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Abstract

This study investigated the effect of processed forms (defatted or hydrolyzed) of black soldier fly larvae (*Hermetia illucens* L., BSFL) as a protein substitute on broilers. Experiment 1 was a feeding experiment, and Experiment 2 was a metabolism experiment. In Experiment 1, a total of 120 day-old Arbor Acres broilers (initial body weight 39.52 ± 0.24 g) were used for 28 days. There were 8 replicate pens, and 5 broilers were assigned to each pen. In Experiment 2, a total of 36 day-old broilers (initial body weight 39.49 ± 0.21 g) were used for the metabolism trial. There were 2 broilers in a metabolism cage and six replicate cages per treatment. The dietary treatments were as follows: a basal diet (CON), a basal diet without fishmeal and substitute with defatted BSFL (T1), a basal diet without fishmeal and a substitute with hydrolyzed BSFL (T2). In Experiment 1, during the entire experimental period, the T2 group significantly increased ($p < 0.05$) body weight gain and feed intake compared to the CON and T1 groups. The feed conversion ratio showed a lower tendency ($p = 0.057$) in the T2 group than in the CON and T1 groups. At week 2, the CON and T2 groups were significantly higher ($p < 0.05$) crude protein (CP) digestibility than the T1 group. At week 4, the total protein level significantly increased ($p < 0.05$) in the CON and T2 groups compared to the T1 group. In Experiment 2, the CP digestibility significantly increased ($p < 0.05$) in the T2 group compared to the CON and T1 group at weeks 2 and 4. At week 4 amino acid digestibility, the T2 group significantly increased ($p < 0.05$) lysine, methionine, tryptophan, and glycine digestibility compared to the T1 group. There was no difference in fecal microbiota among the treatment groups. In conclusion, feeding hydrolyzed BSFL as a fishmeal substitute in broiler diets improved growth performance, CP digestibility, and specific amino acid digestibility. Therefore, it is considered that hydrolyzed BSFL in broiler diets can be sufficiently used as a new protein source.

Keywords (3 to 6): Black soldier fly larvae, Broiler, Fishmeal

Introduction

The environmental trends of global warming, decreasing water availability, and decreasing arable agricultural land are all increasing the importance of finding new feed sources for monogastric animals [1]. Insect meals contain high quality and quantity of protein and also have a high feed-to-protein conversion rate, which has attracted attention to insect meals as a new and promising alternative dietary protein source for monogastric animals [2]. Insects are also easily reared and can promote the reuse of by-products, thus reducing organic waste and waste disposal costs [3, 4].

As a specific example, black soldier fly larvae (*Hermetia illucens* L., BSFL) contain abundant amounts of fat (7-39% on a dry matter basis) and protein (37-63% on a dry matter basis) [5]. The BSFL has great advantages as a protein source, especially as it contains various essential amino acids (Methionine 1.8-2.0%; Valine 2.3-2.8%; Lysine 2.3-2.6%; Arginine 1.8-2.0%) [6, 7]. Lauric acid, which constitutes up to 64% of the total saturated fatty acid composition of BSFL, has been shown to reduce the number of harmful bacteria in feces and to have antibacterial action against harmful bacteria [8-10]. Moreover, chitin—which is part of the BSFL exoskeleton—has been reported to have immunomodulatory effects on the innate and adaptive immune systems in mammals [11]. With this advantage, BSFL is already used today as a protein substitute ingredient in the diets of monogastric animals, including poultry, pigs, and dogs [12]. Previous studies have reported that feeding BSFL as a substitute for soybean meal or fishmeal can improve the broiler feed conversion ratio (FCR) [13, 14]. Also, to use insect meals in animal diets, insects may be processed in various ways and used [15, 16]. When insects are defatted, they can be stored for a longer period by preventing the oxidation of lipids occurring during drying and storage [17, 18]. In the case of using the hydrolysis processing method using enzymes, enzymes can decompose proteins to promote the absorption of nutrients and increase the digestibility of livestock. Cho et al. [15] reported that processing insects by hydrolysis can reduce anti-nutritional factors in insects, and feeding hydrolyzed *Tenebrio molitor* larvae in growing pigs improved the apparent ileal digestibility of dry matter (DM) and crude fat compared to feeding defatted *T. molitor* larvae. Also, the feeding defatted BSFL with a higher protein content at 5 to 19% in a broiler diet, growth performance, carcass quality, and meat quality might be all improved [12, 19]. These previous studies show the possibility that insect meals using various processing methods can replace existing protein sources.

However, the results of existing studies examining the effects of BSFL on immunity and the nutrient digestibility of broilers are still inconsistent, and additional research is needed to elucidate the mechanism of these effects. There is also a relative lack of studies comparing the relative efficacies of different processing forms of BSFL. Therefore, this study was conducted to investigate the effect of the processed form of BSFL (defatted or hydrolyzed) as a protein substitute on growth performance, nutrient digestibility, blood profiles, meat quality, and fecal microbiota in broilers.

Materials and Methods

Ethics approval and consent to participate

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungbuk National University, Cheongju, Korea (approval no. CBNUA-2049-22-02).

Preparation black soldier fly larvae and diets

The BSFL was supplied after being processed in the form of defatted hydrolyzed at Jeju National University (Jeju-si, Korea). Table 1 showed the nutritional components of BSFL in the defatted and hydrolyzed forms. The basal diet contained 3% of fishmeal regardless of the feeding phase, and the BSFL diet replaces all 3% of fishmeal in the basal diet with each BSFL form. All diets were fed over 4 phases: pre-starter (days 0-7; Table 2), starter (days 8-14; Table 3), grower (days 15-21; Table 4), and finisher (days 22-28; Table 5). All diets were formulated to meet or exceed the NRC requirement [20].

