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## *In vivo* immunomodulatory effects of *Trachyspermum ammi*, *Capsicum annuum*, and *Zingiber officinale* alone and in combination in broiler chickens

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### ABSTRACT

**Background:** Phytogetic feed additives have gained considerable attention as natural immunomodulators in poultry production due to increasing restrictions on antibiotic growth promoters. Medicinal plants such as *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* possess bioactive compounds with potential immunostimulatory properties. **Objective:** The present study was designed to investigate the *in vivo* immunomodulatory effects of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* powders, administered individually and in combination, in broiler chickens. **Materials and Methods:** A total of 120-day-old Ven-Cobb broiler chicks were randomly distributed into ten dietary treatment groups, including a basal diet control and a vitamin E-selenium supplemented standard control. Experimental diets consisted of ajwain, capsicum and ginger powders incorporated at 5 and 10 g/kg feed, either alone or in combination and were fed for 35 days. Cell-mediated immunity was evaluated using the cutaneous basophil hypersensitivity (CBH) response to phytohemagglutinin-P, while humoral immunity was assessed by haemagglutination inhibition (HI) antibody titers against Newcastle disease virus. Hematological parameters and histopathological changes in immune organs were also examined. **Results:** Dietary supplementation with phytogetic powders resulted in a significant enhancement of CBH responses at both 100 and 200 µg phytohemagglutinin-P doses compared to the control group, indicating improved cell-mediated immune function. Humoral immune response was significantly elevated on days 21 and 35, particularly in birds receiving *Capsicum annuum* (5 g/kg), *Zingiber officinale* (10 g/kg) and combination treatments, with responses comparable to the vitamin E-selenium group. Hematological findings revealed increased lymphocyte counts and reduced heterophil-to-lymphocyte ratios in supplemented groups. Histopathological evaluation of the bursa of Fabricius, thymus and spleen showed pronounced lymphocytic hyperplasia, reflecting enhanced immune activation. **Conclusion:** The results of the present study demonstrate that dietary inclusion of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale*, particularly at optimal inclusion levels and in combination, effectively enhances both humoral and cell-mediated immune responses in broiler chickens. These phytogetic feed additives may serve as promising natural alternatives to conventional immunomodulatory supplements in poultry nutrition.

**Keywords:** Phytogetic feed additives, Ajwain, Capsicum, Ginger, Immunomodulation, Broiler chickens, Cell-mediated immunity, Humoral immunity.

### INTRODUCTION

The intensification of poultry production has substantially increased the incidence of infectious and stress-related disorders, necessitating effective strategies to enhance immune competence in broiler chickens. Although antibiotic growth promoters were historically used to reduce disease burden and improve productivity, their prolonged use has resulted in antimicrobial resistance, disturbance of gut microbial balance and the presence of drug residues in poultry products, posing serious public health concerns [1,2]. Consequently, the global restriction on antibiotic use has intensified the search for safe, natural alternatives capable of supporting immune function in poultry [3,4]. Phytogetic feed additives derived from medicinal plants have emerged as promising immunomodulatory agents due to their bioactive constituents, including polyphenols, flavonoids and essential oils. These compounds are known to influence both innate and adaptive immune responses by regulating cytokine production, enhancing antibody synthesis, improving leukocyte function and maintaining immune organ integrity [5,6].

Moreover, plant-derived additives can modulate gut-associated lymphoid tissue through stabilization of intestinal microflora, thereby strengthening host defense mechanisms [7]. *Trachyspermum ammi* (Ajwain) has gained attention for its potent immunomodulatory potential attributed to its essential oil constituents, primarily thymol and carvacrol. These compounds exhibit antimicrobial activity against enteric pathogens and contribute to improved gut health, which is closely linked to immune competence in broilers [8]. Ajwain supplementation has also been associated with improvements in hematological indices, antibody production and immune organ function, suggesting its role in enhancing both humoral and cellular immune responses [9]. Similarly, *Capsicum annuum* (hot red pepper) contains capsaicinoids, particularly capsaicin, which have been reported to influence immune regulation through modulation of inflammatory mediators and enhancement of nutrient bioavailability [10,11]. Capsaicin has been shown to stimulate metabolic activity and improve gut function, indirectly supporting immune responses by improving nutrient absorption and reducing pathogenic load. In addition, the high vitamin C content of *Capsicum annuum* contributes to stress mitigation and improved immune resilience in broiler chickens [12]. *Zingiber officinale* (ginger) is widely recognized for its immunostimulatory properties, largely attributed to bioactive compounds such as gingerols and shogaols. Ginger supplementation has been reported to enhance humoral immunity, including antibody response against Newcastle disease and to modulate inflammatory pathways, thereby improving resistance to infectious challenges [13,14]. Furthermore, ginger has been shown to improve immune organ health and leukocyte activity, reinforcing its role as a natural immunomodulator. Despite the documented immunological benefits of these phyto-genic feed additives, comprehensive *in vivo* studies evaluating their individual and combined effects on cell-mediated immunity, humoral immune response, hematological parameters and immune organ histopathology in broiler chickens remain limited. Therefore, the present study was undertaken to investigate the immunomodulatory effects of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale*, administered alone and in combination, in broiler chickens.

**Table 1:** Research protocol to study immunomodulatory activity of *Trachyspermum ammi*, *Capsicum annuum*, *Zingiber officinale* powder alone and its combinations in broilers

Groups	Treatment details	Total number of birds/treatments
I	Control group (Basal diet + no powder)	12
II	Basal diet + vitamin E and selenium (@1.5 grams per 100 birds for first two weeks and 5 grams per 100 birds for next 3 weeks)	12
III	Basal diet + <i>Trachyspermum ammi</i> powder (@ 5 g/kg feed)	12
IV	Basal diet + <i>Trachyspermum ammi</i> powder (@ 10 g/kg feed)	12
V	Basal diet + <i>Capsicum annuum</i> powder (@ 5 g/kg feed)	12
VI	Basal diet + <i>Capsicum annuum</i> powder (@ 10 g/kg feed)	12
VII	Basal diet + <i>Zingiber officinale</i> powder (@ 5 g/kg feed)	12
VIII	Basal diet + <i>Zingiber officinale</i> powder (@ 10 g/kg feed)	12
IX	Basal diet + <i>Trachyspermum ammi</i> , <i>Capsicum annuum</i> , <i>Zingiber officinale</i> (@ 5, 5 and 5 g/kg feed, respectively)	12
X	Basal diet + <i>Trachyspermum ammi</i> , <i>Capsicum annuum</i> , <i>Zingiber officinale</i> (@ 10, 10 and 10 g/kg feed, respectively)	12
Total number of birds = 120		

**Assessment of Cell-Mediated Immunity**

Cell-mediated immune response was assessed on day 14 by the cutaneous basophil hypersensitivity (CBH) test using phytohemagglutinin-P. Phytohemagglutinin-P (100 or 200 µg in 0.1 mL sterile physiological saline) was injected intradermally into the interdigital skin between the third and fourth digits of the right foot.

