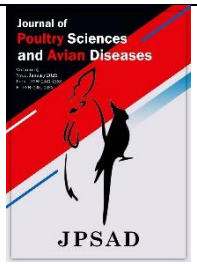
Journal of Poultry Sciences and Avian Diseases

Journal homepage: www.jpsad.com



Effects of Dietary BioHerbal on Growth Performance, Newcastle Disease Antibody Response, and Growth-related Factor in Broiler Chickens

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A r t i c l e I n f o

Article type:
Original Research

How to cite this article:
Khosravi, A. R., Hosseini, A., Hemmati, N., & Karimi, P. (2026). Effects of Dietary BioHerbal on Growth Performance, Newcastle Disease Antibody Response, and Growth-related Factor in Broiler Chickens. *Journal of Poultry Sciences and Avian Diseases, 4*(3), 1-10.
http://dx.doi.org/10.61838/kman.jpsad.187

A B S T R A C T

The withdrawal of antibiotic growth promoters has intensified the need for effective phytogenic alternatives in broiler nutrition. This study evaluated the effects of dietary BioHerbal (2 g/kg feed) on growth performance, humoral immune response, hematological indices, serum biochemical parameters, and circulating growth factors in Ross 308 broiler chickens over a 42-day period. 240 one-day-old male chicks were allocated to two dietary treatments with three replicate pens per treatment. BioHerbal supplementation significantly increased body weight gain and improved feed conversion ratio during both starter and overall growth phases ($p<0.05$). While primary antibody response to Newcastle disease vaccination was unaffected, secondary antibody titers at day 42 were significantly enhanced ($p<0.05$). Supplemented birds exhibited reduced serum glucose, triglycerides, and uric acid concentrations, along with increased globulin and calcium levels. Circulating IGF-1, TGF-β, VEGF, and EGF were significantly elevated ($p<0.05$), whereas growth hormone remained unchanged. These findings indicate that BioHerbal supplementation improves growth efficiency and is associated with increased secondary NDV antibody titers and changes in circulating growth-related factors.

Keywords: *phytogenic feed additive; broiler chickens; immunomodulation; growth factors; BioHerbal.*

Article history:
Received 4 June 2025
Revised 13 July 2025
Accepted 29 July 2025
Published online 01 July 2026

1 Introduction

The global restriction of antibiotic growth promoters (AGPs) in poultry production has accelerated the search for biologically effective and economically sustainable alternatives that maintain productivity without compromising food safety (Bölükbaşi & Erhan, 2007; Dalkiliç & Güler, 2009; Soltan et al., 2008). Among the several alternatives that have been advocated, phytogenic additives have emerged as one of the more promising alternatives because of their wide range of bioactive constituents, including phenolic terpenoids, organosulfur, and flavonoids, which display antimicrobial, antioxidant, and immunomodulatory activity (Lee et al., 2003).

Growth regulation of broiler chickens is regulated by the somatotropic axis, which involves growth hormone (GH) and insulin-like growth factor-1 (IGF-1), which coordinate muscle accretion, protein synthesis, and metabolic efficiency (Breier, 1999; Buyse & Decuypere, 1999; Zhao et al., 2004). In addition, local growth mediators such as epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) play essential roles in epithelial renewal, intestinal development, and tissue vascularization, thereby influencing nutrient utilization and growth potential (Dealy et al., 1998; Kim et al., 2017). Transforming growth factor-β (TGF-β) also regulates the interface between tissue remodeling and immune homeostasis by modulating inflammatory responses and immune tolerance (Lu et al., 2013; Xing et al., 2021). Nutritional regulation of these endocrine and paracrine systems could provide a mechanism by which dietary interventions can enhance growth performance and immune competence.

BioHerbal is a commercial, multi-component phytogenic formulation containing garlic-derived organosulfur compounds (alliin, ajoene, vinyldithiins, and allyl sulfides), thyme phenolic constituents (thymol and carvacrol), and flavonoid-rich botanical extracts including *Melissa officinalis* (Awlqadr et al., 2025; Franco et al., 2018; Iciek et al., 2009; Salehi et al., 2018; Soleimani et al., 2022; Teymouri et al., 2021). Organosulfur metabolites have been associated with modulation of lipid metabolism and inflammatory signaling, while thymol and carvacrol exert antimicrobial and anti-inflammatory effects that may support intestinal integrity (Blachier et al., 2020; Imran et al., 2022; Mączka et al., 2023; Miękus et al., 2020). Flavonoids contribute to antioxidant capacity by attenuating reactive oxygen species and reinforcing endogenous defense systems (Hemmati et al., 2025). The integration of these bioactive compounds into a single formulation provides a biologically plausible basis for the simultaneous regulation of metabolic and physiological processes related to growth and health (Zalfa et al., 2026).

Despite the expanding use of complex phytogenic blends in poultry nutrition, comprehensive in vivo studies integrating productive performance, vaccine-induced humoral immunity, hematological indices, serum biochemical profiles, and circulating growth factor concentrations remain limited. We hypothesized that dietary BioHerbal supplementation would enhance growth performance and immune responsiveness in broiler chickens, potentially by modulating systemic growth factor profiles. Therefore, the present study evaluated the effects of BioHerbal on growth performance, Newcastle disease antibody response, selected hematological and biochemical parameters, and circulating levels of IGF-1, GH, TGF-β, VEGF, and EGF in Ross 308 broilers under controlled feeding conditions.