Experiment 1

Animals and experimental design

A total of 120 one-day-old Arbor Acres broilers (initial body weight of 39.52 ± 0.24 g) were obtained from a local hatchery (Cherrybro Co., Eumseong, Korea) and used in this experiment 1 (feeding trial) for 28 days. All broilers were randomly allocated into three dietary treatments in a randomized complete block design. Each treatment had 8 replicate pens, and 5 broilers were assigned to each pen. The dietary treatments were as follows: a basal diet (CON), a basal diet without fishmeal and substitute with defatted BSFL (T1), a basal diet without fishmeal and substitute with hydrolyzed BSFL (T2). The experiment initiation temperature was 33 ± 1℃, after that, the temperature was gradually lowered to maintain 25 ± 1℃. All broilers were given ad libitum access to diet and water throughout the experiments.

Growth performance and Frequency of diarrhea

All broilers were weighed at the beginning of the experiment, at the 2 weeks, and at the end of the experiment (4 weeks) to calculate the body weight gain (BWG). Feed intake (FI) was calculated by subtracting the remaining amount from the diet supply amount until measuring body weight (BW). The FCR was calculated by dividing FI by BWG.

To measure the frequency of diarrhea, the same person recorded the diarrhea score at 8:00 and 17:00 for each treatment group during the entire experimental period. The diarrhea scores were as follows: 0, normal feces; 1, soft feces; 2, mild diarrhea; 3, severe diarrhea. The frequency of diarrhea was calculated by counting pen days in which the average diarrhea score of each pen was ≥ 2.

Nutrient digestibility

At 2 and 4 weeks, 0.2% chromium oxide (Cr_2O_3) was added as an indigestible indicator in all broiler diets for fecal sampling. While collecting feces, the diet was also collected, and immediately stored in a freezer at -20℃. Before analyzing nutrient digestibility, fecal samples were dried at 70℃ for 72 h and then crushed on a 1 mm screen. The DM, crude protein (CP), and gross energy (GE) of diet and feces samples were all analyzed according to the method of AOAC [21]. The DM analysis of samples was performed in an oven at 105℃ for 16 h. The CP was analyzed according to the Kjeldahl method. An adiabatic oxygen bomb calorimeter (6400 Automatic Isoperibol calorimeter, Parr, USA) was used to measure GE in diets and feces. Chromium levels were determined via UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) using Williams et al. [22] method. The following equation was used to calculate the apparent total tract digestibility (ATTD).

Digestibility = 1 – [(Concentration of nutrient in fecal × Concentration of Cr_2O_3 in the diet)/(Concentration of nutrient in diet × Concentration of Cr_2O_3 in the fecal)] × 100.

Blood profile

Blood samples were collected from the brachial wing vein at 2 and 4 weeks (before slaughter), 8 broilers per treatment. Blood samples were collected into vacuum tubes containing K3EDTA for completed blood count analysis and nonheparinized tubes for serum analysis, respectively. After collection, serum samples were centrifuged at 12,500 × g at 4℃ for 20 min. Red blood cell (RBC), white blood cell (WBC), and lymphocyte were analyzed using an automatic hematology analyzer (XE2100D, Sysmex, Kobe, Japan). Total protein (TP) level was measured using a colorimetric method, and blood urea nitrogen (BUN) level was analyzed using the urease glutamate dehydrogenase method. The TP and BUN in blood were measured using a fully automated chemistry analyzer (Cobas C702, Hofmann-La Roche, Switzerland).

Meat quality

At 4 weeks, all broilers were slaughtered for cervical dislocation and 8 broiler's breast meat was collected per treatment. General component analysis including moisture, fat, protein, and ash was analyzed according to the AOAC method [21]. The pH was measured with a pH meter (Thermo Orion 535A, Thermo, IL, USA) after adding 100 mL of distilled water to 10 g of breast meat and then homogenizing at 68,400 × g for 30 sec using a homogenizer (Bihon seiki, Ace, Osaka, Japan). Water holding capacity (WHC) was analyzed according to the method of Laakkonoen [23]. To analyze the cooking loss (CL), breast meat with a thickness of 3 cm was shaped into a circle, immersed in a 70℃-water bath, and cooled for 30 min. After that, the weight ratio (%) of the initial sample was measured. Drip loss (DL) was calculated as the weight ratio (%) of the initial sample by measuring the amount of loss caused by shaping 2 cm-thick breast meat into a circular shape, vacuum-packing it in a polypropylene bag, and storing it in a refrigerator at 4℃ for 24 h. Shear force was analyzed through a shear force cutting test using a rheometer (Compac-100, Sun Scientific co., Tokyo, Japan). Color measurement of breast meat was performed using a Minolta colorimeter (Konica Minolta CR-410, Osaka, Japan). Meat color characteristics were expressed by the CIE L* (lightness), a* (redness), b* (yellowness) system. Two measurements were taken on the surface and cut area of each meat sample.

Experiment 2

Animals and experimental design

A total of 36 one-day-old mixed-sex Arbor Acres broilers (initial BW of 39.49 ± 0.21 g) were used in this experiment 2 (metabolism trial) for 28 days. All broilers were randomly allocated into three dietary treatments based on the initial BW. Dietary treatments were the same as in Experiment 1. There were 2 broilers in a metabolism cage and six replicate cages per treatment. Each cage was 100 cm in width, 40 cm in depth, and 45 cm in height. The experiment was performed in an environmentally controlled room. During the weeks 1 and 3, the diet was fed ad libitum. During the 2nd and 4th weeks (fecal sampling period), the feed supply amount and the remaining amount were recorded every day. All broilers were given ad libitum access to water throughout the experiments.

