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Effects of plant-derived bioactive supplementation on intestinal morphology and humoral immune response of broiler chickens

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Abstract

With the gradually increasing regulatory restrictions on the prophylactic and growth-promoting use of antibiotics in poultry farming, more interest has been generated in the use of natural alternatives in chickens for optimal gut health and immunity. This study reports the effects of dietary supplementation of *Moringa oleifera* derived bioactive extract on the gut morphology and immune response in broiler chickens. A total of 240 day-old male Cobb-500 chicks were randomly assigned to four treatment groups: a control group with only basal diet and three groups receiving basal diet supplemented with 0.5, 1.0, or 2.0 g/kg of the plant extract. During the feeding trial of 42 days, the birds were raised under strictly controlled environmental conditions. For gut histology, samples were taken from the jejunum to measure villus height, crypt depth, and their ratio. To evaluate the humoral immune status of the birds, the Newcastle disease antibody titer and weights of lymphoid organs were assessed. Birds supplemented with 1.0 and 2.0 g/kg of the plant extract showed significantly improved intestinal morphology with longer villi, a higher villi-to-crypt ratio, and well-preserved gut tissue architecture. The immune system of these birds was enhanced, with higher antibody titers and immune organ weights. A very strong positive correlation was evident between villus height and antibody responses, revealing a close association between gut structure and immune strength. In conclusion, dietary supplementation of broiler chicken with *Moringa oleifera* derived bioactive extract improved their gut health and immune status, supporting its potential as a sustainable alternative to antibiotics in poultry production.

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1. Introduction

The poultry industry continues to face persistent challenges related to animal health management, rising consumer demand for antibiotic-free products, and increasingly restrictive regulations on antimicrobial use. In broiler chickens, gastrointestinal health is the main factor influencing their growth, welfare, nutrient utilization, immunity, and maintaining physiological balance in general. Compromised growth and health are usually the first indicators reflected in response to poor gastrointestinal health (Oni and Oke 2025). Recent studies have focused on different plant-based supplements rich in polysaccharides, polyphenols, and essential oils, as dietary strategies to improve gut health and immune function in broiler chickens. Among these, plant polysaccharides have exhibited significant protection against growth retardation and intestinal damage. For example, Artemisia species helps preserve the intestinal barrier's integrity by enhancing the immune system and elevating antioxidant levels (Guo et al. 2024). On similar lines, curcumin, a main active ingredient of turmeric, improved intestinal morphology and antioxidant levels in broiler chicken, thus underscoring the biological significance of phytogenic compounds in poultry nutrition (Hernndez-Garca et al. 2025).

Phytogenic feed additives rich in flavonoids, alkaloids, and essential oils have multiple effects on gut health by modulating gut microbiota composition. They enhance the growth of beneficial bacteria and reduce pathogen populations, thus maintaining gut health, which in turn improves digestive efficiency and nutrient utilization in broiler chickens (Oni and Oke 2025). Most of the literature, especially experiments focused on licorice (*Glycyrrhiza uralensis*) and echinacea extracts, demonstrated immune response enhancement manifested by higher immunoglobulin levels and a larger number of antioxidant enzymes, which indicates synergistic fortification of intestinal health and the overall immune system (Alhujaili et al. 2025). Collectively, a growing body of evidence indicates that plant-derived bioactive compounds can serve as viable alternatives to conventional synthetic feed additives for sustaining intestinal integrity, modulating gut microbiota, and enhancing immune competence in broiler chickens (Obianwuna et al. 2024; Zhang et al. 2022). Despite such promising evidence, there is a lack of understanding of how different phytochemical mixtures influence gut anatomy and immune system. There remains a critical knowledge gap regarding the synergistic or antagonistic effects of standardized multi-component plant extracts under practical production conditions, as most previous investigations have primarily focused on isolated or purified bioactive constituents rather than complex phytochemical matrices (Sun et al. 2025; Vaou et al. 2022). This limitation is particularly relevant given that extraction techniques substantially influence phytochemical composition and resultant bioactivity (Sun et al. 2025), while interactions among plant-derived compounds may generate additive, synergistic, or even antagonistic antimicrobial effects (Vaou et al. 2022). It is highly desired to conduct *in vivo* experiments to decipher the precise effects of these bioactive phytogenic supplements on parameters such as intestinal

morphology, mucosal immunity, and systemic immune responses to clarify their role in gut health and immunity of broiler chickens.

