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Effect of Replacing Fishmeal with Black Soldier Fly Larvae in Broiler Breeder Feed Based on Different Processing Technique

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ABSTRACT This study investigated the effects of black soldier fly larvae (BSFL) as a replacement for fishmeal (FM) in the diets of broiler breeders. Thirty-six 45-week-old Arbor Acres broiler breeders were randomly assigned to one of three dietary treatments: control (CON, basal diet with FM), T1 (100% FM replacement with defatted BSFL), and T2 (100% FM replacement with hydrolyzed BSFL), with six replicates per treatment. Our results showed no significant differences (P>0.05) among treatments in egg production, egg weight, egg mass, fertility, or hatchability at 4 weeks. At 2 weeks, the T1 showed lower (P<0.001) eggshell thickness than the CON and T2. Also, at 4 weeks, albumin height and Haugh unit were significantly higher (P<0.05) in the T1 and T2 than in the CON. In the evaluation of nutrient digestibility, the T1 showed lower (P = 0.003) dry matter digestibility than the T2 and CON at 2 weeks. Thus, the results of this study suggest that replacing FM with BSFL meal in breeder diets does not compromise laying performance, with defatting proving to be a more effective processing method than hydrolysis.

(Key words: black soldier fly larvae, nutrient digestibility, broiler breeders, egg quality, egg production)

INTRODUCTION

Insects, such as black soldier fly larvae (*Hermetia illucens* larvae, BSFL), are gaining significant attention as sustainable protein sources due to their high protein content, rapid reproduction, and low environmental impact (Wang et al., 2017). The BSFL contains approximately 40−50% protein, essential amino acids (EAA), and antioxidants, making it a promising alternative to fishmeal (FM) in the diet industry (Nam et al., 2022). The nutritional profiles of BSFL are influenced by the processing technique, which can significantly affect nutrient digestibility and utilization efficiency in livestock (Saucier et al., 2022; Zulkifli et al., 2022).

Defatted BSFL has been widely utilized as a valuable ingredient in various livestock diets (Dabbou et al., 2018; Crosbie et al., 2020; Penazzi et al., 2021). Reducing fat content increases relative protein concentration and improves oxidative stability and energy regulation (Zozo et al., 2022; Hurtado-Ribeira et al., 2023). Hydrolysis is a processing technique that uses enzymatic treatment to break down proteins into smaller units, improving digestibility and bioavailability. Previous studies have reported that hydrolyzed BSFL in pig and broiler diets significantly improved performance, nutrient digestibility, and diet efficiency than defatted BSFL (Chang et al., 2023; Lee et al., 2024). However, most of these studies have been conducted on pigs and broilers, while research focusing on laying breeder hens remains limited.

Laying breeders play a crucial role in the poultry industry by producing high-quality hatching eggs essential for meeting market demands and ensuring broiler production efficiency (Afrouziyeh et al., 2021). Xin et al. (2024) reported that defatted BSFL improved nutrient digestibility in laying hens, emphasizing the importance of processing techniques in improving laying performance, including improvements in egg quality and hatchability.

The current study aimed to evaluate the effects of replacing FM in broiler breeder diets with BSFL, focusing on the differences between defatted and hydrolyzed BSFL in terms of hatching rate, egg quality, and nutrient digestibility.

MATERIALS AND METHODS

1. Ethics Approval and Consent to Participate

The protocol for this study received approval after review

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by the Institutional Animal Care and Use Committee at Chungbuk National University in Cheongju, Korea (approval no. CBNUA-2048-22-02).

