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Effect of dietary supplementation of herbal immuno-modulator on growth performance, immune response and serum biochemical indices of broiler chickens

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In poultry production, immune-modulators are used to strengthen the birds' natural defenses against diseases, promote general health, increase productivity, and reduce the need for antibiotics. A 6-weeks study was designed to determine the efficacy herbal immuno-modulator (HIM) on growth performance, immune response and serum biochemical indices of broiler chickens. A total of 240 straight run day old broiler chicks (CARIBRO Dhanraja) were allotted to six treatment groups in a completely randomized design with 5 replicates of 8 birds each and were fed *ad libitum*. The six dietary groups consist of: T_1 (Control): Basal diet; T_2: T_1 + Commercial immunomodulator (SELVIT-E); T_3: T_1 + 1% HIM; T_4: T_1 + 1.5% HIM; T_5: T_1 + 2% HIM; T_6: T_1 + 2.5% HIM. A significantly ($P \le 0.05$) higher body weight gain (0–6 week) was observed in T_5 with 2% HIM and T_2 groups (commercial immune-modulator). The feed intake was significantly ($P \le 0.05$) lower in T_6, whereas feed conversion ratio (FCR) did not differ significantly ($P \ge 0.05$) among the dietary treatment groups. Humoral immune response (HMI) was improved ($P \le 0.05$) on addition of HIM and highest haem-agglutination (HA) titre was observed in group T_6 and T_5 with 2.5 and 2% HIM respectively. Higher cellular response against PHA-P was recorded in T_5 and T_4. Additionally, the relative weight of bursa was significantly greater ($P \le 0.05$) in T_5 group. Significantly ($P \le 0.05$) higher serum albumin level was found in T_5 group, however other serum indices were comparable between dietary and control groups. Although the carcass traits were comparable between the dietary groups, live weight and carcass yield were found to be highest in group T_5. This study concluded that the addition of 2% HIM in broiler diet could enhance the production and immune response and serum biochemical indices in broiler chicken. Immuno-modulators offer a valuable tool for improving poultry production, but careful consideration of their potential benefits and risks is essential. By modulating the immune system, these additives can enhance the birds' ability to fight off infections, reduce the impact of stress, and improve production efficiency.

Keywords Immunomodulator, Biochemical indices, Medicinal plants, Feed additives immune response, Broiler

The poultry industry has established a strong position in the agriculture sector by providing essential animal protein to a rising population. In contemporary poultry farming, providing optimal development, health, and disease resistance in broilers is critical to achieving high productivity. Synthetic antibiotics have long been utilized as growth promoters to improve performance and feed efficiency in intensive poultry farming[1]. However, growing concerns about antibiotic resistance, residual effects on consumers, and the demand for safer, natural alternatives have led to the exploration of herbal extracts as viable solutions in poultry nutrition. This demand

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for safer and viable alternatives was also hastened by the European Union's restriction on the use of antibiotics in feedstuffs for animals only as growth promoters in a number of nations[2]. In order to limit its use, strengthening the immune system by dietary manipulation using herbal combinations can be an efficient strategy.

Herbal combinations as immuno-modulators, derived from medicinal plants have gained attention as potential alternatives due to their ability to strengthen the immune system, promote gut health, and improve metabolic functions in broilers. These natural supplements contain phytochemicals (flavonoids, lycopene, curcumin etc.) described as residue-free and non-toxic plant-derived substances[3] with antimicrobial, antioxidant, and immune-modulatory properties that support overall well-being. By stimulating the immune response and reducing oxidative stress, herbal additives contribute to enhanced disease resistance and improved performance, minimizing the need for conventional antibiotics. Turmeric (*Curcuma longa*), most widely used spice in India, contains curcumin as a major active ingredient has the potential to boost the immune status in broiler chicken through the proliferation of white pulp of spleen, rising the antibody titre against virus and increasing the lymphocytes and heterophils[4]. A remarkable improvement in broiler performance in terms of weight gain, and feed conversion ratio is also witnessed by the dietary addition of turmeric powder[5–8]. Amla (*Phyllanthus emblica* or *Emblica officinalis*), a plant enriched with vitamin C, have significantly boosted the immunity in broilers through raising antibody tires and cell mediated immune responses[9,10]. Dietary inclusion of fenugreek (*Trigonella foenum-graecum*) has considerable impact on functioning of immune system via the regulation of cell mediated and humoral immune response. It activates various immune responsive cells and increases circulating levels of antibody against various pathologic agents[11,12]. Giloy (*Tinospora cordifolia*) also named as Guduchi or Geloy is a widely distributed medicinal plant with antioxidative, antimicrobial, anti-inflammatory effects[13]. Although plant-based compounds offer promising alternatives to antibiotics, challenges remain in optimizing their efficacy and ensuring consistent performance outcomes, particularly concerning the combination form, dosage, and nature of the product. The aim of this study was to evaluate the effects of polyherbal immunomodulators on broiler performance and determine how effectively they promote growth, immunity, feed conversion efficiency, and overall health.