Therefore, this study assesses the effects of a standardized multi-component *Moringa oleifera* extract at graded dietary levels on broiler chickens. The objectives of this study were: (i) to evaluate the impact on jejunal morphology; (ii) to determine the impact on humoral immune response measured in terms of Newcastle disease antibody titers and lymphoid organ development; and (iii) to investigate the association between intestinal morphological parameters and immune responsiveness of broiler chickens.

2. Materials and Methods

This 42-day experimental trial was undertaken at the Integrated Laboratory and Poultry Research Facility of Sumbawa University of Technology, West Nusa Tenggara, Indonesia. All experimental procedures involving the birds were in line with internationally accepted animal care standards. The study was approved by the university animal ethics committee (Approval No. 012/KEPH-UTS/III/2025) and was conducted in accordance with the recognized guidelines for poultry research (Abdelli et al. 2021; Barszcz et al. 2024). In a completely randomized design, 240 one-day-old male Cobb-500 broiler chicks were used. Four dietary treatments were prepared and each was allocated six replicates of ten birds (n = 60). The dietary treatments were as follows:

(1) Control basal diet without any supplementation

(2) Basal diet + 0.5 g/kg plant-derived bioactive extract

(3) Basal diet + 1.0 g/kg plant-derived bioactive extract

(4) Basal diet + 2.0 g/kg plant-derived bioactive extract

The nutritional requirements of broiler chickens during the starter (0-14 days), grower (15-28 days), and finisher (29-42 days) phases were met by formulating the diets from available ingredients with the required supplementations (Cobb-Vantress 2023). The plant-derived bioactive extract was prepared from *Moringa oleifera* Lam. leaves, known for their high levels of polyphenols, flavonoids, and other antioxidant compounds that confer immunomodulatory and gut-protective effects in poultry. The leaves were freshly picked from certified local farms in West Nusa Tenggara, Indonesia, dried naturally without direct sun exposure at room temperature, and ground to a fine powder. The extract was prepared by ethanol-water maceration (70% v/v) at a solid-to-solvent ratio of 1:10 (w/v) for 72 h with periodic agitation. The obtained filtrate was concentrated under reduced pressure with the help of a rotary evaporator at temperatures below 45 C to avoid the loss of thermolabile bioactive compounds. The concentrated extract was then spray-dried to yield a stable powder. The final product was standardized to guarantee batch-to-batch consistency of total polyphenol and flavonoid content and was added to the basal diet at different inclusion levels (0.5, 1.0, and 2.0 g/kg). The extract was very well blended with the feed to ensure even distribution, adhering to standard practices for phytogenic supplementation in broiler nutrition experiments. Birds were housed in environmentally controlled cages under standard management conditions. Temperature, humidity, and ventilation were controlled as per the requirement of commercial broiler chickens to avoid environmental stress influencing the gut health and immune parameters. Feed and water were freely available for the entire 42-day period. At day 0, all birds were vaccinated against Newcastle disease as part of regular flock health practice.