Nutrient digestibility

The total collection method was used to analyze the ATTD of DM, CP, GE, and amino acid. The diet containing 0.5% chromium oxide was fed at the 2 and 4 weeks, and feces were collected for 5 days each. The collected feces were stored at -20℃ until analysis, dried at 70℃ for 72 h at the time of analysis, and then analyzed by crushing with a 1-mm screen. The DM, CP, and GE of diet and feces were analyzed in the same way as in Experiment 1 according to the method of AOAC [21]. Amino acids were analyzed using the high-performance liquid chromatography (HPLC; Shimadzu model LC-10AT, Shimadzu, Kyoto, Japan) method [24]. Cysteine and methionine were oxidized with performic acid for 16 h at 0℃, after that, using cysteic acid and methionine sulfone, respectively, was for analysis.

Fecal microbiota

To analyze fecal microbiota, fresh feces were collected from each cage for each treatment group at the 2 and 4 weeks. Bacterial colonies were counted by the pour plate method. One gram of each fecal sample was diluted with 9 mL of 1×PBS buffer and vortexed for 1 min. Samples were used for measuring the number of viable cells by serial dilution from 10^{-1} to 10^{-8}. To measure the number of colonies, MacConkey agar was used for *Escherichia coli* (*E. coli*), BG sulfa agar was used for *Salmonella*, and de Man, Rogosa and Sharpe agar (MRS) agar was used for *Lactobacillus*. All agars were purchased from

KisanBio (Seoul, Korea). The MacConkey and BG sulfa agar plates were cultured at 37℃ for 24 h. The MRS agar plates were cultured at 37℃ for 48 h. After the incubation periods, the agar plates were immediately removed from the incubator, and the number of each colony was counted. The number of microbial colonies was log-transformed before statistical analysis.

Statistical analysis

All data from Experiments 1 and 2 except for Experiment 1's frequency of diarrhea was analyzed through the general linear model procedure in SAS (SAS Institute, Cary, NC, USA), using each pen as the experimental unit. The frequency of diarrhea was compared with a chi-square test, using the FREQ procedure of SAS. Differences between treatment means were determined using Tukey's multiple range test. A probability level of $p < 0.05$ was indicated to be statistically significant, and a level of $0.05 \leq p < 0.10$ was considered to have such a tendency.

Results

Experiment 1

Growth performance and frequency of diarrhea

There was no difference in initial BW among the treatment groups (Table 6). At 2 and 4 weeks, the T2 group had significantly higher ($p < 0.05$) BW than the T1 group. At weeks 0 to 2, the BWG and FI significantly increased ($p < 0.05$) in the T2 group compared to the T1 group. At weeks 2 to 4, the T2 group had significantly higher ($p < 0.05$) BWG and FI than the CON group. For FCR, the T2 group showed a lower tendency ($p = 0.063$) than the CON and T1 groups. During the entire experimental period, the T2 group significantly increased ($p < 0.05$) BWG and FI compared to the CON and T1 groups. The FCR showed a lower tendency ($p = 0.057$) in the T2 group than in the CON and T1 groups. The frequency of diarrhea was no different among the treatment groups.

Nutrient digestibility

There was no difference in DM digestibility among the treatment groups at weeks 2 and 4 (Table 7). At week 2, the CON and T2 groups were significantly higher ($p < 0.05$) CP digestibility than the T1 group. The GE digestibility was significantly higher ($p < 0.05$) in the T2 group than in the T1 group. At week 4, the CON group had significantly higher ($p < 0.05$) CP digestibility than the T1 group. For GE digestibility, the T2 group showed a similar tendency ($p = 0.068$) to the CON group.

Blood profile

At week 2, there was no difference in RBC, WBC, lymphocyte, TP, and BUN levels among the treatment groups (Table 8). At week 4, the TP level significantly increased ($p < 0.05$) in the CON and T2 groups compared to the T1 group. There was no difference in RBC, WBC, lymphocyte, and BUN levels among the treatment groups at week 4.

Meat quality

The ash content in breast meat had significantly higher ($p < 0.05$) in the T2 group than in the CON group (Table 9). The pH was significantly higher ($p < 0.05$) in the T1 and T2 groups than in the CON group. For WHC, the T1 and T2 groups showed a higher tendency ($p = 0.097$) than the CON group. There was no difference in moisture, fat, protein, CL, DL, shear force, and meat color among the treatment groups.

Experiment 2

Nutrient digestibility

At week 2, the DM digestibility was significantly higher ($p < 0.05$) in the T2 group than in the CON group (Table 10). The CP digestibility significantly increased ($p < 0.05$) in the T2 group compared to the CON and T1 group at weeks 2 and 4. There was no difference in GE digestibility among the treatment groups at weeks 2 and 4.

At week 2 amino acid digestibility, the T2 group had significantly higher ($p < 0.05$) valine and leucine digestibility than the CON and T1 groups (Table 11). The glycine digestibility was significantly higher ($p < 0.05$) in the T2 group than in the CON group. The threonine, phenylalanine, and glutamic acid digestibility showed a higher tendency ($p = 0.058$, $p = 0.072$, and $p = 0.061$, respectively) in the T2 group than in the CON group. At week 4 amino acid digestibility, the T2 group significantly increased ($p < 0.05$) lysine, methionine, tryptophan, and glycine digestibility compared to the T1 group (Table 12). The glutamic acid digestibility was significantly higher ($p < 0.05$) in the T2 group than in the CON group. The phenylalanine digestibility showed a higher tendency ($p = 0.079$) in the T2 group than in the T1 group.

Fecal microbiota

There was no difference in *E. coli*, *Salmonella*, and *Lactobacillus* counts among the treatment groups (Table 13).