Materials and methods

Ethics declarations

The study conducted in accordance with the regulatory framework outlined by the 'Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) 2012', as laid down under the 'Prevention of Cruelty to Animals Act 1960' of the Indian Penal Code. The experimental protocols employed in this investigation received explicit approval from the Institutional Animal Ethics Committee (IAEC) of ICAR-Central Avian Research Institute, Bareilly, 243,122, India. The approval number is 467/GO/S/05/CPCSEA-24/09/2023 (Project code: P-1/2023/1-IAV/L34/3900/6233, Duration: 01-09-2023 to 31-08-2025). The research was done in accordance with the ARRIVE (Animal Research: Reporting of *in- vivo* Experiments) standards.

Animals and experimental design

Following a completely randomized design (CRD), a total of two hundred forty (n = 240) healthy day-old broiler chicks (CARIBRO-Dhanaraja) of same hatch were obtained from institute hatchery (ICAR-Central Avian Research Institute, Izatnagar, India) for this biological feeding trial. Each chick was distinctly identified using wing bands, weighed individually, and randomly assigned to six dietary groups, each consisting of five replicates with 8 birds per replicate. The birds were maintained under uniform husbandry conditions from day one until 42 days of age with a housing system consist of battery brooder equipped with waterer and feeder with a continuous lighting duration of 23 h per day throughout the study period.

Diet formulation

The basal diet was formulated to fulfil the nutrient requirement of ICAR[14] for broiler chicken and the ingredients and nutrient composition of diet is presented in Table 1. The control group (T_1) was fed with basal diet without any supplementation. The herbal immuno-modulator (*Curcuma longa, Phyllanthus emblica*, *Ocimum sanctum*, *Trigonella foenum-graecum, Tinospora cordifolia* and *Foeniculum vulgare* were obtained from institute herbal garden) was added at rate of 1.0, 1.5, 2.0 and 2.5% into basal diet in T_3, T_4, T_5 and T_6 treatment groups, respectively, whereas commercial immunomodulator (SELVIT-E) was used in T_2 group.

Production performance

Weekly and overall body weight gains (BWG) measurements were taken throughout the investigation. Each dietary regimen received a weighed quantity of each diet every morning, and the remainder was weighed the following day to calculate the total amount of feed consumed. Using information from feed intake (FI) and BWG, the weekly and period-wise feed conversion ratio (FCR) of birds was computed. Mortality as and when occurred was monitored on regular basis (Table 2).

Immune response

The humoral immunity was estimated in six birds per treatment by measuring antibody titer to 1% sheep red blood cell (SRBC). A phosphate-buffered (pH = 7.4) suspension of SRBC (1%) was injected (one ml) into the jugular vein of birds at 27 days of age. After 5 days of injection, blood was drawn from the jugular vein and serum was separated for estimation of Haemagglutination agglutination (HA) activity. The antibody titres ($\log_2$) were measured following the standard procedure given by Siegel and Gross[15]. *In-vivo* cell-mediated immune response (CMI) was estimated by using method described by computed Cheng and Lamont[16]. For this, cutaneous basophilic hypersensitivity test was done using Phytohemagglutinin-P (PHA-P) at 35 days (N = 10). The micro-

