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

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

Ojedokun R. O.[1*], Ogunbode S. M.[1], Salawu M. A.[1], Oloyede M. A.[2], Biyaosi S. Y.[2], Olaniyi, K. T.[1], Abd-Salam, T. M.[1] and Majolagbe H. O.[3]

[1]*Biochemistry and Nutrition Unit, Department of Chemical Sciences, Fountain University, Osogbo, Osun State, Nigeria;*
[2]*Microbiology Unit, Department of Biological Sciences, Fountain University, Osogbo, Osun State, Nigeria;*
[3]*Lagos State University of Education, Oto/ Ijanikin, Lagos State, Nigeria.*

*Corresponding Author: ojedokunrahimat@gmail.com; ro.ojedokun@fuo.edu.ng; +2348143273335.

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 optimising nutrition and enhancing poultry performance. An immune response is a physiological reaction for defence. **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 with four replicates of five birds. Treatment 1, which served as the control, utilised a basal diet without humic acid supplementation. Treatments 2 through 5 involved supplementation 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 analysed 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 outperforming those of birds on the basal diet throughout the study. **Conclusion:** The study found that varying concentrations of humic acid in broiler birds' diets significantly influenced their lipid profiles and positively impacted 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 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 defence 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, signalling 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 is available on their simultaneous effects on lipid metabolism and the 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 the 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 acclimatisation 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 lipid profile and overall performance of the birds in this order:

 ISSN: 1595-1693

- 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

Synthesised humic acid supplement 100 g package manufactured by UCORP International, was obtained 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 nutrient 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 samples were analysed for HDL, LDL, triacylglycerol and cholesterol levels using the 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 analysed for immune response markers to Newcastle disease virus (NCDV) using the 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 in 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 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 adversely affect lipid metabolism. This is in tandem with the findings of Šamudovská & Demeterová (2010), who reported lower cholesterol levels ($P < 0.05$) in the Sodium humic acid group.

ISSN: 1595-1693

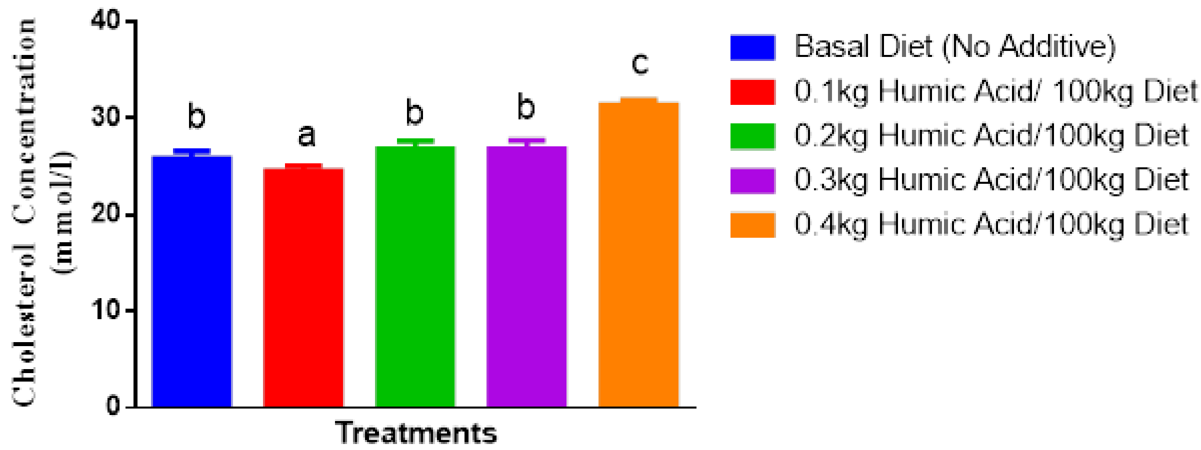

FIG 1. Cholesterol concentrations (mmol/l) of broiler birds placed on varying concentrations of humic acid