**MATERIAL AND METHODS**

**Experimental Location**

The study was conducted at the Department of Veterinary Pharmacology and Toxicology in collaboration with the Poultry Research Station and allied departments, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat.

**Experimental Birds and Ethical Approval**

A total of 120-day-old Ven-Cobb broiler chicks were procured from a commercial hatchery and reared under standard management conditions. The experimental protocol was approved by the Institutional Animal Ethics Committee (Project No. 414/VPT/2023).

**Housing, Feeding and Management**

Birds were maintained under a deep-litter system with proper ventilation, temperature control and biosecurity measures. Broiler pre-starter (1–7 days), starter (8–21 days) and finisher (22–42 days) diets were formulated and provided according to standard nutritional requirements. Clean drinking water was offered ad libitum. All birds were vaccinated against Newcastle disease and infectious bursal disease as per the standard vaccination schedule.

**Experimental Design and Dietary Treatments**

Chicks were randomly allocated into ten treatment groups (n = 12 per group) as detailed in Table 1. Group I received a basal diet (control), Group II received vitamin E and selenium as standard control, Groups III and IV received *Trachyspermum ammi* powder (5 and 10 g/kg feed), Groups V and VI received *Capsicum annuum* powder (5 and 10 g/kg feed), Groups VII and VIII received *Zingiber officinale* powder (5 and 10 g/kg feed), while Groups IX and X received a combination of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* at 5+5+5 and 10+10+10 g/kg feed, respectively, for 35 days.

Skin thickness was measured using a vernier caliper immediately before injection and at 12 and 24 h post-injection and the CBH response was expressed as the increase in skin thickness relative to the pre-injection value.

## Assessment of Humoral Immune Response

Humoral immune response was evaluated by determining antibody titers against the Newcastle disease virus (NDV). Blood samples were collected from the wing vein of birds on days 7, 21 and 35 of the experimental periods. Serum was separated by centrifugation and stored at  $-55^{\circ}\text{C}$  until analysis. Antibody titers were quantified using the haemagglutination inhibition (HI) test following standard procedures. The results were expressed as reciprocal antibody titers and converted to  $\log_2$  values for statistical analysis.

## Hematological Examination

Hematological assessment was performed on day 35 of the experiment. Blood smears were prepared immediately after blood collection, air-dried, fixed and stained using standard staining techniques. Differential leukocyte counts were determined microscopically by counting heterophils, lymphocytes, monocytes and eosinophils under oil immersion. The heterophil-to-lymphocyte (H/L) ratio was calculated to evaluate stress response and immune status in broiler chickens.

## Histopathological Examination of Immune Organs

At the end of the experimental period, birds from each treatment group were humanely sacrificed following standard ethical procedures. Immune organs, including the thymus, spleen and bursa of Fabricius, were carefully excised and immediately fixed in 10% neutral buffered formalin for adequate tissue preservation. Fixed tissues were processed using standard histological procedures, embedded in paraffin wax and sectioned at a thickness of 4–5  $\mu\text{m}$  using a rotary microtome. Tissue sections were mounted on glass slides, deparaffinized and stained with hematoxylin and eosin (H&E). The stained sections were examined under a light microscope for histopathological alterations, including lymphoid follicle development, lymphocytic proliferation and overall tissue architecture.

## Statistical Analysis

A completely randomized experimental design was adopted and data were subjected to one-way analysis of variance (ANOVA) to evaluate differences among treatment groups for immunomodulatory parameters using SPSS statistical software (version 27.0). When overall significance was detected, intergroup comparisons were performed using Duncan's multiple range test at a probability level of  $p < 0.05$ . Initial data processing and graphical representation were carried out using Microsoft Excel. Results are expressed as mean  $\pm$  standard error (SE).

## RESULTS

The cell-mediated immune response was assessed using the cutaneous basophil hypersensitivity (CBH) test following intradermal injection of phytohemagglutinin-P (PHA-P) and the mean toe web skin thickness values (Mean  $\pm$  SE) of the different experimental groups are presented in Table 2 and graphically illustrated in Figure 1. Prior to PHA-P injection (0 h), the mean toe web skin thickness did not differ significantly among the treatment groups, indicating uniform baseline values across all groups. At the 100  $\mu\text{g}$  PHA-P dose, a significant ( $p < 0.05$ ) increase in toe web thickness was observed at 12 h post-injection in all supplemented groups, including birds fed *Trachyspermum ammi*, *Capsicum annuum*, *Zingiber officinale*, their combinations and the vitamin E–selenium group, compared to the control group. Toe web thickness in birds supplemented with *Trachyspermum ammi* at 10 g/kg feed and *Capsicum annuum* at 5 g/kg feed was significantly higher than that observed in the vitamin E–selenium group, whereas most of the remaining phyto-genic-supplemented groups exhibited values comparable to the standard control. At 24 h post-injection, a similar trend was recorded at the 100- $\mu\text{g}$  dose, with significantly higher CBH responses in all

supplemented groups compared to the control group and a dose-dependent increase particularly evident in birds supplemented with *Trachyspermum ammi*. At the 200  $\mu\text{g}$  PHA-P dose, baseline toe web thickness values before injection were also statistically similar across all experimental groups. At 12 h post-injection, birds receiving individual phyto-genic supplements as well as their combinations showed significantly higher CBH responses compared to the control group. Toe web thickness values in most phyto-genic-supplemented groups were comparable to those of the vitamin E–selenium group; however, birds supplemented with *Capsicum annuum* at 5 g/kg feed exhibited significantly higher values than the standard control. At 24 h post-injection, CBH responses remained significantly elevated in all supplemented groups relative to the control group, with dose-dependent increases particularly observed in birds fed *Trachyspermum ammi* and *Zingiber officinale*, indicating a sustained enhancement of cell-mediated immune response.

