GGT, gamma-glutamyl transferase; Mg, magnesium; P, phosphorus; TP, total protein.

for grower and finisher rations were higher in the current study at 3400–3500 kcal/kg compared to Dabbou et al. (2018) at 3099–3199 kcal/kg. The use of corn-based and wheat-based diets contributed to the total ME in the diets. Thus, the growth performances of the birds rely on energy availability in the diets and microbial fermentation of the ingesta in the ceca (Yang et al., 2020).

4.2. Broiler growth performances

A positive performance impact of dietary inclusion of insect meal has also been observed in other research studies in broilers, with increases in body weight, body weight gain, and feed conversion ratio (FCR) observed in at least one phase of the experiment (Dabbou et al., 2018; de Souza Vilela et al., 2021; Loponte et al., 2017). For the growth performance parameters, the FI in the current study was comparable to previous studies (Dabbou et al., 2018; Dengah et al., 2015). It was observed that the average individual FI of the broiler in starter and finisher phases ranged from 39.8 to 44.8 g and from 60.5 to 71.4 g, respectively (Dengah et al., 2015), which were quite similar to the current study. A similar observation on the FI of all three stages were lower compared to that of the current study at 25.6 to 28.5 g (starter stage), 95.2 to 98.7 g (grower stage), and 171.7 to 176.4 g (finisher stage), with T3 in the grower stage and T2 and T3 in finisher stage being exceptions (Dabbou et al., 2018). On the other hand, the birds in the current study displayed lower live weight compared to several previous studies. Higher live weight for broiler chickens compared

to the results of all treatments of the present study at 259.2 to 285.2 g (10 days), 1095.2 to 1227.9 g (24 days), and 2264.1 to 2279.0 g (35 days) (Dabbou et al., 2018). Choi et al. (2012) also found higher live weight for broiler chickens in the finisher stage compared to the current study (1739 to 1837 g). The live weight for the current study was below the normal average for Cobb broilers. This discrepancy may be explained by the occurrence of the rainy season during the feeding trial period, with the average environment temperature being between 18 °C and 21 °C (MET Malaysia, 2019). Moraes et al. (2002) explained that a temperature at 20 °C and below would stress birds and reduce their body weight, especially during brooding. The ADG results of the current study were in line with a previous study at 5.6 to 7.0 g and 18.7 to 27.4 g in both starter and finisher stages, respectively (Dengah et al., 2015). However, Dabbou et al. (2018) found higher ADG in all stages compared to the current study at 21.9 to 24.5 g (starter), 59.1 to 67.3 g (grower), and 88.9 to 97.7 g (finisher). Furthermore, the current study found a higher FCR value in the starter stage compared to Dabbou et al. (2018), which indicated that the FCR of broiler chickens between days 1 and 10 was at 1.2. Similar results were also observed in the grower stage, and Dabbou et al. (2018) defined the FCR value for 10- to 24-day old broiler chickens at 1.4 to 1.6 except for the control group in the current study (1.4). Moreover, the FCR value in the finisher stage was lower compared to Dabbou et al. (2018), which described the FCR value of 24- to 35-day old broiler chicken at 1.8 to 1.9. Choi et al. (2012) also found a relatively similar FCR value in the finisher stages at 1.6 to 1.7. The lower performance of the broiler chickens in the current study can

be explained by the presence of chitin in the BSFL meal, which reduced feed digestibility, thus resulting in increased FI and decreases in both live weight and daily gain (Schiaivone et al., 2018). The findings of the current study can be placed in the context of the wide range of findings reported in the previous studies. This diversity may be related to the nutrient content of the BSFL meal that was used, which can be affected by the insect stages either adult, larva, or pupa. In addition, the BSFL culturing nutrient, the drying and pressing procedure, as well as the period of time during which the chickens were fed.