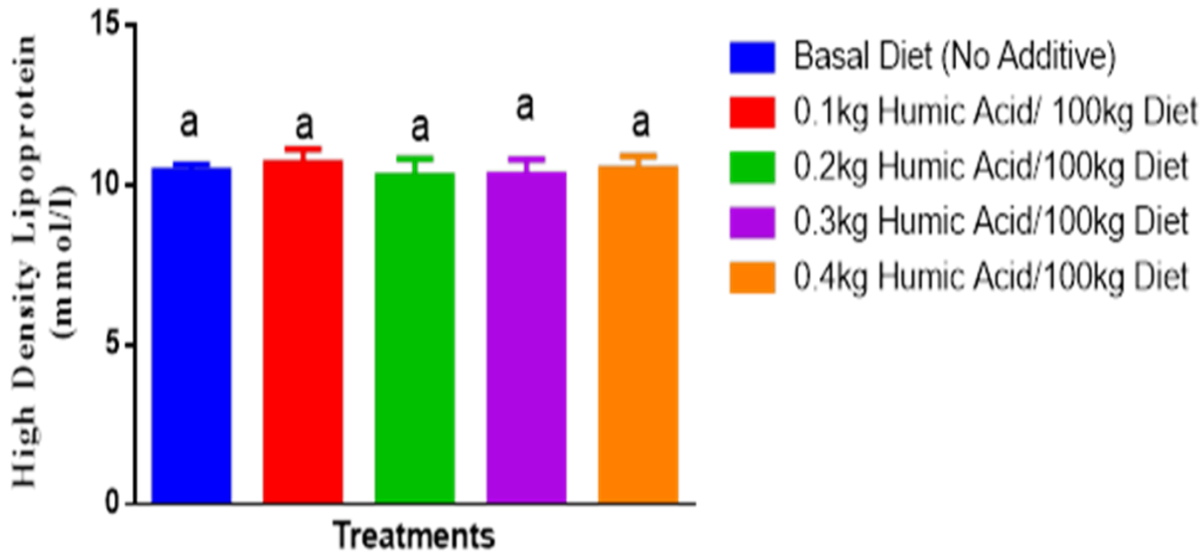

FIG 2. High-density lipoprotein concentrations (mmol/l) of broiler birds placed on varying concentrations of humic acid

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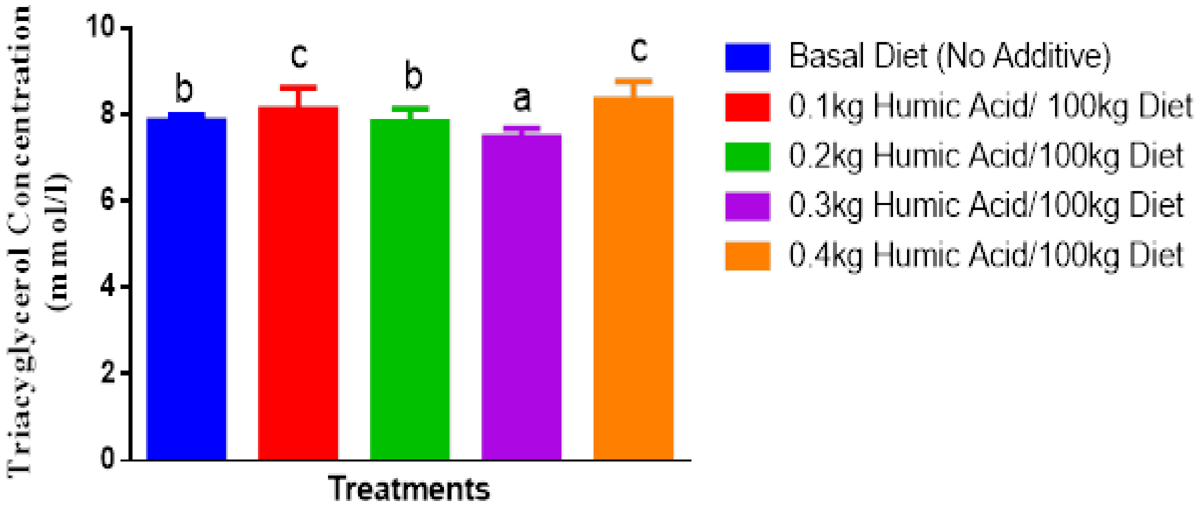