2 Material and Methods

2.1 Experimental Design

The experiment was conducted at the Poultry Research Facility, Faculty of Veterinary Medicine, over a 42-day feeding period. A completely randomized design (CRD) with random allocation was applied. A total of 240 one-day-old male Ross 308 broiler chicks were randomly assigned to two dietary treatments, with three replicate pens per treatment and 20 birds per pen. The dietary treatments consisted of: (1) a basal diet without supplementation (control), and (2) the basal diet supplemented with BioHerbal at a rate of 2 g per kg of feed (2 kg/ton). Birds were housed in floor pens measuring 165×230 cm, bedded with 5 cm of fresh wood shavings. Feed and water were provided ad libitum throughout the experimental period. Diets were formulated according to NRC (1994) recommendations and supplied in mash form. The ingredient composition and calculated nutrient profile of the basal diet are presented in Table 1. Environmental conditions were maintained according to standard commercial management guidelines for Ross 308 broilers. Ambient temperature was set at 33°C during the first week and gradually reduced by approximately 3°C per week until reaching 21°C in week five, where it was maintained for the remainder of the study. A continuous 24-hour lighting schedule was applied throughout the trial. Growth performance parameters were recorded on days 21 and 42. For growth performance

variables (body weight, body weight gain, feed intake, and feed conversion ratio), the pen was considered the experimental unit. For blood-related measurements, including white blood cell (WBC) counts and serum analyses, individual birds sampled within each pen were considered observational units, with pen structure accounted for in the statistical analysis. Notably, BioHerbal (Pras Imen Daru, Iran) is a commercial phytogenic feed additive containing garlic-derived organosulfur compounds, thymol, carvacrol, and flavonoid-rich plant extracts. However, the detailed formulation is proprietary and not publicly disclosed.

Table 1. Effect of dietary BioHerbal supplementation on growth performance of broiler chickens.

Parameter	Age (days)	Control	BioHerbal (2 g/kg)	*p*-value
Body weight gain (g/bird)	1–21	495.2 ±12.4	562.8 ±14.1	<0.001
	1–42	2135.4 ±38.6	2347.9 ±42.3	<0.001
Feed conversion ratio (g/g)	1–21	1.46 ±0.04	1.38 ±0.03	0.03
	1–42	1.70 ±0.05	1.60 ±0.04	0.04

2.2 Growth Performance Measurements

Individual body weight (BW) of birds was recorded at placement (day 1) and subsequently on days 21 and 42 using a calibrated digital scale. Body weight gain (BWG) was calculated for the starter period (day 1–21), finisher period (day 21–42), and the overall experimental period (day 1–42) by subtracting the initial body weight from the final body weight within each respective interval. Feed intake (FI) was determined on a pen basis by recording the amount of feed offered and subtracting feed refusals at the end of each measurement period (days 21 and 42). Mortality was recorded daily, and feed intake was adjusted for mortality when calculating performance indices. Feed conversion ratio (FCR) was calculated for each pen as the ratio of feed intake to body weight gain (FI/BWG) for each period. Performance data were expressed on a pen basis, and the pen was considered the experimental unit for statistical analysis.

2.3 Blood Sampling and Hematological Analysis

At 42 days of age, three birds per pen (n=9 per treatment) were randomly selected for blood sampling. Birds were manually restrained to minimize handling stress, and approximately 4 mL of blood was collected from the jugular vein using sterile disposable 5-mL syringes fitted with 23-gauge needles. Blood was immediately divided into two aliquots: one transferred to plain Vacutainer tubes (without anticoagulant) for serum biochemical analysis, and the other to K2-EDTA tubes for hematological evaluation. Blood collected in plain tubes was allowed to clot at room temperature (22–25°C) for 30 minutes and then centrifuged at 3,000×g for 10 minutes. Serum was carefully separated and stored at −20°C until analysis. All samples were analyzed within 30 days of collection, and repeated freeze–thaw cycles were avoided. Serum concentrations of total protein, albumin, alanine aminotransferase (ALT), alkaline phosphatase (ALP), total cholesterol, triglycerides, uric acid, urea, creatinine, glucose, and calcium were determined using commercially available enzymatic colorimetric kits according to the manufacturer's instructions. Analyses were performed on an automated biochemical analyzer (Mindray BS-380, Mindray, China). Globulin concentration was calculated as the difference between total protein and albumin. All measurements were performed in duplicate, and internal quality control procedures were conducted prior to sample analysis to ensure assay reliability. For hematological assessment, EDTA-treated blood samples were gently mixed and used for total white blood cell (WBC) counting and differential leukocyte analysis. Total WBC counts were manually determined using a Neubauer hemocytometer following 1:20 dilution with Turk's solution and were expressed as cells×10^3/µL. Differential leukocyte counts were performed according to the method described by Schalm (1965). Thin blood smears were immediately prepared after sampling, air-dried, and stained using the May–Grünwald–Giemsa technique. Briefly, May–Grünwald stain diluted 1:1 with distilled water was applied for 1 minute, followed by Giemsa staining for 20 minutes (Piaton et al., 2015). Slides were gently rinsed, air-dried, and examined under oil immersion at 1000×magnification. At least 100 leukocytes were counted per smear to determine the relative percentages of heterophils, lymphocytes, monocytes, eosinophils, and basophils. Absolute leukocyte counts were calculated based on total WBC values. For hematological and biochemical variables, individual birds were considered observational units, with pen structure accounted for in the statistical model.

2.4 *Evaluation of Humoral Immune Response*

Broiler chickens were vaccinated against Newcastle disease (ND) on day 1 of age and revaccinated on day 28 via the ocular route using a live attenuated LaSota strain vaccine (Nobilis® ND Clone 30, Intervet, Boxmeer, The Netherlands), according to the manufacturer's recommendations. To evaluate humoral immune response, blood samples were collected from three randomly selected birds per pen (n=9 per treatment) on days 14 and 42 of age. The sampling at day 14 represented the primary immune response following the initial vaccination, whereas sampling at day 42 corresponded to the secondary (booster) response after revaccination. Serum was separated as previously described and stored at −20°C until analysis. Newcastle disease virus (NDV)-specific antibody titers were determined using a commercial indirect ELISA kit validated for *Gallus gallus* serum (IDEXX NDV Ab Test, IDEXX Laboratories, Westbrook, ME, USA) following the manufacturer's instructions. Optical density was measured at 650 nm using a microplate reader, and antibody titers were calculated from the sample-to-positive (S/P) ratio using the manufacturer's recommended formula. Results were expressed as log10 antibody titers. All samples were analyzed in duplicate. Individual birds were considered observational units, with pen included in the statistical model to account for clustering effects (Adair et al., 1989; Bell et al., 1991).

