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Ahash T
PG Scholar, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal-637002, Tamil Nadu, India

Jagadeeswaran A
Professor and Head, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal-637002, Tamil Nadu, India

Sakthi Priya M
3Assitant professor, Department of Veterinary Pharmacology and Toxicology, Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal-637002, Tamil Nadu, India

Sukumar K
Professor and Head, Department of Veterinary Microbiology, Veterinary College and Research Institute, Namakkal-637002, Tamil Nadu, India

Correspondence:
Dr. A. Jagadeeswaran
Professor and Head, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal-637002, Tamil Nadu, India
Email: drjaggyvpt@gmail.com

Exploration of suitable ratio of inclusive ingredients in Triphala for improved performance in layer cockerels

Ahash T, Jagadeeswaran A, Sakthi Priya M, Sukumar K

ABSTRACT

Background: In poultry, effective immunostimulation not only strengthens defenses against pathogens but also ensures timely resolution of inflammation, allowing nutrients to be redirected to growth and productivity. It helps to counteract immunosuppression caused by stress factors and environmental challenges, thereby maintaining bird health and productivity. Proper immunostimulation can also improve resistance to pathogens, ultimately leading to better overall flock performance. **Objective**: The present study aimed to find out the suitable ration of inclusive ingredients of Triphala powder, viz., *Terminalia chebula, Terminalia bellerica* and *Emblica officinalis* (in order) by assessing their influences on growth, immune responses and antioxidant status in layer cockerels. **Materials and Methods:** A total of 140 BV-300 cockerels were divided into seven groups: control group (fed with basal diet alone) and six treatment groups receiving Triphala powder with three different ratios of ingredients [1:1:1, 1:2:3 and 1:2:4 (in order of ingredients)] at two different (0.5% and 1% of basal diet) inclusion levels. Growth performance was assessed by calculating weekly body weight gains. Humoral immune responses were evaluated by haemagglutination inhibition (HI) titre against Newcastle disease antigen and haemagglutination (HA) titre against Sheep Red Blood Cells (SRBC). Cell mediated immune response was assessed by dinitrochlorobenzene (DNCB) - induced cutaneous delayed hypersensitivity test. Antioxidant status was judged by estimating antioxidant enzyme activities *viz.*, superoxide dismutase (SOD) and catalase (CAT) activities, in the liver tissue. **Results:** Results revealed that supplementation with Triphala powder enhanced growth at all the three different ratios at both the levels of inclusion (0.5% and 1%), with significant effect noticed at 0.5% inclusion level of 1:2:3 ratio. This level also significantly enhanced both humoral and cell-mediated immune responses. Antioxidant enzyme activities were also markedly elevated in groups receiving Triphala powder, with significant effects observed in 1:2:4 ratio (1%) group. No morbidity or mortality were observed during the study period, indicated the inclusion levels or the ratio of ingredients were not obnoxious to the cockerels and proved the safety of the Triphala preparation in the cockerels. **Conclusion:** Triphala supplementation improved growth performance and immune responses at 0.5% inclusion level in 1:2:3 ratio, while 1% inclusion level in 1:2:4 ratio exhibited superior antioxidant potential, suggesting greater benefits under stress conditions such as heat stress or disease challenges.

Keywords: Poultry, Triphala, Three ratios, Growth performance, Immune status, Antioxidant profile.

INTRODUCTION

The Indian poultry sector contributes approximately ₹1.44 lakh crore to the national GDP and 1% of the total national GDP, with its share in the livestock sector being about 14 %. The industry is a major source of employment, providing direct and indirect work to over four million people, and plays a vital role in the nation's food security and economic growth [1]. At present, there is significant concern regarding poultry welfare, sanitation, and disease management that may arise from increased intense genetic pressure to increase egg and meat output. Certainly, the demand to enhance production performance negatively impacts natural immunity and disease resistance [2]. Dietary supplementation with antibiotics to enhance growth and disease resistance has raised public health worries and antibiotic resistance issues. Thus, use of antibiotics in poultry and livestock has been prohibited in numerous countries [3]. Moreover, with extended use of synthetic immunomodulators and antioxidants for enhancing immunity that acts on a particular pathway, there is a risk of reduced effectiveness or exhibiting adverse effects are noticed. Additionally, they are costly and do not possess synergistic effects

like antimicrobial, anti-inflammatory properties. Therefore, to address the drawbacks of synthetic immunomodulators and antioxidants, World Health Organization (WHO) [4] and other regulatory agencies are emphasizing to use herbal alternatives to boost the natural immunity.