4.3. Blood plasma constituents

In the current study, the results from haematological and biochemical blood parameters indicated that feeding Def-BSFL as a replacement for fishmeal did not significantly affect the health status of birds. Moreover, the present study demonstrated that all blood parameters observed fell within the reference ranges for broiler chickens (Lumeij, 1997). In short, all the broiler groups in the current study displayed no toxicity and indicated healthy kidneys, livers, and bile in the birds. The treatments affected the number of RBCs, which might be due to the changes in diets, which altered the synthesis and release of these cells from bone marrow (Abdel-Rahman & Mosaad, 2013). Studies by Marono et al. (2017) and Loponte et al. (2017) have indicated a positive effect for BSFL in the blood profile of layer chickens and Barbary partridges; these findings are in line with the current study. In contrast to observations in the present study, Dabbou et al. (2018) and Kinasih et al. (2018) suggested that feeding birds a BSFL diet did not affect their blood profile. Differences in environmental conditions or diet types could contribute to the differences (Kinasih et al., 2018). A higher value for HGV and RBCs indicates a higher nutrient supply in the body system of the chickens (Alabi et al., 2013). The MCV and MCHC have been related to RBC and HGV results. Higher levels of MCHC observed in the T1 and T2 groups in the current study may indicate blood hemolysis due to high concentrations of HGV inside RBCs. Furthermore, the presence of HGV outside of RBC (due to RBC destruction) will produce high MCHC levels.

Moreover, the MON and BUN levels for all groups were generally based on the reference range. The role of the MON component is to fight foreign materials that enter the bloodstream. There is a direct relationship between protein intake and BUN levels (Szabo et al., 2005) observed in diets T2 and T3 of the current study: Diets containing high protein levels demonstrated significantly high BUN in birds. However, BUN levels higher than the above reference range indicate toxic urea in the liver (Godara et al., 2015). CK is a type of protein responsible for producing enzymes in muscle cells and the brain. Higher CK levels can contribute to diseases related to vital organs (Amaral et al., 2017). The GGT indicates the amount of enzyme in the blood as a chemical reaction. The higher GGT levels observed at the end of the study was due to the increase in growth because of more significant amounts of feed consumed at this stage. Magnesium is an electrolyte that helps to regulate the nervous system and maintain the heart. Studies have indicated that Mg levels are related to potassium levels in blood serum (Sowande et al., 2008). The increase of potassium will trigger the movement of Mg in and out of cells, which will lead to hypo-Mg, which is an imbalance of Mg (Hansen & Bruserud, 2018). A high magnesium concentration can cause renal dysfunction, but this is a rare condition that has only been observed in monogastric animals. Furthermore, the current study proves that the inclusion of BSFL in ration does not increase P serum concentrations as the control group shows higher P compared to other treatments. Contrasting findings suggested by Dabbou et al. (2018) may be explained by the higher bioavailability of P in feed ingredients (Li et al., 2016).

5. Conclusions

The use of Def-BSFL as a protein source significantly affected the FI, total BWG, ADG, and FCR. Overall, the birds fed with 4% Def-BSFL for 6 weeks had the highest performance compared to those fed other treatments. Therefore, it can be concluded that the Def-BSFL is a promising alternative ingredient that can be used to replace FM and SBM to develop a local-based feed ingredient for broiler chickens at the optimal inclusion rate of 4% without compromising performance and health as indicated by the blood parameters of the birds. Furthermore, the new knowledge gained from this research is applicable to the broiler feed industry, and it has the potential to aid Malaysian policymakers in their decision to approve the use of insect meals in poultry feeds.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This study was supported by the Fundamental Research Grant Scheme (R/FRGS/A0700/00122A/002/2019/00701) from the Ministry of Higher Education, Malaysia, the Rising Star Grant Scheme (R/STA/A0700/00130A/003/2021/00933) from the Research Management and Innovation Centre of Universiti Malaysia Kelantan and by Taif University Researchers Supporting Project number (TURSP-2020/76), Taif University, Taif, Saudi Arabia. Special thanks to Mr. Martin Zorilla from Nutrition Technologies Sdn. Bhd. for providing the Defatted BSFL, brand name 'Hi.Protein', for this study.