2.5 *Growth Factor Quantification*

Serum concentrations of insulin-like growth factor-1 (IGF-1), growth hormone (GH), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF) were quantified using commercially available chicken-specific sandwich ELISA kits validated for *Gallus gallus* serum samples (Cloud-Clone Corp., Wuhan, China). All assays were performed strictly according to the manufacturer's instructions. Serum samples were thawed once, equilibrated to room temperature, and gently mixed prior to analysis. Standards and serum samples were assayed in duplicate in 96-well microplates pre-coated with analyte-specific monoclonal antibodies (sandwich ELISA format). After incubation and washing, enzyme-conjugated detection antibodies were applied, and color development was achieved with the supplied chromogenic substrate. Optical density was measured at 450 nm using a microplate reader (Stat Fax 2100, Awareness Technology, USA). Standard curves were constructed using kit-provided calibrators, and sample concentrations were calculated using four-parameter logistic (4-PL) regression. Results were expressed as ng/mL for IGF-1 and GH and as pg/mL for TGF-β, VEGF, and EGF, according to the kit specifications. The analytical sensitivities and detection ranges for each assay were within the manufacturer's reported limits. According to manufacturer specifications, intra-assay coefficients of variation were <8% and inter-assay coefficients of variation were <10%. Samples with absorbance values outside the linear range of the standard curve were appropriately diluted and reassayed. For growth factor analyses, individual birds were considered observational units, with pen included in the statistical model to account for clustering effects (Danielpour, 1993; Inagaki et al., 1990; Shah & Maghsoudlou, 2016).

2.6 *Statistical Analysis*

All statistical analyses were performed using SPSS software (Version 27, IBM Corp., Armonk, NY, USA). The experiment was conducted as a completely randomized design (CRD) with two dietary treatments and three replicate pens per treatment. For growth performance variables (body weight, body weight gain, feed intake, and feed conversion ratio), the pen was considered the experimental unit for performance data to avoid pseudo-replication, and data were analyzed using one-way analysis of variance (ANOVA) with dietary treatment as the fixed effect. For humoral immune response, hematological parameters, serum biochemical indices, and circulating growth factors, individual birds were considered observational units, with pen included in the statistical model to account for clustering (birds nested within pen). Normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. When significant treatment effects were detected, means were compared using Tukey's post hoc test. Results are presented as Mean±SEM, and differences were considered statistically significant at $p \leq 0.05$.

3 Results

Growth performance outcomes are summarized in Table 1. Dietary supplementation with BioHerbal markedly influenced body weight gain (BWG) throughout the experimental period. During the starter phase (day 1–21), birds receiving BioHerbal exhibited greater BWG compared with the control group (562.8±14.1 vs. 495.2±12.4 g/bird)($p<0.001$). During the overall period (day 1–42), supplemented birds demonstrated higher cumulative BWG

relative to controls (2347.9±42.3 vs. 2135.4±38.6 g/bird)(p<0.001). Feed conversion ratio (FCR) was significantly improved in the BioHerbal group during both evaluation intervals. At day 21, FCR was lower in supplemented birds compared with controls (1.38±0.03 vs. 1.46±0.04)(p=0.03). A similar improvement persisted through day 42 (1.60±0.04 vs. 1.70±0.05)(p=0.04) (Figure 1).

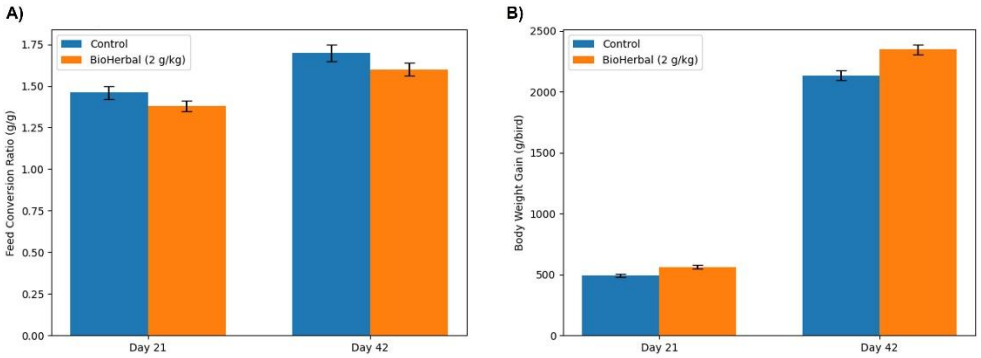

Figure 1. Effect of dietary BioHerbal supplementation (2 g/kg) on growth performance of broiler chickens at days 21 and 42. (A) Feed conversion ratio (FCR). (B) Body weight gain (BWG, g/bird). Values are presented as Mean±SEM (n=3 pens per treatment). The pen was considered the experimental unit for performance analysis. BioHerbal supplementation significantly improved BWG at both day 21 and day 42 (p<0.001) and reduced FCR at both evaluation points (p<0.05) compared with the control group.

