

Effects of graded levels of black soldier fly (*Hermetia illucens*) larvae meal in diet on performance, carcass characteristics, serum biochemical parameters and gut morphology in broiler chickens

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Abstract

This trial evaluated the efficacy of Black Soldier Fly Larvae Meal (BSFLM) as a dietary ingredient in broiler chickens. A total of 160 day-old, straight-run broiler chicks were randomly allocated into four treatment groups, each comprising four replicates of 10 birds. Four iso-caloric and iso-nitrogenous experimental diets were formulated to include BSFLM at graded levels of 0%, 2.5%, 5.0%, and 7.5%, designed to meet the nutrient requirements across three growth stages: pre-starter (0–14 days), starter (15–28 days), and finisher (29–42 days). Birds were fed their respective diet ad lib. across four replicates of 10 chicks each for the full 6-week trial. Dietary incorporation of BSFLM at 5.0 and 7.5% levels had significant influence on body weight gain of broiler chickens during 0–6 weeks of age but feed conversion ratio was significantly improved during initial 3 weeks of age only. Dietary feeding of BSFLM had no influence on any carcass parameters other than breast meat yield. The percent yield of breast meat was significantly ($P < 0.05$) greater in all the dietary groups having different levels of BSFLM, being the highest in 5% BSFLM group. The serum protein content was significantly greater ($P < 0.05$) in all BSFLM feeding groups compared to the control. Serum uric acid remained unaffected up to 5% BSFLM but rose significantly ($P < 0.05$) at the 7.5% level. The serum triglycerides and cholesterol concentration significantly reduced in the serum of broilers fed diets containing 5% and 7.5% BSFLM as compared to control. Intestinal histomorphology showed progressively improved architecture with increasing BSFLM inclusion, with the greatest improvement observed at 7.5%. The width and height of villi, depth of intestinal pits and intervillous distance increased significantly from control to 7.5% BSFLM dietary group. These findings suggest that BSFLM can be included at up to 7.5% in broiler diets, offering a practical route for converting an organic waste stream into a usable poultry feed ingredient.

1. Introduction

Rising costs and inconsistent supply of conventional feed ingredients are compelling poultry nutritionists to explore alternative feed resources. Insects are gaining recognition as promising alternative feed source, owing to their high concentrations of protein, essential amino acids, minerals, and vitamins (1). One such insect is Black Soldier Fly Larvae (BSFL), which has gained attention as a promising alternative feed ingredient for poultry due to their high nutritional value, environmental benefits, and economic advantages (2). The BSF larvae meal typically contains 35–45% protein, making it a potential substitute for conventional protein sources such as soybean and fish meal (3). It is also rich in crude fat (25–35%) and ash (14.6–28.6%) and has high energy content (5277 Kcal/kg GE) (4, 5).

Dietary incorporation of BSF larvae meal has been reported to enhance growth performance and feed conversion efficiency in broilers (6, 2) and to support gut health by favouring beneficial microbiota, improving gut morphology, and boosting immune function (1, 7). At higher inclusion levels, however, performance can decline, as excess amounts may impair palatability and disturb nutrient balance (8).

BSFLM can be produced on organic waste, potentially reducing feed costs and reliance on conventional protein sources (9). Using BSF larvae meal can contribute to a circular economy, reducing waste and environmental footprint of poultry production (10). The information on utilizing BSFLM in poultry diet is very limited in India (1, 2). The present trial was therefore undertaken to evaluate the effect of graded dietary levels of black soldier fly (*Hermetia illucens*) larvae meal on diet performance, carcass characteristics, serum biochemical parameters and gut morphology in broiler chickens. While earlier studies mostly looked at growth performance alone, this study takes a broader approach by examining how graded levels of dietary BSFLM inclusion affect productive performance, carcass traits, serum biochemistry, and intestinal histomorphology, all within one experimental framework.

2. Materials and methods

2.1 Birds and management

A total of 160-day-old commercial broiler chicks were wing-banded and randomly allotted to 16 brooder pens (10 birds/pen). The Brooder temperature was set at $34 \pm 1^\circ\text{C}$ for the first week and gradually decreased to $26 \pm 1^\circ\text{C}$ by day 21, after which birds were maintained at ambient room temperature. Uniform rearing conditions were ensured by applying identical management and vaccination protocols across all pens.