Phyto-additives such as Triphala (where "tri" means three and "phala" means fruits in Sanskrit) are commonly utilized in Indian traditional medicine as a polyherbal formulation. Generally, Triphala supplementation can enhance the growth rate and improve the feed conversion ratio in broiler chickens [5]. This formulation consists of a blend of dried powders from three fruits (in order): *Terminalia chebula* (known as Chebulic myrobalan or Haritaki), *Terminalia bellerica* (also referred to as Belleric myrobalan or Bibhitaki) and *Emblica officinalis* (commonly known as Indian gooseberry or Amalaki). The dried pericarps of these three myrobalans are combined in varying proportions [1:1:1 [5]/ 1:2:3 [6]/ 1:2:4 [7]] which exhibit immunomodulatory, antioxidant, gut microbiome enhancement, hepato-protective, anti-obesogenic, anti-diabetic, anti-aging, anti-inflammatory, and antineoplastic properties [8]. Hence this study was conducted to find out the suitable ration (1:1:1 / 1:2:3 / 1:2:4) of inclusive ingredients of Triphala powder, by assessing their influences on growth, immune responses and antioxidant defense status in layer cockerels.

MATERIAL AND METHODS

This study was sanctioned by the Institutional Animal Ethical Committee of Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Namakkal by IAEC No. 18/VCRI-NKL/2025, dt.: 28.03.2025.

Collection and preparation of Triphala

The myrobalans of *Terminalia chebula, Terminalia bellerica* and *Emblica officinalis* were purchased from the very well-known herbal ingredients' vendor in Tamil Nadu and were authenticated from the plant taxonomist (Dr. P. Subramaniam M. Sc., Ph., D, (Botany) Assistant Professor) of Arignar Anna Government Arts College, Periyar University, Namakkal, Tamil Nadu, India. Voucher specimens were submitted with numbers: for *T. chebula*- AAGA/BOT/003/2025, *T. bellerica*- AAGA/BOT/004/2025 and *E. officinalis*- AAGA/BOT/005/2025.

The seedless fruits of *Terminalia chebula, Terminalia bellerica* and *Emblica officinalis* were shade dried, powdered and mixed in the said different ratios of 1:1:1, 1:2:3 and 1:2:4 respectively and stored under room temperature, away from direct sun light exposure, in air tight containers for further use.

Preparation of aqueous extracts

The dried samples of Triphala powder in three different ratios (1:1:1, 1:2:3 and 1:2:4) were extracted with water. For extraction, ten grams of the Triphala powder was mixed in 100 mL distilled water, in a conical flask and kept in a mechanical shaker for 48 h at room temperature. Extracts were filtered, concentrated, dried and stored in the refrigerator at 4 °C for further use.

Qualitative phytochemical analysis

Qualitative phytochemical analysis of Triphala powder was done as per the methods described by Trease and Evans [9].

Experimental birds and design

A total of 140 No. of layer cockerels (BV 300) was pre - arranged *as gratis* from M/s.Venkateshwara Hatcheries, Namakkal branch and were acclimatized for a period of 45 days. On 46th day, twenty (n=20) birds were randomly assigned to two (2) replicates of seven (7) treatment groups (7 groups x 2 replicates x 10 birds; N = 140). The birds were fed ad libitum and provided free access to drinking water throughout the day.

Climate

During the experimental period, the average temperature and relative humidity during the day time was around 32.9 ºC and 50.1%.

All the birds were vaccinated as per the breeder's schedule as mentioned below:

S. No	Vaccine	Route	Day of administration
1	IB + LaSota	I/o	7th day
2	IBD Intermediate plus	I/o	12th day
3	IBD Intermediate plus	I/o	20th day
4	IB + LaSota	I/o	30th day
5	Infectious coryza	S/c	30th day
6	Fowl pox	I/m	7th week
7	R_2B	I/m	9th week

Treatment groups

The birds in control group were fed only the basal diet (Grower mash) whereas the treatments were assigned to basal diets included with Triphala powder of 1:1:1, 1:2:3 and 1:2:4 ratios of inclusive ingredients, at the inclusion levels of 0.5 and 1.0 percent for each ratio, for the period of 45 days (46 - 90 days old).

Group	Treatment	No. of birds per replicate	Number of replicates	Total No. of birds/ group (n=20)
T1	Basal Diet alone	10	2	20
T2	Basal Diet + Triphala Pdr. 1:1:1 @ 0.5% (i. c)	10	2	20
T3	Basal Diet + Triphala Pdr. 1:1:1 @ 1% (i. c)	10	2	20
T4	Basal Diet + Triphala Pdr. 1:2:3 @ 0.5% (i. c)	10	2	20
T5	Basal Diet + Triphala Pdr. 1:2:3 @ 1% (i. c)	10	2	20
T6	Basal Diet + Triphala Pdr. 1:2:4 @ 0.5% (i. c)	10	2	20
T7	Basal Diet + Triphala Pdr. 1:2:4 @ 1% (i. c)	10	2	20

*i. c- inclusion levels

Assessment of growth performance

All the zootechnic parameters viz., livability, morbidity, mortality, weekly cumulative feed intake, weekly body weight and weight gain per week of all the groups were observed and recorded from 8th week to 13th week.