Ingredients (%)	Pre-starter	Starter	Finisher
Maize	51.435	54.955	61.585
Soybean meal	41.2	38.2	32
Rape seed meal	3	3	3
Rice bran oil	1	0.6	0.4
Limestone	0.9	0.9	0.9
Di-calcium phosphate	1.7	1.6	1.4
Salt	0.3	0.3	0.3
DL-Methionine	0.15	0.13	0.1
*TM Premix	0.1	0.1	0.1
**Vit. Premix	0.15	0.15	0.15
***Vit B complex	0.015	0.015	0.015
Choline chloride	0.05	0.05	0.05
Total	100	100	100

Table 1. Ingredients and nutrient composition of broiler pre-starter, starter and finisher basal diet. *TM Premix: Each gram of total mineral premix provided: 300 mg of Mg, 55 mg of Mn, 4 mg of I, 56 mg of Fe, 30 mg of Zn and 4 mg of Cu. **Vitamin premix: Each gram of Vit A, B2, D3, K (Spectromix, Ranboxy) provided: 540 mg of Vit.A (Retinol), 50 mg of Vit.B2 (Riboflavin), 400 mg of Vit.D3 (Cholecalciferol), 10 mg of Vit K (Menadione). ***Vit. B. Complex: Each gram of B complex provided: 2 mg of Vit.B1(Thiamine), 10 mg Folic acid, 4 mg of Pyridoxine HCl, 10 µg of Cyanocobalamin, 12 mg of Nicotinamide.

	Body weight gain (BWG) (g)			Feed intake (FI) (g)			Feed conversion ratio (FCR)			
Diet	0–3 week	3–6 week	0–6 week	0–3 week	3–6 week	0–6 week	0–3 week	3–6 week	0–6 week	Mortality (%)
T_1	478.63^{ab}	1021.87^{ab}	1500.50^{ab}	765.25^{ab}	2020.59^{a}	2785.84^{a}	1.61	2.01^{a}	1.87	3.25
T_2	483.92^{a}	1052.45^{a}	1536.37^{a}	763.93^{ab}	2028.13^{a}	2792.05^{a}	1.60	1.97^{ab}	1.84	0.75
T_3	465.35^{ab}	974.98^{b}	1440.33^{b}	756.45^{b}	1985.30^{ab}	2741.75^{ab}	1.65	2.07^{a}	1.92	2.50
T_4	454.54^{b}	1031.87^{ab}	1486.40^{ab}	780.31^{a}	1944.99^{b}	2725.30^{b}	1.75	1.91^{ab}	1.84	1.25
T_5	471.25^{ab}	1075.93^{a}	1547.18^{a}	785.25^{a}	1954.99^{b}	2740.24^{ab}	1.69	1.86^{b}	1.80	0.75
T_6	441.66^{c}	1015.45^{ab}	1457.11^{b}	713.19^{c}	2005.40^{ab}	2718.59^{b}	1.65	2.02^{a}	1.90	1.75
SEM	9.06	22.36	25.88	5.64	19.94	19.79	0.04	0.05	0.04	–
P	0.013	0.041	0.023	< 0.001	0.021	0.042	0.051	0.025	0.116	–

Table 2. Effect of herbal immune-modulator on production performance in broiler chickens. Mean values bearing the same superscript in a column did not differ significantly ($P < 0.05$). T_1 = Control, $T_2 = T_1$ + Commercial HI, $T_3 = T_1$ + 1.0% HI, $T_4 = T_1$ + 1.5% HI, $T_5 = T_1$ + 2.0% HI, $T_6 = T_1$ + 2.5% HI. HIM = Herbal Immuno-modulator. SEM = Standard error of mean.

meter measurements recorded at 0 h and 24 h of PHA-P and PBS injections at inter-digital space between 3rd and 4th toe in right and left foot respectively, were used to calculate CMI.

Serum biochemical parameters

Blood samples of 10 birds from each treatment on 42 days were collected from jugular vein in non EDTA containing vial and serum was separated by centrifugation (3000 rpm; 10 min). The concentration of total protein, albumin, triglycerides, cholesterol, AST and ALT were estimated using commercial kits (M/s Coral clinical systems, India) and absorbance for each was measured using spectrophotometer (Eppendorf Bio-Spectrometer® basic, Eppendorf AG, Germany).