Discussion

In Experiment 1, hydrolyzed BSFL showed improvements in both BW and BWG compared to the fishmeal and defatted BSFL throughout the entire experimental period. de Souza Vilela et al. [1] reported significant increases in BW in the grower and finisher phases according to the level of BSFL in broiler diets. Other studies have also reported that feeding BSFL can improve BW and BWG [19, 25]. This is consistent with the present study's findings that feeding hydrolyzed BSFL increased the BW and BWG of broilers. The BSFL is rich in essential nutrients such as protein and fat and is particularly rich in amino acids. Further, chitin, which is a polysaccharide constituting the exoskeleton of insects, can serve as a major energy source for intestinal cells by increasing the production of butyric acid in the cecum [26]. Butyric acid enhances intestinal blood flow, which improves tissue oxygenation and nutrient transport and absorption [27]. Therefore, here it is believed that the abundant nutrients and chitin in hydrolyzed BSFL promote the growth of broilers, ultimately resulting in improved BW and BWG. Moreover, in this study, hydrolyzed BSFL showed higher FI than both fishmeal and defatted BSFL. The FI is used as an indicator to evaluate the palatability of a diet [28]. In this study, the increased FI of hydrolyzed BSFL suggests that it is more palatable than fishmeal and defatted BSFL, and that it does not adversely affect feed consumption. However, to our knowledge, there has yet to be a study examining hydrolysis among the processing methods of BSFL. We hydrolyzed BSFL using an enzyme called alcalase, which is a serine endopeptidase from *Bacillus licheniformis* with an alkaline pH optimum and broad substrate specificity, and which has been reported to be helpful in obtaining peptides with antioxidant activity from various protein sources [29, 30]. When a protein source is hydrolyzed and used, the enzyme decomposes the protein, thus facilitating the absorption of nutrients and increasing the digestibility of livestock. Therefore, hydrolyzed BSFL—which in this study showed CP digestibility similar to that of fishmeal at weeks 2 and 4—is considered to have improved digestibility and growth performance as protein digestion became easier through the hydrolysis process. Also, in Experiment 2, hydrolyzed BSFL showed higher CP digestibility than both fishmeal and defatted BSFL, while in week 2, DM digestibility was also higher than that of fishmeal. It has been reported that if chitin is included in BSFL that is contained in a large amount in a diet, then monogastric animals cannot easily digest it, which can negatively affect protein digestibility [31, 32]. In previous studies, an increase in chitin content when feeding more than 17-29% insect meal has been shown to cause a decrease in protein digestibility [33, 34]. The increase in CP digestibility in our study is believed to be due to the fact that the protein is broken down in advance through the hydrolysis process to facilitate the absorption of nutrients. It is also considered to be the case that the digestibility of broilers was not affected because the chitin content was not high, which was achieved by feeding a lower content (3%) of BSFL than has been fed in previous studies. Insect meals have higher amino acid contents than other animal proteins [35]. In our study, the amino acid digestibility of hydrolyzed BSFL was increased in valine and leucine at week 2, and it was increased at lysine and methionine at week 4. The amino acid digestibility obtained in this study was higher than those of other animal proteins (blood meal, feather meal, etc.) reported in previous studies [36, 37]. In particular, methionine and lysine—which are the limiting amino acids in broilers—showed higher digestibility than other animal proteins when fed with BSFL in this study. This suggests that BSFL has a rich amino acid profile and can be used as a protein source in broiler diets. However, there have been few studies examining the effect of BSFL on amino acid digestibility to this point, so additional research is needed.

In our study, RBC, WBC, lymphocyte, and BUN did not show significant differences among treatment groups, as the outcomes were all within the physiologically normal range for broilers [38], suggesting that BSFL feeding does not affect broiler health. The TP in serum is positively related to tissue synthesis for growth in broilers, and it may reflect protein synthesis and nutritional status [39, 40]. In our study, the TP level at week 4 of hydrolyzed BSFL was significantly similar to that of fishmeal. Therefore, it is believed that hydrolyzed BSFL can play a role similar to fishmeal in tissue synthesis for broiler growth.

In this study, the only general component of broiler breast meat that showed significant differences was ash content. According to Cullere et al. [41], processing insect raw materials can result in higher mineral content than unprocessed insects, particularly when defatted, as the minerals are concentrated and can be even higher. Accordingly, it seems that the ash content of meat was increased by feeding BSFL, which is higher in minerals than fishmeal. Previous studies have shown that the pH of broiler breast meat varies over a wide range of 5.7 to 6.2, with the most cited pH value being 5.8 to 5.9 [42-44]. Popova et al. [45] reported that feeding full-fat BSFL showed higher pH than soybean meal and partially defatted BSFL. Therefore, in this study, it is believed that hydrolyzed BSFL, which has a similar fat content to full-fat BSFL (38.53% vs 31.14%), showed a higher pH than fishmeal. Differences in pH values among treatment groups can affect breast meat color and WHC by increasing WHC and decreasing DL, as proteins that are farther from the isoelectric point bind to more water [13]. Meat color is an important quality indicator for consumers [46]. The paleness of meat is indicated by the L* value, where a high L* value indicates poor meat quality [47]. In this study, WHC tended to increase compared to fishmeal when BSFL was fed due to the difference in pH value, but there was no significant difference in DL and meat color. These results indicate that BSFL feeding does not adversely affect broiler meat quality.

The BSFL has a high content of lauric acid, which is known to be a natural antibacterial agent, and which has been reported to be effective in inhibiting the growth of harmful bacteria in intestines by destroying cell membranes [48]. However, there was no significant difference in fecal microbiota among the treatment groups in our study. This is consistent with the results outlined by Cullere et al. [41], and it is considered that all broilers used in this study exhibited optimal health and did not show any difference in fecal microbiota.