Three birds per replicate were randomly selected and humanely euthanized on days 21 and 42. Birds sampled on day 21 were not included in subsequent measurements at day 42, as intestinal histomorphology and lymphoid organ evaluation required destructive sampling. Therefore, independent sets of birds were used at each time point. Prior to euthanasia, blood samples were collected from the jugular vein for serum separation. After opening the abdominal cavity, jejunal segments were excised, rinsed with saline, and immediately fixed in 10% neutral-buffered formalin for histological examination (Fascina et al. 2017). The fixed jejunal samples were embedded in paraffin, followed by sectioning (5 µ thick) and staining with hematoxylin and eosin (H&E). Villus height (VH), crypt depth (CD), and the VH/CD ratio were measured by digital imaging software. For each sample, ten well-oriented villi were examined for taking measurements in line with prevailing gut health research methods (Obianwuna et al. 2024). Antibody titers in the serum against Newcastle disease virus were measured by the hemagglutination inhibition (HI) assay and expressed as $\log_2$ values, which is a standard method for evaluating humoral immunity in broiler chickens (Cecchini et al. 2019; Hofmann et al. 2020). Immediately after slaughter, the bursa of Fabricius, spleen, and thymus were excised and weighed individually and recorded relative to body weight in percentages.

Data were analyzed using the General Linear Model (GLM) procedure under a completely randomized design. Because birds were sacrificed at each sampling point (destructive sampling), measurements obtained at days 21 and 42 originated from independent experimental units. Therefore, data from each time point were analyzed separately using one-way ANOVA with dietary treatment as the fixed effect. The statistical model applied was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} represents the observed variable, μ is the overall mean, T_i is the fixed effect of dietary treatment ($i = 1–4$), and e_{ij} is the residual error. When ANOVA indicated significant treatment effects ($P < 0.05$), means were separated using Duncan's multiple range test. Pearson correlation and simple linear regression analyses were performed to evaluate the association between villus height and NDV antibody titers. Normality and homogeneity of variance were verified prior to analysis (Mishra et al. 2019).

3. Results and Discussion

Dietary supplementation of plant-derived bioactive extract significantly ($P<0.05$) altered the intestinal histomorphology in chickens (Table 1). Birds receiving extract at the rate of 1.0 or 2.0 g/kg feed exhibited significantly higher villus height (VH), lesser crypt depth (CD), and higher VH/CD ratio compared to control group and group receiving extract at the rate of 0.5 g/kg feed. Significant differences were observed as well between control group and group receiving extract at

Table 1. Effects of plant-derived bioactive supplementation on jejunal morphology of broiler chickens (Day 42)

Treatment	Villus height (µ)	Crypt depth (µ)	VH/CD ratio
Control	1125 ± 48 ^{c}	198 ± 9 ^{a}	5.68 ± 0.21 ^{c}
0.5 g/kg	1214 ± 52 ^{b}	187 ± 8 ^{b}	6.49 ± 0.25 ^{b}
1.0 g/kg	1318 ± 56 ^{a}	176 ± 7 ^{c}	7.49 ± 0.28 ^{a}
2.0 g/kg	1336 ± 59 ^{a}	175 ± 6 ^{c}	7.63 ± 0.31 ^{a}

Data are reported as the mean ± SD. Different superscripts within the column indicate that values differ statistically (P<0.05)

the rate of 1.0 g/kg feed. A higher VH/CD ratio is an indicator of better gut nutrient absorption efficiency and maturity and longer villi signify better renewal of the gut lining and enhanced ability to absorb nutrients. Smaller crypt depths are generally linked to decreased epithelial turnover and lower intestinal stress, which indicates improved gut stability. The dietary supplementation of phytogenics in poultry increases villi length and the effective surface area of the intestine, resulting in a better metabolic efficiency and growth performance of birds (Sharma et al. 2020; Oni et al. 2025). The improvement in villus architecture observed due to the supplementation of Moringa leaf extract is consistent with the findings of previous studies which indicated that phytogenic compounds promote gut cell proliferation and maintain intestinal health, mainly due to their antioxidant and anti-inflammatory properties (Zhang et al. 2022; Ogbuewu et al. 2022). On similar lines, the reduced crypt depth and improved VH/CD ratio have been reported in broiler chickens fed polyphenol-rich plant extracts (Paneru et al. 2022; Urban et al. 2025).

The morphometric quantitative results were additionally confirmed by microscopic observations of jejunum sections. Compared to the control group, broiler chickens supplemented with plant-derived bioactive extract displayed regularly arranged longer villi with intact epithelial lining and had less crypt hyperplasia (Fig. 1). Such architectural changes in the intestine suggest enhanced mucosal protection and intestinal homeostasis due to the bioactive compounds of the extract, which is consistent with the observations made in previous studies (Olfati et al. 2018).