Assessment of Immune response

Humoral Immune response

Humoral immune response of birds of all groups were assessed by estimating the haemagglutination inhibition (HI) titre values against the Newcastle disease (ND) virus inoculated to birds, as per method of OIE [10], microdilution technique. Sera samples were collected on 81st and 90th day for estimation of HI titre.

The Humoral immune response was also assessed by haemagglutination (HA) test against Sheep Red Blood Cells (SRBC). The collected and washed SRBCs were then suspended in sterile Phosphate Buffer Saline (PBS) to prepare a packed cell suspension of 5 %. All the chicks were administered by intramuscular injection of the SRBC suspension into the breast muscle at the rate of 0.1 mL/bird on 75th day for priming, followed by a booster dose on 82nd day. The

sera samples collected on 90th day were used for estimation of HA titre against SRBC [11]. (PBS preparation: The PBS was prepared by dissolving 8.0 g of NaCl, 0.2 g of KCl, 0.2 g of KH_2PO_4, and 1.44 g of Na_2HPO_4 in approximately 800 mL of distilled water. The pH was adjusted to 7.4, and the final volume was made up to 1 litre with distilled water).

Cell mediated immune response

To evaluate the cell-mediated immune response, Dinitrochlorobenzene (DNCB) - induced cutaneous delayed hypersensitivity test was conducted at end of the experimental period on 88th day [12]. The skin thickness in the inter-digital space between the third and fourth digits was measured initially before the test ('0' hour reading) using an electronic digital caliper (M/s. Mitutoyo, Japan). Subsequently, the birds were sensitized by intra-dermal injection of 0.05 mL of 2% solution of DNCB on 88th day. Skin thickness was measured again after 24 h post- injection, at the same site, to assess the cell-mediated immune response.

Estimation of antioxidant enzymes

Tissue Homogenate preparation

Tissue samples (200 mg each) of the liver were weighed and homogenized in 2 mL of ice-cold PBS. Superoxide dismutase (SOD) enzyme activity of the homogenates of liver tissue samples' [12].

The catalase (CAT) activity in supernatant of homogenate of liver tissue samples' was measured spectrophotometrically (M/s. Systronics UV-vis double beam spectrophotometer-2201) at 240 nm by calculating the rate of degradation of hydrogen peroxide which is the substrate of the enzyme [14].

Statistical analysis

Data (mean ± SE of values) obtained on weekly body weight gain (at the end of 8th to 13th weeks), HI titre and HA titre values (on 12th and 13th week), web-index of DNCB induced delayed hypersensitivity test and antioxidant values (SOD and CAT) are presented in table forms. Statistical analysis was carried out through one-way analysis of variance (ANOVA) using IBM SPSS version 20.0 [15] and significant differences among the means from analysis at 5% level of significance were determined by Duncan's post-hoc analysis.

RESULTS

Phytochemical analysis

The results of Phytochemical analysis are presented in Table 1.

Qualitative phytochemical screening of Triphala powders prepared at three ratios (1:1:1, 1:2:3 and 1:2:4) revealed the consistent presence of alkaloids, flavonoids, coumarins, tannins, phenols, saponins, quinones, carbohydrates and Vitamin C in all formulations with higher intensity of Vitamin C, quinones and carbohydrates in the 1:2:4 ratio. Terpenoids and phlobatanin were not detected in any formulation.

Livability, Morbidity and Mortality

Throughout the study period, no birds showed any form of clinical sufferings and no mortality was observed.

Effect of Triphala supplementation on mean body weight (g) in layer cockerels

The mean body weight (BW) of the birds of experimental groups are presented in Table 2 and Figure 1. The cockerels (before treatment) had no significant difference in the mean body weights ranging between 497.02 ± 5.97 g and 507.75 ± 8.36 g at the inception of the experiment (on 46th day).

Throughout the study period, no significant Body weight (BW) differences were observed among T1, T2, T3, T5, T6 and T7 from 10th week onwards. Body weight of layer cockerels of T4 (Triphala @ 1:2:3 ratio at 0.5% inclusion) improved significantly from 8th week onwards compared to the control. By week 13, birds in T4 reached the maximum BW of 989.63 g ± 16.92 compared to 915.90 g ± 18.97 in the control (T1). T6 and T7 showed a significant improvement in body weight in the week 9 in par with T4. However, this effect was not consistently noticed in both the groups (T6 and T7) thereafter, till the last week of the trial.

Effect of Triphala supplementation on mean cumulative weekly body weight gain (g) in layer cockerels

The mean weekly body weight (BW) gain of the birds of experimental groups are presented in Table 3 and Figure 2. Throughout the study period, no significant mean cumulative BW gain differences were observed among T1 (control), T2, T3, T5, T6 and T7 (treated) groups.