CONCLUSION

In conclusion, feeding hydrolyzed BSFL as a fishmeal substitute in broiler diets improved broiler growth performance (increased BW and BWG), improved CP digestibility, and specific amino acid digestibility. Feeding of BSFL did not adversely affect meat quality or blood profiles. Therefore, it is considered that hydrolyzed BSFL in broiler diets can be sufficiently used as a new protein source.

Competing Interests

No potential conflict of interest relevant to this article was reported.

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Tables and Figures

Table 1. Nutrient components of black soldier fly larvae (BSFL) in the defatted and hydrolyzed form[1]

Items, %	Content	
	Defatted BSFL	Hydrolyzed BSFL
Moisture	6.58	6.59
CP	58.76	38.53
EE	11.51	42.91
CF	9.15	5.61
Ash	10.07	7.68
Aspartic acid	5.15	3.38
Threonine	2.00	1.06
Serine	2.09	1.02
Glutamic acid	6.33	4.37
Glycine	3.01	1.85
Alanine	4.25	2.64
Valine	2.72	1.82
Isoleucine	1.63	1.11
Leucine	3.04	1.94
Tyrosine	3.76	2.19
Phenylalanine	2.89	1.37
Lysine	2.84	1.75
Histidine	2.74	1.67
Arginine	2.06	1.16
Cysteine	0.37	0.22
Methionine	2.58	1.74
Proline	3.33	1.87

[1] Abbreviation: BSFL, black soldier fly larvae; CP, crude protein; EE, ether extract; CF, crude fiber.

Table 2. Ingredient composition of experimental diets (phase 1/days 0-7)[1]

Items	Basal diet	Defatted BSFL	Hydrolyzed BSFL
Ingredients, %			
Corn	37.6	39.5	38.7
Wheat fine	15.3	15.3	15.3
Rice pollards	2.4	2.4	2.4
Soybean meal	26.9	25.1	25.9
Cookie wheat flour	1.9	1.9	1.9
DDGS	5.0	5.0	5.0
Animal protein	3.3	3.2	3.2
Fishmeal	3.0	-	-
Defatted BSFL	-	3.0	-
Hydrolyzed BSFL	-	-	3.0
Animal fat	1.7	1.7	1.7
L-lysine	0.6	0.6	0.6
L-methionine	0.4	0.4	0.4
L-threonine	0.2	0.2	0.2
L-tryptophan	0.1	0.1	0.1
Salt	0.2	0.2	0.2
Limestone	0.5	0.5	0.5
MDCP	0.2	0.2	0.2
Liquid-Choline	0.1	0.1	0.1
Vitamin premix[2]	0.3	0.3	0.3
Mineral premix[3]	0.3	0.3	0.3
Total	100.0	100.0	100.0
Chemical composition			
AMEn, Kcal/kg	3,000	3,000	3,000
CP, %	23.3	23.3	23.3
Ether extract, %	5.3	5.3	5.4
Crude fiber, %	3.4	3.4	3.4
Crude ash, %	5.8	5.9	5.8
Calcium, %	0.9	0.9	0.9
Phosphorus, %	0.5	0.5	0.5
Lysine, %	1.5	1.5	1.5
SAA, %	1.1	1.1	1.1

[1] Abbreviation: DDGS, Dried distiller's grains with soluble; MDCP, Mono-dicalcium phosphate; SAA, Sulfur amino acids; AMEn, nitrogen-corrected apparent metabolizable energy; CP, crude protein.

[2] Supplied per kg diet: vitamin A, 9000 IU; vitamin D_3, 3000 IU; vitamin E, 48 mg; vitamin K, 3 mg; thiamin, 1.8 mg; riboflavin, 6 mg; pyridoxine, 3 mg; vitamin B_{12}, 0.012 mg; niacin, 42 mg; folic acid, 1.2 mg; biotin, 0.24 mg; pantothenic acid, 12 mg.

[3] Supplied per kg of diet: manganese, 120 mg; zinc, 100 mg; iron, 80 mg; copper, 20 mg; iodine, 2 mg; selenium, 0.3 mg; cobalt, 0.5 mg.

Table 3. Ingredient composition of experimental diets (phase 2/days 8-14)[1]

Items	Basal diet	Defatted BSFL	Hydrolyzed BSFL
Ingredients, %			
Corn	42.2	44.2	43.2
Wheat fine	15.1	15.1	15.1
Rice pollards	2.5	2.5	2.5
Soybean meal	21.0	19.2	20.1
Cookie wheat flour	2.0	2.0	2.0
DDGS	7.0	7.0	7.0
Animal protein	2.5	2.3	2.4
Fishmeal	3.0	-	-
Defatted BSFL	-	3.0	-
Hydrolyzed BSFL	-	-	3.0
Animal fat	1.9	1.9	1.9
L-lysine	0.6	0.6	0.6
L-methionine	0.3	0.3	0.3
L-threonine	0.1	0.1	0.1
L-tryptophan	0.1	0.1	0.1
Salt	0.2	0.2	0.2
Limestone	0.6	0.6	0.6
MDCP	0.2	0.2	0.2
Liquid-Choline	0.1	0.1	0.1
Vitamin premix[2]	0.3	0.3	0.3
Mineral premix[3]	0.3	0.3	0.3
Total	100.0	100.0	100.0
Chemical composition			
AMEn, Kcal/kg	3020	3020	3020
CP, %	21.3	21.3	21.3
Ether extract, %	5.9	5.9	5.9
Crude fiber, %	3.4	3.4	3.4
Crude ash, %	5.3	5.3	5.3
Calcium, %	0.8	0.8	0.8
Phosphorus, %	0.6	0.6	0.6
Lysine, %	1.3	1.3	1.3
SAA, %	1.0	1.0	1.0

[1] Abbreviation: DDGS, Dried distiller’s grains with soluble; MDCP, Mono-dicalcium phosphate; SAA, Sulfur amino acids; AMEn, nitrogen-corrected apparent metabolizable energy; CP, crude protein.