2.2 Ethical Considerations

The trial was conducted following approval from the Institute Animal Ethics Committee (IAEC/OVC/2023) of the College of Veterinary Sciences and Animal Husbandry, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar, Odisha, India (Ref. No. 1108/CV., dated 12.08.2023). The study was conducted in accordance with the applicable institutional guidelines and regulations for the care, handling, and humane treatment of experimental animals.

2.3 Diets

Powdered black soldier fly (*Hermetia illucens*) larvae meal (BSFLM) was obtained from Insectika Biotech Private Limited, Bhubaneswar, Odisha. Four experimental diets were formulated to incorporate graded levels of BSFLM (0, 2.5, 5.0, and 7.5%; Table 1); all diets were isocaloric and isonitrogenous, formulated on the basis of the analyzed nutrient composition of individual ingredients, and balanced to meet broiler nutrient requirements during the pre-starter (0–14 d), starter (15–28 d), and finisher (29–42 d) phases. Diets were offered ad libitum to four replicates of 10 chicks per treatment throughout the 6-week feeding trial. The amino acid and fatty acid profile of the BSFLM sample was determined at KIIT-TBI-ATF, Bhubaneswar, Odisha, India.

Table 1
Ingredient and Nutrient Composition of Experimental Diets

BSFLM (%)	Pre-Starter (0-14d)				Starter (15–28 d)				Finisher (29.42 d)			
	0	2.5	5	7.5	0	2.5	5	7.5	0	2.5	5	7.5
Maize	55.98	57.69	59.75	60.25	59.26	60.7	61.68	62.62	61.76	63.76	65.91	67.67
Soybean meal	37.9	34.6	31.0	28.3	34.2	31.2	28.65	26.04	31.2	27.3	23.8	20.6
BSF larva meal	0	2.5	5	7.5	0	2.5	5	7.5	0	2.5	5	7.5
Oil	1.7	1	0.2	-	2.6	1.8	1	0.3	3.5	3	2	1
Stone grit	1.45	1.42	1.4	1.38	1.3	1.25	1.2	1.15	1.15	1.15	1.1	1.1
Di-calcium phosphate	1.89	1.75	1.65	1.6	1.65	1.6	1.55	1.5	1.48	1.4	1.32	1.28
DL – methionine	0.22	0.2	0.18	0.16	0.24	0.22	0.2	0.18	0.26	0.24	0.22	0.2
L-Lysine HCl	0.21	0.19	0.17	0.15	0.1	0.08	0.07	0.06	-	-	-	-
Constants	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
TOTAL	100	100	100	100	100	100	100	100.	100	100	100	100
ME (kcal/kg)	2950	2954	2956	2958	3050	3058	3054	3055	3150	3151	3158	3156
Crude Protein (%)	22.51	22.54	22.49	22.52	21.01	20.98	20.96	20.94	19.50	19.51	19.49	19.52
Lysine (%)	1.40	1.39	1.39	1.41	1.15	1.15	1.15	1.15	1.02	1.01	1.01	1.01
Methionine (%)	0.58	0.58	0.57	0.57	0.54	0.54	0.54	0.54	0.52	0.52	0.51	0.51
Calcium (%)	0.95	0.95	0.95	0.95	0.85	0.85	0.85	0.85	0.80	0.80	0.80	0.80
Available Phosphorous (%)	0.45	0.45	0.46	0.47	0.43	0.43	0.44	0.44	0.40	0.40	0.41	0.41

2.4 Parameters studied

Body weights of individual chicks and replicate-wise feed intake were recorded weekly. The feed conversion ratio (FCR) was derived by dividing total feed consumed by weight gained. Mortality was monitored throughout the trial period, with the cause of death determined for each case.

On day 40, blood samples were collected from the brachial vein of three birds per replicate (n = 12 per treatment); serum was separated and stored at -20°C until analysed for total protein, albumin, triglycerides, total cholesterol, calcium, and phosphorus using commercial reagent kits (Coral Clinical Systems, Goa, India).

On day 43, two birds per replicate close to the mean replicate body weight (eight birds/treatment) were humanely euthanized by cervical dislocation followed by rapid decapitation, in accordance with the approved animal ethics protocol and without prior administration of chemical anesthetic. Edible carcass, liver, gizzard, abdominal fat, and breast meat weights were recorded and expressed relative to pre-slaughter live weight.