FIG 3. Triacylglycerol concentrations (mmol/l) of broiler birds placed on varying concentrations of humic acid

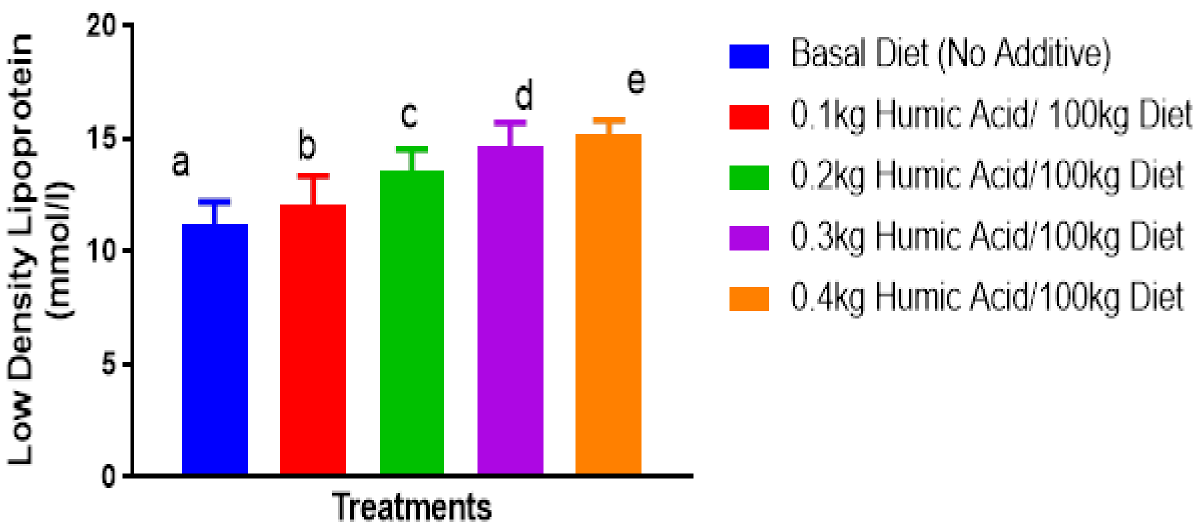

FIG 4. Low-density lipoprotein concentrations (mmol/l) of broiler birds placed on varying concentrations of humic acid

