Fountain Journal of Basic Medical and Health Sciences (FUJBMHES), 2(1), 170-178.

Dose-Dependent Effects of Humic Acid Supplementation on Serum Lipid Profile and Newcastle Disease Antibody Response in Broiler Chickens

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Abstract

Background/Introduction: Humic acid (HA) is a high polyphenol substance. Lipid profiling involves estimation of lipids and in broiler birds, it may be useful for optimizing nutrition and enhancing poultry performance. Immune response is a physiological reaction for the purpose of defense. **Objective:** The study examined the effects of varying concentrations of humic acid on broiler birds, focusing on lipid profiles and immune response. **Methodology:** One hundred (100) one-day-old broiler chicks were distributed into five dietary groups, each consisting of four replicates of five birds were used for the study. Treatment 1, which served as the control, utilized a basal diet without humic acid supplementation. Treatments 2 through 5 involved supplementations of humic acid at levels of 0.1 to 0.4 kg per 100 kg diet respectively. The study spanned 28 days, comprising 7 days of basal diet feeding followed by 21 days of experimental feed. Serum samples collected at the end of the experiment were analyzed for lipid profiling. **Results:** Birds receiving 0.1 kg HA/100 kg exhibited the lowest significant serum cholesterol (24.75 ± 0.42 mmol/l; $p<0.05$) compared to the control (25.88 ± 0.75 mmol/l). Immune response, measured via Hemagglutination Inhibition (HI) titres, peaked in the 0.2 kg group by day 28. Results indicated significant alterations in low-density lipoprotein, very low-density lipoprotein, cholesterol and high-density lipoprotein levels with increasing humic acid concentration in the diet. Birds that received 0.1 kg HA/100 kg dietary supplement exhibited a favourable lipid profile ($p<0.05$). The immune response of the birds showed improvement over time with humic acid supplementation in a concentration dependent manner. Diets supplemented with 0.2 kg and 0.3 kg/100 kg of humic acid showed significantly ($p<0.05$) increased immune response, consistently performing better than those of the birds on the basal diet throughout the duration of the study. **Conclusion:** The study concluded that varying concentrations of humic acid in the diet of broiler birds significantly influenced their lipid profiles and had positive impact on immune response.

Keywords: Humic acid, Lipid profiling, Broiler birds, Immune response, Poultry nutrition

Introduction

Humic acid (HA), a major component of soil organic matter, is a naturally occurring macromolecule formed through the decomposition of plant and microbial residues. Its molecular structure comprises functional groups such as carboxyl, phenolic, hydroxyl, and carbonyl functional groups, which enable HA to interact with soil particles, nutrients, and biological systems (Nabi et al., 2025). Humic acid is a natural organic acid and has been shown to influence digestion, immune response and general performance of broilers (Disetlhe et al., 2019).

Lipid profile is defined as a routine clinical measurement that assesses various lipid levels in the blood, including total cholesterol and triglycerides, to determine the risk of

 ISSN: 1595-1693

cardiovascular disease (Amitava & Amer, 2021). Immunity is the capability of the body to fight against pathogenic microbes in order to prevent us from infectious diseases. Immunity involves 2 types of components i.e., specific and nonspecific components (Deepak & Goyal, 2014). The immune response constitutes the primary biological defense mechanism that safeguards the body from diverse threats, including microorganisms such as bacteria, viruses, fungi, and parasites, as well as complex foreign substances (Sabir & Jan, 2025).

Broiler production is a cornerstone of the global poultry industry, catering to the ever-growing demand for high-quality protein sources (Beski et al., 2015). However, enhancing broiler health and performance while ensuring sustainability remains a significant challenge. In recent years, there has been a growing interest in natural additives as potential supplements to conventional feed formulations (Sugiharto & Ranjitkar, 2019). Humic acid, a complex mixture derived from organic matter, has shown promising immunomodulatory effects in various animal species (Marcinčák et al., 2023). The objective of this study is to investigate the influence of adding humic acid to broiler diets on the lipid profile of broiler chickens and its potential as an immune system booster in these birds. The broiler lipid profile is a key focus because of its vital importance in both health and productivity. Lipids not only act as energy stores but also have critical functions in cell structure, signaling pathways, and immune system functions (Prates, 2025).