Tissue specimens were collected from the duodenum, ileum and caecum of slaughtered birds for histo-morphological study. Specimens were fixed for 72 h in 10% buffered neutral formalin and subsequently processed into 6-µm paraffin sections following the standard technique (11). Sections were floated on a 37°C water bath, mounted on grease-free glass slides, air-dried, and stained by the routine hematoxylin and eosin procedure (11) for assessment of duodenal, ileal, and caecal histomorphology.

2.5 Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) within a completely randomized design (CRD) (12) to assess the effect of graded dietary inclusion of black soldier fly larva meal (BSFLM). Treatment means were separated by Duncan's multiple range tests (13), and results are presented as mean ± SEM, with significance declared at $P < 0.05$. All analyses were performed using IBM SPSS Statistics for Windows, Version 26.0. (14).

3. Results

3.1 Nutrient composition of BSFLM

The nutrient composition of the analyzed BSFLM sample is summarized in Table 2. The crude protein (CP), crude fat (CF) and total ash (TA) content of the analyzed BSFLM sample were 49.4, 36.2 and 7.78%, respectively. Amongst the analyzed amino acid, Arginine content was highest followed by lysine, threonine and histidine. The content of methionine and tryptophan was 0.69 and 0.16%, respectively. The fatty acid profile revealed higher concentration of lauric acid (57.02%) followed by palmitic acid (8.02%). The unsaturated fatty acid linoleic acid and oleic acid comprised 14.64 and 13.52% of total fatty acid in BSFLM, respectively. The resulting ratio of total saturated fatty acids (TSFA) to unsaturated fatty acids (USFA) was 2.41.

Table 2
Composition (% dry matter) of Black Soldier Fly
larva meal (BSFLM)

Nutrients	Composition (%)
Crude protein	49.4
Crude fat	36.2
Total ash	7.78
Amino acids	
Lysine	2.64
Methionine	0.69
Cysteine	0.46
Threonine	2.55
Tryptophan	0.16
Arginine	5.73
Valine	0.70
Histidine	1.14
Leucine	0.59
Isoleucine	0.43
Phenylalanine	0.52
Fatty acid	% of total fatty acids
Caproic acid	0.78
Capric acid	0.72
Lauric acid	57.02
Myristic acid	3.10
Palmitic acid	8.02
Stearic acid	1.04
Total saturated	70.68
Palmitoleic acid	1.16
Oleic acid	13.52
Linoleic acid	14.64
Alpha-Linolenic acid	---
Total unsaturated	29.32

3.2 Growth performance

Effects of dietary BSFLM inclusion on body weight gain, feed intake, and feed conversion ratio during 0–3 and 0–6 week of age are summarized in Table 3. The body weight gain of broilers was significantly influenced by the dietary levels of BSFLM during 0 to 3 weeks of age. The BWG was significantly ($P < 0.05$) higher in the dietary group containing BSFLM at 5% and 7.5% level compared to either control or 2.5% BSFLM diet. No difference in BWG of broiler could be observed between 5% BSFLM and 7.5% BSFLM dietary group. Feed intake did not differ with BSFLM inclusion, whereas FCR improved significantly ($P < 0.05$) with BSFLM

supplementation, although raising inclusion beyond 2.5% conferred no additional benefit. A comparable trend in BWG was observed over the full 0-6-week period. Feed consumption was significantly higher ($P < 0.05$) in broilers fed 5% and 7.5% BSFLM, with the 2.5% group intermediate between these and the control. FCR over 0–6 week, however, was not significantly affected by BSFLM inclusion up to 7.0%.