The mean cumulative BW gain of T4 (Triphala @ 1:2:3 ratio at 0.5% inclusion) were significantly higher throughout the study period in comparison to the control. By week 13, birds in T4 attained maximum cumulative BW gain of 500.05 ± 13.32 g compared to 417.78 ± 16.28 g in the control (T1). On 13th week, though T3 group (455.70 ± 15.42 g) had non-significant mean cumulative BW gain difference when compared with T4 (500.05 ± 13.32g), it was also observed to be non-significant with the T1 group (417.78 ± 16.28 g) and showed numerically higher body weight in comparison to T1 at final week of the study.

Effect of Triphala on Immune response

Effect of Triphala on Humoral Immune Response

The mean HI and HA titre values against Newcastle Disease Virus and against Sheep RBC antigens of the experimental birds are presented in Table 4 and 5 and Figure 3 and 4 respectively.

The mean HI titre values of week 12 were significantly higher in all Triphala - treated groups [(T3 to T7) having Log_2 HI values of 7.40 ± 0.75 to 10.00 ± 0.63], except in T2 (6.40 ± 0.93) and T1 (4.40 ± 1.17), which were having significantly lower antibody titre values. In between them (T2 and T1), no significant difference was noticed. There was no significant difference between T5 (7.40 ± 0.75), T6 (7.80 ± 0.58) and T7 (9.00 ± 0.32). T4 recorded the highest HI titre value (10.00 ± 0.63) which was not significantly different with T7 (9.00±0.32).

The mean HI titre values of week 13 were significantly higher once again in all Triphala - treated groups [(T3 to T7) having Log_2 HI values of 7.80± 0.92 to 9.40±0.60], except T2 (6.40±0.68) and T1 (5.60±0.68), which were having significantly lower antibody titre values. In between them (T2 and T1), no significant difference was noticed. T4 recorded the highest HI titre value (9.40±0.60) which was not significantly different with T7 (9.00 ± 0.84), T3 (8.00 ± 0.95), T5 (7.80 ± 0.74) and T6 (7.80± 0.92).

In total, the treated groups either had numerically or significantly higher antibody titre values, than control group.While assessing the mean Log_2 HA titre values against SRBC antigen of all the groups, significantly higher titre was noticed in all Triphala - treated groups [(T3 to T7) having Log_2 HI values of 3.60 ± 0.43 to 4.50 ± 0.22], except T2 (3.10 ± 0.43) and T1 (2.20 ± 0.44), which were having significantly lower antibody titre values.In between them (T2 and T1), no significant difference was noticed T4 recorded the highest HI titre value (4.50 ± 0.22) which was not significantly different with T7 (4.30 ± 0.26), T3 (4.20± 0.33), T6 (3.80 ± 0.44) and T5 (3.60 ± 0.43).

Effect of Triphala on Cell Mediated Immune Response

The mean web index values of the experimental groups are presented in Table 6 and Figure 5. The highest mean web index value was observed in T4 (1.02 ± 0.12) which was followed by T7 (0.99 ± 0.11). These mean web index values were not significantly different but different from all other groups (T6: 0.61 ± 0.06; T5: 0.53 ± 0.06; T3: 0.50 ± 0.12; T2: 0.33 ± 0.03 and T1: 0.29 ± 0.08). The groups T6, T5, T3, T2 and T1 were not significantly differentiated between them.

Effect of Triphala on Antioxidant Status

The mean enzyme (Catalase and SOD) activity values of the experimental groups are presented in Table 7 and Figure 6 and 7.

Effect of Triphala on Catalase enzyme activity (H_2O_2/min/mg protein)

Antioxidant enzyme activity (Catalase) was significantly higher in all treatment groups than control (T1: 64.20 ± 3.29, the lowest), followed by T2 (83.45 ± 3.12), T3 (97.13 ± 1.56) and T5 (104.60 ± 1.99). All these groups were different from each other significantly.

Among the treated groups T7 has shown the highest enzyme activity with 121.12 ± 1.34 followed by T6 (118.62 ± 1.60) and T4 (116.57 ± 2.41). These values were not significantly different between them.

Effect of Triphala on Super Oxide Dismutase activity (U/mg protein)

Antioxidant enzyme activity (SOD) of T7 (1.05 ± 0.05) was the highest, which was significantly higher than all the other groups except T6 (0.91 ± 0.02). Groups T4 (0.86 ± 0.05) and T5 (0.79 ± 0.04) were having equal values which were not apart between them significantly. But these values were significantly different from still lower groups viz., T3 (0.60 ± 0.01) and T2 (0.60 ± 0.07). The lowest enzyme activity values were observed with group T1 (0.39 ± 0.07).