2. Black Soldier Fly Larvae Meal Sample

The BSFL were provided with a wet mixture of citrus peel and poultry offal at a ratio of 1:1 and reared until five days post-hatching, which corresponds with the second instar larval stage, before undergoing hot air drying. The defatted BSFL powder was produced by mechanically pressing the hot air-dried material to extract fat, followed by pulverizing the resultant product. The hydrolyzed BSFL powder was similarly defatted through a mechanical pressing process to diminish its high fat content, followed by hydrolysis using alcalase enzyme. The resultant hydrolysate was then concentrated and transformed into powder form. All BSFL powders were sourced from Jeju National University. The contents of dry matter (DM; method 930.15), crude protein (CP; method 984.13), ether extract (EE; method 920.39), and ash (method 942.05) for FM, as well as for other defatted and hydrolyzed BSFL were determined using AOAC (2007) methods. Amino acids (AA) were analyzed through high-performance liquid chromatography (HPLC; Shimadzu model LC-10AT, Shimadzu, Kyoto, Japan). The nutritional components of FM, defatted BSFL, and hydrolyzed BSFL are shown in Table 1.

3. Experimental Design, Animals, and Housing

The experiment involved a total of thirty-six 45-week-old Arbor Acre broiler breeders, arranged with 2 birds per cage and divided into three treatment groups, each consisting of six replicates. The birds were randomly assigned to one of three dietary treatments: CON (control, basal diet with FM), T1 (basal diet 100% replacement of FM with defatted BSFL), and T2 (basal diet 100% replacement of FM with hydrolyzed BSFL). The diet formulation adhered to the standards established by the Arbor Acres breeder management guide to fulfill the nutritional requirements of broiler breeders (Table 2). Throughout the experiment, all birds had limited access to diet and water. During this period, the chickens were housed in a room with 16 h of light and 8 h of darkness.

Table 1. Nutrient components of FM and defatted and hydrolyzed BSFL[1]

Items (%)	FM	Defatted BSFL	Hydrolyzed BSFL
DM	93.00	93.42	93.41
GE (kcal/kg)	4,112.20	3,727.80	4,638.90
CP	67.00	58.76	59.97
EE	9.00	10.73	11.41
Ash	10.02	10.07	7.68
Amino acid			
Aspartic acid	6.50	5.15	5.11
Threonine	3.10	2.00	2.00
Serine	2.77	2.09	2.07
Glutamic acid	7.85	6.33	6.14
Glycine	4.70	3.01	3.03
Alanine	4.73	4.25	4.24
Valine	3.48	2.72	3.55
Isoleucine	2.71	1.63	2.41
Leucine	4.42	3.04	3.87
Tyrosine	1.83	3.76	3.66
Phenylalanine	2.22	2.89	2.42
Lysine	5.60	2.84	3.40
Histidine	2.21	2.74	2.48
Arginine	4.37	2.06	2.68
Cystine	0.91	0.37	0.37
Methionine	2.56	2.58	1.74
Proline	2.83	3.33	3.55

[1] FM, fishmeal; BSFL, black soldier fly larvae; DM, dry matter; GE, gross energy; CP, crude protein; EE, ether extract.

4. Measurements and Sampling

1) Egg production

Egg productivity were assessed over 4 weeks. The daily laying rate, calculated as the percentage of eggs laid relative to birds, was recorded alongside weekly averages. Egg weight was measured daily using a digital scale (±0.01 g), and the average weight was calculated using only normal eggs. Egg mass was determined by multiplying the laying rate by the average egg weight, excluding eggs that were broken, deformed, contaminated, or under 48 g. Fertility was evaluated on day 8 of incubation by identifying fertilized

Table 2. Ingredients and chemical composition of the basal experimental diets (as-fed basis)[1]

Items	CON	T1	T2
Corn	54.12	54.12	54.12
Soybean meal (45%)	12.00	12.00	12.00
DDGS (28%)	10.00	10.00	10.00
Corn gluten diet	6.27	6.27	6.27
FM	3.00	-	-
Defatted BSFL	-	3.00	-
Hydrolyzed BSFL	-	-	3.00
Wheat pollards	2.50	2.50	2.50
Rice pollards	1.50	1.50	1.50
Animal fats	0.50	0.48	0.46
L-Lys-SO_4	0.08	0.10	0.10
DL-Methionine	0.12	0.12	0.14
L-Threonine	0.02	0.02	0.02
L-Tryptophan	0.13	0.13	0.13
Salt	0.21	0.21	0.21
Limestone	8.97	8.97	8.97
Mineral premix[2]	0.22	0.22	0.22
Vitamin premix[3]	0.11	0.11	0.11
Choline	0.25	0.25	0.25
Total	100.00	100.00	100.00
Calculated value			
ME (kcal/kg)	2,710	2,710	2,710
CP (%)	15.45	15.42	15.41
EE (%)	3.86	3.86	3.86
Total Lys (%)	0.749	0.749	0.749
Calcium (%)	3.50	3.50	3.50
Available P (%)	0.41	0.41	0.41

