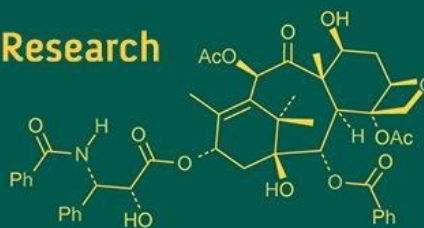


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Evaluation of Biochemical Profile and Antioxidant Activity of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* as Phytogetic Feed Additives in Broiler Chickens

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Abstract

The present study was conducted to evaluate the effects of selected phytogetic feed additives on serum biochemical profile and antioxidant status in broiler chickens. A total of 120-day-old Ven-Cobb broiler chicks were randomly allocated into ten dietary treatment groups and reared for 35 days. The treatments included a basal diet (control), vitamin E and selenium as a standard antioxidant, *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* powders supplemented individually at two inclusion levels (5 and 10 g/kg feed) and their combinations at corresponding low and high doses. Serum biochemical parameters, including total protein, albumin, globulin and albumin-to-globulin ratio, were evaluated on days 7, 21 and 35. Antioxidant status was assessed on day 35 by estimating serum superoxide dismutase (SOD) activity and malondialdehyde (MDA) concentration. The results revealed no significant differences among groups during the first and third weeks. However, at the fifth week, birds supplemented with phytogetic additives exhibited significantly higher serum total protein, albumin and globulin levels compared to the control group. A significant reduction in the albumin-to-globulin ratio was observed in *Capsicum annuum*, *Zingiber officinale*, combination groups and the vitamin E and selenium group. Antioxidant assessment demonstrated a significant increase in serum SOD activity and a concomitant reduction in MDA levels in all phytogetic-supplemented groups. Notably, *Capsicum annuum* at 5 g/kg feed showed the highest SOD activity and the lowest MDA concentration. In conclusion, dietary supplementation with *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale*, either individually or in combination, effectively improves serum biochemical indices or enhances antioxidant defense mechanisms in broiler chickens, suggesting their potential as natural alternatives to synthetic antioxidants in poultry nutrition.

Keywords: Ajwain, *Capsicum annuum*, Ginger, Antioxidant enzymes, Serum biochemical profile, Broiler chickens, Phytogetic feed additives

1. Introduction

Oxidative stress is a major physiological challenge in intensive poultry production systems, arising from high metabolic rates, environmental stressors and nutritional imbalances. Excessive generation of reactive oxygen species can impair cellular integrity, disrupt metabolic processes and negatively affect growth performance, health status and meat quality in broiler chickens (Orhan *et al.*, 2010) [21]. Traditionally, synthetic antioxidants and antibiotic growth promoters were used to mitigate these effects; however, concerns regarding toxicity, residue accumulation and antimicrobial resistance have accelerated the transition toward natural antioxidant sources in poultry nutrition (Castanon, 2007; Van den Bogaard *et al.*, 2002) [4, 32]. Phytogetic feed additives rich in polyphenols, flavonoids and essential oils have demonstrated strong antioxidant potential by scavenging free radicals, reducing lipid peroxidation and enhancing endogenous antioxidant enzyme activity. These natural compounds also play an important role in maintaining biochemical homeostasis by modulating liver function, lipid metabolism and protein synthesis (El-Deek *et al.*, 2012; Sharma *et al.*, 2013) [5, 28]. Consequently, medicinal plants have gained prominence as functional feed additives capable of improving both oxidative balance and biochemical profiles in broiler chickens.

Trachyspermum ammi (Ajwain) is a valuable source of polyphenolic compounds and essential oils with well-documented antioxidant activity. The phenolic constituents of Ajwain contribute to the neutralization of reactive oxygen species and protection against oxidative damage at the cellular level (Ranjan *et al.*, 2011) [22]. Dietary inclusion of Ajwain has been associated with improved antioxidant status and stabilization of biochemical parameters, highlighting its potential role in maintaining metabolic health in poultry. *Capsicum annuum* (hot red pepper) is rich in capsaicinoids and vitamin C, both of which contribute significantly to its antioxidant capacity. Capsaicin has been shown to reduce lipid peroxidation, enhance antioxidant enzyme activity and regulate lipid metabolism, resulting in improved serum biochemical profiles, including reduced cholesterol and triglyceride levels (Obboh *et al.*, 2007; Luqman and Razvi, 2006) [20, 14]. Additionally, *Capsicum annuum* supports energy metabolism and nutrient utilization, thereby contributing to improved physiological efficiency in broiler chickens. *Zingiber officinale* (ginger) is widely recognized for its strong antioxidant and hypolipidemic properties, attributed to bioactive compounds such as gingerols and shogaols. Ginger supplementation has been reported to enhance antioxidant enzyme activities, decrease malondialdehyde levels and improve serum lipid profiles, indicating reduced oxidative stress and improved liver function (Shewita and Taha, 2017; Mohamed *et al.*, 2012) [29, 16]. Furthermore, ginger has been shown to stabilize feed oxidation, thereby preserving nutritional quality during storage.

