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Evaluation of black soldier fly larva on haematology, intestinal morphology and intestinal histomorphology in commercial broiler chicken

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Abstract

A biological experiment was conducted to evaluate the effect of black soldier fly larva on haematology, intestinal morphology and intestinal histomorphology of commercial broiler chicken. A total of 160 broilers were weighed, wing banded and randomly allotted on a weight equalization basis to 4 treatment groups T₁, T₂, T₃ and T₄ with 4 replicates in each treatment with 10 chicks in each replicate. Four treatment rations i.e., broiler pre-starter (0-7 days), broiler starter (8-21 days) and broiler finisher (22- 42 days) containing black soldier fly larva at levels of 0% (T₁), 5% (T₂), 7.5% (T₃) and 10% (T₄) of the total ration at the expense of soya bean meal were formulated to contain nearly isocaloric and iso nitrogenous. All the birds were fed with ad libitum feed and water. Haematological parameters like erythrocyte count, leucocyte count, haemoglobin and packed cell volume were recorded. Intestinal morphology parameters like intestinal length and weight were recorded. Intestinal histomorphology parameters like villus height, crypt depth and villus height crypt depth ratio were recorded. Statistical analysis revealed that there was no significant difference ($p>0.05$) in haematological parameters. There was no significant difference ($p>0.05$) in intestinal length and weight among the treatment groups. Among intestinal histomorphology parameters, there was no significant difference ($p>0.05$) in duodenum and ileum. However, villus height crypt depth ratio of jejunum recorded significantly higher ($p<0.05$) in T₂ (20.09 ± 1.38) compared to other groups. These results indicate that the inclusion of black soldier fly larva up to 10% had brought no negative impacts on haematological parameters, intestinal morphology and intestinal histomorphology compared to that of control. Therefore, it was concluded that black soldier fly larva could be included up to 10% in broiler chicken ration.

Keywords: Black soldier fly larva, haematology, intestinal histomorphology, commercial broiler chicken

Introduction

The poultry industry plays a major role in supplying affordable animal protein to the growing population. In commercial broiler production, feed accounts for the major portion of production cost, creating the need for sustainable and economical alternative feed ingredients. Conventional protein sources such as soybean meal and fish meal are becoming increasingly expensive and limited in availability. In recent years, the larvae of the Black Soldier Fly have gained attention as an alternative protein source in poultry nutrition due to their high protein content, good nutrient profile, and sustainable production system. Black soldier fly larva meal (BSFLM) also contains bioactive compounds that may improve gut health and nutrient utilization in broiler chickens. Intestinal development and histomorphology are important indicators of digestive efficiency and nutrient absorption in broilers. Parameters such as intestinal length, intestinal weight, villus height, and crypt depth help evaluate the functional status of the intestine. Haematological parameters are also useful in assessing the physiological and health status of birds.

Although BSFLM has shown potential as a poultry feed ingredient, limited information is available regarding its effects on intestinal characteristics and haematological parameters in commercial broilers. Therefore, the present study was undertaken to evaluate the effect of black soldier fly larva meal on the production performance of commercial broiler chickens,

with special emphasis on intestinal length and weight, intestinal histomorphology, and haematology.

Materials and Methods

The study was carried out as per guidelines and approval of Institutional Animal Ethical Committee (IAEC) with IAEC number 24/SA/IAEC/2024 (24-12- 2024). A six-week duration biological experiment was conducted to evaluate the effect of dried BSF larvae on the Haematological, Intestinal morphology and Intestinal histomorphology in commercial broilers. The experimental period divided into three phases namely pre starter (0-7 days), starter (8-21 days), and finisher (22-42) days.

A feeding trial was carried out with 160 day-old straight-run commercial broiler chicks (Ven Cobb 430 Y) obtained from Venkateshwara Hatcheries private limited. All the chicks were allotted to four treatment groups viz., T₁, T₂, T₃ and T₄ with four replicates in each treatment with 10 chicks in each replicate as given in Table 1.