NDV-specific antibody titers are presented in Table 2. At day 14, corresponding to the primary immune response following initial vaccination, no significant difference was detected between the BioHerbal and control groups (*p*=0.38). Antibody titers were numerically higher in the supplemented group; however, the magnitude of variation did not reach statistical significance. At day 42, representing the secondary immune response after booster vaccination, birds receiving BioHerbal supplementation exhibited significantly greater NDV-specific antibody titers compared with the control group (3.72±0.27 vs. 3.08±0.24 $\log_{10}$ units)(p=0.02). Antibody titers were analyzed at the individual-bird level, with pen included in the statistical model to account for clustering.

Table 2. Effect of dietary BioHerbal supplementation on Newcastle disease virus (NDV) antibody titers in broiler chickens.

Sampling day	Control	BioHerbal (2 g/kg)	*p*-value
Day 14	1.42 ±0.18	1.58 ±0.21	0.38
Day 42	3.08 ±0.24	3.72 ±0.27	0.02

Serum biochemical profiles at day 42 are presented in Table 3. Dietary BioHerbal supplementation induced distinct metabolic alterations compared with the control group. Markers related to nitrogen metabolism were significantly affected. Serum uric acid concentrations were markedly reduced in the supplemented group (7.48±0.36 vs. 9.02±0.41 mg/dL)(p<0.001). Urea levels were also modestly but significantly lower (13.68±0.55 vs. 14.82±0.63 mg/dL)(p=0.01), while creatinine showed a slight reduction (0.36±0.02 vs. 0.39±0.02 mg/dL)(p=0.04). Energy-related metabolites were similarly influenced. Serum glucose concentration was substantially decreased in the BioHerbal group (101.2±4.1 vs. 135.6±4.8 mg/dL)(p<0.001). Triglyceride levels were also significantly lower (80.6±3.7 vs. 100.4±4.9 mg/dL)(p<0.001). In contrast, total cholesterol was moderately but significantly higher in supplemented birds (76.4±3.6 vs. 68.0±3.2 mg/dL)(p=0.03). Regarding protein fractions, total protein did not significantly differ between groups (*p*=0.07), nor did albumin (*p*=0.42). However, globulin concentration was significantly elevated in the BioHerbal group (2.08±0.12 vs. 1.38±0.09 g/dL)(p=0.006). Liver-associated enzymes showed selective modulation. ALT activity was significantly reduced in supplemented birds (51.4±2.2 vs. 58.2±2.7 IU/L)(p=0.02), whereas ALP activity did not differ between treatments (*p*=0.11). Serum calcium concentration was significantly increased in the BioHerbal group (9.46±0.31 vs. 8.52±0.28 mg/dL)(p=0.02).

Table 3. Effect of dietary BioHerbal supplementation on serum biochemical parameters of broiler chickens at 42 days of age.

Parameter	Control	BioHerbal (2 g/kg)	*p*-value
Creatinine (mg/dL)	0.39 ±0.02	0.36 ±0.02	0.04
Uric acid (mg/dL)	9.02 ±0.41	7.48 ±0.36	<0.001
Urea (mg/dL)	14.82 ±0.63	13.68 ±0.55	0.01
Glucose (mg/dL)	135.6 ±4.8	101.2 ±4.1	<0.001
Total protein (g/dL)	3.12 ±0.14	3.86 ±0.18	0.07
Albumin (g/dL)	1.74 ±0.06	1.78 ±0.07	0.42
Globulin (g/dL)	1.38 ±0.09	2.08 ±0.12	0.006
Cholesterol (mg/dL)	68.0 ±3.2	76.4 ±3.6	0.03
Triglycerides (mg/dL)	100.4 ±4.9	80.6 ±3.7	<0.001
ALT (IU/L)	58.2 ±2.7	51.4 ±2.2	0.02
ALP (IU/L)	36.2 ±1.9	32.4 ±1.8	0.11
Calcium (mg/dL)	8.52 ±0.28	9.46 ±0.31	0.02

Hematological parameters measured at day 42 are summarized in Table 4. Total leukocyte counts were not significantly affected by dietary treatment (19.65±0.82 vs. 21.10±0.91 $\times 10^3/\mu L$ for control and BioHerbal groups, respectively)(p=0.18). Packed cell volume (PCV) similarly showed no significant difference between treatments (p=0.31). Although total WBC counts remained unchanged, leukocyte distribution was significantly modulated. The proportion of lymphocytes was markedly higher in birds receiving BioHerbal (72.9±1.8%) compared with controls (67.7±1.6%)(p=0.002). In contrast, eosinophil percentage was significantly reduced in the supplemented group (3.3±0.3% vs. 4.1±0.4%)(p=0.04). No statistically significant differences were observed for heterophil (p=0.27) or monocyte (p=0.41) proportions.

Table 4. Effect of dietary BioHerbal supplementation on hematological parameters of broiler chickens at 42 days of age.

Parameter	Control	BioHerbal (2 g/kg)	*p*-value
WBC ($\times 10^3/\mu L$)	19.65 ±0.82	21.10 ±0.91	0.18
PCV (%)	33.2 ±1.1	32.1 ±1.0	0.31
Heterophils (%)	23.8 ±1.2	22.7 ±1.0	0.27
Lymphocytes (%)	67.7 ±1.6	72.9 ±1.8	0.002
Monocytes (%)	3.2 ±0.3	3.4 ±0.4	0.41
Eosinophils (%)	4.1 ±0.4	3.3 ±0.3	0.04

Serum growth factor concentrations at day 42 are presented in Table 5. Dietary BioHerbal supplementation resulted in a significant increase in circulating IGF-1 levels compared with the control group (89.7±3.1 vs. 78.4±2.6 ng/mL)(p=0.004). Growth hormone (GH) concentrations showed a modest numerical increase in the supplemented group (5.21±0.34 vs. 4.82±0.29 ng/mL); however, this difference did not reach statistical significance (p=0.118). Serum TGF-β concentrations were significantly higher in BioHerbal-fed birds than in controls (349.6±13.2 vs. 312.5±11.8 pg/mL)(p=0.021). Similarly, VEGF concentrations were significantly elevated in the supplemented group (167.8±7.2 vs. 145.3±6.4 pg/mL)(p=0.017). EGF levels were also significantly higher in BioHerbal-treated birds (114.5±5.3 vs. 96.7±4.9 pg/mL)(p=0.008). Data for circulating growth factors are shown in Figure 2.