Although individual studies have reported beneficial antioxidant and biochemical effects of Ajwain, Capsicum and Ginger in poultry, comparative and combinational evaluations under controlled *in vivo* conditions remain limited. Variability in reported outcomes necessitates further investigation to establish their efficacy and consistency as natural antioxidant alternatives. Therefore, the present study was designed to evaluate the antioxidant activity and biochemical profile of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale*, administered alone and in combination, in broiler chickens.

2. Materials and Methods

2.1 Experimental Location

The experiment was carried out at the Department of Veterinary Pharmacology and Toxicology, with support from the Poultry Research Station and associated departments, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand, Gujarat, India.

2.2 Experimental Birds and Ethical Approval

A total of 120-day-old Ven-Cobb broiler chicks were obtained from a recognized commercial hatchery and maintained under standard husbandry and management practices throughout the study period. The experimental procedures were duly reviewed and approved by the Institutional Animal Ethics Committee (IAEC) under Project No. 414/VPT/2023.

2.3 Experimental Design and Dietary Treatments

The experiment was conducted using a completely randomized design to evaluate the biochemical profile and antioxidant activity of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* in broiler chickens. A total

of 120-day-old Ven-Cobb broiler chicks were randomly allocated into ten dietary treatment groups (n = 12 per group) and reared for a period of 35 days. Group I received a basal diet and served as the control, while Group II received a vitamin E and selenium supplementation as a standard antioxidant control. Groups III and IV were fed a basal diet supplemented with *Trachyspermum ammi* powder at 5 and 10 g/kg feed, respectively. Groups V and VI received *Capsicum annuum* powder at 5 and 10 g/kg feed, respectively and Groups VII and VIII received *Zingiber officinale* powder at the same dose levels. Groups IX and X received a combination of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* at 5+5+5 g/kg feed and 10+10+10 g/kg feed, respectively. The detailed dietary treatment protocol is presented in Table 1. Experimental diets were prepared by uniformly mixing the respective plant powders with the basal feed and were offered throughout the experimental period under standard management conditions.

2.4 Collection of Blood Samples

Blood samples were collected aseptically from the wing vein of birds on days 7, 21 and 35 of the experimental periods into plain collection vials. Samples were allowed to clot at room temperature and centrifuged to separate serum, which was stored at -55 °C until further biochemical and antioxidant analysis.

2.5 Biochemical Analysis

Serum biochemical parameters were estimated using commercially available diagnostic kits following the manufacturer's protocols. Total protein and albumin concentrations were determined using standard kit-based colorimetric methods. Serum globulin concentration was calculated by subtracting albumin values from total protein values and the albumin-to-globulin (A/G) ratio was subsequently derived. Absorbance was measured at specified wavelengths using a spectrophotometer and all estimations were performed in duplicate to ensure analytical accuracy.

2.6 Assessment of Antioxidant Status

2.6.1 Superoxide Dismutase (SOD) Activity

Serum superoxide dismutase activity was estimated using a commercially available assay kit according to the manufacturer's instructions. The method is based on the ability of superoxide dismutase to inhibit the oxidation of hydroxylamine by superoxide anion radicals generated through the xanthine-xanthine oxidase system. In the absence of SOD, superoxide radicals react with hydroxylamine to form nitrite, which produces a colored complex with a chromogenic reagent. The presence of SOD suppresses this reaction, resulting in reduced color development. Absorbance was recorded at 550 nm using a microplate reader and SOD activity was expressed as units per milliliter of serum, where one unit was defined as the amount of enzyme required to inhibit 50% of superoxide radical formation under assay conditions.

2.6.2 Malondialdehyde (MDA) Concentration

Serum malondialdehyde concentration was determined using a commercially available kit following the manufacturer's protocol as an index of lipid peroxidation. The assay is based on the reaction of malondialdehyde with

thiobarbituric acid under acidic and high-temperature conditions to form a colored thiobarbituric acid reactive substance. The intensity of the color formed is directly proportional to the malondialdehyde concentration in the sample. After incubation and centrifugation, absorbance was measured at 532 nm using a microplate reader. Malondialdehyde levels were calculated from a standard calibration curve and expressed as $\mu\text{mol/L}$ of serum.

2.7 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).

3. Result

3.1 Biochemical Investigations

3.1.1 Serum Total Protein

The mean serum total protein concentrations (g/dL) of broiler chickens at the 1st, 3rd and 5th weeks are presented in Table 2 and graphically illustrated in Figure 1. During the 1st and 3rd weeks, no statistically significant differences were observed among the experimental groups. At the 5th week (day 35), serum total protein levels were significantly ($p < 0.05$) higher in all phytogetic-supplemented groups and the vitamin E and selenium group compared to the control group. The serum total protein values of birds supplemented with *Trachyspermum ammi*, *Capsicum annuum*, *Zingiber officinale* and their combinations were statistically comparable to those of the vitamin E and selenium-supplemented group.