[2] Supplied per kg diet: vitamin A, 9000 IU; vitamin D_3, 3000 IU; vitamin E, 48 mg; vitamin K, 3 mg; thiamin, 1.8 mg; riboflavin, 6 mg; pyridoxine, 3 mg; vitamin B_{12}, 0.012 mg; niacin, 42 mg; folic acid, 1.2 mg; biotin, 0.24 mg; pantothenic acid, 12 mg.

[3] Supplied per kg of diet: manganese, 120 mg; zinc, 100 mg; iron, 80 mg; copper, 20 mg; iodine, 2 mg; selenium, 0.3 mg; cobalt, 0.5 mg.

Table 4. Ingredient composition of experimental diets (phase 3/days 15-21)[1]

Items	Basal diet	Defatted BSFL	Hydrolyzed BSFL
Ingredients, %			
Corn	46.1	47.4	47.1
Wheat fine	15.6	15.6	15.6
Rice pollards	2.5	2.5	2.5
Soybean meal	17.7	16.5	16.8
Cookie wheat flour	2.0	2.0	2.0
DDGS	6.0	6.0	6.0
Animal protein	2.5	2.4	2.4
Fishmeal	3.0	-	-
Defatted BSFL	-	3.0	-
Hydrolyzed BSFL	-	-	3.0
Animal fat	1.9	1.9	1.9
L-lysine	0.6	0.6	0.6
L-methionine	0.3	0.3	0.3
L-threonine	0.1	0.1	0.1
L-tryptophan	0.1	0.1	0.1
Salt	0.2	0.2	0.2
Limestone	0.5	0.5	0.5
MDCP	0.2	0.2	0.2
Liquid-Choline	0.1	0.1	0.1
Vitamin premix[2]	0.3	0.3	0.3
Mineral premix[3]	0.3	0.3	0.3
Total	100.0	100.0	100.0
Chemical composition			
AMEn, Kcal/kg	3070	3070	3070
CP, %	20.2	20.2	20.2
Ether extract, %	6.0	5.8	5.9
Crude fiber, %	3.2	3.2	3.2
Crude ash, %	5.1	5.0	5.1
Calcium, %	0.8	0.8	0.8
Phosphorus, %	0.5	0.5	0.5
Lysine, %	1.2	1.2	1.2
SAA, %	1.0	1.0	1.0

[1] Abbreviation: DDGS, Dried distiller's grains with soluble; MDCP, Mono-dicalcium phosphate; SAA, Sulfur amino acids; AMEn, nitrogen-corrected apparent metabolizable energy; CP, crude protein.

[2] Supplied per kg diet: vitamin A, 9000 IU; vitamin D_3, 3000 IU; vitamin E, 48 mg; vitamin K, 3 mg; thiamin, 1.8 mg; riboflavin, 6 mg; pyridoxine, 3 mg; vitamin B_{12}, 0.012 mg; niacin, 42 mg; folic acid, 1.2 mg; biotin, 0.24 mg; pantothenic acid, 12 mg.

[3] Supplied per kg of diet: manganese, 120 mg; zinc, 100 mg; iron, 80 mg; copper, 20 mg; iodine, 2 mg; selenium, 0.3 mg; cobalt, 0.5 mg.

Table 5. Ingredient composition of experimental diets (phase 4/days 22-28)[1]

Items	Basal diet	Defatted BSFL	Hydrolyzed BSFL
Ingredients, %			
Corn	49.7	51.1	50.7
Wheat fine	15.2	15.2	15.2
Rice pollards	2.6	2.6	2.6
Soybean meal	15.5	14.1	14.6
Cookie wheat flour	2.0	2.0	2.0
DDGS	5.0	5.0	5.0
Animal protein	2.4	2.4	2.3
Fishmeal	3.0	-	-
Defatted BSFL	-	3.0	-
Hydrolyzed BSFL	-	-	3.0
Animal fat	1.9	1.9	1.9
L-lysine	0.5	0.5	0.5
L-methionine	0.4	0.4	0.4
L-threonine	0.1	0.1	0.1
L-tryptophan	0.1	0.1	0.1
Salt	0.2	0.2	0.2
Limestone	0.5	0.5	0.5
MDCP	0.2	0.2	0.2
Liquid-Choline	0.1	0.1	0.1
Vitamin premix[2]	0.3	0.3	0.3
Mineral premix[3]	0.3	0.3	0.3
Total	100.0	100.0	100.0
Chemical composition			
AMEn, Kcal/kg	3100	3100	3100
CP, %	19.1	19.1	19.1
Ether extract, %	5.8	5.7	5.8
Crude fiber, %	3.0	3.0	3.0
Crude ash, %	4.8	4.8	4.8
Calcium, %	0.7	0.7	0.7
Phosphorus, %	0.5	0.5	0.5
Lysine, %	1.1	1.1	1.1
SAA, %	1.0	1.0	1.0

[1] Abbreviation: DDGS, Dried distiller's grains with soluble; MDCP, Mono-dicalcium phosphate; SAA, Sulfur amino acids; AMEn, nitrogen-corrected apparent metabolizable energy; CP, crude protein.

[2] Supplied per kg diet: vitamin A, 9000 IU; vitamin D_3, 3000 IU; vitamin E, 48 mg; vitamin K, 3 mg; thiamin, 1.8 mg; riboflavin, 6 mg; pyridoxine, 3 mg; vitamin B_{12}, 0.012 mg; niacin, 42 mg; folic acid, 1.2 mg; biotin, 0.24 mg; pantothenic acid, 12 mg.