Table 5. Effect of dietary BioHerbal supplementation on circulating growth factors in broiler chickens at day 42

Parameter	Control	BioHerbal	*p*-value
IGF-1 (ng/mL)	78.4±2.6	89.7±3.1	0.004
GH (ng/mL)	4.82±0.29	5.21±0.34	0.118
TGF-β (pg/mL)	312.5±11.8	349.6±13.2	0.021
VEGF (pg/mL)	145.3±6.4	167.8±7.2	0.017
EGF (pg/mL)	96.7±4.9	114.5±5.3	0.008

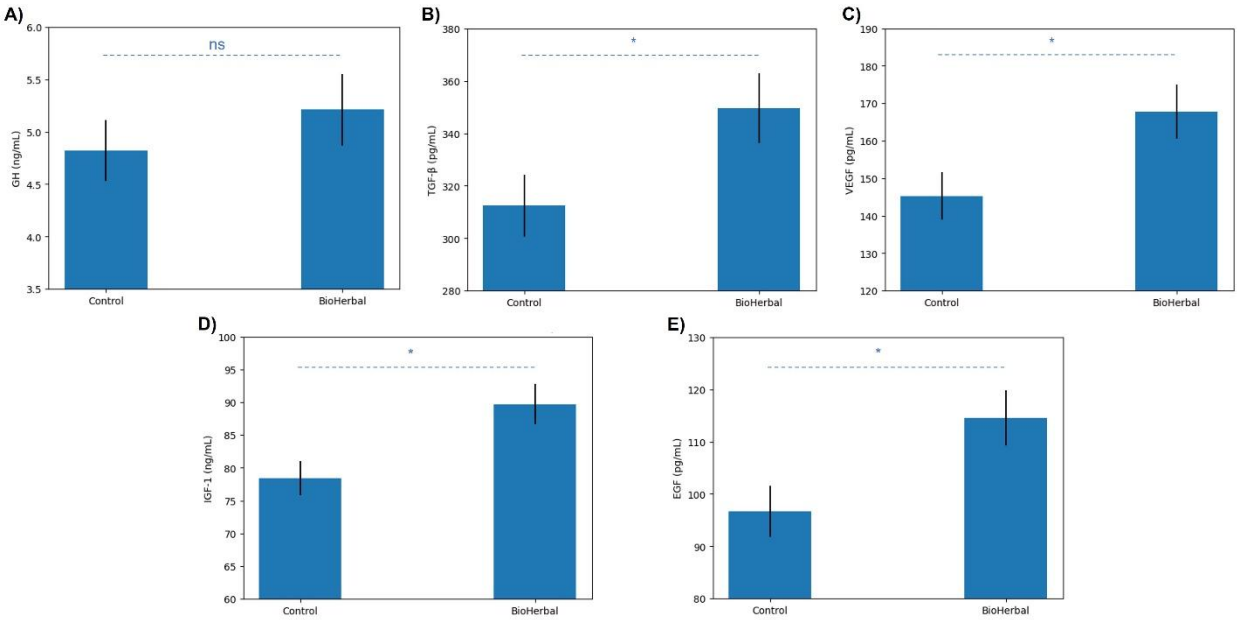

Figure 2. Effect of dietary BioHerbal supplementation on circulating growth factors in broiler chickens at 42 days of age. Serum concentrations of (A) growth hormone (GH), (B) transforming growth factor-β (TGF-β), (C) vascular endothelial growth factor (VEGF), (D) insulin-like growth factor-1 (IGF-1), and (E) epidermal growth factor (EGF) in control and BioHerbal-supplemented groups (2 g/kg). Bars represent Mean±SEM (n=9 birds per treatment). BioHerbal supplementation significantly increased IGF-1, TGF-β, VEGF, and EGF concentrations compared with the control group (* : p≤0.05), whereas GH showed a numerical but non-significant increase (ns : p>0.05).

4 Discussion

Throughout the entire growth period, a consistent and biologically meaningful response was elicited by the supplementation with BioHerbal at a rate of 2 g/kg. This included increased body weight gain during both the starter and total growth periods, accompanied by a reduction in feed conversion ratio and no mortality, which could impact pen-level results. The latter effect suggests that improved nutrient efficiency per unit feed intake, rather than increased feed intake, may have been influenced by the product. Moreover, the sustained improvement from day 1–21 through day 1–42 indicates that the effect was not merely transient. Possible contributing factors could include improvements in digestive efficiency, metabolism, or a reduction in subclinical inflammation, although these were not directly measured. These results, therefore, fit with the general body of literature on phytogenic compounds and antibiotic growth promoters, with essential oil blends and similar interventions showing improvements in BWG and FCR over negative control treatments (Ayalew et al., 2022; Demir et al., 2003; Demir et al., 2005; Gadde et al., 2017; Mehdi et al., 2018; Mokhtari et al., 2016; Noruzi et al., 2022; Nuningtyas et al., 2023; Teymouri et al., 2021).

The immune system results showed that NDV antibody titers were unchanged at day 14 but significantly increased at day 42, suggesting an enhancement of the secondary immune response. The corresponding increase in globulin levels, without changes in albumin or total WBC count, is consistent with possible qualitative modulation of the immune system. The increased proportion of lymphocytes, but not total WBC count, also supports a change in WBC distribution rather than a general increase in leukopoiesis. These trends have also been observed in previous studies with laying hens conducted by BioHerbal, showing increased percentages of lymphocytes and a tendency towards decreased heterophil-to-lymphocyte ratios at higher inclusion levels, although statistical significance was not always achieved.