Table 3
Effect of BSFLM Diet on Growth performance of broiler chickens

Parameters	BSFLM, % IN DIET				SEM	P-Value
	0 (T1)	2.5 (T2)	5 (T3)	7.5 (T4)		
0–3 weeks						
Body weight gain(g)	720.0 ^a	736.6 ^a	768.4 ^b	774.2 ^b	6.542	0.051
Feed intake(g)	864.9	849.0	868.4	884.6	17.572	0.366
Feed conversion ratio	1.21 ^b	1.15 ^a	1.13 ^a	1.14 ^a	0.023	0.043
0–6 weeks						
Body weight gain(g)	2207.6 ^a	2215.3 ^a	2277.0 ^b	2261.5 ^b	11.54	0.044
Feed intake(g)	3487.4 ^a	3564.9 ^{ab}	3594.5 ^b	3584.6 ^b	21.168	0.768
Feed conversion ratio	1.579	1.583	1.578	1.585	0.059	0.142

Table 4
Effect of BSF Larva meal (BSFLM) levels on carcass traits of broiler chickens

Ready to cook yield	BSFLM, % In Diet				SEM	P-Value
	0 (T1)	2.5 (T2)	5 (T3)	7.5 (T4)		
Dressing yield	74.24	74.36	74.30	74.42	0.054	0.668
Liver	2.78	2.82	2.72	2.84	0.112	0.454
Gizzard	2.02	2.14	2.11	2.09	0.088	0.542
Heart	0.59	0.61	0.60	0.61	0.004	0.784
Giblets	5.39	5.57	5.43	5.54	0.104	0.685
Abdominal fat	1.46	1.52	1.54	1.56	0.084	0.768
Breast	21.68 ^a	22.46 ^b	22.62 ^b	22.54 ^b	0.212	0.034
Thigh	15.05	15.26	15.18	15.20	0.132	0.654
Drumstick	12.73	12.84	12.68	12.74	0.042	0.756
Back	18.02	17.66	17.99	17.78	0.224	0.456
Neck	5.44	5.48	5.42	5.34	0.064	0.642
Wing	10.75	10.60	10.48	10.58	0.084	0.212

a and b the means within a row bearing different superscripts differ significantly ($P < 0.05$).; SEM =standard error of the mean

3.3 Carcass traits

In the present investigation, dietary feeding of BSFLM had no influence on any carcass parameters other than breast meat yield. The percent yield of breast meat was significantly ($P < 0.05$) higher in all the dietary groups having different levels of BSFLM,

being the highest in 5% BSFLM group. However, no significant difference was observed amongst different levels of BSF in the diet.

3.4 Serum biochemical parameters

The effect of dietary BSFLM on blood biochemical markers of broiler chickens is shown in Table 5. The serum protein concentration was ($P < 0.05$) lowest in control group. The serum protein concentration increased linearly with levels of BSFLM in diets of broiler chickens, being highest in the dietary group containing 7.5% BSFLM. The serum protein content was significantly greater ($P < 0.05$) in all feeding groups including BSFLM compared to the control group. No difference in the concentration of serum calcium and phosphorus was observed due to dietary inclusion of BSFLM in the present study. Incorporating BSFLM in the diet up to 5% did not affect serum uric acid concentration. However, incorporating BSFLM at 7.5% in the diet significantly increased serum uric acid ($P < 0.05$) concentration of broilers. The serum triglycerides and cholesterol concentration have followed similar trend in different treatments. There exists a significant difference in triglyceride and cholesterol concentration in Control group(T1) compared to BSFLM at 5.0% (T3) and BSFLM at 7.5%(T4) level. However, the difference between control(T1) and BSFLM at 2.5% level(T2) was found to be non-significant.

Table 5
Effect of dietary inclusion of BSF larva meal on serum biochemical profile of broiler chickens

Diets	Protein (g/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)	Uric acid (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)
T1 (0.0)	5.06 ^a	11.24	5.64	4.24 ^a	212.18 ^b	204.64 ^b
T2 (2.5)	5.64 ^b	11.30	5.72	4.28 ^a	204.46 ^{ab}	196.22 ^{ab}
T3 (5.0)	5.72 ^b	11.12	5.46	4.30 ^a	196.64 ^a	188.48 ^a
T4 (7.5)	5.98 ^c	11.44	5.58	4.44 ^b	188.80 ^a	184.24 ^a
SEM	0.03	0.11	0.05	0.03	2.24	3.24
P-value	0.044	0.588	0.645	0.050	0.051	0.049