Carcass traits

On 42th day of experiment, 6 birds from each treatment were sacrificed following a 12-h fasting period, during which they had unrestricted access to drinking water. Birds were humanly slaughtered, allowed to bleed properly, and then de-feathered and eviscerated under proper hygienic conditions. The eviscerated weight and weight of liver, gizzard and heart were recorded and expressed as percentage of live weight. The yield of cut up parts including breast, thighs, wings, neck and drumsticks also expressed as percentage of live weight.

Statistical analysis

The data obtained from the experiment were subjected to analysis of variance for a completely randomized design, using the SPSS software-26 by the methods of Snedecor and Cochran[17]. The mean differences were tested for statistical significance as per Duncan multiple range test (DMRT) as described by Duncan[18] with significance level defined at $P \leq 0.05$.

Results

Growth performance

The results of the herbal immune-modulator supplementation on broiler chicken performance parameters are presented in Table 2. The HIM supplementation had a significant influence ($P \le 0.05$) on body weight gain, feed intake and feed conversion ratio. During the starter phase (0–3 wk), significantly higher ($P \le 0.05$) body weight gain was noticed in group supplemented with SELVIT-E (commercial immune-booster) followed by control and T_5 groups. Group with the highest inclusion level of HIM (2.5%) exhibited the lowest body weight gain and reduced feed intake. Moreover, it was noted that body weight gain was highest ($P \le 0.05$) in T_5 group with 2% HIM supplementation and which was statistically similar to that of T_2 group during the finisher phase (3–6 week). A similar trend was observed for over all body weight gain (0–6 wk) in T_5 and T_2, where as T_3 and T_6 groups had lowest body weight gain, indicates HIM at both lower and higher levels may not be effective in promoting growth improvement in this study. According to the present results, a significant increase ($P \le 0.05$) in feed intake was noticed in T_5 and T_4 groups during the starter phase (0–3 wk), however the FCR was unaffected by the HIM supplementation during this phase. Further, highest ($P \le 0.05$) amount of feed intake was noticed in T_2 and T_1 group birds fed SELVIT-E and basal diet respectively during finisher phase (3–6 wk). A similar pattern was observed for overall feed intake (0–6 week), indicates that HIM supplementation had reduced feed intake in broiler chicken in the present study. However, FCR was significantly lower ($P \le 0.05$) in HIM supplemented groups (T_4 and T_5) during finisher phase and overall FCR was lower in T_4 and T_5 groups with 1.5 and 2% HIM supplementation.

Immune response and immune organ weight

The results of the herbal immunno-modulator supplementation on immune response and immune organ weight are presented in Table 3. Dietary supplementation of HIM shown significant ($P \le 0.05$) impact on both humoral and cell mediated immune response in this study. The highest ($P \le 0.05$) response to SRBC was found in group with 2.5% HIM supplementation followed by 2% HIM supplemented group. However, the groups with lower level of HIM supplementation were comparable with control. Similarly, significantly ($P \le 0.05$) better response against Phytohemagglutinin-P was observed after 24 h of injection in broiler fed 1.5 and 2% of HIM treated group. Although, the supplemented groups were significantly better than control group in cell mediated immune response as indicated by higher foot web index, the groups fed with lowest and highest level of HIM was similar to SELVIT-E fed group. The relative weight of bursa was significantly greater ($P \le 0.05$) in broiler chicken fed 2% HI and SELVIT-E, indicates that 2% HI is similar to that of commercial immune-booster in immune-modulatory activity. However, HI supplementation had no significant effect on relative weight of spleen and thymus.

Serum biochemical indices

The results of the herbal immuno-modulator supplementation on serum biochemical parameters are presented in Table 4. There was no significant effect of HIM supplementation on serum total protein, cholesterol, triglyceride concentration, however serum albumin level differs significantly ($P \le 0.05$) across the treatments. The highest serum albumen concentration was noticed in T_5 followed by T_4, T_3 and T_2 groups in which concentration was comparable with each other. The liver enzymes (AST and ALT) activity in broiler chicken fed with HIM was comparable to that of control group indicating no liver toxicity induced by dietary inclusion of herbal mixture.

