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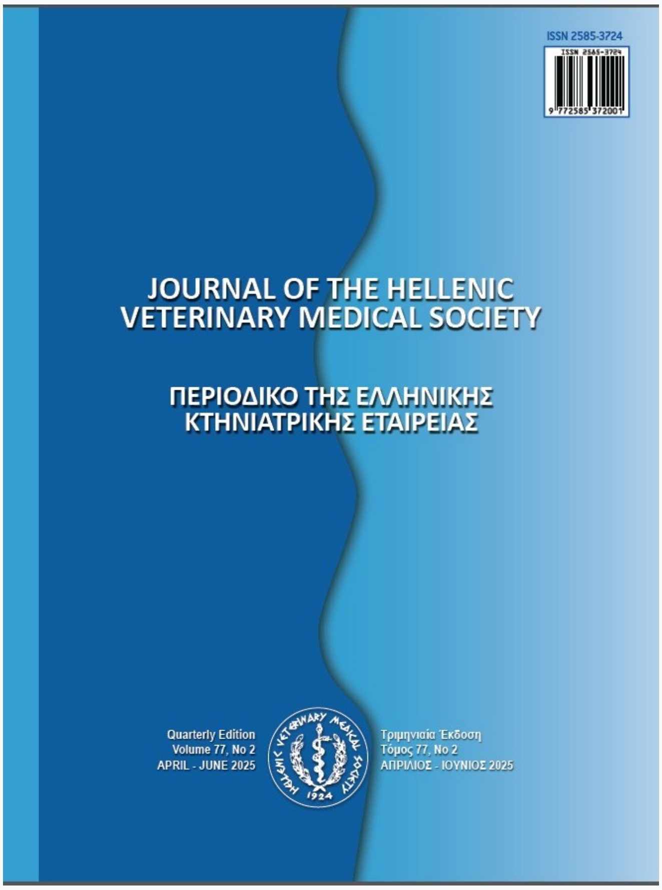


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# Effect of water-based nutritional supplement on growth performance, carcass characteristics, serum biochemistry, and immunological response of broiler chicken

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**ABSTRACT:** Broiler lines are currently rigorously selected for faster growth and greater final body weight. Nutrition has a critical role in maintaining broiler body growth. This study aimed to determine how water-based nutritional supplement affected broiler chickens' growth performance, carcass characteristics, serum biochemistry, and immunological response. A total of 200 newly hatched unsexed broiler chicks (Cobb-500) were divided into four treatment groups, replicated five times with ten birds in each, under a completely randomized design. Different concentrations of the supplement, 0% ($T_1$), 2.5% ($T_2$), 5% ($T_3$), and 7.5% ($T_4$), were added to the water. Growth performance, carcass characteristics, serum biochemical profile, and immune antibody response parameters were evaluated for a span of 35 days. The findings demonstrated that body weight gain and feed efficiency significantly improved in the birds provided nutritional supplements at 5 and 7.5% than in the 2.5% and control group ($T_1$). The 5% and 7.5% nutritional supplement groups displayed lower mortality rates compared to other two groups. However, feed consumption did not significantly differ between treatments. The birds fed nutritional supplement at 5 and 7.5% showed enhanced ($P<0.05$) carcass yield than other groups whereas all other metrics, such as breast yield, thigh yield, wing weight, liver weight, heart weight, gizzard weight, and abdominal fat weight, were not significantly different ($P>0.05$) between treatments. There were no differences in albumin, total protein, globulin, cholesterol, uric acid, triglycerides, or glucose levels in serum between the various treatments. In comparison to the other groups, the birds administered nutritional supplements at 5% and 7.5% had higher immune antibody titers against the Newcastle disease and infectious bronchitis vaccinations. In conclusion, adding 5% of a nutritional supplement to the water may improve broiler overall performance while being more cost-effective.