The resolution of this study design does not lend itself well to a mechanistic interpretation, however, because a general differential WBC count does not distinguish between B-cell or T-cell expansion or a general effect related to corticosterone levels in the stress response.

The biochemical profile also offers further evidence of increased metabolic efficiency. Decreased levels of circulating glucose and triglycerides on day 42 indicate improved peripheral metabolism of energy sources or altered lipid metabolism by the liver. Decreased levels of circulating glucose and triglycerides on day 42 indicate improved peripheral metabolism of energy sources or altered hepatic lipid metabolism. It is also important to note that ALT levels decreased, while ALP levels remained unchanged, indicating no signs of liver distress at this inclusion rate.

The difference was noted between some AGP-alternative studies regarding circulating cholesterol levels, which slightly increased in the BioHerbal group, whereas Teymouri et al. reported that total cholesterol and LDL-related indices were lowered with alternatives such as medium-chain fatty acids (MCFA) (Teymouri et al., 2021). It should be noted that total cholesterol levels reflect a combination of lipoprotein fractions, and without fractionation, it is impossible to determine whether an increase in total cholesterol indicates a positive or negative effect on lipid health.

Hematological data further support selective immunomodulation. The absence of changes in PCV and TLC does not support hematopoietic stimulation. Rather, an increased proportion of lymphocytes and a decreased proportion of eosinophils support the predominance of adaptive immunity, which may also reflect reduced inflammatory responses. Similar trends have been noted in earlier BioHerbal trials on laying hens. Eosinophils, however, are relatively low in number and can vary in poultry, so it is difficult to make definitive inferences based on single-time-point data. The most compelling evidence of immunomodulation comes from the augmented secondary immune response with increased globulin (Chehrei et al., 2011).

The most distinguishing aspect of this study is its demonstration of the profile of endocrine/growth factors. IGF-1, EGF, VEGF, and TGF-β were all significantly increased in the blood of BioHerbal-supplemented birds, with GH remaining unaffected. This is physiologically plausible, as GH secretion is pulsatile and may not be reflected in single-time-point sampling, whereas IGF-1 reflects integrated effects of the GH/IGF-1 axis and hepatic sensitivity. The increase in IGF-1 supports improved growth performance, with no evidence of endocrine overstimulation.

Increased levels of VEGF and EGF support epithelial cell renewal, which could be related to enhanced nutrient assimilation and growth, although intestinal morphometry was not assessed in this research. TGF-β upregulation could be related to immune system modulation, consistent with the enhanced secondary antibody response in the absence of leukocytosis. However, the activation of anti-inflammatory pathways cannot be fully assessed without evaluating cytokines and their expression.

Compared with previous broiler research on BioHerbal, the present research is complementary rather than redundant. Teymouri et al. demonstrated that BioHerbal, in conjunction with other alternatives to AGPs, can improve broiler performance (Teymouri et al., 2021). They also provided supporting evidence from intestinal microbiota and morphometry. Although their research did not include a vaccine response, their work provided a strong foundation for this research, which also demonstrated a performance advantage for broiler chickens given BioHerbal. In a second study, Zalfa et al. also demonstrated that BioHerbal, in both liquid and microencapsulated forms, could improve body weight gain and feed conversion rate, with a corresponding improvement in gut morphology and intestinal microbiota (Zalfa et al., 2026). Although this research did not directly examine the gut, the systemic signals for growth and immune response can be seen as supportive of the enhanced intestinal functionality described in the broader BioHerbal literature.

Certain design limitations should be noted, which include the analysis of performance response with three pens per treatment, analysis of only one inclusion, and single-time point measurement of the biochemical, hematologic, and growth factor response. Additionally, no analysis of intestinal morphology, microbiota, or lipoprotein fractionation was performed. These limitations limit mechanistic evaluation of the response and make it difficult to distinguish among different explanations for the observed FCR and cholesterol responses.

In summary, BioHerbal supplementation at 2 g/kg of diet was associated with improved growth efficiency and enhanced secondary NDV antibody responses. These changes were accompanied by alterations in serum biochemical parameters and upregulation of IGF-1, EGF, VEGF, and TGF-β. While the present findings suggest that BioHerbal may influence metabolic, endocrine, and immune functions, the underlying mechanisms remain to be directly investigated.

5 Conclusion

Dietary BioHerbal supplementation at 2 g/kg feed improved growth performance and feed efficiency in broiler chickens. The secondary NDV antibody response was increased at day 42, while the primary response remained unchanged. BioHerbal supplementation was associated with changes in serum biochemical parameters and increased circulating growth-related factors.

However, the underlying mechanisms were not directly evaluated, and further studies are required to confirm these findings and clarify the biological pathways involved.

Acknowledgements

The authors sincerely appreciate the Department of Genetics and Biotechnology, Animal House, Animal Genetics and Genomics Unit, and all their technical staff who assisted in the experimental setup for this research.

Conflict of Interest

We declare that no conflict of interest.

Author Contributions

Ali Reza Khosravi: Conceptualization, Methodology, Resources, Writing – Review & Editing, Supervision, Project Administration, and Funding Acquisition. **Aida Hosseini:** Investigation, Methodology, Writing – Original Draft. **Negar Hemmati:** Methodology, Software, Validation, Formal Analysis, Data Curation, Writing – Original Draft, and Visualization. **Pegah Karimi:** Formal Analysis, Writing – Original Draft.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical Considerations

All experimental procedures were conducted in accordance with the guidelines of the Research Ethics Committee of the University of Tehran for the care and use of animals in research. The Institutional Animal Care and Use Committee of the University of Tehran approved the protocol (approval code: EUT.7508002518) (date of approval: 15 March 2024).