3.1.2 Serum Albumin

Mean serum albumin concentrations (g/dL) recorded at different experimental intervals are shown in Table 3 and illustrated in Figure 2. No significant differences were observed among treatment groups during the 1st and 3rd weeks. At the 5th week, serum albumin levels were significantly ($p < 0.05$) increased in birds supplemented with *Trachyspermum ammi* at 5 and 10 g/kg feed, *Zingiber officinale* at 10 g/kg feed and the high-dose combination group compared to the control. Serum albumin levels in birds receiving *Capsicum annuum* and the low-dose combination did not differ significantly from the control group.

3.1.3 Serum Globulin

The mean serum globulin concentrations (g/dL) across experimental groups are presented in Table 4 and graphically depicted in Figure 3. Serum globulin levels did not differ significantly among groups during the 1st and 3rd weeks. At the 5th week, a significant ($p < 0.05$) increase in serum globulin concentration was observed in all phytogetic-supplemented groups and the vitamin E and selenium group compared to the control. The highest serum globulin values were recorded in birds receiving the combined supplementation of *Trachyspermum ammi*,

Capsicum annuum and *Zingiber officinale* at the lower dose level.

3.1.4 Albumin to Globulin Ratio (A/G Ratio)

The albumin-to-globulin (A/G) ratio values at different experimental periods are summarized in Table 5 and illustrated in Figure 4. No significant differences were observed among groups during the 1st and 3rd weeks. At the 5th week, a significant ($p < 0.05$) reduction in the A/G ratio was observed in birds supplemented with *Capsicum annuum*, *Zingiber officinale*, their combinations and vitamin E and selenium compared to the control group. Birds supplemented with *Trachyspermum ammi* alone did not show a significant change in A/G ratio compared to the control.

3.2 Assessment of Antioxidant Enzymes

3.2.1 Superoxide Dismutase (SOD)

The serum superoxide dismutase (SOD) activity (U/mL) measured at the 5th week is presented in Table 6 and graphically represented in Figure 5. A significant ($p < 0.05$) increase in SOD activity was observed in birds supplemented with *Trachyspermum ammi* (10 g/kg feed), *Capsicum annuum* (5 and 10 g/kg feed), *Zingiber officinale* (10 g/kg feed), both combination groups and the vitamin E and selenium group compared to the control. Birds supplemented with *Capsicum annuum* at 5 g/kg feed showed the highest SOD activity among all experimental groups.

3.2.2 Malondialdehyde (MDA)

The serum malondialdehyde (MDA) concentrations (μM) are presented in Table 6 and illustrated in Figure 6. The control group recorded the highest MDA levels. A significant ($p < 0.05$) reduction in MDA concentration was observed in all phytogetic-supplemented groups and the vitamin E and selenium group compared to the control. Birds supplemented with *Capsicum annuum* at 5 g/kg feed showed the lowest MDA concentration. Several phytogetic-supplemented groups exhibited MDA values statistically comparable to or lower than those of the vitamin E and selenium-supplemented group.

4. Discussion

The alterations observed in serum biochemical parameters in the present study indicate a positive influence of dietary supplementation with *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* on protein metabolism and physiological status of broiler chickens. The significant increase in serum total protein, albumin and globulin concentrations by the fifth week of age is in close agreement with earlier findings reported for phytogetic feed additives in poultry. Similar improvements in serum total protein levels have been reported in broilers supplemented with ajwain powder or its extracts (Shroha *et al.*, 2016; Saei *et al.*, 2021; Mahala *et al.*, 2023) [30, 26, 15]. Increased protein levels suggest enhanced hepatic synthetic activity and improved utilization of dietary amino acids. Consistent findings were also reported in quails fed ajwain-supplemented diets, where increased serum total protein and albumin were observed (Kheiri *et al.*, 2018) [11]. In contrast, Tayeb *et al.* (2015) [31] reported a reduction in serum protein parameters in red pepper-supplemented quails, indicating that the response to phytogetic additives may vary depending on species, dose and experimental conditions.

The elevation in serum albumin observed particularly in *Trachyspermum ammi*-supplemented groups reflect improved liver function, as albumin synthesis occurs exclusively in hepatocytes. Similar increases in albumin concentration following ajwain and ginger supplementation have been documented earlier (Rathor and Meel, 2023; El-Kashef, 2022) [23, 7]. Albumin also serves as an important antioxidant and transporter protein, suggesting improved metabolic homeostasis. Serum globulin concentration, a key indicator of immune status, was significantly increased in phyto-genic-supplemented groups. This observation aligns with previous reports where ginger supplementation resulted in elevated globulin levels, reflecting enhanced immunoglobulin synthesis (Morsy *et al.*, 2013; Abdel-Fatt *et al.*, 2008) [17, 1]. Increased globulin levels may indicate improved humoral immune competence and antibody production. Consequently, the observed reduction in the albumin-to-globulin ratio in supplemented groups is considered beneficial, as it reflects enhanced immune protein synthesis rather than impaired albumin production. The combined supplementation groups showed more pronounced improvements in protein profiles compared to individual treatments, suggesting a possible synergistic effect among bioactive constituents of ajwain, capsicum and ginger. These findings indicate that phyto-genic feed additives positively modulate protein metabolism, liver function and immune-related biochemical indices in broiler chickens.