3.5 Intestinal histo-morphology (Duodenum, Ileum and caecum)

Across the duodenum, ileum and caecum, intestinal morphology improved steadily with rising dietary BSFLM (Fig. 1A-L). The control group (T1) set a fairly uniform baseline in all three segments: villi were short and crypts of Lieberkühn sparse in the duodenum (Fig. 1A) and ileum (Fig. 1E), while the caecal mucosa showed a comparatively simple architecture with few crypts (Fig. 1I); Peyer's patches were scarce throughout, and squamous M cells were essentially absent. Even the lowest inclusion level began to shift this picture at 2.5% BSFLM (T2), villi in both the duodenum (Fig. 1B) and ileum (Fig. 1F) were noticeably more elongated and crypts more prominent, and Peyer's patches started to appear more regularly, including in the ileum (Fig. 1F) and caecum (Fig. 1J). By 5.0% BSFLM (T3), villi in the duodenum were further elongated with abundant lymphocytes in the lamina propria (Fig. 1C), the ileum showed well-developed villi with deeper crypts and dense lymphocyte populations (Fig. 1G), and the caecum carried numerous crypts of Lieberkühn alongside well-formed Peyer's patches (Fig. 1K). The most developed architecture, however, was consistently in T4 (7.5% BSFLM): duodenal villi were the longest and broadest of any group, with distinct infoldings running along their length and conspicuous Peyer's patches (Fig. 1D); ileal villi and crypts reached their greatest depth, again with prominent Peyer's patches (Fig. 1H); and the caecum showed the most extensively developed crypts and the most abundant Peyer's patches among all treatments (Fig. 1L). Goblet cell numbers and crypt density rose visibly from T1 through T4 in all three segments, and M cells absent in the control became progressively more frequent from T2 onward. Higher-magnification images of T4 confirmed this: well-formed Peyer's patches in both the duodenum and ileum, and numerous M cells in the caecal epithelium (Fig. 2A-C).

4. Discussion

The CP, lysine and EE, TSFA and TUSFA content of BSFLM sample tested in the present study was comparable to a similar study (2) wherein the researchers reported 49.8% CP, 2.4% Lys and 35.2% EE, 71.6% TSFA and 28.37% TUSFA in BSFLM sample. However, lower methionine, and higher threonine and tryptophan content was observed in the BSFLM sample tested in the present study. In another BSFLM sample analyzed in India, an author (1) reported a lower value for both CP (44.8%) and EE (21.2%) in BSFLM sample. But, the lysine (2.31%) and methionine (0.74%) content was comparable to the BSFLM sample of the current investigation. A wide variation in the nutrient composition of BSFLM was reported in the literature (3, 15) which could be attributed mainly to the insect rearing substrate. The substrate used for the production of BSF larvae of the present study was mainly kitchen waste and this could be the reason that the nutrient profile of BSFLM sample of the current experiment closely resembled that reported previously (1).

In the present study, body weight gain of broilers was significantly ($P < 0.05$) higher in the 5% and 7.5% BSFLM groups than in either the control or the 2.5% BSFLM diet during 0–3 week of age. Feed conversion ratio (FCR) likewise improved significantly across all BSFLM-supplemented groups, regardless of inclusion level. This finding is consistent with earlier work (7), which reported improved growth performance in broiler chickens fed diets containing 3% BSFLM. A significant FCR improvement during the first 3 week has similarly been reported in broilers fed 5% house fly larvae meal relative to a soybean-based control diet (6). Elsewhere (2), broilers fed BSFLM up to 5% showed higher body weight gain and improved FCR during 0–3 week of age, although this advantage was not sustained over the subsequent 4-6-week period. In contrast, the present study found higher body weight gain persisting in the 5% and 7.5% BSFLM groups, while FCR was no longer influenced by BSFLM inclusion at 6 weeks of age. The favorable effects on BWG and FCR observed here both during the initial 3 week and across the overall 6-wk period for BWG may be attributable to improved diet palatability (8) and a preference among broilers for feed containing insect meal over conventional diets (5).

Among the carcass traits examined, only breast meat yield was influenced by dietary BSFLM inclusion. Breast meat percentage increased significantly with BSFLM incorporation relative to the control, although no significant differences emerged among the three inclusion levels tested (2.5, 5, or 7.5%). This result aligns with a previous report (6), in which broilers fed 5% house fly larvae meal exhibited significantly higher breast meat yield than controls. Similarly, another study (2) found no significant differences in slaughter yield or organ weights in broiler chicks fed up to 5% BSFLM compared with control birds, consistent with the present findings. In quail, dressed yield and organ weights were likewise unaffected by BSFLM inclusion up to 15% of the diet (5).