Understanding the immunostimulatory properties of humic acid in broiler birds could offer practical implications for the poultry industry. If proven effective, humic acid supplementation could represent a sustainable and cost-effective strategy to enhance broiler immunity, thereby reducing the reliance on antibiotics and other pharmaceutical interventions (Ahfeethah et al., 2023). While humic substances are known to improve growth, limited information exists regarding their simultaneous impact on lipid metabolism and Newcastle Disease Virus (NDV) antibody response in broiler chickens under tropical production systems. In this paper, we present the findings of our study, which explored the effects of humic acid supplementation on broiler lipid profile and its implications for immunostimulatory activity in broiler birds. Through a combination of biochemical analyses and immunological assessments, we aimed to provide valuable insights into the potential of humic acid as a novel feed additive for improving broiler health and welfare which indirectly influences health of humans as the consumers of these birds.

Materials and Methodology

The experiment was carried out at the Rearing Section of the Poultry Unit Biochemistry and Nutrition Laboratory, Department of Chemical Sciences Fountain University, Osogbo located in the tropical forest zone of Nigeria, Latitude 7°46' North and Longitude 4°34' East at an altitude of 320 m (1,050ft) above sea level drained by the Osun rivers and its tributaries.

Birds, Housing and Feeding

The study involved one hundred (100) unsexed one-day-old broiler birds, which were divided into five dietary treatment groups with four replicates of five birds each. This design allows for a controlled comparison of the effects of varying concentrations of humic acid on the birds' health and performance. One hundred 100-day-old broiler birds, sourced from GS Farmers' Centre, Owode, Ede, Nigeria, were housed in well-ventilated, standard poultry cages equipped with proper lighting and temperature control. Prior to the experimental diet, the birds were fed a standard commercial starter feed, formulated to meet their nutritional requirements, for an acclimatization period of seven days. The control group received a basal diet without humic acid, while the other groups received diets supplemented with 0.1 to 0.4 kg of humic acid per 100 kg of feed.

This range of supplementation enables the assessment of dose-dependent effects on the

 ISSN: 1595-1693

lipid profile and overall performance of the birds in this order:

- T1 – Control
- T2 – 0.1 kg HA /100 kg feed
- T3 – 0.2 kg HA /100 kg feed
- T4 – 0.3 kg HA /100 kg feed
- T5 – 0.4 kg HA /100 kg feed

Chemicals and Reagents Used

Synthesized humic acid supplement 100 g package manufactured by UCORP International, was gotten from Pasig Philippines, purified Agar or Agarose, (sodium chloride, sodium azide, distilled water) and normal saline were all of analytical grades and were obtained from the Veterinary Medicine Department, University of Ibadan, Ibadan Nigeria.

Experimental Feed Diets

Isonitrogenous which are the diets containing equal crude protein levels and isocaloric diets which are the diets containing equal metabolizable energy were formulated to meet the nutrient requirements recommended by NRC (1994) for broiler pre-starter, starter, and finisher diets. Experimental diets in each phase have the same ingredients and nutrients composition with the addition of varied concentrations of humic acid to the already formulated diets.

Duration of the Experiment

The experiment lasted for 28 days, with the first 7 days on the basal diet and the subsequent 21 days on the experimental diets. This duration is sufficient to observe the effects of dietary changes on growth and lipid metabolism.

Lipid Profile and Immune Response Analysis

Serum samples were collected at the end of the experimental period for lipid profiling and immune response analysis. Serum sample were analyzed for HDL, LDL, triacylglycerol and cholesterol levels using procedure by Barter et al. (2012) for HDL, Friedewald et al. (1972) for LDL, Foster and Dunn (1973) for triacylglycerol and Rifai et al. (2007) for cholesterol. Serum samples were analyzed for immune response markers to Newcastle disease virus (NCDV) using Hemagglutination Inhibition (HI) test for NDV antibodies according to the procedure described by (OIE, 2000).

Data analysis

Data were analyzed using one-way ANOVA in GraphPad Prism 6.0. Significant differences among treatment means were separated using Tukey's multiple comparison test at $p \leq 0.05$. Results are presented as mean ± Standard Error of Mean (SEM).