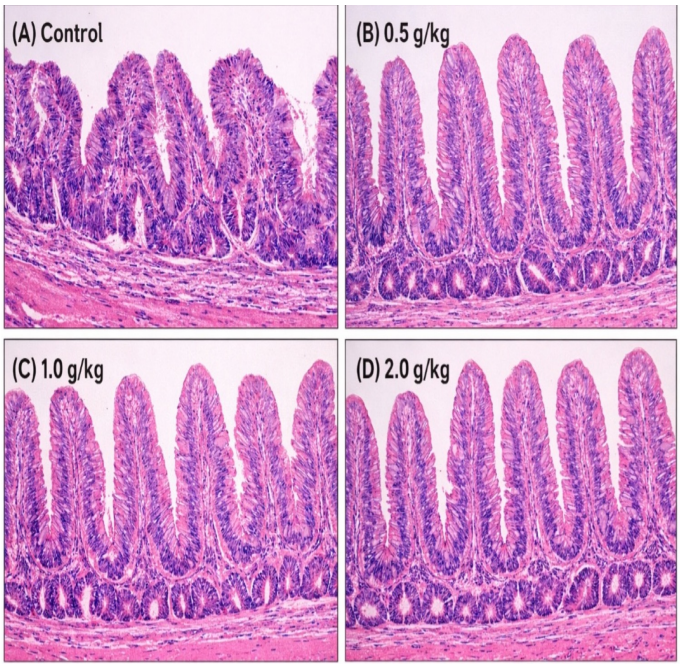


Fig. 1. *Jejunal tissue sections from broiler chickens fed either the control diet or a diet supplemented with a Moringa-derived bioactive extract, stained with H&E, and examined at 100× magnification.*
Control A: No supplementation; Group B: 0.5 g/kg extract; For condition C, the dosage was 1.0 g/kg, and the

The dietary supplementation of Moringa-derived bioactive extract significantly (P<0.05) improved the humoral immune response and lymphoid organ weights (Table 2). Birds receiving extract at the rate of

Table 2. Effects of plant-derived bioactive supplementation on immune response of broiler chickens

Treatment	ND antibody titer (log_2)	Bursa (% BW)	Spleen (% BW)
Control	4.8 ± 0.4 ^{c}	0.19 ± 0.02 ^{c}	0.11 ± 0.01 ^{c}
0.5 g/kg	5.6 ± 0.5 ^{b}	0.22 ± 0.02 ^{b}	0.13 ± 0.01 ^{b}
1.0 g/kg	6.4 ± 0.6 ^{a}	0.25 ± 0.03 ^{a}	0.15 ± 0.02 ^{a}
2.0 g/kg	6.6 ± 0.6 ^{a}	0.26 ± 0.03 ^{a}	0.16 ± 0.02 ^{a}

Data are reported as the mean ± SD. Different superscripts within the column indicate that values differ statistically (P<0.05)
ND: Newcastle disease; BW: Body weight

1.0 or 2.0 g/kg feed exhibited significantly higher Newcastle disease virus (NDV) antibody titers, as well as higher weights of bursa and spleen compared to control group and group receiving extract at the rate of 1.0 g/kg feed. Enlargement of primary and secondary lymphoid organs is generally associated with enhanced immune competence and lymphocyte proliferation. Significant differences were observed as well between control group and group receiving extract at the rate of 1.0 g/kg feed. These results are corroborate previous studies demonstrating that phytogenic compounds improve humoral immunity through modulation of cytokine signaling and stimulation of lymphocyte proliferation (Ravi et al. 2025; Alhujaili et al. 2025). Polyphenols and flavonoids are especially effective at macrophage activation and antibody production stimulation which subsequently results in increased broiler chicken resistance against diseases (Biswas et al. 2024; Abd El-Ghany 2020).