Working solution of Nambiar's fluid	Ringers' solution
2% Sodium Citrate solution -1ml	Sodium chloride -0.7 g
Gentian violet 0.1% in Ringer's solution - 1ml	Sodium bicarbonate -0.03 g
Brilliant cresyl blue 0.1% in Ringer's solution- 1ml	Potassium chloride - 0.026 g
Neutral formalin 3 drops	Calcium chloride - 0.003 g
	Distilled water -100ml

iii. Haemoglobin and Packed Cell Volume (PCV)

Haemoglobin concentration in blood was examined by Sahli's acid haematin method. Packed Cell Volume (PCV) was estimated by Win Trobe's microhematocrit method.

Intestinal morphology

A total of 12 birds comprising of three birds per treatment were randomly selected for measuring the Small intestinal length and weight. The collected intestines were flushed with Phosphate buffer saline (PBS) to remove all the feed contents. The total intestinal length was measured using a measuring tape in centimeter scale and weighed in grams.

Intestinal histomorphology

Small intestine (middle part of jejunum, duodenum, ileum) samples were taken from three birds per treatment after the experimental period of 42 days and were flushed with phosphate buffer saline and preserved in 10% neutral buffer formalin for processing. Later, it was dehydrated cleaned and embedded in paraffin wax. Serial histology sections of 4 to 5 μ m thickness were cut on a microtome and mounted on slides. The slides were stained with Hematoxylin and Eosin stain as

Table 1: Experimental design

Experimental group	Treatment
T ₁	Control diet 0% BSFL meal
T ₂	Basal Diet with 5% BSFL meal
T ₃	Basal Diet with 7.5% BSFL meal
T ₄	Basal Diet with 10% BSFL meal

Feeding standards as per BIS (2024) [1] Specification

Collection of samples and analysis

Haematological Parameters

i. Collection of blood: Three milliliters of blood was collected from two males and two females from each treatment in EDTA vacutainer vials for blood analysis.

ii. Total count: Total Erythrocyte Count (TEC) and Total Leucocyte Count (TLC) were estimated by Neubauer counting chamber by using Nambiar's diluting fluid (Bancroft and Marilyn, 2008) [2].

described by Bancroft and Marilyn (2008) [2] using image analysis software (Mag vision). Villus height and crypt depth were measured in micrometer (μ m) per sample and mean values were used in statistical analysis. The villus height (VH) was measured from the tip of the villus to the villus crypt junction; Crypt depth (CD) was defined as the depth of the invagination between adjacent villi, and epithelial thickness (ET) considered as the distance from the epithelial surface to the basement membrane of the epithelial cell. VH:CD was determined as the ratio of VH to CD.

Statistical analysis

The data pertaining to various parameters were analysed statistically using Analysis of Variance (ANOVA) by software (IBM SPSS version 25.0 for Windows) as per Snedecor and Cochran (1990) [3]. Significant mean differences were determined at 5% probability level ($P < 0.05$) to identify the fixed effect of treatment using Duncan's Multiple Range Test (DMRT) as modified by Kramer (1956) [4].

Results

Table 2: Effect of feeding different levels of Black Soldier Fly Larva in commercial broiler chicken on hematological parameters at 6th week of age (Mean $\pm$ SE)

Parameters	Haematological Parameters				F Value
	T ₁ (control)	T ₂ (5%)	T ₃ (7.5%)	T ₄ (10%)	
PCV (%)	31.45 $\pm$ 0.83	29.95 $\pm$ 0.46	29.85 $\pm$ 1.13	31.40 $\pm$ 1.37	0.761 ^{NS}
Haemoglobin (g/dl)	10.48 $\pm$ 0.27	9.98 $\pm$ 0.15	9.95 $\pm$ 0.37	10.46 $\pm$ 0.45	0.761 ^{NS}
Total Erythrocyte Count (10 ⁶ / μ l)	2.44 $\pm$ 0.58	2.62 $\pm$ 0.16	2.75 $\pm$ 0.29	2.63 $\pm$ 0.09	0.140 ^{NS}
Total Leucocyte Count (10 ³ / μ l)	14266 ^a $\pm$ 1346	12253 ^b $\pm$ 422	12475 ^b $\pm$ 1254	12316 ^b $\pm$ 476	1.900 ^{NS}