Results and Discussion

The results of lipid profiling as shown above from Figures 1- 4 provide insights into the effects of humic acid supplementation on various lipid parameters in broiler birds. From Fig.1, the study found that broiler birds supplemented with 0.1 kg of humic acid per 100 kg of diet had the lowest significant serum cholesterol levels ($p < 0.05$). This indicates that this level of humic acid may effectively reduce cholesterol accumulation in the bloodstream, which is beneficial for both poultry health and consumer safety. Conversely, birds receiving 0.4 kg of humic acid exhibited the highest cholesterol levels, suggesting that excessive supplementation may have adverse effects on lipid metabolism. This is in tandem with the findings of the work by Šamudovská & Demeterová (2010) which revealed lower cholesterol levels ($P < 0.05$) in the Sodium humic acid group.

ISSN: 1595-1693

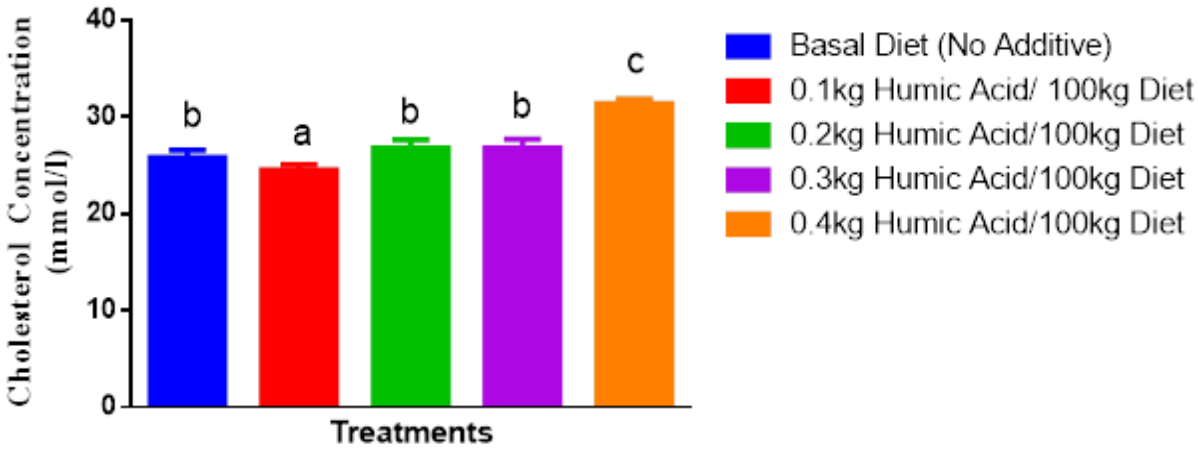

FIG 1. Cholesterol concentrations (mmol/l) of broiler birds placed on varying concentration of humic acid

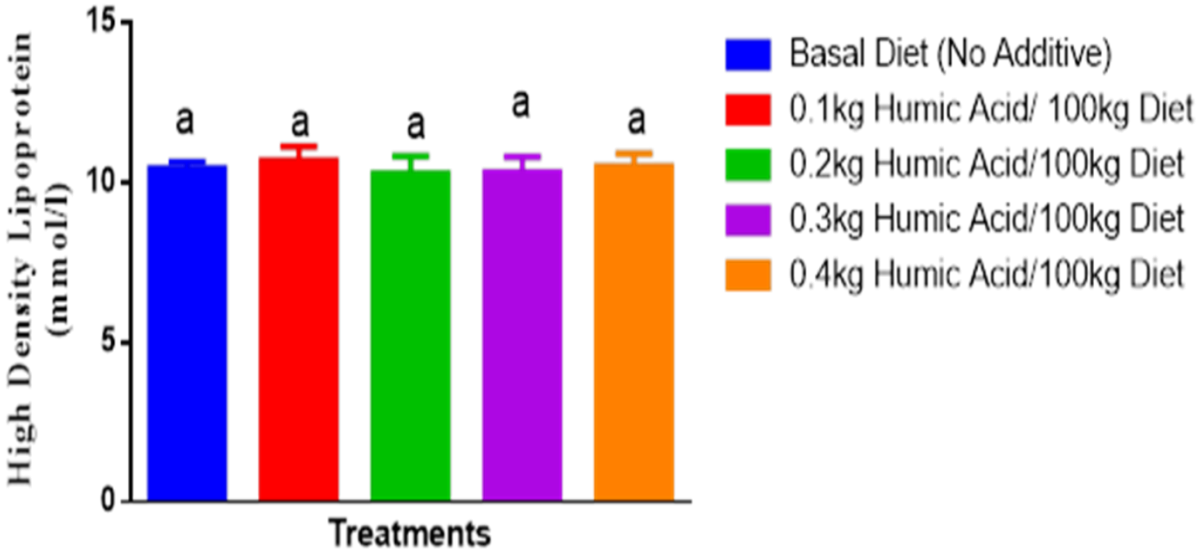

FIG 2. High density lipoprotein concentrations (mmol/l) of broiler birds placed on varying concentration of humic acid

