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Effect of feeding black soldier fly larvae meal on gut morphology and gut microbial load in broilers

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Abstract
Increasing world population has led to a very high demand of food and feed resources. Livestock forms a very large part of India and poultry forms a very large part of livestock industry. Poultry feed industry today is facing challenges such as limited availability of raw materials to be used in poultry feed. This study was conducted to study the effect of black soldier fly larvae meal, a potential protein source on gut microbial load and gut morphology in broilers. Four treatment groups T_1, T_2, T_3 and T_4 with three replicates each containing ten birds were made and T_1, T_2, T_3 and T_4 were fed with control diet, diet with 5% black soldier fly larvae meal, 10% black soldier fly larvae meal and 15% black soldier fly larvae meal, respectively. At the end of the experiment studies revealed that feeding of black soldier fly larvae meal had no significant effect on gut microbial load and gut morphology in broilers.

Keywords: Protein, black soldier fly, gut microbial load, gut morphology

1. Introduction
A major problem being faced by Poultry industry in India is the limited availability of conventional feed resources that are used in poultry feeds. Soyabean is the most commonly used protein source in poultry feeds. The major problem with using Soyabean meal in poultry feeds is the increased cost fluctuations in the price of soyabean meal, its limited availability, deforestation and limited availability of land for soyabean meal cultivation. Other protein sources such as fish meal bone meal faces problem such as limited inclusion, possible anti-nutritional factors and higher costs. These problems have led to the quest of searching new protein sources that can be used in poultry.

According to Bovera *et al*. (2015) [3], the natural inclination of chickens to consume insects underscores the potential of insects as a viable protein source in poultry feed. This observation suggests that incorporating insects into the diet of poultry may not only align with their natural dietary preferences but also offer nutritional benefits. Furthermore, insects offer significant advantages in terms of resource utilization and environmental sustainability compared to conventional protein sources.

Insects, such as the Black soldier fly (BSF), have garnered attention in poultry nutrition due to their ability to thrive in various environmental conditions with minimal space requirements. This adaptability makes them an attractive option for protein production, especially in regions where space and resources are limited. Additionally, insects possess the remarkable ability to convert organic waste into valuable nutrients, thereby reducing waste accumulation and associated environmental impacts.

Research by Chavez *et al*. (2023) [4] highlights the environmental benefits of insect-based protein production, demonstrating that insects can substantially reduce nitrogen excretion, odors, and methane emissions compared to traditional protein sources. This reduction in environmental footprint presents a compelling case for integrating insects into poultry feed formulations.

Among the commonly used insect species in poultry feed, the Black soldier fly stands out for its nutritional profile and ease of cultivation. Originating from South America, the Black soldier fly has spread to tropical and subtropical regions worldwide, facilitated by human activities. Unlike some other insect species, such as the housefly, the Black soldier fly is not considered a pest due to its non-aggressive nature and inability to transmit zoonotic diseases

(de Baets, 2017) [5]. The nutritional composition of Black soldier fly larvae makes them particularly valuable as a dietary ingredient in poultry feed. With protein content ranging from 40% to 60% CP, Black soldier fly larvae provide a substantial protein source for poultry. Additionally, they are rich in fats, calcium (5-8%), and phosphorus (0.6-1.5%), making them a well-rounded nutritional supplement for poultry diets.

Given these nutritional attributes and environmental benefits, Black soldier fly larvae have attracted interest not only as a feed ingredient for poultry but also as a potential novel ingredient in human diets. This growing interest underscores the versatility and potential of insects as a sustainable protein source for both animal and human nutrition.

Chitin which forms the exoskeleton of the BSF larvae and its derivatives are known to have potential antimicrobial activity by recruiting new antibodies and promoting good gut health (Uushona *et al.*, 2015) [10]. Attia *et al.* (2023) [1] reported that the caecal microbial count of *clostridial*, total coliform and *Escherichia coli* were not discrete among the groups fed with Black soldier fly larvae meal at 3% and 5% during the starter, growing and finishing periods. Dietary fully fatted BSF larvae meal inclusion had a positive impact on ileal mucosal immunity in layers at 42 days of age (Edea *et al*., 2022) [6].

Hence, this study was conducted to study the effect of feeding Black solider fly larvae meal on gut morphology and gut microbial load in broilers.

2. Materials and Methods

The research outlined in this study was conducted at the Department of Poultry Science, Veterinary College Hebbal, Bengaluru. A total of 120 one-day-old broiler chicks were obtained from Venkateshwara Hatcheries Pvt. Ltd., Bengaluru. Specifically selecting Cobb birds was motivated by the aim to explore alternative protein sources beneficial to farmers for poultry feed production.