References

- Abdel-Rahman, M.A., Mosaad, G.M., 2013. Effect of propolis as additive on some behavioural patterns, performance and blood parameters in Muscovy broiler ducks. *J. Adv. Veterinary Res.* 3 (2), 64–68.
- Al-Qazzaz, M.F.A., Ismail, D., Akit, H., Idris, L.H., 2016. Effect of using insect larvae meal as a complete protein source on quality and productivity characteristics of laying hens. *Revista Brasileira de Zootecnia* 45 (9), 518–523.
- Alabi, J.O., Arigbede, M., Ng'ambi, J., Norris, D., Shiawoya, E., Onyekachi, S., 2013. Effects of urea-treated fibrous diets on nutrient intake, digestibility, performance and haematological parameters of Yankasa rams. *Tropical Animal Health and Production*, 45(6), 1349–1355. <https://doi.org/DOI.10.1007/s11250-013-0368-5>.
- Amaral, P., Zimmermann, C., Santos, L., Noro, M., Prá, M., Pilotto, F., Rodrigues, L., Dickel, E., 2017. Evaluation of physiological parameters of broilers with dorsal cranial myopathy. *Brazilian J. Poultry Sci.*, 19, 69–74. <https://doi.org/https://dx.doi.org/10.1590/1806-9061-2016-0377>.
- AOAC, C., 2005. Official methods of analysis of the Association of Analytical Chemists International. Official Methods: Gaithersburg, MD, USA.
- Cerrate, S., Wang, Z., Coto, C., Yan, F., Waldroup, P., 2009. Effect of pellet diameter in broiler starter diets on subsequent performance. *J. Appl. Poultry Res.*, 18(3), 590–597. <https://doi.org/https://doi.org/10.3382/japr.2009.00041>.
- Choi, W.H., Yun, J.H., Chu, J.P., Chu, K.B., 2012. Antibacterial effect of extracts of *Hermetia illucens* (Diptera: Stratiomyidae) larvae against Gram-negative bacteria. *Entomological Res.*, 42(5), 219–226. <https://doi.org/10.1111/j.1748-5967.2012.00465.x>.
- Cockcroft, B.L., 2018. An evaluation of defatted black soldier fly (*Hermetia illucens*) larvae as a protein source for broiler chicken diets. Stellenbosch University, Stellenbosch.
- Dabbou, S., Gai, F., Biasato, I., Capucchio, M.T., Biasibetti, E., Dezzutto, D., Meneguz, M., Plachà, I., Gasco, L., Schiaivone, A., 2018. Black soldier fly defatted meal as a dietary protein source for broiler chickens: Effects on growth performance, blood traits, gut morphology and histological features. *J. animal science and biotechnology*, 9(1), 1–10. <https://doi.org/https://doi.org/10.1186/s40104-018-0266-9>.
- de Souza Vilela, Jessica, Andronicos, Nicholas M., Kolakshyapati, Manisha, Hilliar, Matthew, Sibanda, Terence Z., Andrew, Nigel R., Swick, Robert A., Wilkinson, Stuart, Ruhnke, Isabelle, 2021. Black Soldier Fly larvae in broiler diets improve