Table 1: Phytochemical analysis of aqueous extract of Triphala powder

Phytochemicals	Result		
	Triphala @1:1:1	Triphala @1:2:3	Triphala @1:2:4
Alkaloids	+	+	+
Carbohydrates	+	+	++
Coumarins	+	+	+
Flavonoids	+	+	+
Phenol	+	+	+
Phlobatannin	-	-	-
Quinones	+	+	++
Saponin	+	+	+
Tannins	+	+	+
Terpenoids	-	-	-
Vitamin C	+	+	++

+ Present, ++ Higher intensity, - Absent

Table 2: Effect of Triphala supplementation on body weight (g) (mean ± SE) in layer cockerels (n=20)

Treatment	Initial BW- 46th Day	Age in weeks (at the end of every week)					
		8	9	10	11	12	13
T1 Control	498.13 ± 9.64	571.50^{a}± 11.58	623.50^{a} ± 10.77	717.35^{a} ± 15.25	777.33^{a} ± 16.87	819.50^{a} ± 14.78	915.90^{a} ± 18.97
T2 - 1:1:1 @ 0.5%	507.75 ± 8.36	599.93^{ab}± 11.46	651.30^{ab} ± 14.62	738.05^{ab} ± 14.91	798.53^{ab} ± 17.34	844.15^{a} ± 18.94	923.10^{a} ± 17.65
T3- 1:1:1 @ 1%	497.02 ± 5.97	570.68^{a}± 7.00	630.80^{a} ± 11.96	737.65^{ab} ± 15.26	807.90^{ab} ± 20.16	860.53^{ab} ± 20.18	952.43^{ab} ± 20.33
T4- 1:2:3 @ 0.5%	499.58 ± 13.24	604.53^{b}± 9.71	684.37^{b} ± 11.39	771.80^{b} ± 13.58	831.15^{b} ± 16.60	900.9^{b} ± 14.70	989.63^{b} ± 16.92
T5- 1:2:3 @ 1%	502.65 ± 10.52	593.33^{ab}± 10.82	659.25^{ab} ± 13.20	735.95^{ab} ± 15.37	799.10^{ab} ± 19.88	845.11^{a} ± 20.55	940.68^{a} ± 20.65
T6- 1:2:4 @ 0.5%	502.63 ± 18.00	595.25^{ab}± 8.97	680.48^{b} ± 12.73	753.75^{ab} ± 15.62	816.98^{ab} ± 16.92	864.50^{ab} ± 17.54	953.85^{ab} ± 23.23
T7- 1:2:4 @ 1%	506.18 ± 6.59	596.38^{ab}± 8.18	671.18^{b} ± 9.56	732.38^{ab} ^{b}± 13.56	800.85^{ab} ± 13.33	856.03^{a} ± 13.14	962.55^{ab} ± 13.95

*Means within column bearing common superscript did not vary significantly (p>0.05)

Table 3: Effect of Triphala supplementation on cumulative weekly body weight gain (g) (mean ± SE) in layer cockerels (n=20)

Treatment	Age in weeks (at the end of every week)					
	8	9	10	11	12	13
T1 Control	73.38^{a} ± 5.36	125.43^{a} ± 6.19	219.23^{a} ± 9.64	279.20^{a} ±11.60	321.38^{a} ± 11.09	417.78^{a} ± 16.28
T2 - 1:1:1 @0.5 %	92.18^{ab} ± 9.97	143.55^{ab} ± 12.17	230.30^{a} ± 13.15	290.78^{a} ± 15.99	336.40^{a} ±17.24	415.35^{a} ± 16.66
T3- 1:1:1 @1 %	73.60^{a} ± 5.64	133.75^{ab} ± 9.78	240.63^{ab} ± 11.08	310.88^{ab} ± 15.63	363.50^{a} ±15.72	455.40^{ab} ± 15.42
T4- 1:2:3 @0.5 %	104.95^{b} ± 9.40	184.80^{c} ± 11.12	272.23^{b} ± 12.01	346.58^{b} ± 10.68	411.35^{b} ±11.57	500.05^{b} ± 13.32
T5- 1:2:3 @1 %	90.68^{ab} ± 6.61	156.60^{abc} ± 8.17	233.30 ^{a} ± 9.66	296.45^{a} ± 13.67	342.46^{a} ±15.54	438.02^{a} ± 15.26
T6- 1:2:4 @0.5 %	92.63^{ab} ± 18.38	177.85^{c} ±15.57	251.13^{ab} ± 15.57	314.35^{ab} ± 17.16	361.88^{a} ± 18.46	451.23 ^{ab} ± 23.37
T7- 1:2:4 @1%	90.20^{ab} ± 3.67	165.00^{bc} ± 6.80	226.20^{a} ± 9.69	294.68^{a} ± 10.05	349.85^{a} ±13.22	456.38^{ab} ± 12.41

*Means within column bearing common superscript did not vary significantly (p>0.05)

Table 4: Effect of Triphala supplementation on HI antibody titre (log2) values (mean ± SE) against ND vaccine in layer cockerels (n=6)