Funding

The authors did not receive any financial support from either government or non-governmental organizations for this research or its publication.

References

Adair, B., McNulty, M., Todd, D., Connor, T., & Burns, K. (1989). Quantitative estimation of Newcastle disease virus antibody levels in chickens and turkeys by ELISA. *Avian Pathology*, *18*(1), 175-192. [PMID: 18679847] [DOI]

Awlqadr, F. H., Altemimi, A. B., Qadir, S. A., Mohammed, O. A., Saeed, M. N., Hesarinejad, M. A., & Lakhssassi, N. (2025). Bioactive Compounds, Medicinal Benefits, and Contemporary Extraction Methods for Lemon Balm (Melissa officinalis). *Food Science & Nutrition*, *13*(9), e70864. [PMID: 40927050] [PMCID: PMC12415070] [DOI]

Ayalew, H., Zhang, H., Wang, J., Wu, S., Qiu, K., Qi, G., Tekeste, A., Wassie, T., & Chanie, D. (2022). Potential feed additives as antibiotic alternatives in broiler production. *Frontiers in Veterinary Science*, *9*, 916473. [PMID: 35782570] [PMCID: PMC9247512] [DOI]

Bell, J., El Hakim El Alaoui, M., & Jaouzi, T. (1991). An ELISA kit for antibodies against Newcastle disease virus. *World Animal Review*, *69*, 59-63.

Blachier, F., Andriamihaja, M., & Blais, A. (2020). Sulfur-containing amino acids and lipid metabolism. *The Journal of Nutrition*, *150*, 2524S-2531S. [PMID: 33000164] [DOI]

Bölükbaşi, Ş. C., & Erhan, M. K. (2007). Effect of Dietary Thyme (Thymus vulgaris) on Laying Hens Performance and Escherichia coli (E. coli) Concentration in Feces. *International Journal of Natural & Engineering Sciences*, *1*(2).

Breier, B. (1999). Regulation of protein and energy metabolism by the somatotropic axis. *Domestic Animal Endocrinology*, *17*(2-3), 209-218. [DOI]

Buyse, J., & Decuypere, E. (1999). The role of the somatotrophic axis in the metabolism of the chicken. *Domestic Animal Endocrinology*, *17*(2-3), 245-255. [DOI]

Chehrei, A., Nobakht, A., & Shahir, M. (2011). The effects of different levels of biohebal® feed supplement (contains thymus and garlic extracts) on performance, egg traits and blood biochemical and immunity parameters of laying hens. *Veterinary Research & Biological Products*, *24*(1), 58-65.

Dalkiliç, B., & Güler, T. (2009). The effects of clove extract supplementation on performance and digestibility of nutrients in broilers. *FÜ Sağ. Bil. Vet. Derg*, *23*(3), 161-166.

Danielpour, D. (1993). Improved sandwich enzyme-linked immunosorbent assays for transforming growth factor β1. *Journal of immunological methods*, *158*(1), 17-25. [PMID: 8429213] [DOI]

Dealy, C. N., Scranton, V., & Cheng, H.-C. (1998). Roles of transforming growth factor-α and epidermal growth factor in chick limb development. *Developmental biology*, *202*(1), 43-55. [PMID: 9758702] [DOI]

Demir, E., Sarica, Ş., Özcan, M., & Sui Mez, M. (2003). The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diets. *British Poultry Science*, *44*(S1), 44-45. [DOI]

Demir, E., Sarica, Ş., Özcan, M., & Suicmez, M. (2005). The use of natural feed additives as alternative to an antibiotic growth promoter in broiler diets. *European Poultry Science*, *69*(3), 110-116. [DOI]

Franco, J. M., Pugine, S. M. P., Scatoline, A. M., & de Melo, M. P. (2018). Antioxidant capacity of Melissa officinalis L. on biological systems. *Eclética Química*, *43*(3), 19-29. [DOI]

Gadde, U., Kim, W., Oh, S., & Lillehoj, H. S. (2017). Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. *Animal Health Research Reviews*, *18*(1), 26-45. [PMID: 28485263] [DOI]

Hemmati, N., Khaleghi, M., Afzadi, H. A., Hashemi, Z., & Bagherian, M. (2025). Flavonoids as Anti-Metastatic agents: targeting the Epithelial-Mesenchymal transition (EMT) in breast cancer treatment: flavonoids as Anti-Metastatic agents. *International pharmacy acta*, *8*(1), e4: 1-15.

Iciek, M., Kwiecień, I., & Włodek, L. (2009). Biological properties of garlic and garlic-derived organosulfur compounds. *Environmental and molecular mutagenesis*, *50*(3), 247-265. [PMID: 19253339] [DOI]

Imran, M., Aslam, M., Alsagaby, S. A., Saeed, F., Ahmad, I., Afzaal, M., Arshad, M. U., Abdelgawad, M. A., El-Ghorab, A. H., & Khames, A. (2022). Therapeutic application of carvacrol: A comprehensive review. *Food Science & Nutrition*, *10*(11), 3544-3561. [PMID: 36348778] [PMCID: PMC9632228] [DOI]

Inagaki, H., Katoh, M., Kurosawa-Ohsawa, K., & Tanaka, S. (1990). A new sandwich enzyme-linked immunosorbent assay (ELISA) for tranforming growth factor α (TGFα) based upon conformational modification by antibody binding. *Journal of immunological methods*, *128*(1), 27-37. [PMID: 2324504] [DOI]