- broiler performance and modulate the immune system. *Animal Nutrition* 7 (3), 695–706. <https://doi.org/10.1016/j.aninu.2020.08.014>.
- Dengah, S.P., Umboh, J., Rahasia, C., Kowel, Y.H., 2015. Pengaruh penggantian tepung ikan dengan tepung maggot (*Hermetia illucens*) dalam ransum terhadap performans broiler. *Zootec* 36 (1), 51–60.
- Department Veterinary Service, M., 2020. Laporan perangkaan ternakan 2018/2019.
- Godara, R.S., Naskar, S., Das, B.C., Godara, A.S., Ghosh, M.K., Mondal, M., Bhat, S.A., 2015. Effect of area specific mineral supplementation on biochemical profile in female Black Bengal goats. *J. Animal Res.* 5 (2), 263–268. <https://doi.org/10.5958/2277-940X.2015.00045.5>.
- Hansen, B.-A., Bruslerud, Ø., 2018. Hypomagnesemia in critically ill patients. *J. Intensive Care* 6 (1), 1–11.
- Hong, J.-C., Steiner, T., Aufy, A., Lien, T.-F., 2012. Effects of supplemental essential oil on growth performance, lipid metabolites and immunity, intestinal characteristics, microbiota and carcass traits in broilers. *Livestock Sci.*, 144(3), 253–262. <https://doi.org/10.1016/j.livsci.2011.12.008>.
- Inrae-Cirad-Afz, F.t., 2021. Composition and nutritive values of feeds for cattle, sheep, goats, pigs, poultry, rabbits, horses and salmonids.
- Jansen, Z., 2018. The nutritional potential of black soldier fly (*Hermetia illucens*) larvae as a protein source for broiler chicken diets. Stellenbosch University, Stellenbosch.
- Kinasih, I., Putra, R.E., Permana, A.D., Gusmara, F.F., Nurhadi, M.Y., Anitarsari, R.A., 2018. Growth performance of black soldier fly larvae (*Hermetia illucens*) fed on some plant based organic wastes. *HAYATI J. Biosci.*, 25(2), 79–79.
- Kooienga, E.M., Baugher, C., Currin, M., Tomberlin, J.K., Jordan, H.R., 2020. Effects of bacterial supplementation on black soldier fly growth and development at benchtop and industrial scale. *Frontiers Microbiol.*, 11, 2907. <https://doi.org/10.3389/fmicb.2020.587979>.
- Li, X., Zhang, D., Yang, T. Y., & Bryden, W. L. (2016). Phosphorus bioavailability: a key aspect for conserving this critical animal feed resource with reference to broiler nutrition. *Agriculture*, 6(2), 25. <https://doi.org/https://doi.org/10.3390/agriculture6020025>.
- Loponte, R., Nizza, S., Bovera, F., De Riu, N., Fliegerova, K., Lombardi, P., Vassalotti, G., Mastellone, V., Nizza, A., Moniello, G., 2017. Growth performance, blood profiles and carcass traits of Barbary partridge (*Alectoris barbara*) fed two different insect larvae meals (*Tenebrio molitor* and *Hermetia illucens*). *Res. Veterinary Sci.*, 115, 183–188. <https://doi.org/10.1016/j.rvsc.2017.04.017>.
- Lumeij, J., 1997. *Avian clinical biochemistry*. In: *Clinical biochemistry of domestic animals*. Elsevier, pp. 857–883.
- Marono, S., Loponte, R., Lombardi, P., Vassalotti, G., Pero, M.E., Russo, F., Gasco, L., Parisi, G., Piccolo, G., Nizza, S., Di Meo, C., Attia, Y.A., Bovera, F., 2017. Productive performance and blood profiles of laying hens fed *Hermetia illucens* larvae meal as total replacement of soybean meal from 24 to 45 weeks of age. *Poult. Sci.* 96 (6), 1783–1790.
- Mat, K., Mohamad, N., Rusli, N., Rahman, M., Harun, H., Al-Amsyar, S., Mahmud, M., 2021. Preliminary study on the effect of feeding Black Soldier Fly Larvae (BSFL) on growth and laying performance of Japanese Quail (*Coturnix japonica*). *J. Agricultural Technol.* 17 (3), 977–986.
- MET Malaysia, M.M.D., 2019. Ministry of Environment and Water.
- Moraes, V., Malheiros, R., Furlan, R.L., Bruno, L., Malheiros, E., Macari, M., 2002. Effect of environmental temperature during the first week of brooding period on broiler chick body weight, viscera and bone development. *Brazilian J. Poultry Sci.*, 4. <https://doi.org/10.1590/S1516-635X2002000100003>.
- National Research Council, P., 1994. *Nutrient Requirement of Poultry*. 9th revised edn National Academic Press. Washington, DC.