Treatment	HI titre (log2) week 12	HI titre (log2) week 13
T1	4.40^{a} ± 1.17	5.60^{a} ± 0.68
T2	6.40^{ab} ± 0.93	6.40^{a} ± 0.68
T3	8.40^{bcd} ± 0.87	8.00^{bc} ± 0.95
T4	10.00^{d} ± 0.63	9.40^{b} ± 0.60
T5	7.40^{bc} ± 0.75	7.80^{bc} ± 0.74
T6	7.80^{abc} ± 0.58	7.80^{bc} ± 0.92
T7	9.00^{cd} ± 0.32	9.00^{b} ± 0.84

*Means within column bearing common superscript did not vary significantly (p>0.05)

Table 5: Effect of Triphala supplementation on HA antibody titre (log2) values (mean ± SE) against SRBC of layer cocekrels (n=10)

Treatment	HA against SRBC week 13 (Log2)
T1	2.20^{a} ± 0.44
T2	3.10^{ab} ± 0.43
T3	4.20^{bc} ± 0.33
T4	4.50^{c} ± 0.22
T5	3.60^{bc} ± 0.43
T6	3.80^{bc} ± 0.44
T7	4.30^{c} ± 0.26

*Means within column bearing common superscript did not vary significantly (p>0.05)

Table 6: Effect of Triphala supplementation on web index values (mean ± SE) in DNCB induced delayed type dermal hypersensitivity test in layer cockerels (n=10)

Treatment	Web Index (mm)
T1	0.29^{a} ± 0.08
T2	0.33^{a} ± 0.03
T3	0.50^{ab} ± 0.12
T4	1.02^{c} ± 0.12
T5	0.53^{ab} ± 0.06
T6	0.61^{b} ± 0.06
T7	0.99^{c} ± 0.11

*Means within column bearing common superscript did not vary significantly (p<0.05)

Table 7: Effect of Triphala supplementation on antioxidant enzymes' activity (mean ± SE) in

Treatment	Catalase (H_2O_2/min /mg protein)	SOD (U/mg protein)
T1	$64.20^{a} \pm 3.29$	$0.39^{a} \pm 0.07$
T2	$83.45^{b} \pm 3.12$	$0.60^{b} \pm 0.07$
T3	$97.13^{c} \pm 1.56$	$0.60^{b} \pm 0.01$
T4	$116.57^{e} \pm 2.41$	$0.86^{c} \pm 0.05$
T5	$104.60^{d} \pm 1.99$	$0.79^{c} \pm 0.04$
T6	$118.62^{e} \pm 1.60$	$0.91^{cd} \pm 0.02$
T7	$121.12^{e} \pm 1.34$	$1.05^{d} \pm 0.05$

*Means within column bearing common superscript did not vary significantly ($p<0.05$)

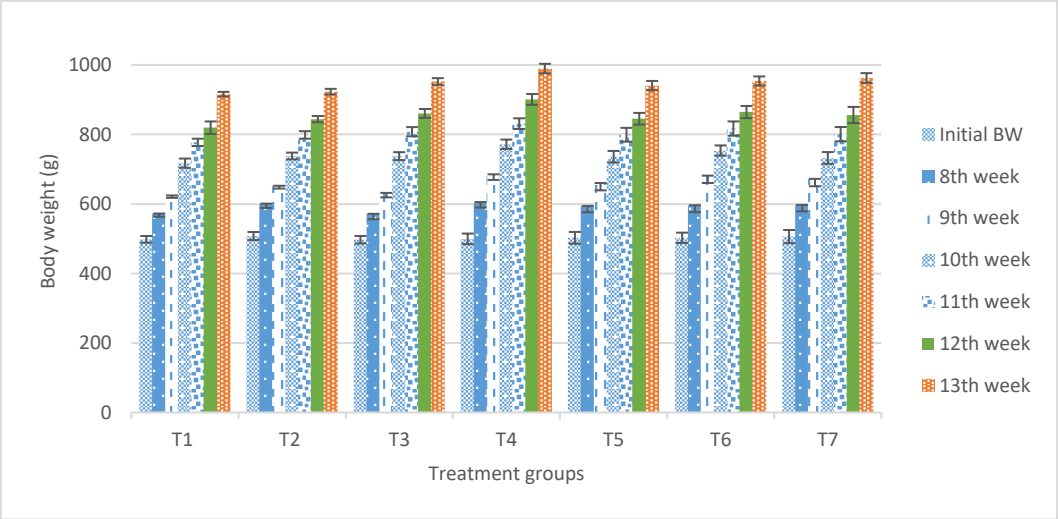

Figure 1: Effect of Triphala supplementation on body weight (g) (mean ± SE) in layer cockerels