ISSN: 1595-1693

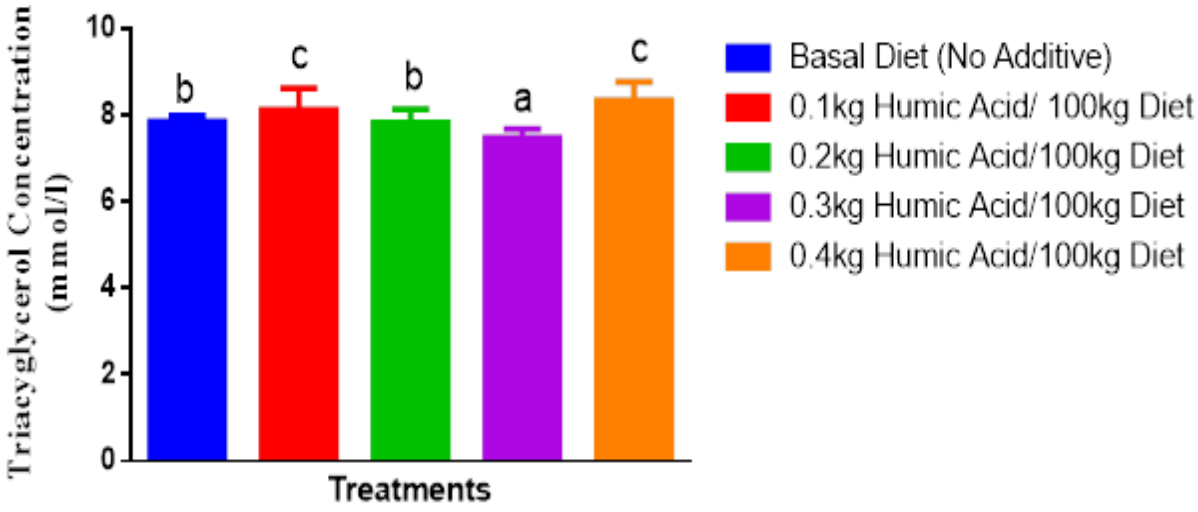

FIG 3. Triacylglycerol concentrations (mmol/l) of broiler birds placed on varying concentration of humic acid

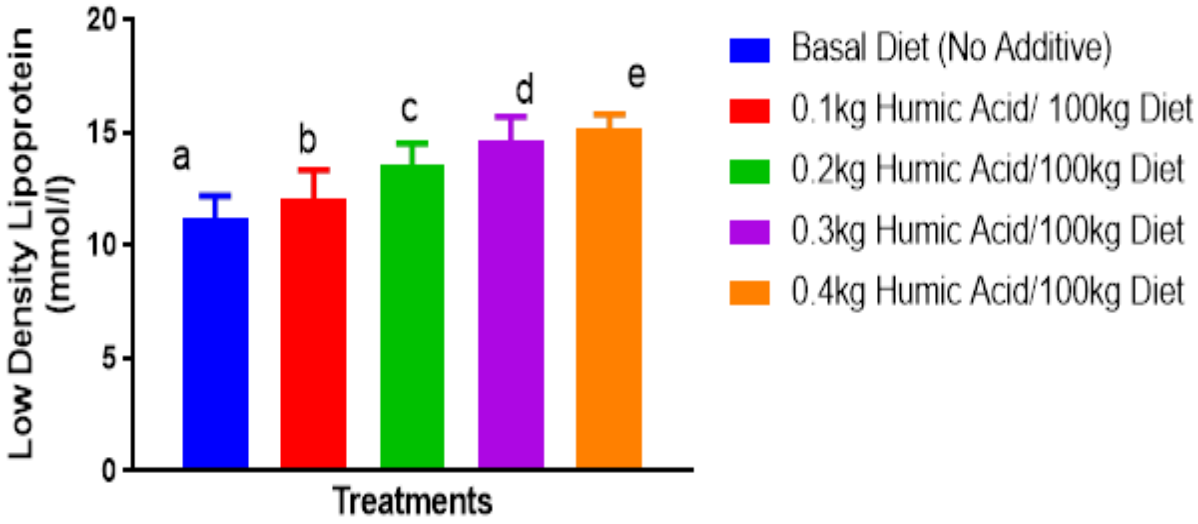

FIG 4. Low density lipoprotein concentrations (mmol/l) of broiler birds placed on varying concentration of humic acid