***Keyword:*** nutritional supplement; growth; carcass characteristics; blood serum profile; immune response.

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## INTRODUCTION

Poultry farming plays a crucial part in the economic growth of any nation since it may generate better quality proteins for human diet and serve as a source of income for several people (Tarhyel *et al*., 2012). In poultry farming, broiler meat requirement is rising quickly due to rising income levels, population expansion, and urbanization. Therefore, it appears that a significant portion of meat production and consumption in any country comes from broiler farming. At present, broiler lines are rigorously selected for faster growth and greater final body weight (Petracci & Cavani, 2011). Maintaining broiler body growth depends heavily on nutrition (Wang *et al*., 2017). Antibiotics have been regularly used on poultry farms for many years for both therapeutic and growth-promoting goals to enhance chicken development and preserve the ecological balance in the stomach (Karavolias *et al.,* 2018). This method has several disadvantages, including the emergence of antibiotic resistance and the presence of residues in chicken products (Lutful Kabir, 2009). Antibiotic alternatives must be found for sustainable chicken farming (Upadhayay & Vishwa, 2014; Alagawany *et al*., 2018; El-Hack *et al*., 2020). Nutritional supplements or feed additives have recently received more attention in poultry research because of the physiological and nutritional advantages of feed ingredients as well as the drawbacks of chemical medications (Elnesr *et al*., 2019, 2020; Yilmaz & Gul, 2023).

The streamlined form of dietary nutritional supplements, when compared to traditional forms, can encourage better digestion, absorption, utilization, metabolism, and advantageous effects on health (Rinttilä & Apajalahti, 2013; Sugiharto, 2016; Yadav & Jha, 2019). Nutritional supplements, which are commonly present in poultry meals (Moghaddam & Emadi, 2014; Horváth & Babinszky, 2018), can include amino acids, vitamins, and minerals (Ahmad *et al.* 2019, Ghoreyshi *et al.* 2019, Khatun *et al.* 2019) and combinations of these. For appropriate general health and physiological processes including development, growth, maintenance, and reproduction, vitamins are vital nutritional supplement. Vitamins have catalytic effects that make it easier for nutrients to be made, which regulate metabolism and influence poultry performance and health (Whitehead., 2002). Minerals are crucial nutritional supplements needed for maximum health and physiological wellness. Post-hatch nutrients can have a long-lasting impact on tissue mineralization, broiler performance, and overall health. Therefore, if antibiotics are fully excluded from diets, the usage of supplement like minerals is even more crucial (Nollet *et al*., 2007). The addition of supplement in feeding enhances bird growth performance (Kidd & Kerr, 1996).

Commercially available product HANSOLVET, which includes fat, crude fiber, proteins, several enzymes (protease, amylase, lipase, and pectinase), amino acids (lysine, methionine and threonine), minerals (calcium gluconate, and phosphorus), as well as vitamins A, D3, E, K, C, and B12, is recommended to be utilized in poultry ration to enhance performance. There is limited information available on the use of this commercial product in broiler diets. The purpose of this study was to compare the effects of various levels of a water-based nutritional supplement on the growth performance, carcass characteristics, serum biochemistry, and immune response of broiler chickens.

## MATERIALS AND METHODS

### Experimental Birds and Husbandry

The study was carried out at Allah Waris Protein Farm in Lodhran, Punjab, Pakistan. A total of 200 newly hatched unsexed broiler chicks (Cobb-500) were divided into four treatment groups, replicated five times with ten birds in each, under a completely randomized design (**CRD**). Commercially obtainable product HANSOLVET (Al-Asar Enterprises), which includes fat, crude fiber, proteins, several enzymes (protease, amylase, lipase, and pectinase), amino acids (lysine, methionine and threonine), minerals (calcium gluconate, and phosphorus), as well as vitamins A, $D_3$, E, K, C, and $B_{12}$, was used as supplement in this study. Different concentrations of the supplement, 0% ($T_1$), 2.5% ($T_2$), 5% ($T_3$) and 7.5% ($T_4$), were added to the water. Treatment that does not include any supplementation was taken as the control ($T_1$). In the hatchery, the chicks got vaccinations against infectious bronchitis (**IB**) and Newcastle disease (**ND**). The chicks were placed in 20 floor pens, using a deep litter system with rice husk as bedding material. Birds were offered an *iso-caloric* and *iso-nitrogenous* diet. In order to suit the nutritional needs of broilers at each stage of development—starter (1 to 11 days), grower (12 to 21 days), and finisher (22 to 35 days)—the diet was carefully balanced (NRC, 1994). In each pen (1×1 $m^2$), there were two round feeders and a nipple drinker for *ad libitum* consumption of feed and fresh water. The brooding temperature and relative humidity (**RH**) were maintained at 34 ± 1.1 °C and 62 ± 3%, respectively, throughout