Pearson correlation analysis revealed a significant positive correlation between jejunal villus height and the NDV antibody titers (r = 0.72, P< 0.01) (Fig. 2). Regression analysis revealed a linear increase in villus height with increased humoral immune response (R^2 = 0.83). This relationship emphasizes and supports the concept of the gut-immune axis, whereby a healthy gut facilitates efficient antigen sampling, nutrient absorption, and immune signaling (Zhao et al. 2024; Al-Garadi et al. 2025).

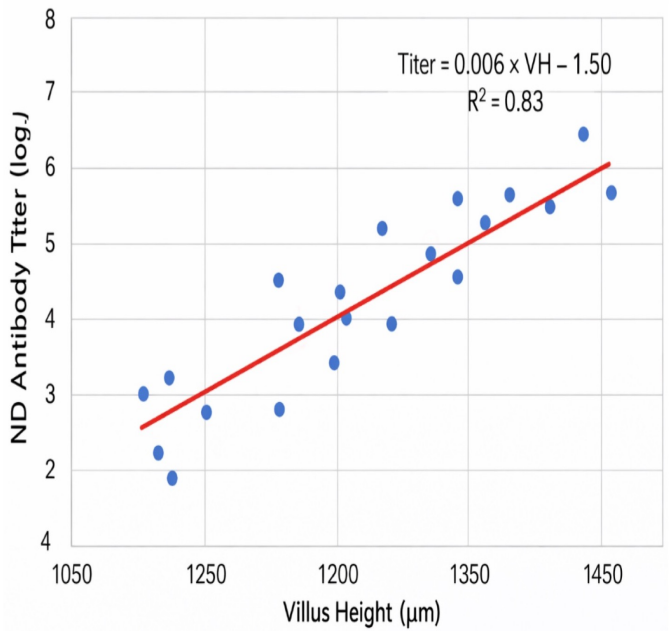


Fig. 2. *Association between intestinal villus height and Newcastle disease antibody titers in broiler chickens*

Such findings confirm that the dietary bioactive extracts are not merely growth enhancers, but essentially modulators of intestinal-immune homeostasis, a concept that modern poultry biology research is increasingly highlighting (Fagnon et al. 2020; Singh and Gaikwad 2020). The observed positive association between villus height and humoral immune response further supports the functional integration of intestinal structural integrity and systemic immunity. Improved villus architecture enhances nutrient absorption efficiency, including amino acids and micronutrients essential for immunoglobulin synthesis and lymphocyte proliferation. This mechanistic linkage strengthens the biological plausibility of phytogenic supplementation as a dual modulator of digestive and immune functions. However, the present study was limited to morphological and humoral immune parameters without integrating growth performance indices such as feed conversion ratio or carcass yield, which may further substantiate production relevance. Moreover, although the extract was standardized for total phenolic and flavonoid content, detailed phytochemical profiling using advanced analytical techniques (e.g. HPLC or LC–MS) was not performed. Consequently, the specific bioactive compounds responsible for the observed biological responses remain to be elucidated. Future investigations integrating metabolomic profiling and mechanistic immune assays are warranted.

4. Conclusions

Dietary supplementation of broiler chickens with plant-derived bioactive extract significantly improved both intestinal structural integrity and immune responsiveness. Improved villus height, reduced crypt depth, and higher villus to crypt ratio indicated improved gut health and absorptive capacity. Elevated NDV antibody titers and higher lymphoid organ weights suggest an improved immune system of birds. Furthermore, a close association was observed between gut health and humoral immunity in birds. Overall, the results suggest that plant-derived bioactive extracts are a promising antibiotic-free nutritional strategy for maintaining gut health and immunity of broiler chicken. However, further studies including biochemical characterization of the extracts and production performance parameters are required.

Declarations

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Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study

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Ethics approval: All experimental procedures involving animals were reviewed and approved by the Animal Research Ethics Committee of Sumbawa University of Technology (Approval No. 012/KEPH-UTS/III/2025) and were conducted in accordance with internationally accepted guidelines for the care and use of animals in research

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