Carcass traits and cut up parts

The results of the herbal immuno-modulator supplementation on carcass traits and cut up parts are presented in Tables 5 and 6. Dietary treatment had a significant ($P \le 0.05$) effect on live weight and carcass percentage, where highest value was observed in group supplemented with 2% HIM (T_5). The dressing percentage, relative weight of abdominal fat, heart, liver, gizzard, intestine and intestine length was unaffected by the dietary supplementation. The HIM addition significantly increased ($P \le 0.05$) the percent yield of cutup parts (thighs, drumsticks, back and neck) except for breast and wings in present study. The highest percentage of thigh yield was observed in

	Immune response		Immune organ weight (% of live weight)		
Diet	Foot web index (mm)	HA Titre ($\log_2$)	Spleen	Bursa	Thymus
T_1	0.41^{b}	2.88^{c}	0.20	0.15^{ab}	0.40
T_2	0.46^{ab}	3.18^{b}	0.26	0.18^{a}	0.43
T_3	0.48^{ab}	2.82^{c}	0.17	0.14^{b}	0.42
T_4	0.53^{a}	2.87^{c}	0.16	0.16^{ab}	0.44
T_5	0.55^{a}	3.33^{bc}	0.28	0.19^{a}	0.43
T_6	0.48^{ab}	3.51^{a}	0.24	0.17^{ab}	0.39
SEM	0.01	0.06	0.01	0.01	0.03
P	0.023	0.001	0.085	0.018	0.725

Table 3. Effect of herbal immune-modulator on immune response and immune organ weight in broiler chickens. Mean values bearing the same superscript in a column did not differ significantly ($P \le 0.05$). T_1 = Control, $T_2 = T_1$ + Commercial HI, $T_3 = T_1$ + 1.0% HI, $T_4 = T_1$ + 1.5% HI, $T_5 = T_1$ + 2.0% HI, $T_6 = T_1$ + 2.5% HI. HIM = Herbal Immuno-modulator. SEM = Standard error of mean.

Treatment	Total protein (g/dl)	Albumin (g/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	AST (U/L)	ALT (U/L)
T_1	4.32	1.73^{b}	91.51	74.08	129.10	14.70
T_2	4.28	1.88^{ab}	90.66	74.77	123.40	13.97
T_3	4.43	1.78^{ab}	89.19	71.24	125.90	14.99
T_4	4.18	1.91^{ab}	78.60	71.13	128.08	14.40
T_5	4.36	2.03^{a}	84.07	73.27	135.65	14.99
T_6	4.35	1.69^{b}	79.93	69.85	130.82	15.28
SEM	0.06	0.03	2.51	1.30	2.08	0.26
P value	0.93	0.005	0.55	0.89	0.66	0.77

Table 4. Effect of herbal immune-modulator on serum biochemical parameters in broiler chickens. Mean values bearing the same superscript in a column did not differ significantly ($P<0.05$). T_1 = Control, $T_2 = T_1$ + Comercial HI, $T_3 = T_1$ + 1.0% HI, $T_4 = T_1$ + 1.5% HI, $T_5 = T_1$ + 2.0% HI, $T_6 = T_1$ + 2.5% HI. HIM = Herbal Immuno-modulator.

Diet	Live weight (g)	Dressing percentage	Carcass yield	Abd. fat	Heart	Liver	Gizzard	Intestine	Intestine length (cm)
T_1	1520.50^{b}	72.68	64.72^{b}	1.63	0.45	2.58	2.75	5.56	171.50
T_2	1564.27^{ab}	70.25	64.65^{b}	1.73	0.51	2.57	2.53	5.26	183.50
T_3	1537.33^{b}	71.28	65.72^{ab}	1.68	0.49	2.43	2.64	5.46	189.17
T_4	1565.79^{ab}	71.10	65.84^{ab}	1.66	0.52	2.43	2.32	5.79	189.67
T_5	1605.67^{a}	70.44	66.89^{a}	1.89	0.46	2.67	2.59	5.94	186.67
T_6	1539.25^{b}	71.46	65.87^{ab}	1.64	0.53	2.62	2.44	5.73	187.00
SEM	21.65	0.22	0.21	0.09	0.01	0.05	0.05	0.09	2.14
P	0.006	0.073	0.008	0.974	0.124	0.614	0.274	0.349	0.129

Table 5. Effect of herbal immune-modulator on carcass traits (% of live weight) in broiler chicken. Mean values bearing the same superscript in a column did not differ significantly ($P<0.05$). T_1 = Control, $T_2 = T_1$ + Commercial HI, $T_3 = T_1$ + 1.0% HI, $T_4 = T_1$ + 1.5% HI, $T_5 = T_1$ + 2.0% HI, $T_6 = T_1$ + 2.5% HI. HI = Herbal Immuno-modulator. SEM = Standard error of mean.

