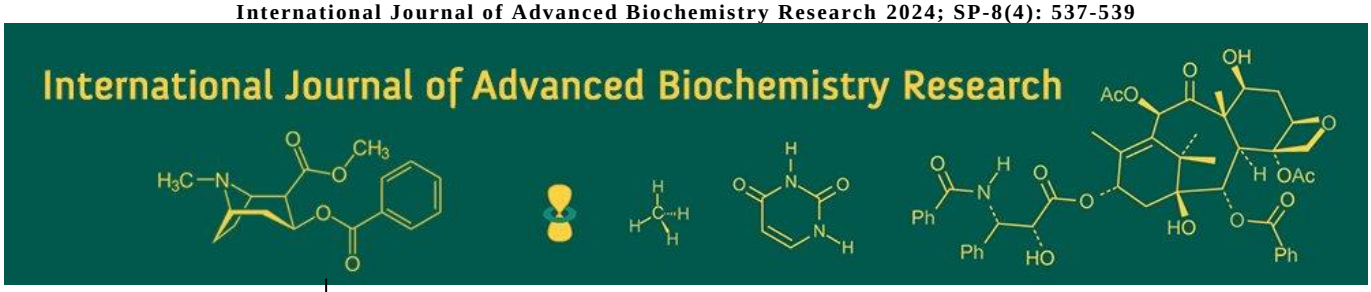

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Effect of feeding back soldier fly larvae meal on immune response and serum biochemistry profile in broilers

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Abstract
Poultry sector is the largest amongst the livestock sector. Feed quality is very important to sustain a profitable poultry business while lack of conventional protein sources is a major problem being faced by the poultry industry. Insects are the natural diet of poultry as birds with access to outdoors pick up insects and eat them. This study was conducted to study the effect of feeding Black soldier fly larvae meal on immune response and serum biochemistry profile in broilers. Four treatment groups T1, T2, T3 and T4 with three replicates each containing ten birds were made and T1, T2, T3 and T4 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 the results showed that inclusion of black soldier fly larvae meal had no significant difference on immune titres against Newcastle and Infectious bursal disease, immune organ weight and serum biochemistry profile in comparison to control stating that black soldier fly larvae meal can be included in poultry without affecting immune response and serum biochemistry profile.

Keywords: Black soldier fly larvae meal, insects, protein, immune response, serum biochemistry profile

Introduction
Rapidly growing world population is a boon for food industry as the industry is set to produce food, feed and fuel to substantiate this growing population. Livestock forms a major part of the food industry and poultry industry forms a very large part of livestock industry. India today is the third largest producer of egg and eighth largest producer of meat and increased awareness about consumption of protein will increase the demand for poultry products in the future as egg and meat are the most affordable source of protein.

Poultry feed forms a very large part of expenses faced by a poultry farmer. Protein is a very important micronutrient in a poultry feed formulation and is very important for growth of birds especially broilers. Soyabean meal is very commonly used source of protein in poultry feed formulation because of its high protein content (42 to 45%). The major problem with soyabean meal is the availability of soyabean meal, its fluctuating costs and the limited availability of land for soyabean cultivation. Fish meal is another major protein source in poultry feed industry and the problems with fish meal are limited level of inclusion, antinutritional factory "gizzarosine" which is known to cause erosions in gizzard and marine exploitation because of excessive usage of fish meal. This clearly illustrates that there is a need to find new unconventional sources of protein that can be used in poultry feeds.

Insects can potentially be used as a replacement of protein sources in poultry feeds as chicken with access to outdoor areas pick up insects at all life stages and eat them voluntarily, which indicates that they are voluntarily adapted to insects as natural part of their diet (Bovera *et al*., 2015) [2]. The amount of space required to grow insects is very minimal when compared to other conventional sources of protein. Insects convert waste into protein and reduce total nitrogen excretion, odours and methane emission, thus reduce up to 80% of waste mass (Chavez *et al*., 2023) [3].

Some of the common insect species used in poultry feed are:

1. Black soldier fly (*Hermitia illucens*)
2. Housefly (*Musca domestica*)

3. Mealworm (*Tenebrio molitor*)
4. Termites (*Mastotermes darwiniensis*)
5. Earthworm (*Lumbricus terrestris*)
6. Grasshoppers (*Locust migratoria*)
7. Cricket (*Gryllus testaceus walker*)
8. Silkworm (*Bombyx Mori*)

The Black soldier fly (BSF) is a common and widespread fly in many tropical regions, but BSF originates from South America, because of the human actions the fly was able to spread other tropical, subtropical and warmer areas. The BSF has the ability to adjust to different circumstances very quickly, but in contrast to housefly BSF is not a pest (de Baets.2017) [5]. This is because they do not a sting or bite and are not able to transfer any zoonotic diseases.

Black soldier fly larvae or Black soldier fly pre pupae is a very rich source of both fats and protein. The protein content of Black soldier fly larvae meal can range from 40 to 60% CP, the protein content majorly depends on the substrate on which the insects are reared and the steps of processing involved. The larvae meal can also be an excellent source of fats because of their rich ether extract content, they are also a very good source of calcium (5 to 8%) and phosphorus (0.6 to 1.5%). Because of all the considerations made about Black soldier fly, they have recently gained a lot of interest as being used as a dietary ingredient in not only animal feeds but also to be used as a novel ingredient in human diet also.