The humoral immune response was evaluated by measuring haemagglutination inhibition (HI) antibody titers against Newcastle disease (ND) vaccine. The mean HI antibody titers ( $\log_2$  values; Mean  $\pm$  SE) recorded at 7, 21 and 35 days are presented in Table 3 and depicted in Figure 2. On day 7, no significant difference in HI antibody titers was observed among treatment groups, indicating comparable maternal antibody levels at the start of the experiment. On day 21, birds supplemented with *Capsicum annuum* (5 g/kg feed), *Zingiber officinale* (10 g/kg feed) and the higher-dose phyto-genic combination (10+10+10 g/kg feed) exhibited significantly ( $p < 0.05$ ) higher HI titers compared to the control group. The vitamin E–selenium group also showed a significant increase relative to control and HI titers of selected phyto-genic-supplemented groups were comparable to the standard control. On day 35, a significant ( $p < 0.05$ ) increase in HI antibody titers was observed in birds supplemented with *Trachyspermum ammi* (10 g/kg feed), *Capsicum annuum* (5 g/kg feed), *Zingiber officinale* (5 and 10 g/kg feed) and both combination groups, compared to the control group. The HI titers of these groups were statistically similar to those of the vitamin E–selenium group, indicating sustained enhancement of humoral immune response.

Differential leukocyte count was performed on day 35 of the experiment and the effects of dietary treatments on heterophils, lymphocytes, monocytes, eosinophils and the heterophil-to-lymphocyte (H/L) ratio are presented in Table 4 and Figures 3 and 4. A significant ( $p < 0.05$ ) reduction in heterophil counts was observed in all supplemented groups, including birds receiving *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* powders individually and in combination, as well as in the vitamin E and selenium-supplemented group, compared to the control group. Heterophil counts in birds supplemented with *Trachyspermum ammi* (both doses), *Capsicum annuum* at the higher dose, *Zingiber officinale* at the lower dose and the higher-dose combination were statistically comparable to those of the vitamin E and selenium group, whereas birds supplemented with *Capsicum annuum* at the lower dose, *Zingiber officinale* at the higher dose and the lower-dose combination exhibited significantly lower heterophil counts than the vitamin E and selenium group. Lymphocyte counts were significantly ( $p < 0.05$ ) increased in all supplemented groups compared to the control group, including the vitamin E and selenium group. Lymphocyte percentages in birds receiving *Trachyspermum ammi* (both doses), *Capsicum annuum* at the higher dose, *Zingiber officinale* at the lower dose and the combination treatments were comparable to the vitamin E and selenium group, while birds supplemented with *Capsicum annuum* at the lower dose and *Zingiber officinale* at the higher dose showed significantly higher lymphocyte counts than the standard control. A dose-dependent increase in lymphocyte counts was evident in birds supplemented with *Trachyspermum ammi* and *Zingiber officinale*. No significant differences were observed in monocyte or eosinophil counts among any of the treatment groups when compared with the control group. The heterophil-to-lymphocyte ratio was significantly ( $p < 0.05$ ) reduced in all supplemented groups, including the vitamin E and selenium group, compared to the control group. The H/L ratios of birds supplemented with *Trachyspermum ammi* (both doses),

*Capsicum annuum* at the higher dose, *Zingiber officinale* at the lower dose and the combination treatments were statistically comparable to those of the vitamin E and selenium group, whereas birds receiving *Capsicum annuum* at the lower dose and *Zingiber officinale* at the higher dose exhibited significantly lower H/L ratios than the vitamin E and selenium group.

Histopathological evaluation of lymphoid organs revealed distinct morphological differences between the control and dietary supplemented groups, as illustrated in Figures 5 to 7. The bursa of Fabricius from the control group exhibited normal histoarchitecture, characterized by well-defined follicles with normal corticomedullary differentiation. In contrast, birds supplemented with *Trachyspermum ammi* powder (5.0 and 10.0 g/kg feed), *Capsicum annuum* powder (5.0 and 10.0 g/kg feed), *Zingiber officinale* powder (5.0 and 10.0 g/kg feed) and their combinations at both lower (5.0 + 5.0 + 5.0 g/kg feed) and higher (10.0 + 10.0 + 10.0 g/kg feed) inclusion levels exhibited marked enlargement of bursal follicles along with prominent lymphocytic hyperplasia on the 35th day of the experiment. A similar

pattern of follicular enlargement and lymphocytic hyperplasia was also observed in birds supplemented with vitamin E and selenium (Group II), indicating enhanced lymphoid activity compared to the control group. Histopathological examination of the thymus in the control group revealed normal cortical and medullary organization. In contrast, birds receiving *Trachyspermum ammi*, *Capsicum annuum*, *Zingiber officinale* powders, either individually or in combination at both inclusion levels, demonstrated marked lymphocytic hyperplasia on day 35. Comparable thymic lymphocytic proliferation was also evident in the vitamin E and selenium-supplemented group, suggesting stimulation of thymocyte activity in response to dietary supplementation. The spleen of control birds showed normal histological features with distinct red and white pulp regions. However, birds supplemented with phytochemical powders individually or in combination at both lower and higher doses exhibited marked lymphocytic hyperplasia in the white pulp region on day 35. Similar splenic white pulp hyperplasia was observed in the vitamin E and selenium group, indicating enhanced immune activation relative to the control group.