[3] Supplied per kg of diet: manganese, 120 mg; zinc, 100 mg; iron, 80 mg; copper, 20 mg; iodine, 2 mg; selenium, 0.3 mg; cobalt, 0.5 mg.

Table 6. Effect of replacement dietary of fishmeal with black soldier fly larvae (BSFL) on growth performance in broilers (Experiment 1)[1]

Items	CON	T1	T2	SE	*p*-value
BW, kg					
Initial	39.52	39.51	39.52	0.415	0.986
2 w	440.50^{ab}	431.00^{b}	465.00^{a}	7.454	0.012
4 w	1542.00^{b}	1541.00^{b}	1669.00^{a}	26.384	0.003
0-2 w					
BWG, g	400.99^{ab}	391.49^{b}	425.48^{a}	7.456	0.012
FI, g	479.40^{b}	474.55^{b}	512.85^{a}	3.828	<0.001
FCR	1.20	1.21	1.21	0.030	0.828
2-4 w					
BWG, g	1101.50^{b}	1110.00^{b}	1204.00^{a}	26.094	0.020
FI, g	1803.20^{b}	1838.20^{ab}	1861.35^{a}	13.085	0.017
FCR	1.64	1.66	1.55	0.035	0.063
0-4 w					
BWG, g	1502.49^{b}	1501.49^{b}	1629.48^{a}	26.393	0.003
FI, g	2282.60^{b}	2312.75^{b}	2374.20^{a}	12.994	<0.001
FCR	1.52	1.54	1.46	0.024	0.057
Frequency of diarrhea[2], %	35.71	30.36	35.72	-	0.670

[1] Abbreviation : CON, basal diet; T1, basal diet without a fishmeal and substitute with defatted BSFL; T2, basal diet without a fishmeal and substitute with hydrolyzed BSFL; BW, body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; SE, standard error.

[2] Frequency of diarrhea = (number of pens with diarrhea/number of pen days) × 100.

[a,b] Means with different letters are significantly differ ($p < 0.05$).

Table 7. Effect of replacement dietary of fishmeal with black soldier fly larvae (BSFL) on nutrient digestibility in broilers (Experiment 1)[1]

Items, %	CON	T1	T2	SE	*p*-value
2 w					
DM	78.11	78.22	78.05	0.300	0.920
CP	70.92^{a}	69.56^{b}	70.62^{a}	0.278	0.006
GE	78.55^{ab}	78.17^{b}	79.00^{a}	0.159	0.005
4 w					
DM	78.54	78.67	78.55	0.278	0.930
CP	75.12^{a}	73.61^{b}	74.25^{ab}	0.247	0.001
GE	78.68	77.94	78.47	0.219	0.068

[1] Abbreviation : CON, basal diet; T1, basal diet without a fishmeal and substitute with defatted BSFL; T2, basal diet without a fishmeal and substitute with hydrolyzed BSFL; DM, dry matter; CP, crude protein; GE, gross energy; SE, standard error.

[a,b] Means with different letters are significantly differ ($p < 0.05$).

Table 8. Effect of replacement dietary of fishmeal with black soldier fly larvae (BSFL) on blood profile in broilers (Experiment 1)[1]

Items	CON	T1	T2	SE	*p*-value
2 w					
RBC, 10^6/µl	2.31	2.32	2.27	0.181	0.979
WBC, 10^3/µl	22.91	23.09	22.66	1.125	0.963
Lymphocyte, %	65.15	65.03	67.35	1.630	0.535
TP, g/dL	3.23	2.95	2.68	0.296	0.436
BUN, mg/dL	3.75	3.50	3.75	0.278	0.767
4 w					
RBC, 10^6/µl	2.26	2.27	2.34	0.142	0.914
WBC, 10^3/µl	23.86	24.05	24.06	1.137	0.990
Lymphocyte, %	65.05	65.68	66.03	3.026	0.974
TP, g/dL	2.93[a]	2.65[b]	3.03[a]	0.068	0.002
BUN, mg/dL	2.50	3.00	2.75	0.374	0.646

[1] Abbreviation : CON, basal diet; T1, basal diet without a fishmeal and substitute with defatted BSFL; T2, basal diet without a fishmeal and substitute with hydrolyzed BSFL; RBC, red blood cell; WBC, white blood cell; TP, total protein; BUN, blood urea nitrogen; SE, standard error.

[a,b] Means with different letters are significantly differ ($p < 0.05$).

Table 9. Effect of replacement dietary of fishmeal with black soldier fly larvae (BSFL) on meat quality in broilers (Experiment 1)[1]

Items	CON	T1	T2	SE	*p*-value
Approximate composition of meat, %					
Moisture	75.76	75.98	75.81	0.140	0.528
Ash	1.03^{b}	1.12^{ab}	1.28^{a}	0.047	0.012
Fat	3.48	2.83	2.59	0.279	0.121
Protein	19.74	20.07	20.31	0.355	0.537
Meat quality, %					
pH	5.85^{b}	5.99^{a}	6.03^{a}	0.022	0.001
WHC	54.41	55.99	55.34	0.454	0.097
CL	17.54	17.81	17.36	0.622	0.881
DL	4.73	3.95	3.91	0.299	0.147
Shear force, g	2583.75	2421.25	2277.50	124.823	0.273
CIE L*	51.95	53.72	55.24	0.983	0.113
CIE a*	5.49	4.31	4.82	0.528	0.329
CIE b*	17.15	16.25	17.18	0.594	0.481

[1] Abbreviation : CON, basal diet; T1, basal diet without a fishmeal and substitute with defatted BSFL; T2, basal diet without a fishmeal and substitute with hydrolyzed BSFL; WHC, water holding capacity; CL, cooking loss; DL, drip loss; SE, standard error.