[1] CON, basal diet with 3% FM; T1, basal diet without FM and substitute with defatted black soldier fly larvae; T2, basal diet without FM and substitute with hydrolyzed black soldier fly larvae; DDGS, dried distiller's grains with soluble; FM, fishmeal; BSFL, black soldier fly larvae; ME, metabolizable energy; CP, crude protein; EE, ether extract; Lys, lysine; P, phosphorus.

[2] Provided per kg of diet: 37.5 mg Zn (as $ZnSO_4$), 37.5 mg of Mn (MnO_2), 37.5 mg of Fe (as $FeSO_4 \cdot 7H_2O$), 3.75 mg of Cu (as $CuSO_4 \cdot 5H2O$), 0.83 mg of I (as KI), and 0.23 mg of Se (as $Na_2SeO_3 \cdot 5H_2O$).

[3] Provided per kg of diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D_3, 37.5 mg of vitamin E, 2.55 mg of vitamin K_3, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of vitamin B_6, 24 µg of vitamin B_{12}, 51 mg of niacin, 1.5 mg of folic acid, 0.2 mg of biotin and 13.5 mg of pantothenic acid.

eggs through candling, and hatchability was calculated as the percentage of hatched chicks relative to incubated eggs using an MX-1000CD incubator (Gimhae, Republic of Korea). Excluded eggs in fertility assessments were also omitted from hatchability calculations.

2) Egg quality

Egg quality was assessed during 2 and 4 weeks of the experimental period. A total of 18 eggs (6 eggs per group) were collected and stored frozen until analysis. Before measurements, eggs were thawed at 4℃ for 24 h. Eggshell thickness was measured at three points (top, middle, and bottom) using a digital caliper, and the average value was recorded. Eggshell strength was determined using a texture analyzer (model 081002, FHK, Fujihara Ltd., Tokyo, Japan), measuring the compressive force per unit surface area. The whole egg weight was measured with a digital scale, and albumen height was determined using a tripod micrometer (AMES, Waltham, MA, USA). The Haugh unit (HU) was calculated based on albumen height and egg weight using the formula:

$$HU = 100 \times \log(\text{Albumen Height} - 1.7 \times \text{Egg Weight}^{0.37} + 7.6)$$

3) Nutrient digestibility

At 2 and 4 weeks, 0.2% chromium oxide (Cr_2O_3) was included in all broiler breeder diets as an indigestible marker for ileal digesta collection. Following CO_2 gas euthanasia of six broiler breeders per treatment group, ileal digesta samples were promptly collected and frozen at −20℃ for preservation. Prior to nutrient digestibility analysis, the digesta samples were dried at 70℃ for 72 h and ground using a 1 mm sieve. DM, CP, and GE in both diet and ileal digesta samples were determined following the AOAC (2007) methods.

AA analysis was performed using high-performance liquid chromatography (HPLC; Shimadzu LC-10AT, Shimadzu, Kyoto, Japan). Methionine and cysteine were pre-oxidized with performic acid at 0℃ for 16 h to produce methionine sulfone and cysteic acid, respectively, before analysis. Chromium concentration was measured through UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan), using the method described by Williams et al. (1962). Apparent total tract digestibility (ATTD) was calculated using the following formula:

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.

4) Statistical analysis

All data in this study were analyzed utilizing JMP (JMP® Pro version 16.0.0, SAS Institute Inc., Cary, NC, USA). A one-way analysis of variance (ANOVA) was conducted to evaluate differences among treatment groups, and the significance of treatment means was determined using Tukey's multiple range test to assess differences among treatment groups, with significance set at $P<0.05$. A tendency toward differences among treatment groups was indicated at $0.05 \leq P<0.10$.