ISSN: 1595-1693

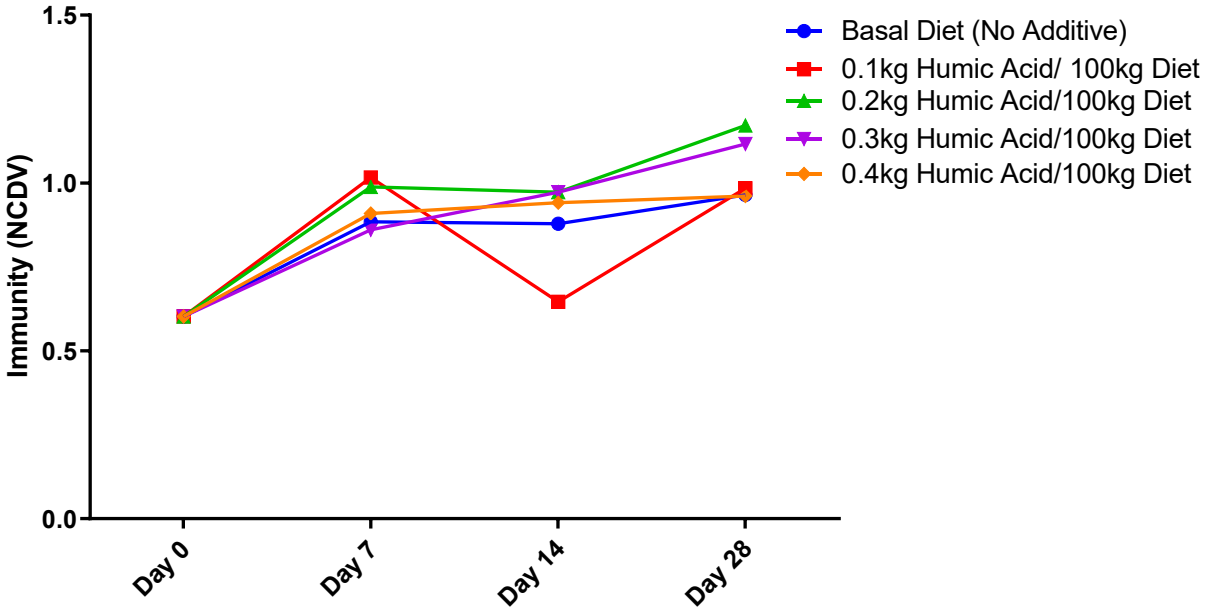

Fig 5. Effect of varying concentration of humic acid supplemented diet on immune response of broiler birds.

From Fig. 2, the results indicated that serum triacylglycerol levels were lowest in birds fed a diet containing 0.3 kg of humic acid per 100 kg. This finding aligns with study by Lacková et al. (2022) that reported humic acid's ability to exert a positive effect in the reduction in the intramuscular fat content in rabbits.

The significant decrease in triacylglycerol concentrations at this supplementation level suggests that humic acid may play a role in lipid metabolism regulation, potentially reducing the risk of lipid-related health issues.

From Fig. 3, the study reported non-significant changes ($p > 0.05$) in HDL levels among the different dietary treatments. This indicates that while humic acid supplementation may influence other lipid parameters, it does not significantly affect HDL concentrations in the serum of broiler birds. HDL is known for its role in transporting lipids from peripheral tissues to the liver for metabolism, and the stability of HDL levels suggests that humic acid does not negatively impact this protective lipid profile component (Jomard & Osto, 2020). From Fig. 4, the results showed non-significant values for LDL and VLDL in birds fed diets with 0.1 to 0.3 kg of humic acid per 100 kg. This finding is consistent with research by Lacková et al. (2022) indicating that humic acid can positively influence the lipid profile by reducing harmful lipoprotein levels. The lack of significant changes suggests that moderate supplementation of humic acid does not lead to an increase in these potentially harmful lipoproteins, which is favourable for overall health.