**Table 2:** Effect of dietary supplementation of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* powder alone and in combination on Haemagglutination inhibition (HI) antibody titer log<sub>2</sub> value against ND vaccine in broiler (n=6)

Groups	HI Antibody titer		
	1 <sup>st</sup> week (Day 7)	3 <sup>rd</sup> week (Day 21)	5 <sup>th</sup> week (Day 35)
I	5.00 ± 0.26	5.17 ± 0.60 <sup>a</sup>	5.33 ± 0.33 <sup>a</sup>
II	5.50 ± 0.43	7.00 ± 0.73 <sup>b</sup>	7.50 ± 0.56 <sup>bc</sup>
III	5.67 ± 0.33	6.17 ± 0.31 <sup>ab</sup>	6.33 ± 0.33 <sup>ab</sup>
IV	5.33 ± 0.49	6.00 ± 0.37 <sup>ab</sup>	6.67 ± 0.21 <sup>bc</sup>
V	5.83 ± 0.70	7.33 ± 0.21 <sup>b</sup>	7.83 ± 0.40 <sup>c</sup>
VI	5.33 ± 0.21	6.00 ± 0.45 <sup>ab</sup>	6.33 ± 0.42 <sup>ab</sup>
VII	5.50 ± 0.43	6.17 ± 0.48 <sup>ab</sup>	6.83 ± 0.40 <sup>bc</sup>
VIII	5.17 ± 0.75	7.33 ± 0.33 <sup>b</sup>	7.67 ± 0.33 <sup>bc</sup>
IX	5.50 ± 0.56	6.33 ± 0.49 <sup>ab</sup>	6.83 ± 0.48 <sup>bc</sup>
X	5.67 ± 0.56	7.00 ± 0.26 <sup>b</sup>	7.50 ± 0.56 <sup>bc</sup>

Values (Mean ± S.E.) bearing different superscripts (a, b, c) in a column differ significantly (P < 0.05).

**Table 3:** Effect of dietary supplementation of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* powder alone and in combination on serum total protein in broiler (n=6)

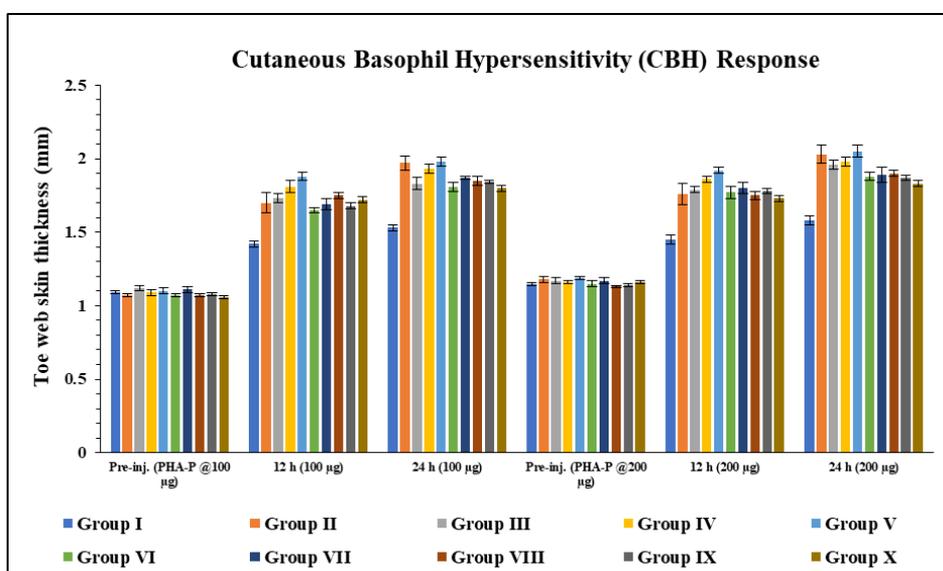
Groups	Serum total protein (g/dl)		
	1 <sup>st</sup> week (Day 7)	3 <sup>rd</sup> week (Day 21)	5 <sup>th</sup> week (Day 35)
I	2.68 ± 0.14	2.88 ± 0.12	3.20 ± 0.25 <sup>a</sup>
II	3.03 ± 0.10	3.49 ± 0.10	4.47 ± 0.16 <sup>bc</sup>
III	2.84 ± 0.12	2.99 ± 0.07	4.43 ± 0.16 <sup>bc</sup>
IV	2.87 ± 0.17	3.05 ± 0.18	4.60 ± 0.14 <sup>bc</sup>
V	2.81 ± 0.09	3.07 ± 0.13	4.01 ± 0.09 <sup>b</sup>
VI	2.86 ± 0.15	3.09 ± 0.11	4.07 ± 0.07 <sup>b</sup>
VII	2.70 ± 0.12	3.17 ± 0.25	4.12 ± 0.30 <sup>b</sup>
VIII	2.77 ± 0.14	3.22 ± 0.08	4.49 ± 0.23 <sup>bc</sup>
IX	3.02 ± 0.19	3.51 ± 0.34	4.96 ± 0.22 <sup>c</sup>
X	2.82 ± 0.10	3.05 ± 0.11	4.56 ± 0.24 <sup>bc</sup>

Values (Mean ± S.E.) bearing different superscripts (a, b, c) in a column differ significantly (P < 0.05).

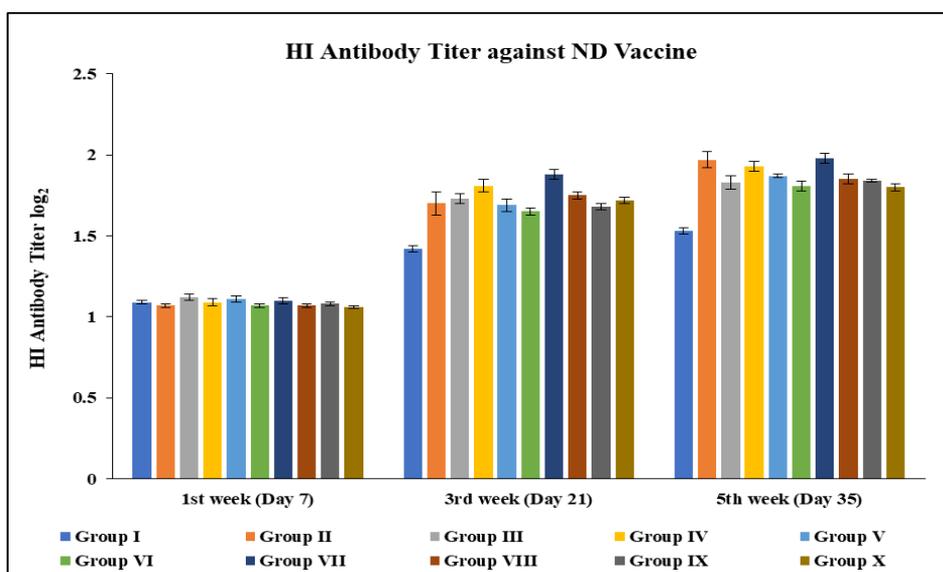
**Table 4:** Effect of dietary supplementation of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* powder alone and in combination on differential leukocyte counts and heterophil to lymphocyte (H/L) ratio in broiler (n=6)