**Table 1.** Ingredient composition of basal diet for different development phases.

| Ingredients (%) | Development Phases | | |
|---|---|---|---|
| | Starter (1-11 days) | Grower (12- 21 days) | Finisher (22-35 days) |
| Corn | 52.00 | 54.00 | 57.00 |
| Rice tips | 4.90 | 5.20 | 5.34 |
| Canola meal | 4.05 | 4.90 | 5.00 |
| Corn gluten 60 % | 2.00 | 2.00 | 2.00 |
| Soybean meal | 30.00 | 26.00 | 22.00 |
| Canola oil | 2.00 | 3.21 | 4.20 |
| $CaCO_3$ | 1.20 | 1.00 | 0.90 |
| $DCP.2H_2O$ | 2.20 | 2.15 | 2.10 |
| Lysine-$SO_4$ | 0.60 | 0.50 | 0.40 |
| DL-Methionine | 0.15 | 0.13 | 0.12 |
| Threonine | 0.10 | 0.06 | 0.04 |
| Sodium chloride | 0.20 | 0.25 | 0.30 |
| Sodium bicarbonate | 0.10 | 0.10 | 0.10 |
| Vitamin premix | 0.20 | 0.20 | 0.20 |
| Minerals premix | 0.30 | 0.30 | 0.30 |
| Total | 100.00 | 100.00 | 100.00 |

[1]Provided per kg of diet: vitamin A, 11,000 IU; vitamin $D_3$, 2,560 IU; vitamin E, 44 IU; vitamin K, 4.2 mg; riboflavin, 8.5 mg; niacin, 48.5 mg; thiamine, 3.5 mg; d-pantothenic, 27 mg; choline, 150 mg; vitamin $B_{12}$, 33 µg.

[2]Provided per kg of diet: copper, 8 mg; zinc, 75 mg; manganese, 55 mg; iodine, 0.35 mg; selenium, 0.15 mg.

**Table 2.** Nutrient composition of basal diet (% as fed basis) for different development phases.

| Nutrients | Phases of development | | |
|---|---|---|---|
| | Starter (1-11 days) | Grower (12- 21 days) | Finisher (22-35 days) |
| Crude protein | 23.00 | 22.00 | 21.00 |
| Metabolizable energy E (kcal/kg) | 3000 | 3100 | 3200 |
| Digestible Lysine | 1.21 | 1.21 | 1.01 |
| Digestible Methionine | 0.54 | 0.50 | 0.45 |
| Digestible Methionine + Cysteine | 0.88 | 0.81 | 0.76 |
| Arginine | 1.25 | 1.16 | 1.07 |
| Threonine | 0.80 | 0.74 | 0.68 |
| Tryptophan | 0.19 | 0.18 | 0.16 |
| Calcium | 0.90 | 0.85 | 0.80 |
| Available phosphorus | 0.50 | 0.48 | 0.46 |
| Sodium | 0.16 | 0.16 | 0.16 |
| Potassium | 0.55 | 0.55 | 0.55 |
| Chloride | 0.30 | 0.30 | 0.30 |

the first week following hatching. After that, the temperature dropped gradually till it reached 24°C and 65% RH on day 21. Throughout the investigation, a lighting regimen of 23L: 1D was used.