From Fig. 5. The immune response was assessed using serum HI titres against Newcastle Disease Virus (NDV). On the first day, there were no significant variances in HI titres across the groups. By the 7th day, birds on the 0.1kg and 0.2kg humic acid diets showed a significant increase ($p < 0.005$) in HI titres compared to the basal diet. This indicates that early supplementation of humic acid can enhance the immune response shortly after dietary changes. On the 14th day, birds on the 0.2kg, 0.3kg, and 0.4kg diets exhibited higher HI titres ($p < 0.05$) compared to the basal diet, while the 0.1kg group showed a lower significant difference.

ISSN: 1595-1693

This suggests that higher concentrations of humic acid may further enhance immune responses as the birds mature. By the 28th day, the 0.2kg/100kg group had the highest HI titre, indicating a robust immune response against NDV. The 0.3kg group also showed a significant response but was slightly lower than the 0.2kg group. This reinforces the idea that a specific concentration of humic acid can optimize immune function. The study by Marcinčák et al. (2023) noted that humic acid also exert a beneficial effect on the immune system of poultry.

The findings of this study are consistent with previous research, such as that of Nagaraju et al. (2014) which noted an improvement in the production parameters and the immune status of broilers after the addition of humic substances to antibiotic-free feed. This supports the notion that humic acid can be beneficial in poultry nutrition. The significant reduction in cholesterol at the 0.1 kg HA/100 kg level suggests that humic acid may effectively modulate lipid metabolism at low concentrations. This mechanism is likely linked to humic acid's ability to improve gut microbiota and enhance bile acid sequestration, which prevents cholesterol reabsorption in the small intestine. However, the increase in cholesterol at 0.4 kg suggests a dose-dependent saturation point where excessive HA may interfere with normal lipid clearing mechanisms. Regarding immune response, the peak HI titre observed in the 0.2 kg group indicates an optimal immunostimulatory effect. Humic substances are known to act as natural antioxidants and immunomodulators, potentially stimulating the production of cytokines and enhancing the B-lymphocyte response to viral antigens like NDV.

Conclusion

The lipid profiling results indicated that humic acid supplementation, particularly at 1 kg and 3 kg per 100 kg of diet, can lead to favourable changes in the lipid profile of broiler birds. The significant reduction in cholesterol and triacylglycerol levels suggests that humic acid may be beneficial for managing lipid metabolism and reducing the risk of cardiovascular issues. However, higher levels of supplementation (4 kg) may have adverse effects, highlighting the importance of moderation in dietary formulations. The stability of HDL, LDL, and VLDL levels further supports the potential of humic acid as a safe dietary additive in poultry nutrition.

Dietary supplementation of humic acid at 0.1 kg per 100 kg of feed results in the most favourable cholesterol reduction in broiler birds. For optimal immune enhancement against Newcastle Disease Virus, a concentration of 0.2 kg per 100 kg is recommended. Humic acid can serve as a viable, organic alternative to synthetic growth promoters. The authors therefore recommend using humic acid as additive in poultry diets, emphasizing its potential to enhance both health and productivity.

Professional implications of the study

The findings of this study offer practical applications for the poultry industry and animal nutritionists seeking organic alternatives to synthetic growth promoters. By demonstrating the impact of humic acid on lipid profiles and immune responses, the research provides a scientific basis for using high-polyphenol substances to optimize broiler performance and meat quality.

Limitations of the study

The study was conducted over a relatively short period of 28 days, which may not fully capture the long-term physiological effects or the cumulative impact of humic acid through the entire life cycle of the bird.

Funding/Sponsorship/Grant: Nil.

Informed Consent

All participants provided informed consent for publication of this paper.

ISSN: 1595-1693

Acknowledgements

The authors express their gratitude for the contributions of laboratory staff of Fountain University Osogbo for their immense contribution in the course of this study.

References

Ahfeethah, F., Elazomi, A., & Kammon, A. (2023). Effect of humic acid and probiotics on immunity of broiler chickens. *Open veterinary journal*, *13*(7), 839–845.

Amitava D., & Amer W. (2021). Chapter 6 - Lipid metabolism and disorders, Clinical Chemistry, Immunology and Laboratory Quality Control (Second Edition), Elsevier, 105-126, ISBN 9780128159606.