Groups	Differential leukocyte counts (%)				H/L Ratio
	Heterophils	Lymphocytes	Monocytes	Eosinophils	
I	40.33 ± 1.31 <sup>c</sup>	51.17 ± 1.01 <sup>a</sup>	4.83 ± 0.31	3.67 ± 0.33	0.79 ± 0.04 <sup>c</sup>
II	31.50 ± 1.93 <sup>cd</sup>	60.50 ± 2.03 <sup>bc</sup>	4.67 ± 0.33	3.33 ± 0.21	0.53 ± 0.05 <sup>cd</sup>
III	34.17 ± 1.08 <sup>d</sup>	58.33 ± 0.80 <sup>b</sup>	4.33 ± 0.33	3.17 ± 0.31	0.59 ± 0.03 <sup>d</sup>
IV	29.17 ± 2.09 <sup>bc</sup>	63.17 ± 1.92 <sup>bcd</sup>	4.17 ± 0.31	3.50 ± 0.43	0.47 ± 0.05 <sup>abcd</sup>
V	25.83 ± 1.70 <sup>ab</sup>	66.17 ± 1.74 <sup>dc</sup>	4.33 ± 0.42	3.67 ± 0.49	0.40 ± 0.04 <sup>ab</sup>
VI	28.67 ± 1.02 <sup>bc</sup>	63.83 ± 1.76 <sup>cde</sup>	4.17 ± 0.48	3.33 ± 0.61	0.45 ± 0.03 <sup>abc</sup>
VII	30.17 ± 1.87 <sup>bcd</sup>	61.67 ± 2.08 <sup>bcd</sup>	4.50 ± 0.76	3.67 ± 0.49	0.50 ± 0.05 <sup>bcd</sup>
VIII	23.83 ± 0.95 <sup>a</sup>	67.83 ± 0.60 <sup>c</sup>	4.67 ± 0.49	3.67 ± 0.33	0.35 ± 0.02 <sup>a</sup>
IX	26.33 ± 1.12 <sup>ab</sup>	65.17 ± 1.60 <sup>cde</sup>	4.83 ± 0.48	3.67 ± 0.49	0.41 ± 0.03 <sup>abc</sup>
X	31.33 ± 1.93 <sup>cd</sup>	60.83 ± 1.47 <sup>bc</sup>	4.67 ± 0.49	3.17 ± 0.40	0.52 ± 0.05 <sup>bcd</sup>

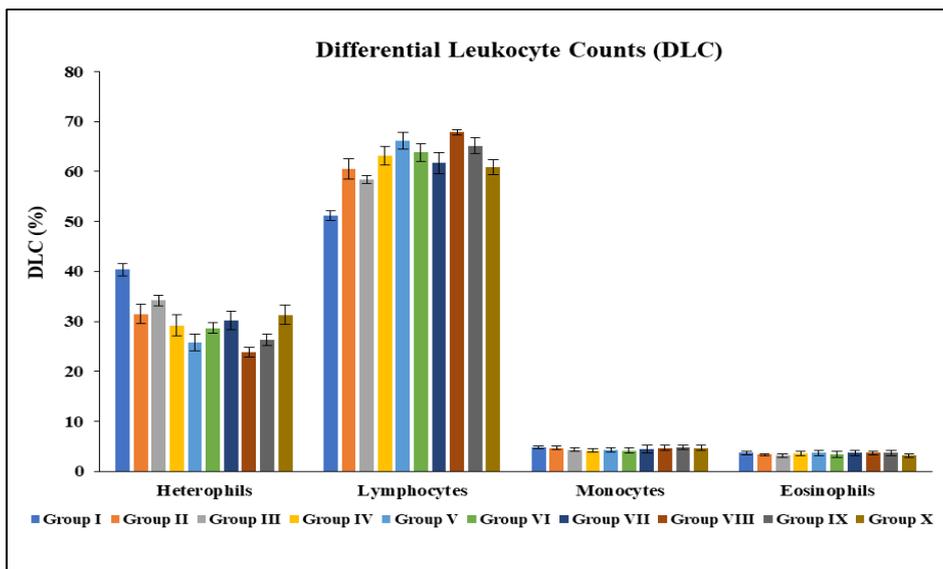
Values (Mean ± S.E.) bearing different superscripts (a, b, c, d, e) in a column differ significantly (P < 0.05).



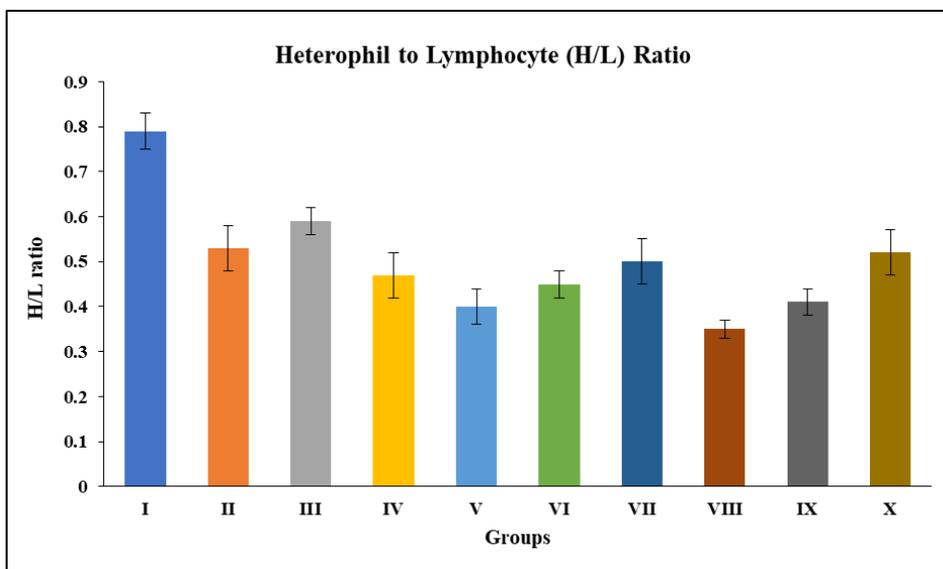
**Figure 1:** Effect of dietary supplementation of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* powder alone and in combination on cutaneous basophil hypersensitivity response against phytohemagglutinin-P (100 and 200 µg) in broiler (n=6)