Marano *et al.*, (2017) [6] stated that the inclusion of black soldier fly larvae meal had no significant effect on antibody titres against Newcastle and Infectious bursal disease in broilers. Dabbou *et al.* (2018) [4] reported that the dietary inclusion of black soldier fly larvae meal at any levels had no effect on lymphoid organs like bursa, spleen and thymus weight. Van Huis *et al.* (2020) [8] reported that the inclusion of black soldier fly larvae meal at 20% inclusion did not have any significant difference on serum protein levels.

Material and Methods

The present study was conducted at Department of Poultry Science, Veterinary college Hebbal, Bengaluru. A total of one-hundred-and-twenty-day old broiler chicks were procured from Venkateshwara Hatcheries Pvt. LTD., Bengaluru. The main reason of selecting cobb birds was to explore and find alternative protein sources that can be used in producing poultry feeds that can be beneficial to the farmers. The chicks were weighed and allocated randomly to four experimental groups consisting of three replicates with ten chicks each.

The treatment groups T1, T2, T3 and T4 were fed with control diet, diet with 5% black soldier fly larvae meal, diet with 10% black soldier fly larvae meal and diet with 15% black soldier fly larvae meal, respectively as per BIS 2007 specifications. Chicks were reared under deep litter system up to six weeks of age with the supply of *ad libitum* feed and water. Standard managemental practice was followed during the experiment.

Marek's disease vaccine (HVT strain), ND vaccine (Live BI strain) and Infectious bursal disease of intermediate strain were procured from Ventri biologicals, Bengaluru. Black soldier fly larvae meal was procured from Sri Kalleshwari feeds Pvt LTD, Kerala.

At the end of the experiment (42nd day) blood samples were collected from two birds from each replicate at the end of the experiment. Serum was separated from the blood collected from the wing vein of the birds and antibody titre against Newcastle disease virus and Infectious bursal disease virus was estimated by Haemagglutination followed by Heaminhibition (Allan and Gough, 1975) [1] and using indirect ELISA kit, respectively at the end of the trial in treatment groups. Two birds from each replicate in each treatment groups were slaughtered et the end of experiment. The weight of lymphoid organs like spleen, thymus and bursa of Fabricius were recorded which were expressed as the per cent of pre slaughter weight (% of live weight).

Samples were collected from two birds from each replicate on 42nd day (at the end of the experiment. Serum was separated and total protein, levels of albumin and globulin were estimated.

The design of the experiment was complete randomized design (CRD) with one way analysis. All the data pertaining to various parameters of the biological trial was analysed by standard procedure described by Snedecor and Cochran (1994) [7] and by using SPSS 20 statistical software. Differences between the means were tested using Tukey's Range Test at ($P \leq 0.05$).

Results

The results showed that the inclusion of black soldier fly larvae meal at different levels of 5, 10 and 15% had no significant effect ($P < 0.05$) on immunological response and serum biochemistry profile in comparison to control in broilers. It was observed that the antibody titre against ND and IBD in different treatment groups T1, T2, T3 and T4 were 1.20, 1.15, 1.20 and 1.15 and 2.31, 2263.83, 2317.67 and 2.303.17 respectively. The weight of spleen in different treatment groups T1, T2, T3 and T4 were 0.162, 0.163, 0.170 and 0.160, respectively. The weight of bursa in different treatment groups T1, T2, T3 and T4 were 0.142, 0.148, 0.147 and 0.146, respectively. The weight of thymus in different treatment groups T1, T2, T3 and T4 were 0.420, 0.433, 0.452 and 0.443 respectively. The serum protein levels in treatment groups T1, T2, T3 and T4 were 2.93, 2.87, 2.98 and 2.91 respectively, albumin levels were 1.36, 1.40, 1.38 and 1.43 respectively and globulin levels were 1.57, 1.47, 1.59 and 1.47 respectively.

Discussion

The findings of the present study revealed that there was no significant ($P < 0.05$) difference in immune response and serum biochemistry profile with inclusion of black soldier fly larvae meal among different treatment groups in comparison to control.

The findings of the present study were in agreement with Marono *et al.* (2017) [6] who reported that the inclusion Black soldier fly larvae meal had no significant impact on Newcastle disease and Infectious bronchitis disease (IBD) titre in broilers at the end of the experiment. The results of the present study were in accordance with Dabbou *et al.* (2018) [4] who reported that the dietary inclusion of Black soldier fly larvae meal at any levels had no significant effect on lymphoid organs like bursa, spleen and thymus weight.

The results of the present study align with Van Huis *et al.* (2020) [8] who conducted an experiment by feeding Black soldier fly larvae meal at 20% inclusion and reported that there was no significant difference in serum protein levels between the treatment group and control group.

Conclusion

Inclusion of Black soldier fly larvae meal at different levels of 5, 10 and 15% had no significant effect on both immune response and serum biochemistry profile in comparison to control in broilers, hence it was concluded that Black soldier fly larvae meal can be included up to 15% in broilers without affecting immune response and serum biochemistry profile.

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