Oxidative stress is a major challenge in intensive poultry production and antioxidant enzymes play a pivotal role in maintaining redox balance. In the present study, dietary supplementation with *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* significantly enhanced antioxidant defense, as evidenced by increased serum superoxide dismutase (SOD) activity and decreased malondialdehyde (MDA) levels. The increase in SOD activity observed in phyto-genic-supplemented groups corroborates earlier reports demonstrating enhanced antioxidant enzyme activity following capsicum and ginger supplementation (Abdelnour *et al.*, 2018; Liu *et al.*, 2021;

Sadeghi and Moghaddam, 2018) [2, 13, 25]. SOD constitutes the first line of enzymatic defense against superoxide radicals and its elevation reflects improved cellular protection against oxidative damage. Al-Khalaifah *et al.* (2022) [3] similarly reported increased hepatic SOD activity in broilers receiving ginger-supplemented diets. Malondialdehyde, a marker of lipid peroxidation, was significantly reduced in all phyto-genic-supplemented groups, indicating lower oxidative stress. These findings are consistent with previous studies reporting reduced MDA levels following supplementation with ajwain essential oil, red pepper oil and ginger powder (Hazrati *et al.*, 2020; Reda *et al.*, 2020; Safiullah *et al.*, 2019; Namdeo *et al.*, 2022). [9, 24, 27, 19] Reduced lipid peroxidation suggests effective scavenging of free radicals and stabilization of cell membranes. The antioxidant potential of *Trachyspermum ammi* is largely attributed to thymol-rich essential oil, which exhibits strong free radical-scavenging activity and inhibits lipid peroxidation (Vitali *et al.*, 2016) [33]. *Capsicum annuum* contains capsaicinoids, carotenoids, flavonoids and vitamin C, which collectively contribute to its potent antioxidant capacity (El-Ghorab *et al.*, 2013; Jayaprakasha *et al.*, 2012) [6, 10]. Capsaicin, in particular, has been shown to neutralize free radicals and suppress oxidative degradation of lipids and DNA (Liu *et al.*, 2015; Zhang *et al.*, 2017) [12, 34]. Furthermore, improved absorption of sulfur-containing amino acids may enhance glutathione synthesis, strengthening intracellular antioxidant defenses (Elnesr *et al.*, 2019) [8]. The antioxidant effects of *Zingiber officinale* are associated with its ability to regulate cytoprotective gene expression and enhance endogenous antioxidant systems (Mountzouris *et al.*, 2020) [18]. Gingerols and shogaols present in ginger effectively suppress lipid peroxidation and improve oxidative stability, leading to reduced MDA concentrations. Overall, the combined improvement in SOD activity and reduction in MDA levels demonstrate that supplementation with ajwain, capsicum and ginger particularly at appropriate inclusion levels effectively enhances antioxidant status and mitigates oxidative stress in broiler chickens.

Table 1: Experimental Design for Evaluating Biochemical profile and Antioxidant Effects of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* Supplementation in Broiler Chickens

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		

Table 2: 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 st week (Day 7)	3 rd week (Day 21)	5 th week (Day 35)
I	2.68 ± 0.14	2.88 ± 0.12	3.20 ± 0.25 ^a
II	3.03 ± 0.10	3.49 ± 0.10	4.47 ± 0.16 ^{bc}
III	2.84 ± 0.12	2.99 ± 0.07	4.43 ± 0.16 ^{bc}
IV	2.87 ± 0.17	3.05 ± 0.18	4.60 ± 0.14 ^{bc}
V	2.81 ± 0.09	3.07 ± 0.13	4.01 ± 0.09 ^b
VI	2.86 ± 0.15	3.09 ± 0.11	4.07 ± 0.07 ^b
VII	2.70 ± 0.12	3.17 ± 0.25	4.12 ± 0.30 ^b
VIII	2.77 ± 0.14	3.22 ± 0.08	4.49 ± 0.23 ^{bc}
IX	3.02 ± 0.19	3.51 ± 0.34	4.96 ± 0.22 ^c
X	2.82 ± 0.10	3.05 ± 0.11	4.56 ± 0.24 ^{bc}

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 albumin in broiler (n=6)

Groups	Serum albumin (g/dl)		
	1 st week (Day 7)	3 rd week (Day 21)	5 th week (Day 35)
I	1.19 ± 0.06	1.21 ± 0.04	1.23 ± 0.04 ^a
II	1.23 ± 0.03	1.28 ± 0.05	1.43 ± 0.05 ^b
III	1.22 ± 0.05	1.25 ± 0.03	1.66 ± 0.03 ^c
IV	1.20 ± 0.05	1.23 ± 0.04	1.63 ± 0.03 ^c
V	1.30 ± 0.02	1.33 ± 0.03	1.33 ± 0.03 ^{ab}
VI	1.27 ± 0.05	1.32 ± 0.03	1.35 ± 0.06 ^{ab}
VII	1.14 ± 0.03	1.24 ± 0.02	1.33 ± 0.03 ^{ab}
VIII	1.13 ± 0.03	1.26 ± 0.02	1.38 ± 0.03 ^b
IX	1.18 ± 0.04	1.23 ± 0.02	1.35 ± 0.03 ^{ab}
X	1.21 ± 0.05	1.28 ± 0.04	1.43 ± 0.04 ^b