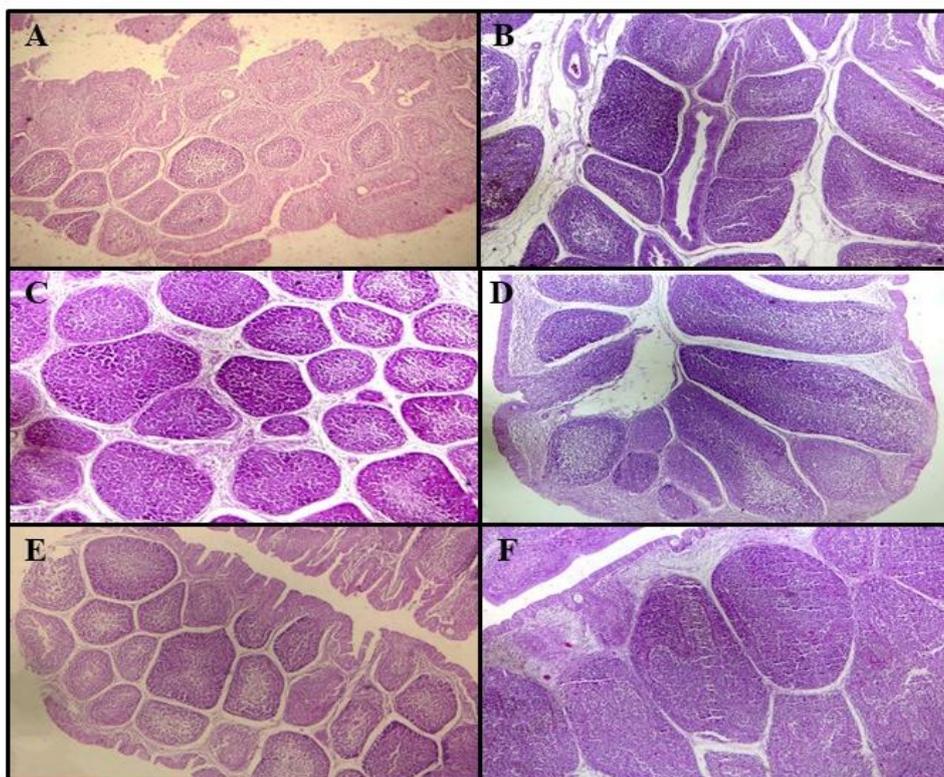
**Figure 2:** Effect of dietary supplementation of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* powder alone and in combination on HI antibody titer against ND vaccine in broiler



**Figure 3:** Effect of dietary supplementation of *Trachyspermum ammi*, *Capsicum annum* and *Zingiber officinale* powder alone and in combination on differential leukocyte counts (%) in broiler

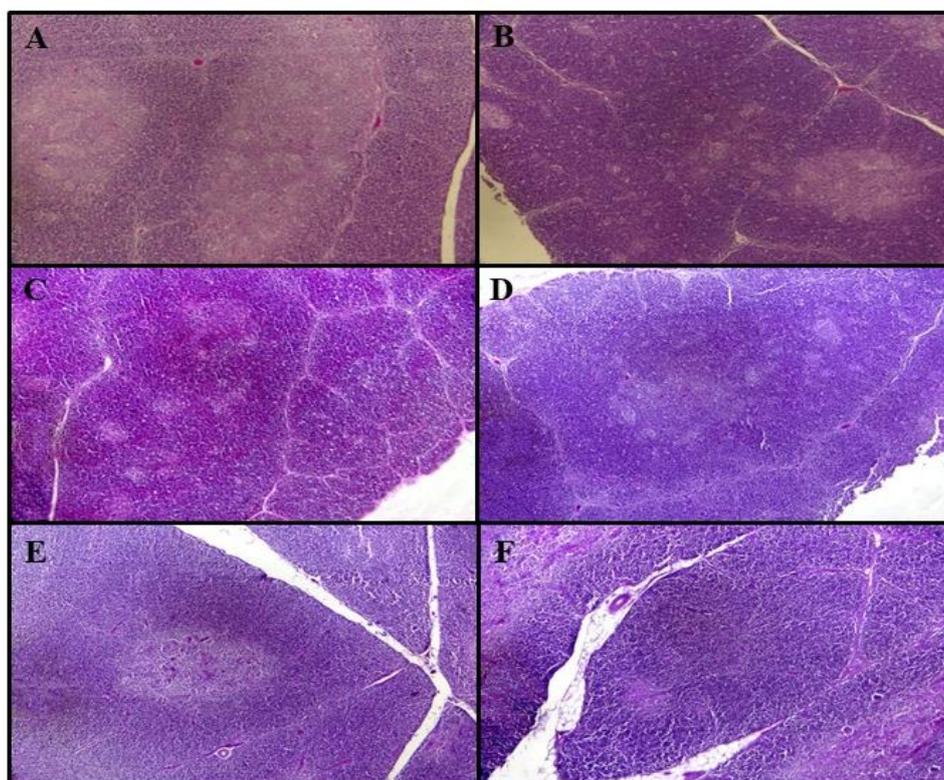


**Figure 4:** Effect of dietary supplementation of *Trachyspermum ammi*, *Capsicum annum* and *Zingiber officinale* powder alone and in combination on heterophil to lymphocyte (H/L) ratio in broiler