Means bearing different superscripts within a row differ Significantly ($P < 0.05$)

No of observations (n) =16 (4 birds x 4 treatments)

**Significant ($P < 0.01$), *Significant ($P < 0.05$), NS-Not significant.

Haematological parameters

Effect of feeding different levels of Black Soldier Fly Larva in commercial broilers on haematological parameters (Mean $\pm$ SE) is presented in Table 2.

The haematological parameters evaluated, namely packed cell volume (PCV), haemoglobin concentration, total erythrocyte count (TEC), and total leucocyte count (TLC), did not differ significantly ($P > 0.05$) among the treatment groups. The PCV

was highest in T₁ followed closely by T₄ while T₂ and T₃ recorded lower values; Similarly, haemoglobin concentration was highest in T₁ closely followed by T₄ whereas T₂ and T₃ showed slightly lower values, with no significant variation among treatments. Total erythrocyte count was numerically

highest in T₃ followed by T₄, T₂ and lowest in T₁. Total leucocyte count was highest in T₁ whereas T₂, T₃ and T₄ recorded comparatively lower values. Despite numerical variation, the differences were statistically non-significant.

Table 3: Effect of feeding different levels of Black Soldier Fly Larva in commercial broiler chicken on intestinal length and weight at 6th week of age (Mean ± SE)

Sl. No.	Parameters	Intestinal Morphology				F VALUE
		T ₁ (0%)	T ₂ (5%)	T ₃ (7.5%)	T ₄ (10%)	
1	Intestinal length (cm)	198.33±1.66	200.00±2.88	197.66±2.02	202.66±4.05	0.627 ^{NS}
2	Intestinal weight (g)	98.66±1.75	101.36±1.82	97.56±0.97	96.73±1.27	1.818 ^{NS}

Means bearing different superscripts within row differ significantly ($P<0.05$)

No of observations (N) = 12 (3 birds x 4 treatments)

**Significant ($P<0.01$), *Significant ($P<0.05$), NS-Not significant.

Intestinal morphology

Effect of feeding different levels of Black Soldier Fly Larva in commercial broilers on Small Intestinal morphology parameters (Mean ± SE) is presented in Table 3.

i. Intestinal length

No significant difference was observed among the treatment groups T₁, T₂, T₃ and T₄ and all groups were statistically comparable. Numerically, T₄ (10%) recorded the highest intestinal length followed by T₂ and T₁ while the lowest value was recorded in T₃.

ii Intestinal weight

No significant difference was observed among the treatment groups T₁, T₂, T₃ and T₄ and all groups were statistically comparable. Numerically, T₂ recorded the highest intestinal weight followed by T₁ and T₃ while the lowest value was recorded in T₄.

Intestinal Histomorphology

Effect of feeding different levels of Black Soldier Fly Larva

in commercial broilers on Intestinal Histomorphology parameters (Mean ± SE) is presented in Table 4.

i. Duodenum

Villi height, Crypt depth and Villus Height Crypt Depth ratio of duodenum did not differ significantly ($P>0.05$) among the control and treatment groups.

ii. Jejunum

Villus Height Crypt Depth ratio of jejunum differed significantly ($P<0.05$) among the control and treatment groups. T₂ recorded the highest villus height to crypt depth ratio, which was statistically comparable with T₁ and T₄. However, Villi height and Crypt depth did not differ significantly ($P>0.05$) among the control and treatment groups.

iii. Ileum

Villi height, Crypt depth and Villus Height Crypt Depth ratio of ileum did not differ significantly ($P>0.05$) among the control and treatment groups.

