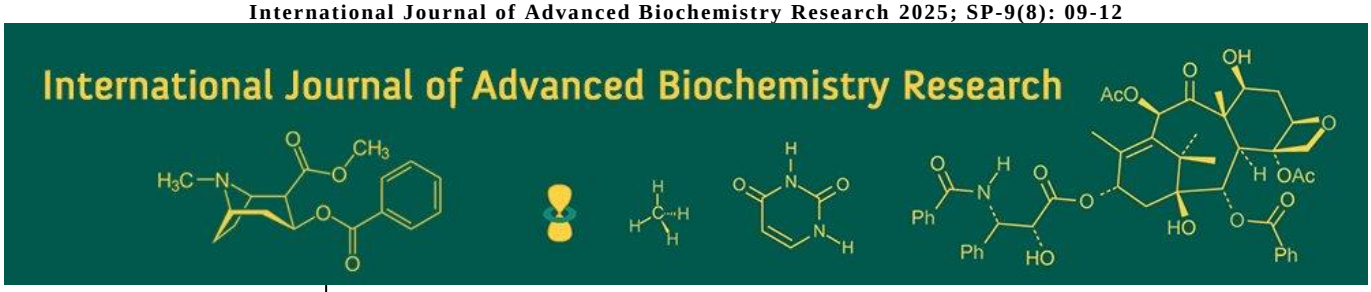

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Effect of supplementing flaxseed powder on immune status in broilers

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Abstract
An experiment was carried out to evaluate the impact of flaxseed powder supplementation on the serum lipid profile and immune status of broiler chickens. A total of 150 Cobb chicks were assigned to five dietary treatment groups and reared until 42 days of age. The treatments included: T_1-control group (basal diet formulated as per BIS, 2007), T_2-basal diet with 5% flaxseed powder, T_3-basal diet with 7% flaxseed powder, T_4-basal diet with 10% flaxseed powder, and T_5-basal diet with 12% flaxseed powder. The findings indicated that the inclusion of flaxseed powder had no statistically significant effect ($p>0.05$) on the immune response to Newcastle Disease (ND) and Infectious Bursal Disease (IBD) when compared to the control group. Additionally, there were no significant differences ($p>0.05$) in the relative weights of immune organs across all treatment groups at the conclusion of the trial (42 days). It was therefore concluded that dietary flaxseed powder, even up to a 12% inclusion level, did not influence the immune status of broilers under the conditions of this study.

Keywords: Flaxseed powder, immunity, broilers, antibody titer, lymphoid organs, omega-3 fatty acids

Introduction
The poultry sector plays an integral role in meeting the global demand for high-quality animal protein. With the world population projected to exceed 9.7 billion by 2050, the demand for poultry products, especially meat and eggs, is expected to rise sharply. This anticipated growth emphasizes the urgent need for sustainable intensification of poultry production systems to ensure food security (FAO, 2018). Broiler chickens, owing to their fast growth rate, efficient feed conversion, and relatively short production cycle, form the backbone of the commercial poultry industry. However, intensive production conditions often pose challenges in maintaining optimal health and immune function in birds.

Ensuring robust immune function in broilers is essential not only for disease resistance but also for achieving consistent growth performance. In light of increasing concerns over antimicrobial resistance, there is a global movement to reduce the reliance on antibiotics in animal farming. This shift has accelerated research into natural and sustainable alternatives that can enhance immune performance in poultry. Among the most promising candidates are functional feed additives rich in omega-3 fatty acids, which are known to positively influence immune modulation (Calder, 2013) [6].

Flaxseed (*Linum usitatissimum*), also known as linseed, has emerged as a valuable feed ingredient due to its impressive nutritional composition. Approximately 40% of flaxseed is oil, with alpha-linolenic acid (ALA)—an essential omega-3 fatty acid—constituting the majority. ALA acts as a precursor to longer-chain polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), both of which play crucial roles in regulating immune cell membrane integrity and the synthesis of eicosanoids—bioactive compounds involved in immune signaling. In addition to omega-3s, flaxseed also contains lignans, high-quality protein, and dietary fiber, making it a multifunctional feed additive for poultry (Bhatty, 1995) [5].

Several studies have explored the immunological effects of flaxseed in poultry. Mashaan and Meisaa (2014) [8] evaluated the inclusion of flaxseed in broiler diets and found that it enhanced red and white blood cell counts—parameters that are closely linked to immune competence.