## Data Collection

### *Growth Performance and Carcass Characteristics*

Feed supplied on a daily basis, body weight on a weekly basis, and weekly mortality were all recorded for growth performance. The recorded data were utilized to calculate weekly feed intake, weekly weight gain, and weekly mortality percentage following the method used by Khan *et al.* (2019). Three broilers from each replicate that were closest to the average weight of that replicate at the end of the experiment were chosen, held off-feed for four hours, and then slaughtered in line with Halal laws, with bleeding permitted for about three to four minutes. Data for carcass attributes (carcass yield, breast yield, thigh yield, and relative weights of gizzard, heart, wing, and abdominal fat) were collected.

### Blood Biochemistry and Immune Response

Three birds per experimental unit—15 birds per treatment—had blood samples (3 ml/sample) obtained at the time of slaughter using a 5 ml disposable syringe without anticoagulant. Following the method used by Khan *et al.* (2019), blood samples were processed, and serum was separated for the analysis of albumin, total protein, glucose, cholesterol, triglycerides, uric acid, and globulin. All of the birds received the ND and IB vaccinations one week before to sampling, and the immune response was assessed using the technique used by Rehman *et al.* (2017).

### Statistical Analysis

The data were analyzed using one-way ANOVA in compliance with the CRD using the Statistical Analysis System (SAS Institute Inc., Cary, NC, 2002-2003). The means of the various treatments were compared using the Duncan's Multiple Range (DMR) test at a 5% probability level.

## RESULTS

### Growth Performance

The findings for the growth performance parameters are shown in Table 3. There were notable differences between the birds for weight gain (**WG**), feed efficiency (**FE**), and mortality (**M**). In comparison to the 2.5% and control group, WG rose and FE was considerably higher (P<0.05) in the groups receiving nutritional supplements at 5 and 7.5%. The 5% and 7.5% nutritional supplement groups displayed lower mortality rates compared to other two groups. However, there was no significant change in feed intake (**FI**) between the regimens.

### Carcass Characteristics

Data on carcass attributes are shown in Table 4. For carcass yield (**CY**), there were significant variations among the birds. In comparison to other groups, the birds that received nutritional supplements at 5 and 7.5% displayed improved (P<0.05) CY. The differences between treatments for all other measures, including breast yield (**BR**), thigh yield (**TH**), wing weight (**W**), liver weight (**L**), gizzard weight (**G**), heart weight (**H**), and abdominal fat weight (**ABF**), were not statistically significant (P>0.05).

### Blood Biochemistry

Data on blood profiles are shown in Table 5. There were no significant variations in the levels of serum total protein (**TP**), albumin (**AB**), globulin (**GB**), glucose (**GL**), cholesterol (**CH**), triglyceride (**TR**), and uric acid (**UA**) between treatments (P>0.05). Although the birds who got the 5 and 7.5% supplements had somewhat lower blood TR readings than the other groups, this difference was not significantly different from the other groups (P>0.05).

### Immune Response

Data on immune responses are shown in Table 6. For ND and IB titers, there were significant variations among the birds. The birds who received nutrition-

**Table 3.** Effect of water-based nutritional supplement on growth performance of broiler chicken[1]

| Treatments[3] | Parameters[2] | | | |
|---|---|---|---|---|
| | CFI (g/bird) | WG (g/bird) | FE (g/g) | M (%) |
| $T_1$ | 3288.60 | 1980.00$^b$ | 0.60$^b$ | 3.20$^a$ |
| $T_2$ | 3280.80 | 1993.60$^b$ | 0.61$^b$ | 3.20$^a$ |
| $T_3$ | 3274.80 | 2138.00$^a$ | 0.65$^a$ | 1.60$^b$ |
| $T_4$ | 3271.80 | 2154.40$^a$ | 0.65$^a$ | 1.60$^b$ |
| SEM | 4.903 | 18.657 | 0.005 | 0.275 |
| P-value | 0.669 | 0.0001 | 0.0001 | 0.022 |

$^{a-b}$Treatment means within a column bearing the different letters are significantly different (P<0.05).