Table 4: Effect of feeding different levels of Black Soldier Fly Larva in commercial broiler chicken on Intestinal histomorphology at 6th week of age (Mean ± SE)

Sl. No.	Parameters	Intestinal histomorphology				F VALUE
		T ₁ (0%)	T ₂ (5%)	T ₃ (7.5%)	T ₄ (10%)	
1	Duodenum					
	Villi Height (µm)	586.25±64.36	797.18±94.87	621.23±59.35	748.69±67.44	1.91 ^{NS}
	Crypt depth (µm)	40.55±0.50	47.28±1.50	45.96±2.31	43.70±3.19	1.92 ^{NS}
	Villus Height: Crypt Depth	14.42±1.40	16.77±1.44	13.65±1.77	17.13±0.88	1.47 ^{NS}
2	Jejunum					
	Villi Height (µm)	713.41±31.09	852.25±82.59	663.53±6.31	844.64±48.57	2.58 ^{NS}
	Crypt depth (µm)	44.49 ±4.08	42.38±4.90	52.51±4.75	43.83±1.04	1.71 ^{NS}
	Villus Height: Crypt Depth	16.36±1.99 ^{ab}	20.09±1.38 ^a	12.69±0.78 ^b	19.31±1.33 ^a	5.41 [*]
3	Ileum					
	Villi Height (µm)	836.06±80.49 ^a	794.10 ±38.74 ^a	552.55±50.28 ^b	663.72 ±85.75 ^{ab}	3.72 ^{NS}
	Crypt depth (µm)	42.97±0.95	48.70±5.01	50.91±8.12	43.00±2.21	0.67 ^{NS}
	Villus Height: Crypt Depth	19.48±1.86 ^a	16.59 ±1.53 ^{ab}	11.10±0.79 ^b	15.63±2.53 ^{ab}	3.74 ^{NS}

Means bearing different superscripts within row differ significantly ($P<0.05$)

No of observations (N) = 12 (3 birds x 4 treatments)

**Significant ($P<0.01$), *Significant ($P<0.05$), NS-Not significant.

Discussion

haematological parameters

The findings of the present study are in agreement with those reported by Belleza *et al.* (2021) [5], who observed that supplementation of live BSF larvae in broiler diets did not influence hematological and serum parameters. Similarly, Dabbou *et al.* (2018) [6] reported that inclusion of defatted BSF larvae meal up to 15% in broiler diets did not significantly affect blood and serum parameters.

Contrastingly, Mat K *et al.* (2021) [7] observed significant effects for several hematological parameters when broilers were fed diets containing defatted BSF larvae meal, although leucocytes, granulocytes and haematocrit remained unaffected.

Intestinal morphology

In accordance with present study, Elangovan *et al.* (2020) [8] observed no significant differences in the length or weight of

different intestinal segments such as the duodenum, jejunum, ileum and caecum between broilers fed diets containing BSF prepupae meal and the control group. Comparable findings were also reported by Belleza *et al.* (2021) [5], who noted that the inclusion of live BSF larvae in broiler diets did not influence intestinal weight. However, some studies have reported variations in intestinal characteristics with the inclusion of BSF larvae meal. Attivi *et al.* (2020) [9] reported that intestinal length increased significantly in broilers fed higher levels of BSF larvae meal (50%, 75% and 100%) compared to lower inclusion levels.

Intestinal histomorphology

The findings of the present study are consistent with the results reported by Dabbou *et al.* (2018) [6], who observed that inclusion of defatted BSF larvae meal up to 15% did not significantly influence most intestinal morphometric indices in broiler chickens. Similarly, Moula *et al.* (2017) [10] reported no significant difference in intestinal histomorphology between control birds and those fed diets containing BSF larvae. Contrastingly, Changqing He *et al.* (2021) [11] reported significantly higher villus height in the duodenum of birds fed moderate levels of BSF larvae meal, although crypt depth and VH:CD ratio were not significantly affected.

Conclusion

Based on the present study, inclusion of Black Soldier Fly Larva up to 10% had brought no negative impacts on haematological parameters, Intestinal morphology and Intestinal histomorphology to that of control which indicates Black Soldier Fly Larva can be included as one of the potential protein sources in broiler chicken diet.

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Conflict of Interest

Not available.

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Not available.

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