Likewise, Abdulwahid and Mudheher (2017) [1] demonstrated that flaxseed oil, especially when combined with probiotics, improved key immune functions such as phagocytic activity, cytokine production, and natural killer cell response. Their results also indicated that birds supplemented with flaxseed showed stronger antibody responses to Newcastle Disease (ND) vaccination and had improved blood lipid profiles, suggesting a dual benefit for both immunity and metabolism.

Taher (2018) [11] reported that dietary flaxseed oil at a level of 0.6% enhanced packed cell volume and red blood cell counts, suggesting a positive influence on the hematological and immune status of broilers. This aligns with the broader body of research indicating that flaxseed may act as an immunostimulant under appropriate inclusion levels.

On the other hand, some studies have shown no significant effects on immune traits. Ahmed *et al.*, (2009) [2] examined the effect of high levels of heat-treated flaxseed on immune organ development in pre-laying hens and found no measurable changes. Similarly, Moslehi *et al.*, (2019) [9] assessed the immune responses of laying hens fed diets containing up to 100 g/kg of flaxseed and found no performance or organ weights. These discrepancies could be attributed to differences in bird age, flaxseed processing, or experimental conditions.

In contrast, Fiky *et al.*, (2020) [7] observed that broilers fed a diet supplemented with 3% flaxseed oil exhibited significantly higher relative weights of lymphoid organs, including the spleen, thymus, and bursa of Fabricius, compared to birds fed sunflower oil. This suggests that flaxseed oil may confer specific advantages in promoting immune organ development.

Given the variability in research outcomes, it is essential to identify the most effective inclusion levels of flaxseed that can enhance immune responses without adversely affecting performance. The current study aims to assess the impact of different dietary levels of flaxseed powder on immune parameters in broilers, with a specific focus on antibody titers and the development of lymphoid organs.

Materials and Methods

A total of 150 day-old broiler chicks were procured from Venkateshwara Hatcheries Pvt. Ltd., Bengaluru, for the experimental study. The chicks were individually weighed, wing-banded, and randomly allocated into five treatment groups following a completely randomized design. They were reared under a deep litter system for a period of six weeks, with free access to feed and water. Standard management practices were maintained throughout the experimental period. The study protocol was approved by the Institutional Animal Ethics Committee of KVAFSU, Bidar, Karnataka. Flaxseed used in the experiment was sourced from the commercial market in Bengaluru.

Based on the BIS (2007) [4] guidelines, standard broiler pre-starter, starter, and finisher rations were formulated using commonly available feed ingredients. The control group (T_1) was offered a basal diet without flaxseed supplementation. The treatment groups T_2, T_3, T_4, and T_5 received the basal diet supplemented with 5%, 7%, 10%, and 12% flaxseed powder, respectively.

1. Immunological response

A. Antibody titres against Newcastle disease (ND) and Infectious bursal disease (IBD)

At the end of the experimental period, blood samples were collected from two birds per replicate via the wing vein. Serum was separated and used to assess antibody titers against Newcastle disease virus (NDV) and infectious bursal disease virus (IBDV). Antibody response to NDV was evaluated using the hemagglutination followed by hemagglutination inhibition test as described by Allan and Gough (1974) [4], while titers against IBDV were determined using an indirect ELISA kit.

a) Newcastle disease

Hemagglutination (HA) and Hemagglutination Inhibition (HI) assays were employed to determine antibody titers against Newcastle disease virus (NDV). The HI titers were assessed using the micro-test method described by Allan and Gough (1974) [4], to evaluate the humoral immune response in relation to different dietary treatments. The HI test was conducted manually using the B-procedure in 'U'-bottom microplates. Serum samples were subjected to serial two-fold dilutions in normal saline, with 25 µl of each dilution dispensed into individual wells, followed by the addition of 4 HA units of NDV antigen. After a 45-minute incubation at room temperature, 50 µl of 0.8% chicken erythrocyte suspension was added to each well, and the plates were further incubated for one hour at room temperature. The antibody titer, expressed as the log10 of the reciprocal of the highest serum dilution that completely inhibited hemagglutination (indicated by button formation), was recorded.

b) Infectious bursal disease

Antibody titers against Infectious Bursal Disease Virus (IBDV) were determined using an indirect ELISA kit, following the manufacturer's protocol. The assay was performed at the Poultry Diagnostic and Research Centre (PDRC).