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 serum globulin in broiler (n=6)

Group	1 st Week (Day 7)	3 rd Week (Day 21)	5 th Week (Day 35)
I	1.49 ± 0.09	1.68 ± 0.11	1.97 ± 0.26 ^a
II	1.80 ± 0.10	2.21 ± 0.13	3.04 ± 0.19 ^{bc}
III	1.62 ± 0.10	1.74 ± 0.08	2.77 ± 0.17 ^b
IV	1.68 ± 0.12	1.82 ± 0.15	2.97 ± 0.14 ^{bc}
V	1.51 ± 0.07	1.73 ± 0.14	2.68 ± 0.08 ^b
VI	1.59 ± 0.12	1.77 ± 0.11	2.73 ± 0.07 ^b
VII	1.56 ± 0.11	1.94 ± 0.26	2.80 ± 0.30 ^b
VIII	1.64 ± 0.13	1.95 ± 0.09	3.10 ± 0.24 ^{bc}
IX	1.85 ± 0.20	2.27 ± 0.35	3.61 ± 0.21 ^c
X	1.61 ± 0.11	1.77 ± 0.12	3.13 ± 0.27 ^{bc}

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

Table 5: Effect of dietary supplementation of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* powder alone and in combination on albumin to globulin ratio (A/G ratio) in broiler (n=6)

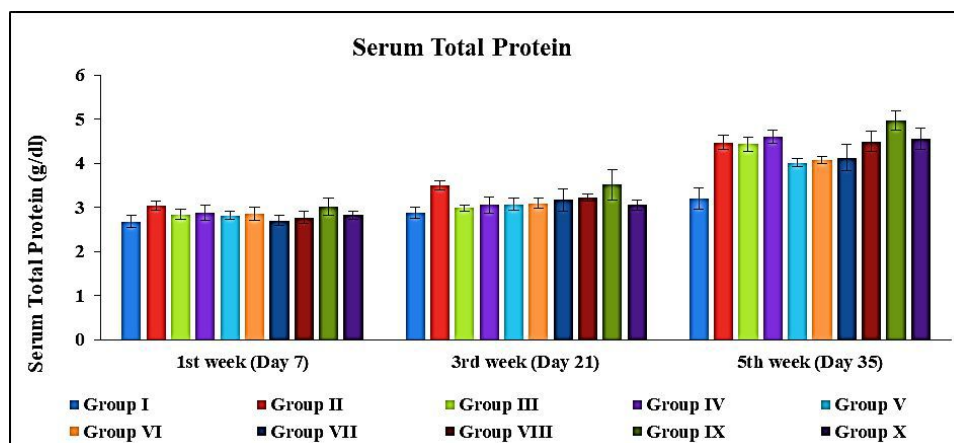
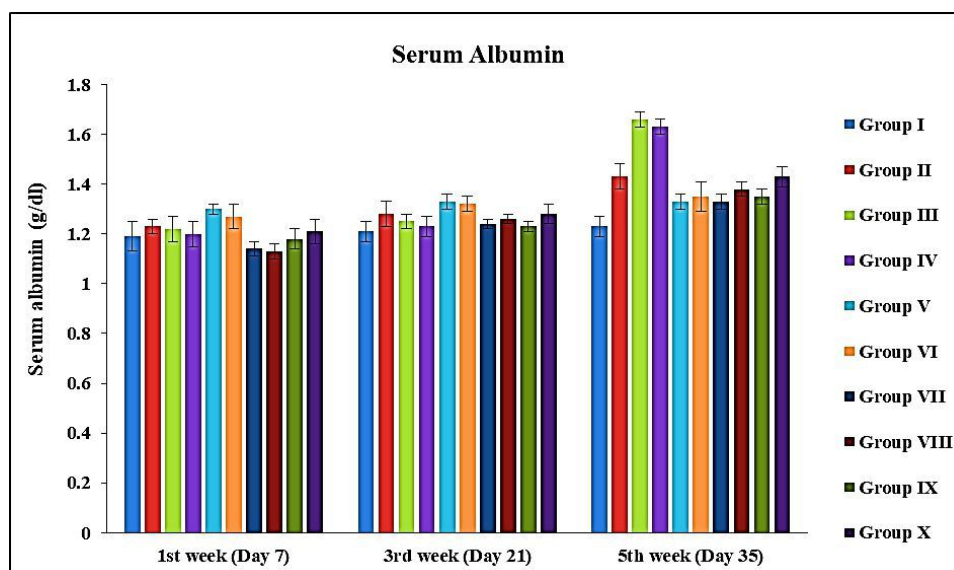
Groups	Albumin/Globulin ratio		
	1 st week (Day 7)	3 rd week (Day 21)	5 th week (Day 35)
I	0.80 ± 0.04	0.73 ± 0.04	0.68 ± 0.09 ^c
II	0.70 ± 0.06	0.60 ± 0.06	0.48 ± 0.05 ^{ab}
III	0.76 ± 0.05	0.73 ± 0.04	0.61 ± 0.04 ^{bc}
IV	0.72 ± 0.02	0.69 ± 0.04	0.55 ± 0.03 ^{bc}
V	0.87 ± 0.03	0.80 ± 0.08	0.50 ± 0.02 ^{ab}
VI	0.81 ± 0.04	0.76 ± 0.06	0.50 ± 0.03 ^{ab}
VII	0.75 ± 0.04	0.70 ± 0.10	0.51 ± 0.07 ^{ab}
VIII	0.71 ± 0.06	0.65 ± 0.04	0.46 ± 0.04 ^{ab}
IX	0.67 ± 0.07	0.60 ± 0.08	0.38 ± 0.02 ^a
X	0.77 ± 0.06	0.74 ± 0.06	0.48 ± 0.05 ^{ab}