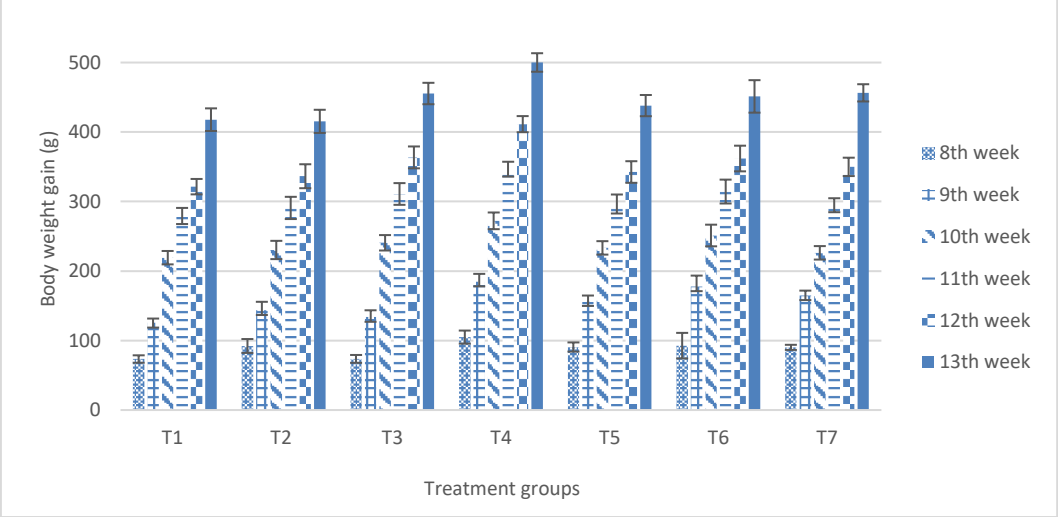

Figure 2: Effect of Triphala supplementation on cumulative weekly body weight gain (g) (mean ± SE) in layer cockerels

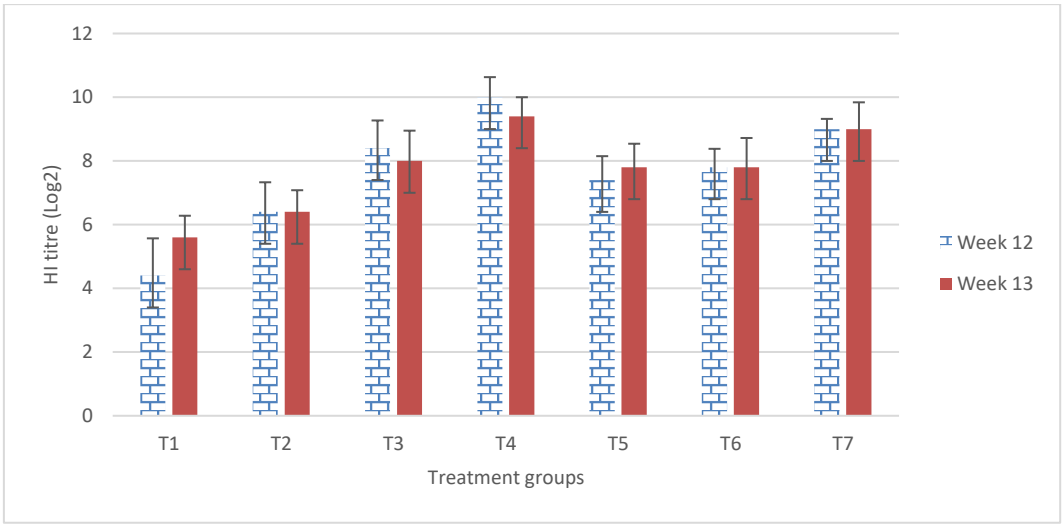

Figure 3: Effect of Triphala supplementation on HI antibody titre (log2) values (mean ± SE) against ND vaccine in layer cockerels