Kim, E., Leung, H., Akhtar, N., Li, J., Barta, J., Wang, Y., Yang, C., & Kiarie, E. (2017). Growth performance and gastrointestinal responses of broiler chickens fed corn-soybean meal diet without or with exogenous epidermal growth factor upon challenge with Eimeria. *Poultry Science*, *96*(10), 3676-3686. [PMID: 28938785] [PMCID: PMC5850350] [DOI]

Lee, K.-W., Everts, H., Kappert, H., Frehner, M., Losa, R., & Beynen, A. (2003). Effects of dietary essential oil components on growth performance, digestive enzymes and lipid metabolism in female broiler chickens. *British Poultry Science*, *44*(3), 450-457. [PMID: 12964629] [DOI]

Lu, Y., Chen, S., & Yang, N. (2013). Expression and methylation of FGF2, TGF-β and their downstream mediators during different developmental stages of leg muscles in chicken. *PloS one*, *8*(11), e79495. [PMID: 24260234] [PMCID: PMC3832633] [DOI]

Mączka, W., Twardawska, M., Grabarczyk, M., & Wińska, K. (2023). Carvacrol—A natural phenolic compound with antimicrobial properties. *Antibiotics*, *12*(5), 824. [PMID: 37237727] [PMCID: PMC10215463] [DOI]

Mehdi, Y., Létourneau-Montminy, M.-P., Gaucher, M.-L., Chorfi, Y., Suresh, G., Rouissi, T., Brar, S. K., Côté, C., Ramirez, A. A., & Godbout, S. (2018). Use of antibiotics in broiler production: Global impacts and alternatives. *Animal Nutrition*, *4*(2), 170-178. [PMID: 30140756] [PMCID: PMC6103476] [DOI]

Miękus, N., Marszałek, K., Podlacha, M., Iqbal, A., Puchalski, C., & Świergiel, A. H. (2020). Health benefits of plant-derived sulfur compounds, glucosinolates, and organosulfur compounds. *Molecules*, *25*(17), 3804. [PMID: 32825600] [PMCID: PMC7503525] [DOI]

Mokhtari, A., Akbari, M. R., & Asadi Khoshoei, E. (2016). Effect of Dietary Garlic Powder or Fresh Ground Garlic on Performance and Immune Response of Broiler Chickens. *Research on Animal Production*, *7*(13), 31-24. [DOI]

Noruzi, S., Torki, M., & Mohammadi, H. (2022). Effects of supplementing diet with Thyme (Thymuas vulgaris L.) essential oil and/or selenium yeast on production performance and blood variables of broiler chickens. *Veterinary Medicine and Science*, *8*(3), 1137-1145. [PMID: 35077017] [PMCID: PMC9122464] [DOI]

Nuningtyas, Y. F., Natsir, M. H., Widyastuti, E. S., Sjofjan, O., Susilo, A., Widiati, A. S., & Lestari, S. P. (2023). Laying Hens Growth Performance in the Peak Production Phase Offered Bio-Herbal as a Feed Additive. *Jurnal Ilmu-Ilmu Peternakan*, *33*(1). [DOI]

Piaton, E., Fabre, M., Goubin-Versini, I., Bretz-Grenier, M.-F., Courtade-Saidi, M., Vincent, S., Belleannee, G., Thivolet, F., Boutonnat, J., & Debaque, H. (2015). Technical recommendations and best practice guidelines for May-Grünwald-Giemsa staining: literature review and insights from the quality assurance. Annales de pathologie,

Salehi, B., Mishra, A. P., Shukla, I., Sharifi-Rad, M., Contreras, M. d. M., Segura-Carretero, A., Fathi, H., Nasrabadi, N. N., Kobarfard, F., & Sharifi-Rad, J. (2018). Thymol, thyme, and other plant sources: Health and potential uses. *Phytotherapy Research*, *32*(9), 1688-1706. [PMID: 29785774] [DOI]

Shah, K., & Maghsoudlou, P. (2016). Enzyme-linked immunosorbent assay (ELISA): the basics. *British journal of hospital medicine*, *77*(7), C98-C101. [PMID: 27388394] [DOI]

Soleimani, M., Arzani, A., Arzani, V., & Roberts, T. H. (2022). Phenolic compounds and antimicrobial properties of mint and thyme. *Journal of Herbal Medicine*, *36*, 100604. [DOI]

Soltan, M., Shewita, R., & El-Katcha, M. (2008). Effect of dietary anise seeds supplementation on growth performance, immune response, carcass traits and some blood parameters of broiler chickens. *International Journal of Poultry Science*, *7*(11), 1078-1088. [DOI]

Teymouri, P., Jafari Khorshidi, K., Rezaeipour, V., & Assadi Soumeh, E. (2021). Efficacy of natural alternatives to antibiotic on the growth performance, gut microbial population, intestinal morphology, and serum biochemical metabolites of broiler chickens. *Italian Journal of Animal Science*, *20*(1), 1801-1809. [DOI]

Xing, T., Zhao, Z., Zhao, X., Xu, X., Zhang, L., & Gao, F. (2021). Enhanced transforming growth factor-beta signaling and fibrosis in the pectoralis major muscle of broiler chickens affected by wooden breast myopathy. *Poultry Science*, *100*(3), 100804. [PMID: 33516474] [PMCID: PMC7936165] [DOI]

Zalfa, N. A., Nuningtyas, Y. F., Hermanto, F. E., Permata, F. S., Sjofjan, O., & Natsir, M. H. (2026). Influence of liquid and encapsulated bioherbal additives on broiler performance and quality. *European Poultry Science*, *90*(1-2), 100011. [DOI]

Zhao, R., Muehlbauer, E., Decuypere, E., & Grossmann, R. (2004). Effect of genotype–nutrition interaction on growth and somatotropic gene expression in the chicken. *General and Comparative Endocrinology*, *136*(1), 2-11. [PMID: 14980790] [DOI]