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

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

Groups	Serum antioxidant enzymes (5 th Week)	
	SOD enzyme (U/ml)	MDA (μ M)
I	283.77 $\pm$ 3.47 ^a	11.17 $\pm$ 0.49 ^f
II	320.17 $\pm$ 6.28 ^e	8.25 $\pm$ 0.35 ^{de}
III	294.36 $\pm$ 2.70 ^{ab}	8.51 $\pm$ 0.30 ^e
IV	310.12 $\pm$ 3.09 ^{de}	6.19 $\pm$ 0.27 ^b
V	338.20 $\pm$ 5.51 ^f	4.24 $\pm$ 0.17 ^a
VI	296.94 $\pm$ 3.09 ^{bc}	6.81 $\pm$ 0.30 ^{bc}
VII	292.47 $\pm$ 5.84 ^{ab}	6.62 $\pm$ 0.33 ^{bc}
VIII	309.38 $\pm$ 2.30 ^{cde}	7.88 $\pm$ 0.46 ^{de}
IX	299.48 $\pm$ 2.93 ^{bcd}	6.91 $\pm$ 0.15 ^{bc}
X	300.19 $\pm$ 3.61 ^{bcd}	7.35 $\pm$ 0.08 ^{cd}

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

**Fig 1:** Effect of dietary supplementation of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* powder alone and in combination on serum total protein (g/dl) in broiler**Fig 2:** Effect of dietary supplementation of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* powder alone and in combination on serum albumin (g/dl) in broiler

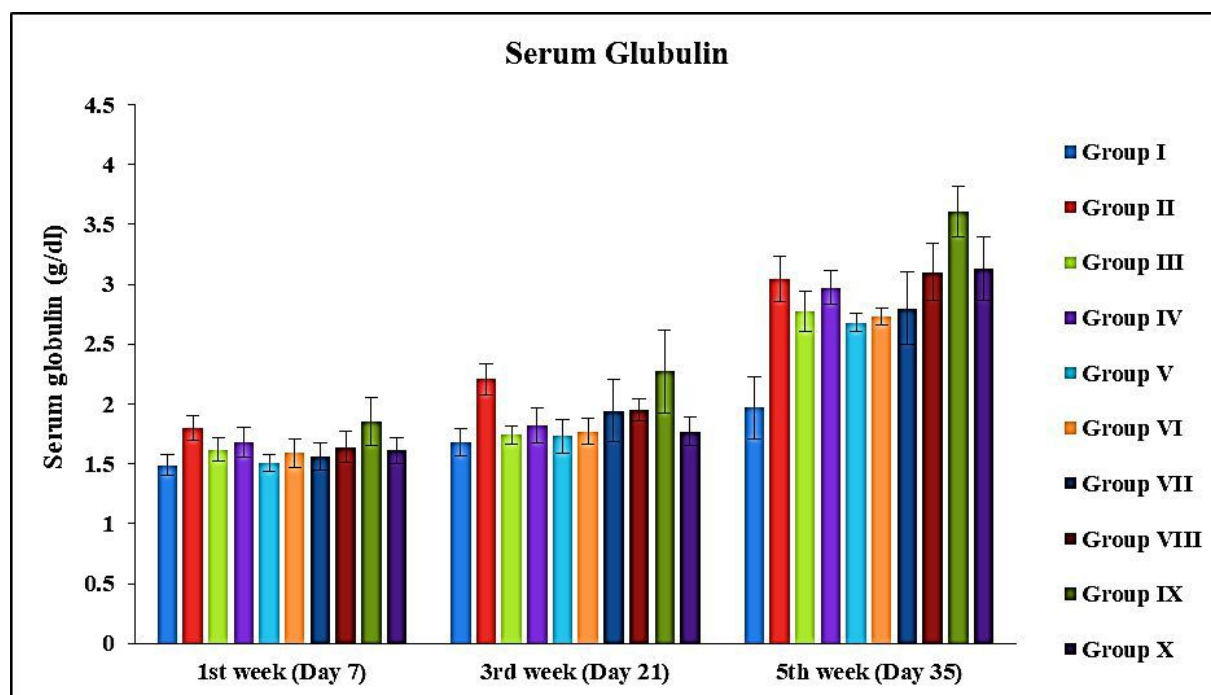


Fig 3: Effect of dietary supplementation of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* powder alone and in combination on serum globulin (g/dl) in broiler