Barter, P., Gotto, A. M., LaRosa, J. C., Maroni, J., Szarek, M., Grundy, S. M., Kastelein, J. J., Bittner, V., & Fruchart, J. C. (2007). HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. *New England Journal of Medicine*, *357*(13), 1301–1310. https://doi.org/10.1056/NEJMoa064278

Beski, S. S. M., Swick, R. A., & Iji, P. A. (2015). Specialized protein products in broiler chicken nutrition: A review. *Animal nutrition (Zhongguo xu mu shou yi xue hui)*, 1(2), 47–53.

Deepak, T., & Goyal, K. (2014). The role of helminths in immunity. *Journal of Allergy & Therapy*, *7*, 231. https://doi.org/10.4172/2155-6121.1000231

Disetlhe, A. R. P., Marume, U., Mlambo, V., & Hugo, A. (2019). Effects of dietary humic acid and enzymes on meat quality and fatty acid profiles of broiler chickens fed canola-based diets. *Asian-Australasian journal of animal sciences*, *32*(5), 711–720. https://doi.org/10.5713/ajas.18.0408

Foster, L. B., & Dunn, R. T. (1973). Stable reagents for determination of serum triglycerides by a colorimetric Hantzsch condensation method. *Clinical Chemistry*, *19*(3), 338–340.

Friedewald, W. T., Levy, R. T., & Frederickson, D. S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clinical Chemistry*, *18*(6), 499–502.

Jomard, A., & Osto, E. (2020). High Density Lipoproteins: Metabolism, Function, and Therapeutic Potential. *Frontiers in cardiovascular medicine*, *7*, 39.

Lacková, Z., Zigo, F., Farkašová, Z., & Ondrašovičová, S. (2022). The Effect of Humic Substances as an Organic Supplement on the Fattening Performance, Quality of Meat, and Selected Biochemical Parameters of Rabbits. *Life (Basel, Switzerland)*, *12*(7), 1016.

Marcinčák, S., Semjon, B., Marcinčáková, D., Reitznerová, A., Mudroňová, D., Vašková, J., & Nagy, J. (2023). Humic Substances as a Feed Supplement and the Benefits of Produced Chicken Meat. *Life (Basel, Switzerland)*, *13*(4), 927.

Nabi, F., Sarfaraz, A., Kama, R., Kanwal, R., & Li, H. (2025). Structure-Based Function of Humic Acid in Abiotic Stress Alleviation in Plants: A Review. *Plants (Basel, Switzerland)*, *14*(13), 1916.

Nagaraju, R., Reddy, B. S., Gloridoss, R., Suresh, B. N., & Ramesh, C. (2014). Effect of dietary supplementation of humic acids on performance of broilers. *Indian Journal of Animal Sciences*, *84*(4), 447–452.

National Research Council. (1994). *Nutrient requirements for poultry* (9th rev. ed.). National Academy Press.

OIE (Office International des Epizooties) (2000). Newcastle disease. Manual of standards for diagnostic tests and vaccines, 4th edition. OIE, Paris.. 221 – 232.

Prates, J. A. M. (2025). The Role of Meat Lipids in Nutrition and Health: Balancing Benefits and Risks. *Nutrients*, *17*(2), 350.

Rifai, N., Horvath, T., & Wittwer, C. (2012). *Tietz textbook of clinical chemistry and molecular diagnostics*. Elsevier Health Sciences.

Sabir, S., & Jan, A. (2025). Physiology, immune response. In *StatPearls*. StatPearls

Publishing. https://www.ncbi.nlm.nih.gov/books/NBK539801/

Šamudovská, A. & Demeterová, M. (2010). Effect of Diet Supplemented with Natural Humic Compounds and Sodium Humate on Performance and Selected Metabolic Variables in Broiler Chickens. *Acta Veterinaria Brno*. 79.

Sugiharto, S., & Ranjitkar, S. (2019). Recent advances in fermented feeds towards improved broiler chicken performance, gastrointestinal tract microecology and immune responses: A review. *Animal nutrition (Zhongguo xu mu shou yi xue hui)*, *5*(1), 1–10.

Citation*:*

Ojedokun R. O., Ogunbode S. M., Salawu M. A., Oloyede M. A., Biyaosi S. Y., Olaniyi, K. T., Abd-Salam, T. M. and Majolagbe H. O. (2026). Dose-Dependent Effects of Humic Acid Supplementation on Serum Lipid Profile and Newcastle Disease Antibody Response in Broiler Chickens. *Fountain Journal of Basic Medical and Health Sciences (FUJBMHES), 2(1),170*-178.

 ISSN: 1595-1693