[1]Data are means ± SEM of 5 replicates (n=5) with ten birds per replicate.

[2]CFI: cumulative feed intake, WG: weight gain, FE: feed efficiency, M: mortality.

[3]$T_1$: 0% supplement (control), $T_2$: 2.5% supplement, $T_3$: 5% supplement, $T_4$: % supplement.

**Table 4.** Effect of water-based nutritional supplement on carcass characteristics of broiler chicken[1]

| Treatments[3] | Parameters[2] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | CY (%) | BR (%) | TH (%) | W (%) | L (%) | G (%) | H (%) | ABF (%) |
| $T_1$ | 67.02[b] | 25.75 | 20.26 | 7.96 | 2.32 | 1.80 | 0.48 | 0.89 |
| $T_2$ | 67.18[b] | 25.47 | 20.29 | 7.98 | 2.31 | 1.81 | 0.47 | 0.87 |
| $T_3$ | 69.54[a] | 24.38 | 21.35 | 8.02 | 2.30 | 1.79 | 0.45 | 0.85 |
| $T_4$ | 69.78[a] | 24.20 | 21.49 | 8.04 | 2.28 | 1.79 | 0.46 | 0.86 |
| SEM | 0.405 | 0.302 | 0.233 | 0.015 | 0.009 | 0.007 | 0.007 | 0.006 |
| P-value | 0.006 | 0.174 | 0.097 | 0.180 | 0.503 | 0.775 | 0.393 | 0.321 |

[a-b]Treatment means within a column bearing the different letters are significantly different (P<0.05).
[1]Data are means ± SEM of 5 replicates (n=5) with ten hens per replicate.
[2]CY: carcass yield, BR; breast yield, TH: thigh yield, W; wing weight, L: liver weight, G: gizzard weight, H: heart, ABF: abdominal fat.
[3]$T_1$: 0% supplement (control), $T_2$: 2.5% supplement, $T_3$: 5%g supplement, $T_4$: 7.5% supplement.

**Table 5.** Effect of water-based nutritional supplement on serum biochemistry of broiler chicken[1]

| Treatments[3] | Parameters[2] | | | | | | |
|---|---|---|---|---|---|---|---|
| | TP (g/dL) | AB (g/dL) | GB (g/dL) | GL (mg/dL) | CH (mg/dL) | TR (mg/dL) | UA (g/dL) |
| $T_1$ | 4.12 | 2.26 | 1.53 | 133.28 | 163.45 | 91.07 | 3.99 |
| $T_2$ | 4.11 | 2.28 | 1.53 | 133.86 | 162.90 | 90.61 | 3.98 |
| $T_3$ | 4.10 | 2.29 | 1.52 | 132.98 | 161.87 | 89.78 | 3.97 |
| $T_4$ | 4.09 | 2.27 | 1.53 | 132.55 | 161.47 | 89.63 | 3.96 |
| SEM | 0.007 | 0.008 | 0.006 | 0.352 | 0.342 | 0.403 | 0.010 |
| P-value | 0.375 | 0.638 | 0.823 | 0.643 | 0.142 | 0.575 | 0.678 |

[a-b]Treatment means within a column bearing the same letters are not significantly different (P>0.05).
[1]Data are means ± SEM of 5 replicates (n=5) with ten hens per replicate.
[2]TP: total protein, AB: albumin, GB: globulin, GL: glucose, CH: cholesterol, TR: triglyceride, UA: uric acid.
[3]$T_1$: 0% supplement (control), $T_2$: 2.5% supplement, $T_3$: 5% supplement, $T_4$: 7.5% supplement.

al supplements at 5 and 7.5% had higher levels of immunological antibodies against the ND and IB vaccinations than the other groups.