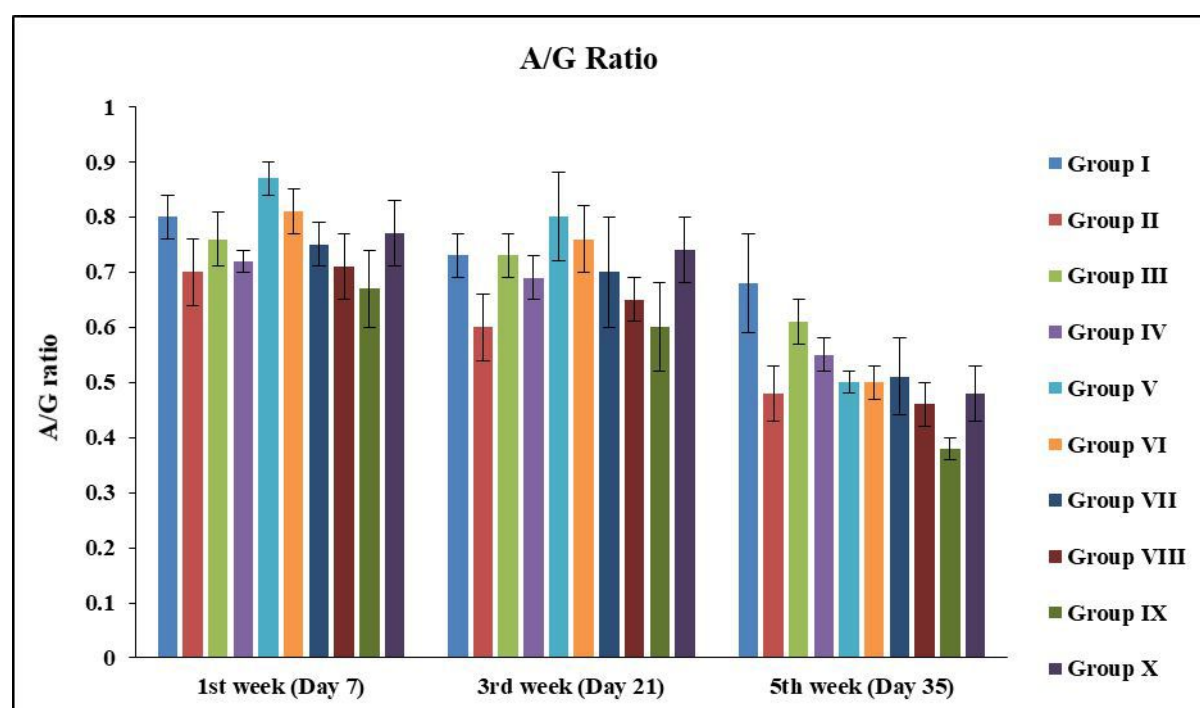


Fig 4: Effect of dietary supplementation of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* powder alone and in combination on albumin to globulin ratio (A/G ratio) in broiler

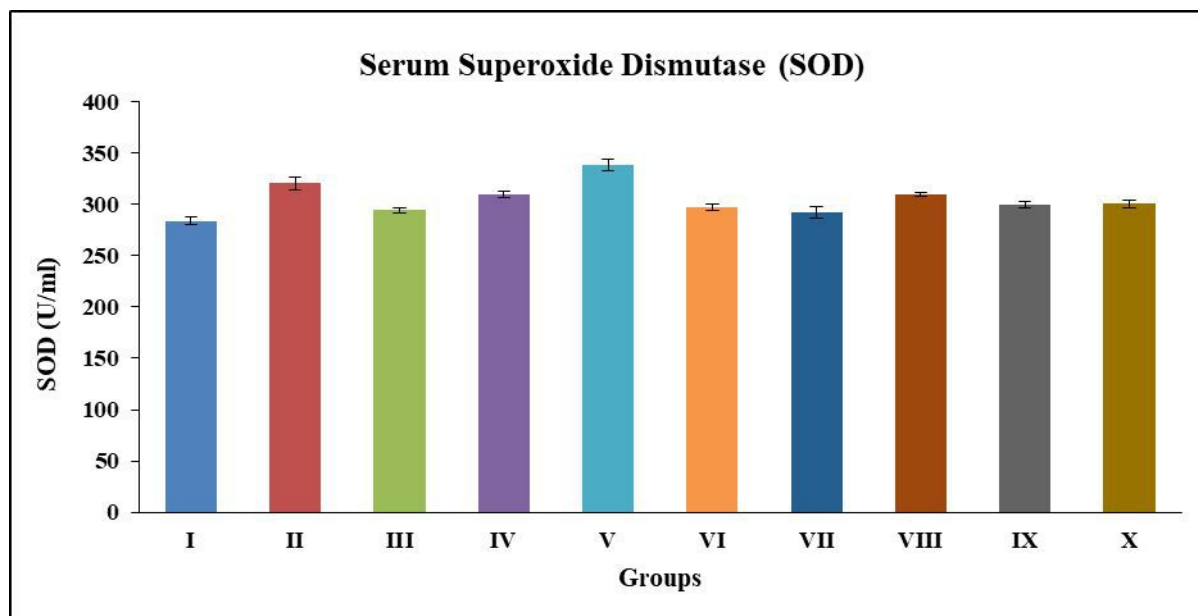


Fig 5: Effect of dietary supplementation of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* powder alone and in combination on serum SOD enzyme (U/ml) in broiler