- Nekrasov, R.V., Zelenchenkova, A.A., Chabaev, M.G., Ushakova, N.A., 2018. Melanine protein-energy additive from *Hermetia illucens* larvae in nutrition of calves. *Sel'skokhozyaistvennaya Biologiya* 53 (2), 374–384.
- Onsongo, V., Osuga, I., Gachuiiri, C., Wachira, A., Miano, D., Tanga, C., Ekesi, S., Nakimbugwe, D., Fiaboe, K., 2018. Insects for income generation through animal feed: effect of dietary replacement of soybean and fish meal with black soldier fly meal on broiler growth and economic performance. *J. Econ. Entomol.*, 111(4), 1966–1973. <https://doi.org/10.1093/jeetov118>.
- Pati, S., Sumedha, C., Chatterji, A., Dash, B.P., 2016. A novel food formulation for the juveniles of *Penaeus monodon* (Fabricius). *Turkish J. Fisheries Aquatic Sci.* 16 (2), 481–488. https://doi.org/10.4194/1303-2712-v16_2_29.
- Rahman, M.M., Mat, K., Ishigaki, G., Akashi, R., 2021. A review of okara (soybean curd residue) utilization as animal feed: Nutritive value and animal performance aspects. *Animal Sci. J.*, 92(1), e13594. <https://doi.org/10.1111/asj.13594>.
- Schiavone, A., Dabbou, S., De Marco, M., Cullere, M., Biasato, I., Biasibetti, E., Capucchio, M.T., Bergagna, S., Dezzutto, D., Meneguz, M., Gai, F., Dalle Zotte, A., Gasco, L., 2018. Black soldier fly larva fat inclusion in finisher broiler chicken diet as an alternative fat source. *Animal* 12 (10), 2032–2039. <https://doi.org/10.1017/S1751731117003743>.
- Shumo, M., Osuga, I.M., Khamis, F.M., Tanga, C.M., Fiaboe, K.K., Subramanian, S., Ekesi, S., van Huis, A., Borgemeister, C., 2019. The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya. *Sci. Rep.*, 9(1), 1–13. <https://doi.org/10.1038/s41598-019-46603-z>.
- Sowande, O., Odufowora, E., Adalokun, A., Egbeyale, L., 2008. Blood minerals in wad sheep and goats grazing natural pastures during wet and dry seasons. *Archivos de zootecnia* 57 (218), 275–278.
- Szabó, A., Mézes, M., Horn, P., Sütő, Z., Bázár, Gy., Romvári, R., 2005. Developmental dynamics of some blood biochemical parameters in the growing turkey (*Meleagris gallopavo*). *Acta Vet. Hung.* 53 (4), 397–409.
- Widjastuti, T., Wiradimadja, R., Rusmana, D., 2014. The effect of substitution of fish meal by Black Soldier Fly (*Hermetia illucens*) maggot meal in the diet on production performance of quail (*Coturnix coturnix japonica*). *Anim. Sci.* 57, 125–129.
- Xiao, X., Jin, P., Zheng, L., Cai, M., Yu, Z., Yu, J., Zhang, J., 2018. Effects of black soldier fly (*Hermetia illucens*) larvae meal protein as a fishmeal replacement on the growth and immune index of yellow catfish (*Pelteobagrus fulvidraco*). *Aquaculture Res.* 49(4), 1569–1577. <https://doi.org/10.1111/are.13611>.
- Yan, F., Coto, C., Wang, Z., Cerrate, S., Watkins, S.E., Waldroup, P.W., 2010. Comparison of nutrient recommendations for broilers. *Int. J. Poultry Sci.* 9 (11), 1006–1014.
- Yang, Z., Pirgozliev, V., Rose, S., Woods, S., Yang, H., Wang, Z., Bedford, M., 2020. Effect of age on the relationship between metabolizable energy and digestible energy for broiler chickens. *Poultry Sci.*, 99(1), 320–330. <https://doi.org/10.3382/ps/pez495>.
- Zulhisyam, A.K., Kabir, M.A., Dawood, M.A., Razab, M.K.A.A., Ariff, N.S.N.A., Sarkar, T., Pati, S., Edinur, H.A., Mat, K., Ismail, T.A., 2021. Effect of fish meal substitution with fermented soy pulp on growth performance, digestive enzyme, amino acid profile, and immune-related gene expression of African catfish (*Clarias gariepinus*). *Aquaculture*, 737418. <https://doi.org/10.1016/j.aquaculture.2021.737418>.
- Zulhisyam, A.K., Kabir, M.A., Razab, M.K.A.A., Munir, M.B., Lim, P.T., Wei, L.S., 2020. A replacement of plant protein sources as an alternative of fish meal ingredient for African catfish, *Clarias gariepinus*: A review. *J. Tropical Resour. Sustain. Sci. (JTRSS)* 8 (1), 47–59.