The concentration of protein, uric acid, triglycerides and cholesterol in serum was influenced by feeding BSFLM up to 7.5% in the diet of broiler chickens. Dietary inclusion of BSFLM larvae linearly increased serum protein and uric acid concentration but decreased serum triglycerides and cholesterol concentration. Consistent with the present findings, a study (16) reported a linear decrease in serum triglyceride and cholesterol concentrations with increasing dietary inclusion levels of BSFLM up to 9% in Muscovy ducks. Similar reductions in serum cholesterol and triglyceride concentrations in birds fed BSFLM were reported in a previous study (17). The significant reductions in serum cholesterol and triglyceride concentrations in broilers fed BSFLM could be attributed to the presence of short-chain fatty acids and chitin in BSF, which exert hypocholesterolemia impact by reducing lipid absorption (18).

The intestinal histomorphology revealed better histoarchitecture in 7.5% BSFLM group followed by 5%, 2.5% and control group in relation to absorption and assimilation of nutrients and immunity of the birds. From the present histo-morphological results, it was evident that the occurrence of goblet cells, distribution of intestinal villi, depth of intestinal pit, population of 'M' cells, Peyer's patches and crypts of Liberkuhn in the intestine played significant role in the absorption of nutrients and imparting immunity to the birds in different treatment groups under the study (7). It has been reported that medium chain fatty acids present in BSF could improve gut health through their positive effects on intestinal morphology (19). The presence of longest intestinal villi with deepest and wider intestinal pits and numerous in foldings of the mucosa all along the length of the intestinal villi in the duodenum and ileum of 7.5% BSFLM group indicated the increase in surface area of absorption of nutrients leading to higher growth rate of birds in this group. The higher population of 'M' cells in the lining epithelium with numerous lymphocytes and higher density of Peyer's patches in the lamina propria of duodenum, ileum and caecum in 7.5% BSFLM group could be attributable to the higher immunity of these birds as compared to the other groups under present study. Similarly, the increased populations of goblet cells and high density of crypts of Liberkuhn in the lamina propria of duodenum, ileum and caecum in 7.5%

BSFLM group contributed significantly to the secretion of intestinal juice leading to better absorption of nutrients and higher growth rate in the 7.5% BSFLM group.

By jointly examining productive performance, carcass traits, serum biochemistry, and intestinal histomorphology in response to graded BSFLM feeding, this study contributes an integrated perspective to the growing body of literature on insect-derived feed ingredients and supports the biological suitability of BSFLM as an alternative protein source for broilers.

5. Conclusions

Feeding BSFLM at 5.0% and 7.5% significantly improved body weight gain in broilers across the 0–6-week period without adversely affecting carcass traits, apart from a significant ($P < 0.05$) rise in breast meat yield across all BSFLM-supplemented groups, which peaked at the 5% inclusion level. These results support the effective incorporation of BSFLM at up to 7.5% in broiler diets and highlight its promise as a sustainable, nutritionally viable alternative protein source for poultry feeding programs

Declarations

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Author contributions

Conceptualization, methodology and visualization: MPS, LKB and AKP; **supervision:** LKB; **software and investigation:** AKP and SP; **writing, review and editing:** AKP, SP; **validation:** SKD and KB; **formal analysis:** KB; **resources:** SKD and RNB; **data curation:** SKD and KB; **writing & original draft preparation:** MPS; All authors made substantive contributions to the work and have approved the final version of the manuscript for submission.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

The original datasets generated and analysed in the current study are included in the manuscript further inquiries can be directed to the corresponding authors.