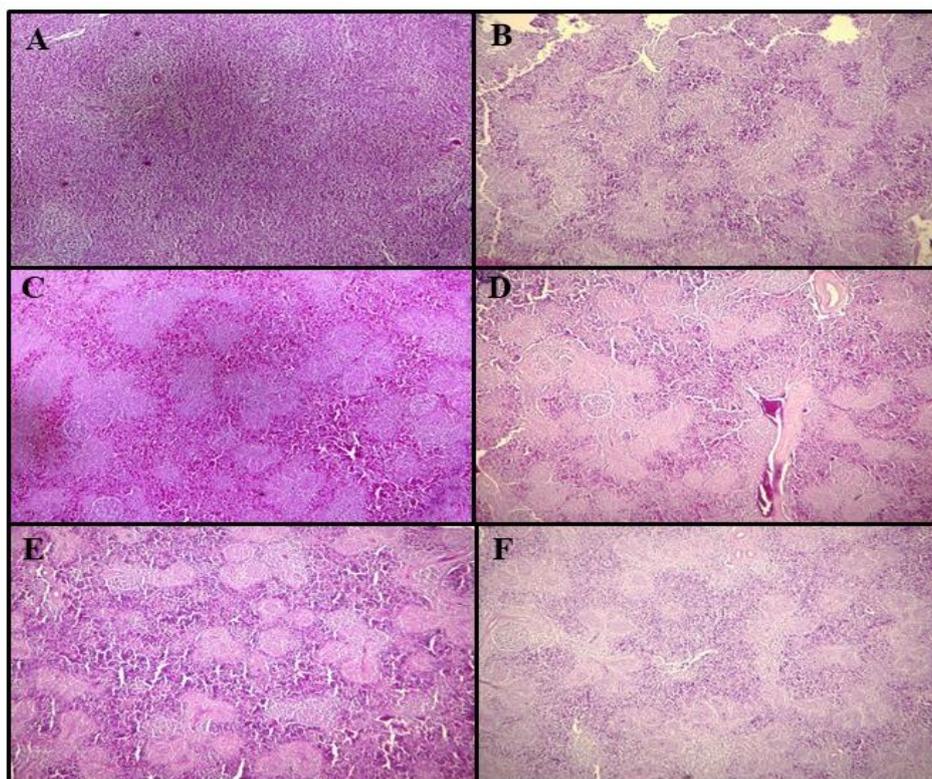
**Figure 5:** Histopathology of the bursa of Fabricius in broilers on day 35 (H&E, ×40).

(A) Control group showing normal bursal architecture. (B) Vitamin E and selenium-supplemented group showing marked enlargement of bursal follicles with prominent lymphocytic hyperplasia. (C) *Trachyspermum ammi* powder-supplemented group (10 g/kg feed) showing mild to moderate follicular enlargement with moderate lymphocytic hyperplasia. (D) *Capsicum annuum* powder-supplemented group (10 g/kg feed) showing marked follicular enlargement with prominent lymphocytic hyperplasia. (E) *Zingiber officinale* powder-supplemented group (10 g/kg feed) showing mild to moderate follicular enlargement with moderate lymphocytic hyperplasia. (F) Polyherbal mixture-supplemented group (5 g/kg feed each of *T. ammi*, *C. annuum*, and *Z. officinale*) showing marked enlargement of bursal follicles with prominent lymphocytic hyperplasia.



**Figure 6:** Histopathology of the thymus in broilers on day 35 (H&E, ×40).

(A) Control group showing normal thymic architecture. (B) Vitamin E and selenium-supplemented group showing marked lymphocytic hyperplasia. (C) *Trachyspermum ammi* powder-supplemented group (10 g/kg feed) showing mild to moderate lymphocytic hyperplasia. (D) *Capsicum annuum* powder-supplemented group (5 g/kg feed) showing marked lymphocytic hyperplasia. (E) *Zingiber officinale* powder-supplemented group (10 g/kg feed) showing mild to moderate lymphocytic hyperplasia. (F) Polyherbal mixture-supplemented group (5 g/kg feed each of *T. ammi*, *C. annuum*, and *Z. officinale*) showing marked lymphocytic hyperplasia.



**Figure 7:** Histopathology of the spleen in broilers on day 35 (H&E, ×40).

(A) Control group showing normal splenic architecture. (B) Vitamin E and selenium-supplemented group showing marked white pulp lymphocytic hyperplasia. (C) *Trachyspermum ammi* powder-supplemented group (10 g/kg feed) showing moderate white pulp lymphocytic hyperplasia. (D) *Capsicum annuum* powder-supplemented group (5 g/kg feed) showing marked white pulp lymphocytic hyperplasia. (E) *Zingiber officinale* powder-supplemented group (10 g/kg feed) showing moderate white pulp lymphocytic hyperplasia. (F) Polyherbal mixture-supplemented group (5 g/kg feed each of *T. ammi*, *C. annuum*, and *Z. officinale*) showing marked white pulp lymphocytic hyperplasia.

## DISCUSSION

The cutaneous basophil hypersensitivity (CBH) response is a well-established indicator of cell-mediated immunity in poultry, reflecting T-lymphocyte activation and local inflammatory responses following mitogen stimulation. In the present study, dietary supplementation with *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale*, either individually or in combination, significantly enhanced the CBH response at both 100 µg and 200 µg PHA-P doses, indicating a pronounced stimulation of cell-mediated immune function. These findings are consistent with earlier reports demonstrating improved CBH responses in broilers supplemented with ginger [15]. Similarly, supplementation with garlic, onion and chili has been reported to significantly enhance cell-mediated immune responses in broiler chickens [16]. Enhanced delayed-type hypersensitivity reactions following immunostimulatory interventions further support the reliability of CBH as a marker of immune competence [17]. Among the tested phytochemicals, *Capsicum annuum* at the lower inclusion level (5 g/kg feed) elicited the most pronounced CBH response, comparable to or exceeding that of the vitamin E and selenium-supplemented group. This enhanced response may be attributed to capsaicin and related phenolic compounds, which are known to stimulate immune cell activation and cytokine release. The dose-dependent improvement observed with *Trachyspermum ammi* and *Zingiber officinale* further suggests that optimal inclusion levels of these herbs can maximize immune responsiveness.