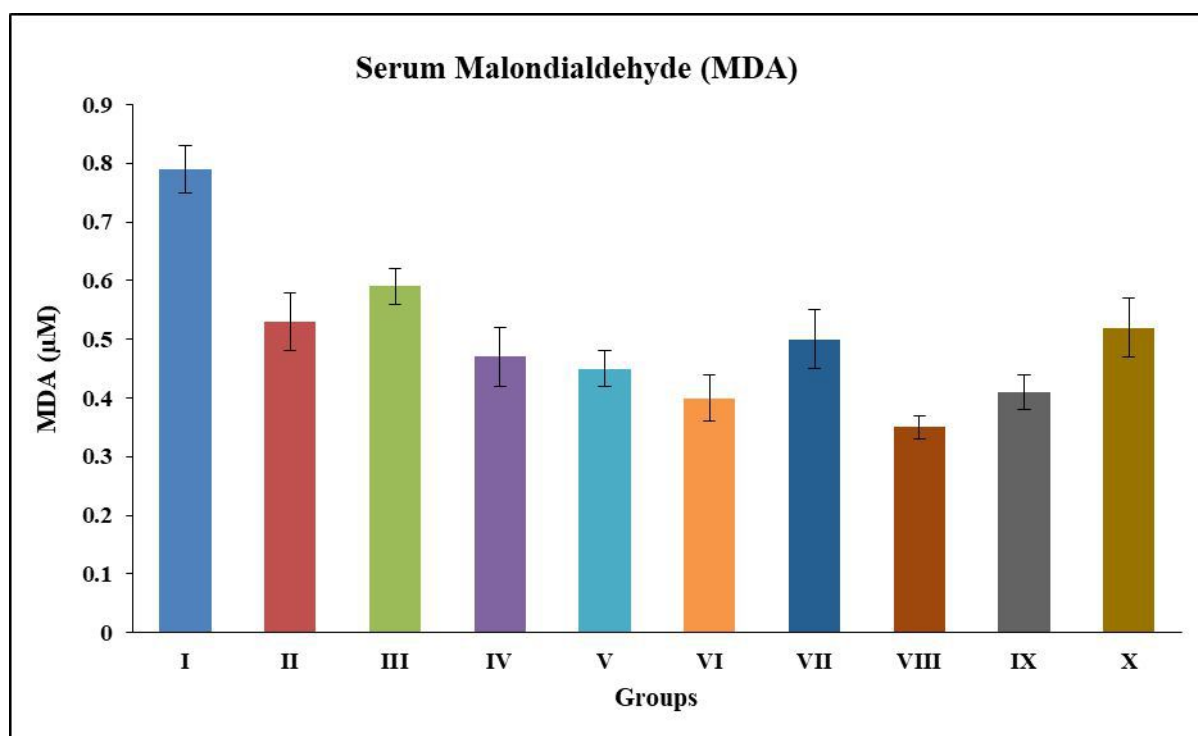


Fig 6: Effect of dietary supplementation of *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* powder alone and in combination on serum MDA (μM) in broiler

4. Conclusion

Based on the findings of the present study, it can be concluded that dietary supplementation with *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale* positively influences serum biochemical parameters and antioxidant status in broiler chickens. The significant increase in serum total protein, albumin and globulin levels indicates improved protein metabolism, hepatic function and immune-related biochemical responses. The reduction in albumin-to-globulin ratio observed in several supplemented groups further suggests enhanced immune protein synthesis. The antioxidant evaluation demonstrated that phytogetic supplementation significantly

elevated serum superoxide dismutase activity while reducing malondialdehyde concentration, reflecting improved antioxidant defense and reduced lipid peroxidation. Among the tested additives, *Capsicum annuum* at 5 g/kg feed exhibited the most pronounced antioxidant effect, comparable to or superior to vitamin E and selenium. Overall, the results indicate that *Trachyspermum ammi*, *Capsicum annuum* and *Zingiber officinale*, particularly when used at appropriate inclusion levels, can serve as effective natural alternatives to synthetic antioxidants for improving biochemical homeostasis and oxidative stability in broiler chickens. Their use may contribute to sustainable poultry production by enhancing

bird health while reducing reliance on conventional antioxidant supplements.

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7. Conflict of interest

The authors declared no conflict of interest.

8. Financial Support

None declared.

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