Serum antibody titers against Infectious Bursal Disease Virus (IBDV) were measured using an indirect ELISA kit, following the manufacturer's instructions. The assay was performed in antigen-precoated microplate wells. Positive and negative control sera (100 dl each) were added in duplicate to their designated control wells. Subsequently, 100 dl of each test serum sample, appropriately diluted in sample buffer, was added in duplicate to the corresponding wells, excluding the control wells. The plate was incubated at 37 °C for one hour to facilitate antigen-antibody binding.

Following the initial incubation, the plate was washed thoroughly using the wash buffer provided in the kit to remove unbound antibodies. Subsequently, 100 dl of mouse anti-chicken IgG conjugated with horseradish peroxidase (HRP), diluted in wash buffer, was added to each well. The plate was incubated for an additional one hour at 37 °C. After incubation, a second washing step was performed to remove any unbound conjugate.

Following the second wash, 100 dl of freshly prepared chromogen-substrate solution—containing o-phenylenediamine dihydrochloride (OPD) and 3% hydrogen peroxide (H_2O_2) as the substrate, at a concentration of 4 dl/ml—was added to each well. The plate was then incubated at room temperature for 15 minutes to allow for color development.

To stop the enzyme-substrate reaction, 50 dl of 2.5 N hydrochloric acid (HCl) was added to each well. Absorbance was then measured using an ELISA reader (Bio-Rad) equipped with a 492 nm interference filter. The

instrument was zeroed using wells containing only the substrate-chromogen solution and HCl as blanks prior to reading the test samples.

B. Lymphoid organ weight

At the conclusion of the trial, two birds from each replicate within each treatment group were slaughtered for evaluation. The lymphoid organs—spleen, thymus, and bursa of Fabricius—were excised and weighed. The organ weights were expressed as a percentage of the live body weight prior to slaughter (i.e., percent live weight).

The percentage relative weight of each lymphoid organ was calculated using the following formula:

$$\text{Lymphoid organ weight (\%)} = \frac{\text{Lymphoid organ weight (g)}}{\text{Pre slaughter live weight (g)}} \times 100$$

Statistical analysis

The experimental data were analyzed using a completely randomized design (CRD) with one-way analysis of variance (ANOVA). All biological parameters were statistically evaluated following the standard procedures outlined by Snedecor and Cochran (1994) [10], using SPSS version 20. Differences between treatment means were assessed using Tukey's Range Test, with statistical significance considered at (p<0.05).

Results

Immunological response

1. Antibody titres against Newcastle Disease and Infectious Bursal Disease

At the end of the experiment, the antibody titers against Newcastle disease virus (expressed as $\log_{10}$ HI titers) for treatment groups T_1, T_2, T_3, T_4, and T_5 were 1.36, 1.49, 1.52, 1.51, and 1.42, respectively. Statistical analysis showed no significant differences (p>0.05) in HI titers among the treatment groups.

Similarly, the ELISA-based antibody titers against Infectious Bursal Disease Virus (IBDV) for T_1, T_2, T_3, T_4, and T_5 were 2158.67, 2142.83, 2362.50, 2225.33, and 2440.33, respectively. These differences were also found to be statistically non-significant (p>0.05) across the treatment groups.

2. Immune organ weights

At the end of the experiment, the relative weight of the spleen (as a percentage of live body weight) in treatment groups T_1, T_2, T_3, T_4, and T_5 was 0.171, 0.168, 0.171, 0.176, and 0.177, respectively. Statistical analysis indicated no significant differences (p>0.05) in spleen weight among the treatment and control groups.

The relative weight of the thymus in T_1, T_2, T_3, T_4, and T_5 was recorded as 0.384, 0.376, 0.383, 0.406, and 0.409, respectively. These values did not differ significantly (p>0.05) across treatments.

Similarly, the relative weight of the bursa of Fabricius was 0.138, 0.134, 0.140, 0.142, and 0.148 in T_1, T_2, T_3, T_4, and T_5, respectively, with no significant differences (p>0.05) observed among the groups.

Discussion

At the end of the 42-day feeding trial, the results showed no statistically significant (p>0.05) differences in the immune response to Newcastle Disease and Infectious Bursal Disease, nor in the relative weights of lymphoid organs among broilers supplemented with flaxseed powder, compared to those in the control group.