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Figures

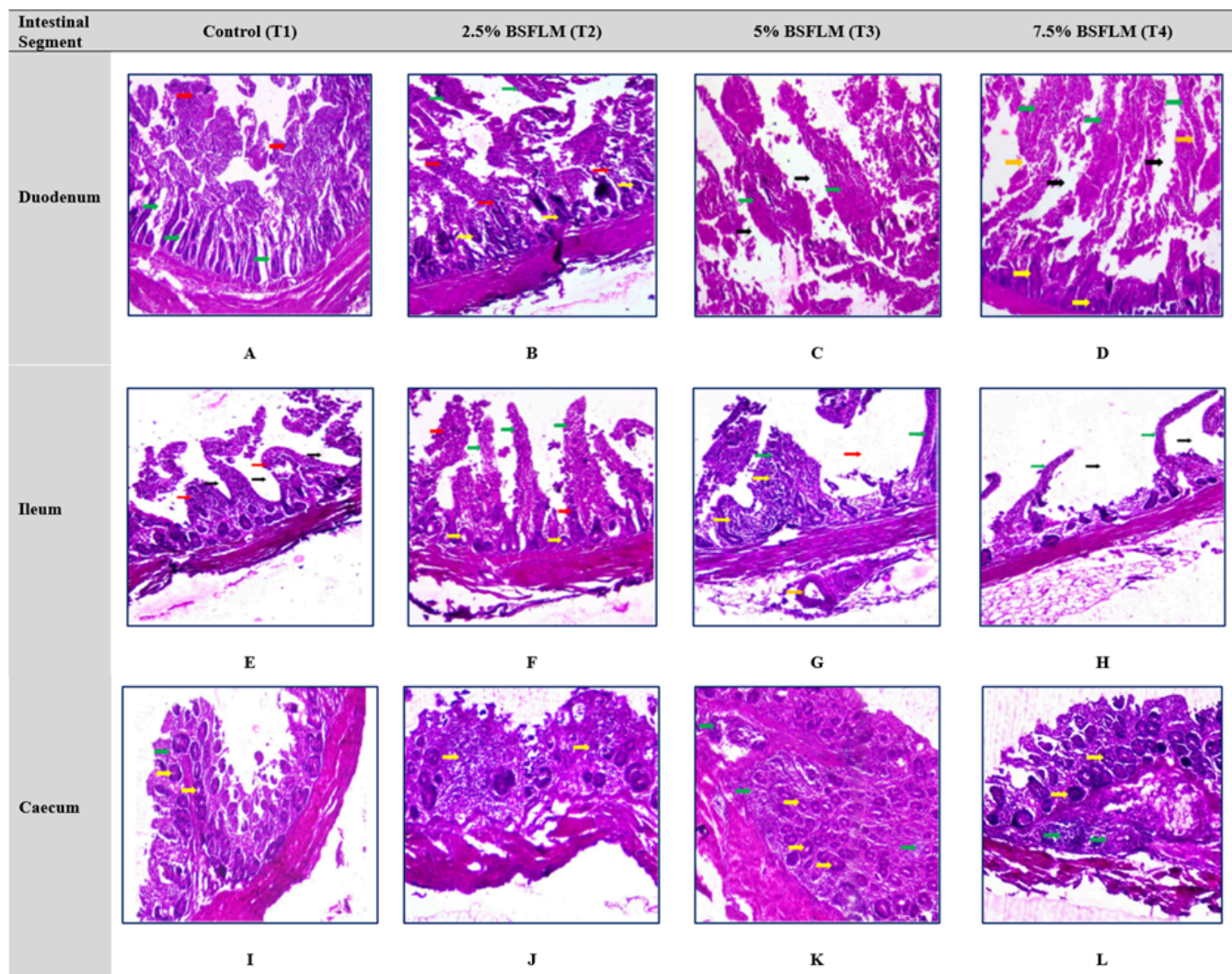


Figure 1

(A-L). Representative histo-morphological architecture of the duodenum, ileum and caecum of broiler chickens fed graded levels of black soldier fly larva meal (BSFLM).

(A) Duodenum of the control group (T1; 0% BSFLM) showing normal villi, crypts of Lieberkühn and scattered lymphocytes. (B) Duodenum of broilers fed 2.5% BSFLM (T2) showing elongated villi with prominent crypts of Lieberkühn. (C) Duodenum of broilers fed 5.0% BSFLM (T3) showing well-developed villi and numerous lymphocytes. (D) Duodenum of broilers fed 7.5% BSFLM (T4) showing well-developed villi with villus infoldings and prominent Peyer's patches.

(E) Ileum of the control group (T1; 0% BSFLM) showing shorter villi, fewer crypts of Lieberkühn and scattered lymphocytes. (F) Ileum of broilers fed 2.5% BSFLM (T2) showing elongated villi, prominent crypts of Lieberkühn and Peyer's patches. (G) Ileum of broilers fed 5.0% BSFLM (T3) showing well-developed villi, increased crypt development and abundant lymphocytes. (H) Ileum of broilers fed 7.5% BSFLM (T4) showing elongated villi with deeper crypts of Lieberkühn and prominent Peyer's patches.