Treatment	Breast	Thigh	Drumstick	Back	Wings	Neck
T_1	18.95	10.07^{b}	9.47^{ab}	14.22^{b}	8.53	1.82^{b}
T_2	18.10	10.28^{b}	10.03^{a}	15.82^{ab}	8.32	2.79^{a}
T_3	17.78	10.95^{ab}	9.17^{b}	14.34^{b}	8.56	2.03^{ab}
T_4	18.44	11.02^{a}	9.37^{ab}	14.59^{ab}	8.62	2.11^{ab}
T_5	19.13	11.35^{a}	9.52^{b}	16.60^{a}	8.38	2.82^{a}
T_6	18.90	10.96^{ab}	10.08^{a}	14.38^{b}	8.46	2.10^{ab}
SEM	0.45	0.17	0.14	0.42	0.16	0.13
P value	0.282	0.021	0.002	0.005	0.686	0.006

Table 6. Effect of herbal immune-modulator on cut up parts yield (% of live weight) in broiler chickens. Mean values bearing the same superscript in a column did not differ significantly ($P<0.05$). T_1 = Control, $T_2 = T_1$ + Comercial HI, $T_3 = T_1$ + 1.0% HI, $T_4 = T_1$ + 1.5% HI, $T_5 = T_1$ + 2.0% HI, $T_6 = T_1$ + 2.5% HI. HIM = Herbal Immuno-modulator. SEM = Standard error of mean.

T_4 and T_5 groups with 1.5 and 2% HIM respectively, whereas drumstick yield (%) was highest in T_6 with 2.5% HIM. The percentage yield for back and neck were higher in groups fed with 2% HIM and commercial immuno-modulator.

Discussion

The results suggest that, at high concentration (2%) HIM shown a tendency for growth promotion due to better feed intake and nutrient utilization. However, group with the highest inclusion level (2.5%), indicating that the HIM addition at this level may be undesirable to broiler chickens. The higher weight gain in HIM supplemented groups is suggestive of enhanced digestive function and nutrient absorption due to presence of active chemicals in the herbs. Similar results were noticed by Omar et al.[1] and Giannenas et al.[19], and they reported that herbs in a combined form added in to diet could improve the body weight gain in broiler chickens. Herbal supplementation shows a tendency for better overall FCR compared to control group in consistent to reports of Giannenas et al.[19].

The improved FCR in HIM groups may be due to activation of digestive enzymes in the gastrointestinal tract which resulted in improved nutrient utilization and overall growth performance. Although, the finisher phase feed intake results in present study are contradictory, the starter phase results are consistent with results of Omar et al.[1], who found increased feed intake in herbal supplemented group as compared to control. The variations in results may be due to the difference in herbs and their dosage regimen. In agreement with current study, Eevuri and Putturu[20] reported reduced feed intake due to dietary addition of turmeric, tulsi, amla and aloe vera in broiler's diet.

According to results of the present experiment, the polyherbal immuno-modulator might be enhanced the functioning of immune system. The improved immunity may be due the increasing avian T and B-cell multiplication stimulated by herbal metabolites which is known to have antibacterial and immune-modulatory activity. The results are consistent with result stated by Saleh et al.[21], who found that new castle disease (ND) titers at 28 days and after infection were significantly higher in herb mixture-supplemented groups (0.5, 1 and 1.5 kg/ton). Similarly, Gilani et al.[22] observed a higher antibody titre against ND in broiler chicken supplemented with phytobiotics (ginger, liquorice, ashwagandha, green tea and black seed). Further, Marimuthu et al.[9] observed that, CMI response was significantly increased in phytogenic feed additive group under extreme heat exposure which is in agreement with current study. In addition, potential of turmeric in immunomodulation was identified as enhanced CMI response by Choudhary et al.[23] by incorporating 1% turmeric in to broiler diet.