These outcomes are in line with the findings of Moslehi *et al.*, (2019) [9], who reported that dietary inclusion of flaxseed up to 100 g/kg, in combination with selenium, had no significant influence on the immune organ weights of laying hens. Similarly, Ahmed *et al.*, (2009) [2] concluded that high levels of heat-treated flaxseed had no measurable effect on immune organ development in local layer chickens. These findings support the hypothesis that flaxseed powder, even at higher inclusion levels, may have a limited impact on the anatomical development of immune organs in poultry.

Conversely, Fiky *et al.*, (2020) [7] demonstrated that the addition of 3% flaxseed oil to broiler diets led to a significant increase in the relative weights of the spleen, thymus, and bursa compared to sunflower oil supplementation, indicating a potential role for flaxseed oil in immune organ development. Similarly, Taher (2018) [11] and Abdulwahid and Mudheher (2017) [1] reported that flaxseed oil enhanced several immunological mechanisms in broilers, including phagocytic activity, natural killer cell function, and cytokine production. These differences may stem from variations in the form of flaxseed used (oil versus powder), as well as differences in experimental design, bird strain, or environmental conditions.

Additionally, Al-Nasser and Hanan Al-Khalaifah (2020) [3] observed that flaxseed supplementation positively affected the cellular immune response, as indicated by phytohemagglutinin (PHA) skin test results. Their findings contrast with the present study, suggesting that the immunomodulatory effects of flaxseed may depend on specific immunological endpoints, dosage levels, and the presence of other dietary components.

In summary, while several studies support the immune-enhancing potential of flaxseed particularly in oil form the present results indicate that dietary supplementation with flaxseed powder up to 12% does not significantly influence humoral immune responses or immune organ development in broilers. Further research is needed to clarify the influence of flaxseed form, processing methods, and synergistic additives on poultry immune function.

Conclusion

Based on the findings of this study, it can be concluded that dietary inclusion of flaxseed powder had no statistically significant effect (p>0.05) on the immune titers of broilers to Newcastle Disease and Infectious Bursal Disease by day 42 of the experiment. Additionally, there were no significant differences (p>0.05) in the relative weights of lymphoid organs between the flaxseed-supplemented groups and the control group. These results suggest that flaxseed powder when included in the diet up to 12%, does not exert any notable influence on the immune status of broiler chickens under the conditions of this study.

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References

1. Abdulwahid MT, Mudheher SJ. Influence of probiotic and flaxseed oil supplementation on some physiological parameters and immune response of broilers. Journal of Entomology and Zoology Studies. 2017;5:1836-1842.
2. Ahmed AS, Al-Yousif YM, Najib H. Effect of dietary high levels of heat treated locally produced flaxseeds on body weight gain and immune response of pre-layer local chicken. Egyptian Poultry Science Journal. 2009;29(3):821-835.
3. Al-Khalaifah H, Al-Nasser A. Dietary supplementation with various fat oils affect phytohemagglutinin skin test in broiler chickens. Frontiers in Immunology. 2020;11:551443.
4. Allan WH, Gough RE. A standard haemagglutination inhibition test for Newcastle disease. (2) Vaccination and challenge. The Veterinary Record. 1974 Aug 17;95(7):147-149.
5. Bhatty RS, Cuannane SC, Thompson LU. Flaxseed in Human Nutrition. Champaign (IL): AOSC Press; 1995. p. 22.
6. Calder PC. Omega-3 polyunsaturated fatty acids and inflammatory processes: nutrition or pharmacology? British Journal of Clinical Pharmacology. 2013 Mar;75(3):645-662.
7. Fiky AE, Zanaty GA, El-Naga MKA. Influence of adding linseed or sunflower oils to diets on the performance of broiler chicks. Menoufia Journal of Animal, Poultry and Fish Production. 2020;4(6):115-125.
8. Mashaan A, Al-Zuhairy M, Taher G. Effects of feeding different levels of flaxseed on performance traits and blood parameters in broiler. Diyala Agricultural Sciences Journal. 2014;6(2):1-10.
9. Moslehi H, Navidshad B, Sharifi SD, Aghjegheshlagh FM. Effects of selenium and flaxseed on selenium content and antioxidant properties of eggs and immune response in hens. South African Journal of Animal Science. 2019;49(4):770-780.
10. Snedecor GW, Cochran WG. Statistical methods. 6th ed. Ames (IA): Iowa State University Press; 1994.
11. Taher MG. Effects of feeding flaxseed oil on performance and some blood biochemical traits of broiler. Diyala Agricultural Sciences Journal. 2018;10(2):20-28.