The haemagglutination inhibition (HI) antibody response against Newcastle disease virus is a reliable indicator of humoral immune competence and vaccine responsiveness in broiler chickens. The enhanced antibody titers observed following dietary supplementation with *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale*, either individually or in combination, indicate a clear immunomodulatory influence of these phytochemical feed additives. Similar improvements in HI antibody titers have been reported in broilers supplemented with ajwain, red pepper and ginger, supporting

the present findings [18-24]. The immunoenhancing effect of *Zingiber officinale* has been attributed to its potent antioxidant properties and the presence of bioactive compounds such as gingerols and shogaols, which protect immune cells from oxidative damage and promote antibody synthesis [25,26]. Likewise, *Trachyspermum ammi* contains thymol, a compound known for its antimicrobial and immunostimulatory activity that supports humoral immune responses [27]. While *Capsicum annuum* is rich in capsaicin, which enhances circulation, modulates inflammatory mediators and exerts antioxidant effects, thereby indirectly stimulating immune function [28]. The comparable antibody responses observed between phytochemical-supplemented groups and the vitamin E and selenium-supplemented group further highlight the potential of these plant-based additives to serve as effective natural alternatives to conventional immunomodulators. Collectively, these findings suggest that the inclusion of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* in broiler diets can enhance humoral immune responses and improve vaccine-induced antibody production, thereby contributing to improved immune health in poultry.

Hematological parameters, particularly differential leukocyte counts and the heterophil-to-lymphocyte (H/L) ratio, are widely recognized as sensitive indicators of immune status and physiological stress in poultry. In the present study, dietary supplementation with *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale*, either individually or in combination, significantly influenced leukocyte profiles, indicating enhanced immune competence in broiler chickens. The observed increase in lymphocyte counts and reduction in the H/L ratio in phytochemical-supplemented groups are in agreement with the findings of Waheed et al. [18], who reported significantly higher lymphocyte counts in birds supplemented with ajwain extract, reflecting improved adaptive immune responses. In contrast, Al-Khalafah et al. [30] reported increased heterophil percentages in broilers fed ginger powder at higher inclusion levels and Ademola et al. [29] also observed elevated heterophil counts in chicks fed ginger-supplemented diets, suggesting that heterophil responses may vary

depending on dosage, duration of supplementation and physiological state of the birds. Notably, in the present study, broilers receiving *Capsicum annuum* at 5 g/kg feed and *Zingiber officinale* at 10 g/kg feed exhibited the lowest H/L ratios and highest lymphocyte counts, even compared to the vitamin E and selenium-supplemented group, indicating reduced physiological stress and improved immune balance. A lower H/L ratio is widely considered a reliable indicator of enhanced immune status and stress resilience in poultry. These favorable hematological responses may be attributed to the bioactive constituents of the phyto-genic feed additives, such as capsaicin in *Capsicum annuum* and gingerols and shogaols in *Zingiber officinale*, which possess anti-inflammatory, antioxidant and immunomodulatory properties that protect leukocytes from oxidative damage and promote immune cell activity. Overall, the modulation of leukocyte profiles and significant reduction in the H/L ratio observed in the present study provide strong evidence that dietary inclusion of these phyto-genic powders effectively enhances immune function and maintains immune homeostasis in broiler chickens.

The histopathological alterations observed in the lymphoid organs of broiler chickens supplemented with phyto-genic feed additives provide strong morphological evidence of their immunomodulatory potential. Lymphocytic hyperplasia and follicular enlargement in the bursa of Fabricius are indicative of enhanced B-lymphocyte proliferation and differentiation, which are essential for effective humoral immune responses [17]. The marked bursal follicular development observed in birds supplemented with *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* suggests stimulation of antibody-producing cells and is in agreement with the enhanced haemagglutination inhibition antibody titers recorded in the present study, as well as previous reports on phyto-genic immunostimulation in poultry [11,18,19]. The thymus plays a central role in the maturation and differentiation of T-lymphocytes, which are critical for cell-mediated immune responses [16]. The pronounced lymphocytic hyperplasia observed in the thymus of birds receiving phyto-genic supplements either individually or in combination indicates increased thymocyte proliferation and activation of T-cell-mediated immune pathways. Similar thymic lymphoid activation has been reported in broilers supplemented with ginger, ajwain and other herbal immunomodulators, supporting the present findings [20-23]. These histological changes corroborate the enhanced cutaneous basophil hypersensitivity responses observed in the supplemented groups, confirming stimulation of cell-mediated immunity. The spleen, a major secondary lymphoid organ involved in antigen processing and systemic immune surveillance, exhibited marked lymphocytic hyperplasia in the white pulp region of birds supplemented with phyto-genic powders. White pulp expansion reflects activation and proliferation of lymphoid populations responsible for both humoral and cellular immune responses. Similar splenic histological changes have been reported in broilers receiving herbal feed additives and antioxidant supplements, indicating improved immune readiness and antigen-handling capacity [14,22,30].

## CONCLUSION

The present investigation clearly demonstrates that dietary supplementation with *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale*, either individually or in combination, exerts a significant immunomodulatory effect in broiler chickens. Enhanced cutaneous basophil hypersensitivity responses confirmed the stimulation of cell-mediated immunity, while elevated haemagglutination inhibition antibody titers against Newcastle disease vaccine reflected improved humoral immune responsiveness. Phyto-genic supplementation also favorably modulated hematological parameters, characterized by increased lymphocyte counts and reduced heterophil-to-lymphocyte ratios, indicating enhanced immune competence and reduced stress. Furthermore, histopathological findings of marked lymphocytic hyperplasia in primary and secondary lymphoid organs provided strong morphological evidence of immune activation in supplemented birds. Notably, *Capsicum annuum* at 5 g/kg feed and *Zingiber officinale* at 10 g/kg feed exhibited the most

pronounced immunostimulatory effects, while combination treatments produced responses comparable to the vitamin E-selenium standard control, suggesting potential synergistic interactions among phyto-genic constituents. In conclusion, these phyto-genic feed additives represent effective, natural and safe alternatives to conventional immunomodulators and antibiotic growth promoters in poultry nutrition, with substantial potential to improve immune health and vaccine responsiveness in broiler production systems.

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## Conflict of interest

The authors declared no conflict of interest.

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