The results of the present study are consistent with Al-Homidan et al.[11] who found that, addition of fenugreek, mung bean, and garden cress into the diets of broiler chickens improved the relative weight of bursa of Fabricius. In addition, Islam and Sultana[24] found phytobiotics inclusion (clove and tulsi) in wholesome drinking water could improve the relative weight of spleen, thymus and bursa in broiler chickens. In contrast, supplementation of amla, turmeric, tulsi or combination[25], amla powder[26] and turmeric and ginger[27] had no appreciable changes in relative weight of immune organs. The better bursa weight in T_5 similar to that of T_2 might be owing to the combined effect of active components in the herbs at higher levels. The active ingredients may exert its effects by regulating the activity of various immune cells such as T-cells, B-cells, NK cells, and macrophages[28].

The results of the serum biochemical indices are consistent with results of Gilani et al.[22] and Saleh et al.[21] who also reported that no significant difference were observed after dietary supplementation of polyherbal supplementation. Higher concentration of albumin in herbal mixture supplemented group can be associated with better nutrient intake and greater absorption of amino acid which is the end products of protein metabolism eventually results in higher serum albumen concentration. In contrast to present results, serum triglycerides and cholesterol levels were reduced in herbal mixture supplemented groups[21]. The dietary addition showed no significant effect on AST and ALT activity in broiler chicken. Similar results in AST and ALT activity were observed by Singh et al.[29] who fed combination of *Allium sativum* bulbs, *O. sanctum* leaves and *N. sativa* seeds in broiler chicken.

The results of the current study are in agreement with Ali et al.[30] who observed similar results in broiler chickens, where the inclusion of fenugreek seed powder at 1.5% level in the diet contributed to increased carcass yield. Additionally, Atay[31] reported that herbal mixture containing garlic, ginger, and turmeric enhanced carcass yield more effectively than individual supplementation. The highest live weight and carcass yield in the HIM supplemented group could be attributed to the growth-promoting properties of herbal ingredients. The antimicrobial compounds present in the herbal mixture play a key role in modifying gastrointestinal function, thereby improving digestion, nutrient metabolism, and overall growth performance[32,33].

In alignment with present study, Tanwar et al.[34] and Gaikwad et al.[35] reported non-significant effect on dressing percentage in groups fed with aloe vera, tulsi or their combination and amla respectively. Similarly, dressing yield and internal organ weight (liver, heart, gizzard and intestine) was unaffected by turmeric supplementation in broiler chicken[36]. According to Patel et al.[37], garlic and fenugreek or their combination in broiler diet had shown no interaction with carcass traits such as dressing percentage, relative weight of liver, heart and gizzard, and length of small intestine.

These positive results of cutup parts are suggestive of better growth performance in HIM supplemented groups as their ingredients show antibacterial, immune-modulatory and growth promoting properties. Similar results for significant increment in cutup parts yield were observed in fenugreek seed powder (3%) fed broilers and mixture of gugal and fenugreek (50:50) fed broilers[38]. In contrast, Choudhury et al.[32] found no significant difference in percentage yield of cutup parts in turmeric fed broiler which aligns with breast and wing yield results of present study.

Conclusions
The results of the present research suggested that the addition of 2% herbal immune-modulator in broiler diet could enhance the production performance and immune response in broiler chicken and the combined use of different herbal extracts could create synergistic effects and have better overall results.

Data availability
The data produced during and/or analyzed during present study are obtainable from the corresponding author on sensible request.

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SR, KG and PP: trial, investigation, laboratory work writing original draft preparation; AB: conceptualization, designing, feed formulation, supervision, and manuscript review. CD, JJR: data evaluation, revising critically and providing comments on manuscript, editing; GK: data analysis, editing; data collection.

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Competing interests
We hereby declare that none of our financial or personal relationships with other individuals or groups could unduly influence our work, nor do we have any personal or professional interest in any good, service, or business that might be interpreted as influencing the content of this paper.

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