## DISCUSSION

### Growth Performance

The nutrients used in the poultry industry consist of both chemical and natural compounds (Abdulameer & Alwan, 2022). The financial viability of large-scale farms is directly impacted by FI, WG, FE, and M, which are key indicators of live performance. In the present study, nutritional supplement at concentrations of 5% and 7.5% led to improvements in weight gain and feed efficiency, as well as reduced mortality rates (P<0.05), when compared to the groups receiving 2.5% concentration and the control group. Importantly, these improvements were observed without any impact on the birds' feed consumption. It can be predicted that the higher levels of vitamins and minerals in the nutritional supplement provided at concentrations of 5% and 7.5% might have improved digestion, absorption, and nutrient utilization, leading to faster growth and enhanced feed efficiency. A low feed conversion rate is an indicator of efficient feed consumption in broilers, indicating that the broiler chicken benefited greatly from the feeds. Our findings indicate that the 5% supplement level is optimal and more cost-effective since both the 5% and 7.5% levels resulted in similar performance gains.

Similar findings were reported by other researchers, who found that broilers provided with a nutritional supplement in their feed (Islam *et al.,* 2004; Bolu & Balogun, 2009; Adegbeye *et al.,* 2019; Adegbeye *et al.,* 2020; Grioui *et al.,* 2021) or drinking water (Del Barrio *et al.,* 2020; Abdulameer & Alwan, 2022) showed improved growth performance. However, Ri-

**Table 6.** Effect of water-based nutritional supplement on immune response of broiler chicken[1]

| Treatments[3] | Antibody titers[2] | |
|---|---|---|
| | ND (HI titer, $log_2$) | IB (ELISA titer) |
| $T_1$ | 4.29[b] | 3527.00[b] |
| $T_2$ | 4.29[b] | 3529.75[b] |
| $T_3$ | 4.34[a] | 3540.02[b] |
| $T_4$ | 4.35[a] | 3543.12[b] |
| SEM | 0.009 | 1.682 |
| P-value | 0.007 | 0.0001 |

[a-b]Treatment means within a column bearing the different letters are significantly different (P<0.05).

[1]Data are means ± SEM of 5 replicates (n=5) with ten hens per replicate.

[2]Hens were vaccinated via drinking water using commercially available ND (La Sota) and IB (H 120) vaccines, one week before blood samples were taken.

[3]$T_1$: 0% supplement (control), $T_2$: 2.5% supplement, $T_3$: 5% supplement, $T_4$: 7.5% supplement.

pon et al. (2019) indicated that the inclusion of natural herbs did not affect broiler growth performance.

## Carcass Characteristics

Alongside the rising demand for poultry production, there is also a growing emphasis on the composition and quality of chicken carcasses (Carmen & Mountney, 1998; Leeson & Summers, 1999). According to Li *et al.* (2014), the dressing % is regarded as the primary factor in determining carcass quality. The primary edible component in meat broilers is muscle meat. For broiler producers, better breast and thigh muscle yields translate into higher economic value. There is proof that poor feed impacts the growth of the birds as well as the quality of the carcass (Lima *et al.,* 2016). However, the current data show that the carcass yield of broilers improved (P<0.05) when nutritional supplements were added to their drinking water at 5 and 7.5%. The enhanced carcass yield observed in the 5% and 7.5% groups may be attributed to the increased weight gain in these groups (Table 3), as carcass yield tends to increase with slaughter weight (Narinc *et al.,* 2015). In line with these findings, Corzo *et al.* (2003) came to the conclusion that carcass and breast meat both improved linearly as nutritional supplementation increased. Kidd *et al.* (2004) concluded that to increase final broiler weight and breast meat production, diets with a higher nutritional density were required. Anwar *et al.* (2022) found that thigh meat output was higher in diets with low levels of P supplemented with phytase relative to diets with low levels of P without phytase supplementation. The absolute and relative weight of the breast increased significantly (P<0.05) in broiler chicks fed meals with methionine levels above NRC (Ahmed & Abbas, 2011). According to the current research, the relative weight of abdominal fat was statistically unrelated to dietary intervention (P>0.05). However, Upadhaya *et al.* (2016) found that a water-soluble nutritional supplement was associated with increased levels of abdominal fat.