(I) Caecum of the control group (T1; 0% BSFLM) showing normal mucosal architecture with fewer crypts of Lieberkühn. (J) Caecum of broilers fed 2.5% BSFLM (T2) showing increased crypt development and prominent Peyer's patches. (K) Caecum of broilers fed 5.0% BSFLM (T3) showing numerous crypts of Lieberkühn and well-developed Peyer's patches. (L) Caecum of broilers

fed 7.5% BSFLM (T4) showing well-developed crypts of Lieberkühn with abundant Peyer's patches, indicating improved intestinal morphology.

Green arrows indicate intestinal villi; yellow arrows indicate crypts of Lieberkühn; red arrows indicate Peyer's patches or lymphoid tissue; and black arrows indicate villus infoldings, lymphocytes or M cells, as applicable. Sections were stained with hematoxylin and eosin (H&E); original magnification $\times 100$.

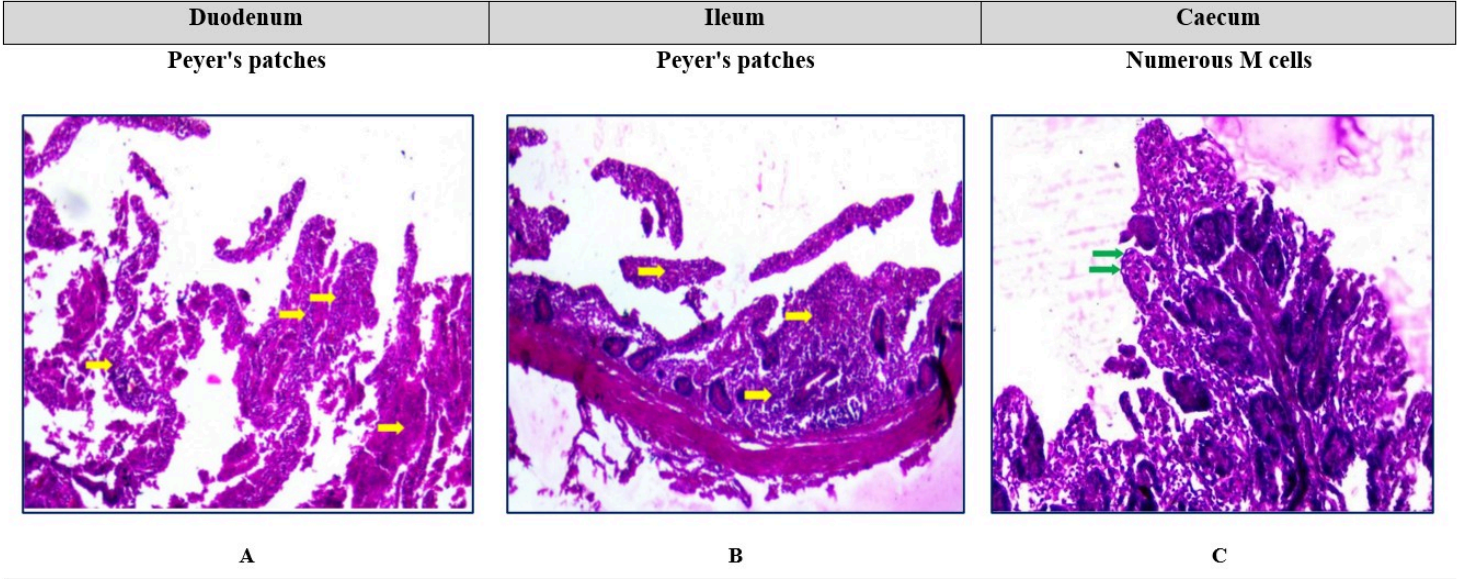


Figure 2

Higher-magnification photomicrographs highlighting gut-associated lymphoid tissue in broiler chickens fed 7.5% black soldier fly larva meal (BSFLM).

(A) Duodenum showing prominent Peyer's patches. (B) Ileum showing well-developed Peyer's patches. (C) Caecum showing numerous M cells within the intestinal epithelium. Sections were stained with hematoxylin and eosin (H&E); original magnification $\times 200$ (or $\times 100$, if applicable).