Upon arrival, the chicks were individually weighed and then randomly assigned to one of four experimental groups, each comprising three replicates with ten chicks in each. These experimental groups were designated as T_1, T_2, T_3, and T_4. The feeding regimen for each group followed the specifications outlined in the Bureau of Indian Standards (BIS) 2007 [2] guidelines. T_1 received the control diet, while T_2, T_3, and T_4 were provided with diets containing 5%, 10%, and 15% black soldier fly larvae meal, respectively.

Throughout the six-week duration of the experiment, the chicks were reared using the deep litter system and had access to feed and water ad libitum. Standard management practices were adhered to diligently to ensure the welfare and health of the experimental subjects.

Vaccines for Marek's disease (HVT strain), Newcastle disease (Live BI strain), and Infectious bursal disease (intermediate strain) were sourced from Ventri Biologicals, Bengaluru. The black soldier fly larvae meal utilized in the experimental diets was obtained from Sri Kalleshwari Feeds Pvt Ltd, Kerala. This comprehensive setup aimed to investigate the effects of incorporating different levels of black soldier fly larvae meal into broiler chick diets on gut microbial load and gut morphology in broilers.

Microbiological parameters were assessed by using the *Lactobacillus* count and *Escherichia coli* count. At the end of the experiment, two birds from each replicate from the treatment groups, T_1 to T_4 were slaughtered. Intestinal contents from small intestine were collected aseptically. The samples were collected in sterile container and were further subjected to enumeration of gut microbes as per standard plate method (Postgate, 1969) [9]. Specific media such as MacConkey agar was used for assessing *E. coli* count, whereas brain heart infusion agar was used for assessing *Lactobacillus* by pour plate method (Mackie and McCartney, 1996) [8]. Ten-fold serial dilution of intestinal contents was used to assess the bacterial count. The bacterial counts were expressed as logs colony forming units/gm. of the sample from intestinal content.

Tissue samples of duodenum, jejunum and ileocecal junction were collected on the 42nd day of the experiment from the sacrificed birds, flushed with buffered saline and fixed in 10% neutral buffered formalin for histopathological studies *viz*., villus height and crypt depth.

3. Results

The findings of the present study revealed that there was no significant (p<0.05) difference in gut microbial load and gut morphology in broilers when fed with Black soldier fly larvae meal at different levels.

The villi height of duodenum (µm) in groups T_1, T_2, T_3 and T_4 were 1139.50, 1145.00, 1137.00 and 1132.00, respectively and the crypt depth (µm) were 126.83, 131.33, 128.17 and 125.17, respectively at the end of 42nd day.

The villi height of jejunum (µm) in groups T_1, T_2, T_3 and T_4 were 940.17, 930.67, 938.83 and 944.83, respectively and the crypt depth (µm) were 123.33, 124.00, 128.33 and 127.17 respectively, at the end of 42nd day.

The villi height of ileocaecocolic junction (µm) in groups T_1, T_2, T_3 and T_4 were 640.00, 636.17, 648.17, 657.17, respectively and the crypt depth (µm) were 116.83, 122.17, 116.17 and 118.17, respectively at the end of 42nd day.

At the end of the experiment, the intestinal *E. coli* count (log_{10} CFU/ g) in groups T_1, T_2, T_3 and T_4 were 7.055, 7.035, 7.048 and 7.052, respectively and at the end of the experiment, the intestinal Lactobacillus count (log_{10} CFU/ g) in groups T_1, T_2, T_3 and T_4 were 6.58, 6.60, 6.61 and 6.59, respectively.

4. Discussion

The results of the present study revealed no significant difference between control and different treatment groups in gut morphology and gut microbial load when broilers were fed with Black soldier fly larvae meal at different inclusion levels of 5, 10 and 15%.

The results of the present study are in agreement with He *et al*. (2023) [7] who reported that there were no significant differences in gut microbial count when Xuefeng Black-Bone chickens were fed with Black soldier fly larvae meal at inclusion levels of 1%, 3% and 5% and also the finding of the present study are in accordance with Yan *et al*. (2023) [12] who conducted an experiment on laying hens at the inclusion levels of 1%, 2% and 3% and reported that there were no significant changes in gut microbial count with the inclusion of Black soldier fly larvae meal.

The results of the present study incline with findings of He *et al.* (2023) [7] who reported that there was no significant difference in gut morphology when Xuefeng Black-Bone chickens were fed with Black soldier fly larvae meal at inclusion levels of 1%, 3% and 5%.

5. Conclusion

The present study revealed that there was no significant difference in gut morphology and gut microbial count between control and among different treatment, hence it was concluded that black soldier fly larvae meal may be included

up to 15% in broiler diets without causing any adverse effect on gut morphology and gut microbial count.

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