## Blood Biochemistry

Blood is crucial for the transport of hormones, metabolic waste, and nutrients (Khan *et al.,* 2013, 2014). Birds' blood composition provides insight on their state of health (Qiu *et al.,* 2021). In the current investigation, addition of nutritional supplement to broilers diet at any level did not affect (P>0.05) blood serum profile. In line with these findings, Abdulameer & Alwan (2022) revealed no significant effect of nutritional supplement on blood serum parameters. Similarly, Ojabo *et al.* (2013) found that adding a nutritional supplement to the feed did not result in any changes in the biochemical blood parameters. However, Islam *et al.* (2004) discovered that the group getting the highest nutrient supplements had significantly (P<0.05) greater levels of total serum protein, glucose, and albumin. According to Toghyani *et al.* (2010), 2% nanoclay minerals added weekly or every other week at day 28 of age statistically improved albumen, total protein, and globulin. But when compared to other dietary interventions, the concentration of total protein and globulin was higher at day 36 of age (P<0.05). According to Bolu & Balogun (2009), natural vitamin premix increases blood uric acid concentration while lowering glucose and total serum protein concentrations. The Deyhim *et al.* (1995) discovered that nutritional supplementation significantly enhanced the serum glucose concentration (P<0.05), but total protein and albumin levels declined.

**Immunological Response**

Promoting the rapid development and maturation of the immune system after hatching is crucial for the early health of chickens. Broilers frequently exhibit improved growth performance due to an improvement in immune response as a result of dietary manipulation. In the present study, the birds fed nutritional supplement at 5 and 7.5% showed greater immune antibody titers against ND and IB vaccines than those of the other groups. An effective immune response in birds is heavily influenced by their nutritional status (Kogut, 2009). Poor diets or diets containing toxic substances affect the immune system negatively (Faluyi *et al.,* 2015). Infectious diseases, such as infectious bronchitis, are prevalent worldwide and hold significant economic implications (Fayyaz *et al.,* 2023). In the current investigation, the addition of nutritional supplements at 5 and 7.5% levels increased antibody titers against NDV and IBV when compared to the control or 2.5% group. The rise in antibody levels against ND and IB might be attributed to an increase in neutrophil activity in the bloodstream after vaccination, which could be due to the nutritional supplement. This enhanced neutrophil activity is thought to play a vital role in developing the immune system (Guo *et al.,* 2003).

In consistent with these findings, Elham *et al.* (2010) found that broilers receiving a higher dose of nutritional supplement had significantly higher ND and IBD antibody titers than those of other treatment groups. Swain *et al.* (2000) also reported that supplementing with 150–300 mg/kg of vitamin E prior to NDV vaccination raised blood antibody titres. Khan *et al.* (2018) found that groups receiving nutritional supplements (L-threonine) had higher antibody titers for ND and IBD. According to Raza *et al.* (1997), Vitamin E supplementation at a level of 300 IU/kg boosted the blood antibody titres against the NDV vaccine. According to Toghyani *et al.*'s (2007), feeding 1,000 and 1,500 ppb of chromium picolinate to broiler chickens at 30 days of age showed greater immunological response against the Newcastle disease virus. According to Lee *et al.* (2003), feeding 400 ppb Cr picolinate to broiler chicks enhanced antibody titers against infectious bronchitis.

## CONCLUSIONS

Based on these findings, it can be concluded that adding 5% of a nutritional supplement to the drinking water may enhance overall broiler performance while being cost-effective. Future research could explore the long-term impact of water-based nutritional supplements on broiler chickens, including their influence on overall health, productivity, and economic viability. Additionally, studies could investigate how these supplements affect meat quality attributes, such as flavor, texture, and nutrient composition, in order to address consumer demands.

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## CONFLICT OF INTEREST

The authors did not disclose any conflicts of interest in relation to the publication of this study.

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