ISSN: 1595-1693

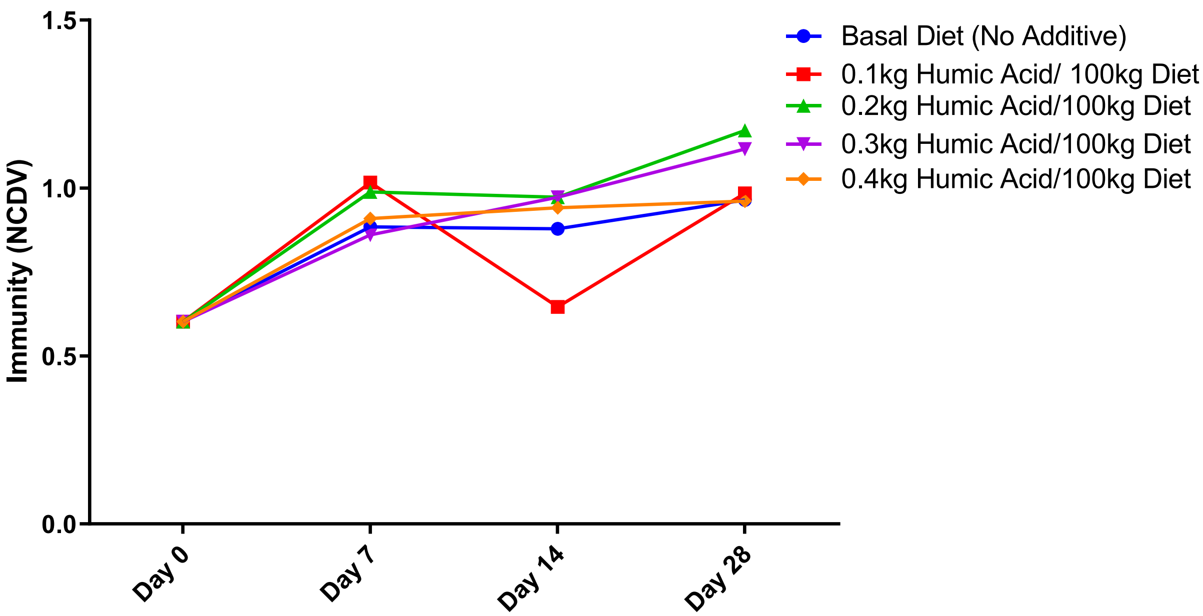

Fig 5. Effect of varying concentrations of humic acid-supplemented diet on the immune response of broiler birds.

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 a study by Lacková et al. (2022) that reported humic acid's ability to exert a positive effect in the reduction of 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 no significant changes ($p > 0.05$) in HDL levels across the different dietary treatments. This indicates that while humic acid supplementation may influence other lipid parameters, it does not significantly affect serum HDL concentrations in broiler birds. HDL is known for transporting lipids from peripheral tissues to the liver for metabolism, and the stability of HDL levels suggests that humic acid does not negatively affect 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 with humic acid does not increase 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. 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 it was slightly lower than that of the 0.2kg group. This reinforces the idea that a specific concentration of humic acid can optimise immune function. The study by Marcinčák et al. (2023) noted that humic acid also exerts 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 reported improvements in broiler production parameters and immune status 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 cytokine production and enhancing B-lymphocyte responses to viral antigens such as NDV.

Conclusion

The lipid profiling results indicated that humic acid supplementation, particularly at 0.1 kg and 0.3 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 (0.4 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. Although direct clinical effects on humans were not investigated, the improved lipid profile observed in the broilers may translate into healthier meat products for consumers by potentially lowering the deposition of undesirable lipids in poultry meat. Furthermore, enhanced immune competence in the birds may contribute to improved poultry productivity and reduced disease burden, indirectly supporting food safety and public health. The authors therefore recommend using humic acid as an additive in poultry diets, emphasising its potential to enhance both health and productivity.

Professional implications of the study

The study professionally implies that humic acid possesses significant potential as a functional and sustainable feed additive in broiler production, due to its ability to favourably modulate lipid metabolism and enhance immune responsiveness in a concentration-dependent manner. Its application in poultry nutrition may support the production of healthier birds and meat products with improved lipid characteristics, thereby offering possible health benefits to humans as final consumers. Furthermore, the observed enhancement of immune response against NCDV suggests that humic acid could serve as a natural alternative to antibiotic growth promoters, contributing to improved poultry health management, reduced disease burden, and safer poultry products.

 ISSN: 1595-1693

These findings are of considerable relevance to professionals in Nutritional Biochemistry, Animal Nutrition, Veterinary Medicine, and public health, while also presenting economic, industrial, and research opportunities toward sustainable poultry production and the development of functional animal-derived foods for improved consumer wellbeing.

Limitations of the study

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

Funding/Sponsorship/Grant: Nil.

Informed Consent

All participants provided informed consent for publication of this paper.

Acknowledgements

The authors express their gratitude to the laboratory staff of the Department of Chemical Science, Fountain University, Osogbo, for their immense contribution in the course of this study.

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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), 1(2),170*-178.

 ISSN: 1595-1693