RESULTS

1. Egg Production

The egg production results are shown in Table 3. Dietary supplementation of BSFL showed no differences in egg production, egg weight, egg mass, fertility, and hatchability when compared to the CON ($P>0.05$).

2. Egg Quality

At 2 weeks, the eggshell thickness of the T1 group was significantly lower ($P<0.001$) than that of the CON and T2 groups (Table 4). At 4 weeks, the HU of the T2 group was significantly higher (P = 0.012) than that of the T1 group. Albumin height tended to be higher (P = 0.056) in the T1 and T2 groups than in the CON group.

Table 3. Effects of BSFL as FM substitutes on egg production of 45 w broiler breeders[1]

Items	CON	T1	T2	SE	*P*-value
Egg production (%)	72.62	75.00	72.62	2.085	0.653
Egg weight (g/egg)	62.48	62.65	63.72	0.843	0.544
Egg mass (g/d)	45.21	47.00	46.34	1.529	0.711
Fertility (%)	91.67	94.44	93.75	1.268	0.302
Hatchability (%)	82.64	86.11	84.03	2.240	0.557

[1] CON, basal diet with FM; T1, basal diet without FM and substitute with defatted BSFL; T2, basal diet without FM and substitute with hydrolyzed BSFL; FM, fishmeal; BSFL, black soldier fly larvae; SE, standard error.

Table 4. Effects of BSFL as FM substitutes on egg quality of 45 w broiler breeders[1]

Items	CON	T1	T2	SE	*P*-value
2 w					
Eggshell thickness (mm)	0.42[a]	0.36[b]	0.42[a]	0.005	<0.001
Eggshell strength (kg/cm²)	4.02	3.84	4.29	0.258	0.469
Albumin height (cm)	6.75	6.88	6.94	0.078	0.240
Haugh unit	86.61	82.30	82.77	0.544	0.342
4 w					
Eggshell thickness (mm)	81.61	82.30	82.77	0.544	0.342
Eggshell strength (kg/cm²)	3.86	3.71	4.20	0.196	0.226
Albumin height (cm)	6.21[b]	6.84[a]	6.81[a]	0.054	<0.001
Haugh unit	80.26[b]	82.01[a]	81.92[a]	0.457	0.012

[1] CON, basal diet with FM; T1, basal diet without FM and substitute with defatted BSFL; T2, basal diet without FM and substitute with hydrolyzed BSFL; FM, fishmeal; BSFL, black soldier fly larvae; SE, standard error.
[a,b] Means within a row with different letters are significantly different at $P<0.05$.

3. Nutrient Digestibility

At 2 weeks, the DM digestibility of the T1 group was significantly lower (P = 0.003) than the CON and T2 groups (Table 5). At 4 weeks, the DM digestibility of the T1 group was significantly lower ($P<0.05$) than the CON and T2 groups. Additionally, at 4 weeks, the CP digestibility of the T1 group tended to be higher (P = 0.062) than the CON and T2 groups.

DISCUSSION

BSFL have gained significant attention as a sustainable protein source, with their potential extensively evaluated in various studies (Lu et al., 2022; Siddiqui et al., 2022). The CP content of BSFL varies depending on the processing technique with previous research reporting CP levels of 44.47% in defatted BSFL and 50.12% in hydrolyzed BSFL (Mshayisa et al., 2022). In our study, defatted and hydrolyzed

Table 5. Effects of BSFL as FM substitutes on nutrient digestibility of 45 w broiler breeders[1]

Items (%)	CON	T1	T2	SE	*P*-value
2 w					
DM	62.67[a]	63.63[a]	60.47[b]	0.537	0.003
CP	60.91	62.77	59.42	1.026	0.102
GE	65.17	66.59	62.67	2.048	0.413
4 w					
DM	61.74[a]	59.19[b]	60.99[a]	0.371	0.001
CP	61.16	62.24	60.35	0.514	0.062
GE	60.35	62.46	60.10	0.827	0.121

[1] CON, basal diet with FM; T1, basal diet without FM and substitute with defatted BSFL; T2, basal diet without FM and substitute with hydrolyzed BSFL; FM, fishmeal; BSFL, black soldier fly larvae; DM, dry matter; CP, crude protein; GE, gross energy; SE, standard error.