[a,b] Means with different letters are significantly differ ($p < 0.05$).

Table 10. Effect of replacement dietary of fishmeal with black soldier fly larvae (BSFL) on nutrient digestibility in broilers (Experiment 2)[1]

Items, %	CON	T1	T2	SE	*p*-value
2 w					
DM	77.88^{b}	79.10^{ab}	79.75^{a}	0.437	0.039
CP	74.29^{b}	74.21^{b}	76.15^{a}	0.310	0.003
GE	77.78	78.92	78.02	0.583	0.382
4 w					
DM	76.83	75.75	76.20	0.633	0.506
CP	72.78^{b}	72.73^{b}	73.87^{a}	0.272	0.026
GE	79.30	79.08	79.63	0.527	0.763

[1] Abbreviation : CON, basal diet; T1, basal diet without a fishmeal and substitute with defatted BSFL; T2, basal diet without a fishmeal and substitute with hydrolyzed BSFL; DM, dry matter; CP, crude protein; GE, gross energy; SE, standard error.

^{a,b} Means with different letters are significantly differ ($p < 0.05$).

Table 11. Effect of replacement dietary of fishmeal with black soldier fly larvae (BSFL) on amino acid digestibility in broilers at 2 w (Experiment 2)[1]

Items, %	CON	T1	T2	SE	*p*-value
Indispensable amino acids					
Threonine	85.54	86.63	86.49	0.273	0.058
Valine	80.26^{b}	79.85^{b}	81.99^{a}	0.369	0.014
Isoleucine	84.27	83.82	86.08	0.866	0.227
Leucine	89.00^{b}	89.07^{b}	90.10^{a}	0.132	0.002
Phenylalanine	88.42	88.42	89.75	0.374	0.072
Histidine	83.15	83.49	85.47	0.876	0.209
Lysine	90.65	90.66	91.04	0.277	0.565
Arginine	92.36	92.17	93.34	0.453	0.226
Methionine	93.78	94.12	93.47	0.534	0.705
Tryptophan	84.85	86.98	87.45	2.147	0.678
Dispensable amino acids					
Aspartic acid	85.49	85.65	86.19	0.822	0.824
Serine	86.12	86.64	86.49	0.890	0.913
Glutamic acid	89.98	90.22	90.90	0.222	0.061
Proline	83.14	83.05	83.79	0.586	0.641
Glycine	81.25^{b}	82.01^{ab}	84.32^{a}	0.578	0.022
Alanine	88.85	89.21	89.41	0.406	0.633
Tyrosine	90.73	91.05	91.65	0.471	0.425
Cysteine	71.47	75.71	75.44	2.475	0.449

[1] Abbreviation : CON, basal diet; T1, basal diet without a fishmeal and substitute with defatted BSFL; T2, basal diet without a fishmeal and substitute with hydrolyzed BSFL; SE, standard error.

^{a,b} Means with different letters are significantly differ ($p < 0.05$).

Table 12. Effect of replacement dietary of fishmeal with black soldier fly larvae (BSFL) on amino acid digestibility in broilers at 4 w (Experiment 2)[1]

Items, %	CON	T1	T2	SE	*p*-value
Indispensable amino acids					
Threonine	82.23	83.45	84.15	0.579	0.136
Valine	78.73	79.22	81.01	0.773	0.170
Isoleucine	85.20	83.69	86.09	0.682	0.116
Leucine	87.35	87.09	88.20	0.435	0.248
Phenylalanine	86.39	85.77	87.30	0.385	0.079
Histidine	81.35	81.31	82.84	1.693	0.775
Lysine	90.17^{ab}	89.86^{b}	90.66^{a}	0.174	0.045
Arginine	89.20	90.28	90.33	0.379	0.134
Methionine	91.37^{a}	89.33^{b}	91.52^{a}	0.467	0.028
Tryptophan	86.78^{a}	84.01^{b}	86.88^{a}	0.216	<0.001
Dispensable amino acids					
Aspartic acid	78.14	79.83	80.43	0.684	0.125
Serine	79.79	80.57	81.79	0.704	0.210
Glutamic acid	86.84^{b}	87.76^{ab}	88.20^{a}	0.304	0.048
Proline	77.85	78.35	79.64	1.154	0.559
Glycine	69.16^{ab}	68.05^{b}	72.81^{a}	1.066	0.045
Alanine	82.90	84.43	85.15	0.628	0.106
Tyrosine	87.47	88.14	88.30	1.058	0.847
Cysteine	66.37	64.48	70.38	3.227	0.466

[1] Abbreviation : CON, basal diet; T1, basal diet without a fishmeal and substitute with defatted BSFL; T2, basal diet without a fishmeal and substitute with hydrolyzed BSFL; SE, standard error.

[a,b] Means with different letters are significantly differ ($p < 0.05$).

Table 13. Effect of replacement dietary of fishmeal with black soldier fly larvae (BSFL) on fecal microbiota in broilers at 4 w (Experiment 2)[1]

Items, $\log_{10}$CFU/g	CON	T1	T2	SE	*p*-value
2 w					
E. coli	5.97	6.08	6.10	0.082	0.483
Salmonella	2.18	2.28	2.32	0.076	0.427
Lactobacillus	7.53	7.52	7.41	0.078	0.456
4 w					
E. coli	5.97	6.04	6.08	0.066	0.511
Salmonella	2.29	2.28	2.24	0.064	0.830
Lactobacillus	7.49	7.42	7.52	0.099	0.769

[1] Abbreviation : CON, basal diet; T1, basal diet without a fishmeal and substitute with defatted BSFL; T2, basal diet without a fishmeal and substitute with hydrolyzed BSFL; *E. coli*, *Escherichia coli*; SE, standard error.