[a,b] Means within a row with different letters are significantly different at $P<0.05$.

BSFL showed CP contents of 58.76% and 59.97%, respectively. While these values are lower than the 67% CP content of traditional FM, BSFL's superior digestibility and well-balanced AA profile suggests its viability as a FM substitute (Van Huis et al., 2013).

Especially, hydrolyzed BSFL exhibited a higher AA profile than defatted BSFL, with elevated levels of arginine, valine, and lysine. However, other processing technique resulted in reductions in lysine and methionine, likely due to thermal degradation during processing (Makkar et al., 2014).

Our study indicated that substituting FM with BSFL in diets showed no significant differences in egg production, egg weight and egg mass, regardless of processing technique. This result may be attributed to the high nutrient digestibility and balanced AA profile of BSFL (Finke, 2013). BSFL is rich in EAA such as tyrosine, phenylalanine, and histidine which are critical for immune function, growth, and muscle metabolism (Zulkifli et al., 2022; Kannan et al., 2023). These characteristics may help BSFL meet the nutrient requirements for egg production and reproductive performance, even with its slightly lower CP content than FM (Attivi et al., 2022). Our results indicate that replacing FM with BSFL in broiler breeder diets does not negatively affect reproductive performance.

The results of this study on egg quality revealed noteworthy variations. At 2 weeks, the T1 group showed thinner eggshells than the CON and T2 groups, which was attributed to potential mineral loss during the defatting process (Zozo et al., 2022). Also, The T1 and T2 groups showed significantly higher albumin height and HU than the CON group in 4 weeks. Albumin concentration and HU are important indicators of egg freshness. In this study, we found that BSFL has the potential to improve both egg freshness and quality (Heiman and Carver, 1936). Similarly, a previous study reported a numerical increase in HU values when 1.5% of FM in layer diets was replaced with BSFL (Zhao et al., 2022). This increase in HU suggests a positive effect on egg quality, likely due to the bioactive peptides and medium-chain fatty acids in BSFL, which can enhance nutrient absorption and protein metabolism (Van Huis et al., 2013). Additionally, AA in the diet can improve albumin quality through its antioxidant properties, and in this study, defatted BSFL groups showed higher AA content than FM (Udenigwe et al., 2011; Benede and Molina, 2020). Therefore, this study suggests that replacing FM with BSFL can improve egg quality regardless of processing techniques.

At 2 weeks, the T2 group showed lower DM digestibility than the CON and T1 groups, which may be attributed to residual chitin resulting from reduced chitin content during the defatting process and incomplete hydrolysis (De Marco et al., 2015; Dabbou et al., 2018). Chitin, a structural polysaccharide found in insect exoskeletons, is resistant to enzymatic degradation in monogastric animals, potentially lowering nutrient digestibility (Finke, 2013; Rumpold and Schlüter, 2013). At 4 weeks, the T2 group showed an increase in DM digestibility than the T1 group. Chitin can potentially impair digestion, while it also has the potential to improve gut microbiota balance and enhance gut health (Biasato et al., 2020; Liu et al., 2023). Over time, chitin may have contributed to the improving of the gut environment, which could have positively influenced DM digestibility (Spranghers et al., 2017). Beyond the chitin-related findings, no significant differences in CP and GE digestibility were observed among the groups at 2 and 4 weeks. This suggests that the AA composition of BSFL is relatively comparable to that of FM, ensuring sufficient protein quality (Xin et al., 2024).

SUMMARY

This study suggests that replacing FM with defatted and hydrolyzed BSFL did not negatively affect egg production, fertility, and hatchability in broiler breeders, while improving internal egg quality in later stages. Two forms of BSFL are effective alternative protein sources for FM. However, further research is needed to optimize processing technique and nutrient profiles to achieve consistent results.

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