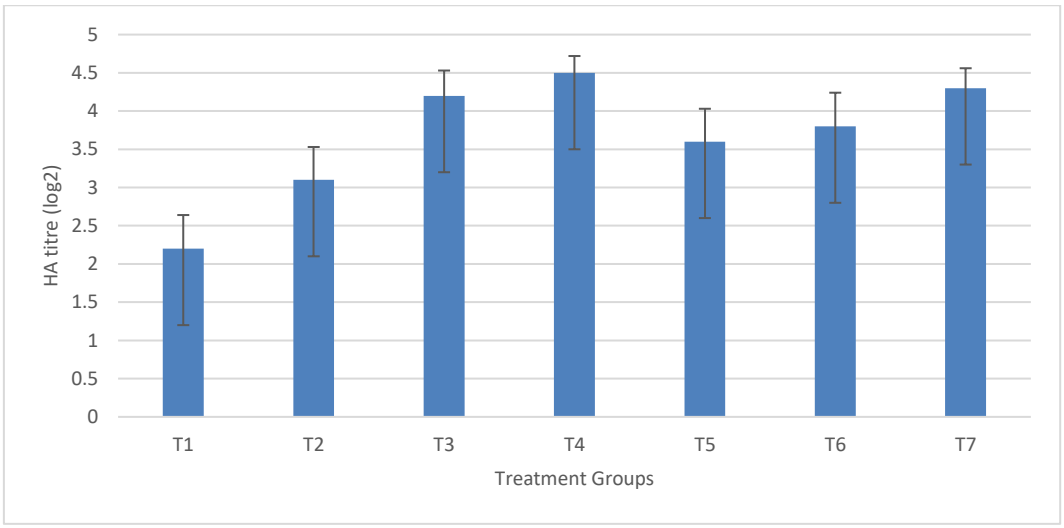

Figure 4: Effect of Triphala supplementation on HA antibody titre (log2) values (mean ± SE) against SRBC in layer cockerels

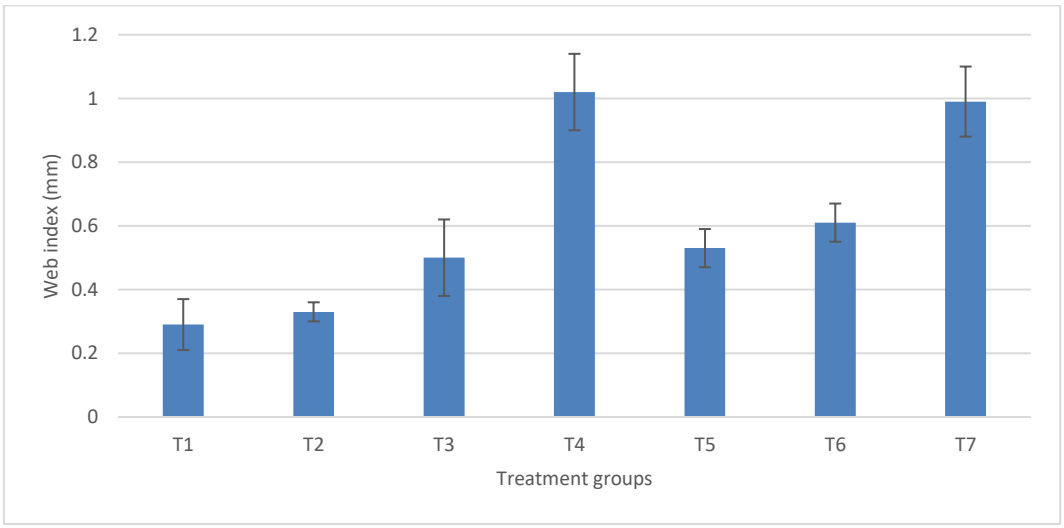

Figure 5: Effect of Triphala supplementation on web index values (mean ± SE) in DNCB induced delayed dermal hypersensitivity test in layer cockerels

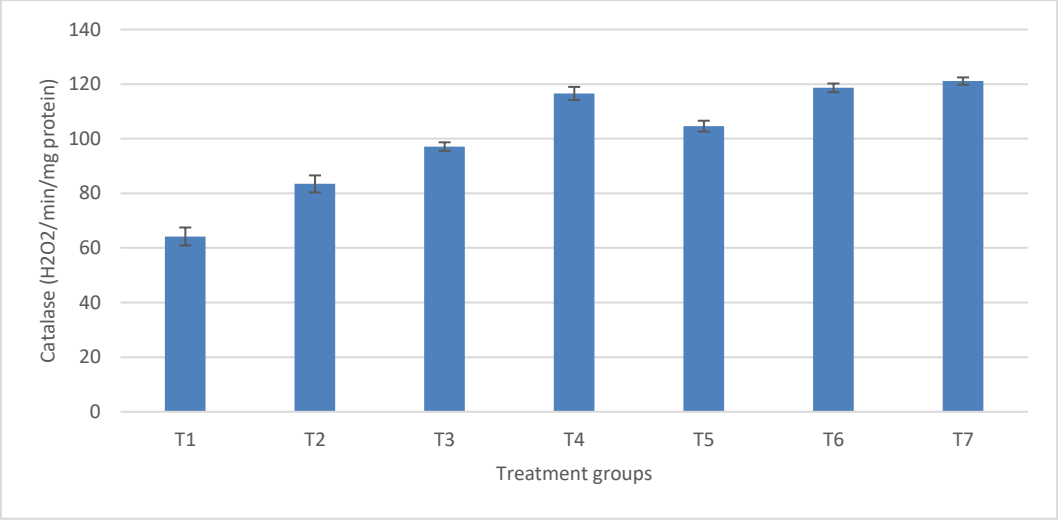

Figure 6: Effect of Triphala supplementation on Catalase (CAT) enzyme activity (mean ± SE) in layer cockerels

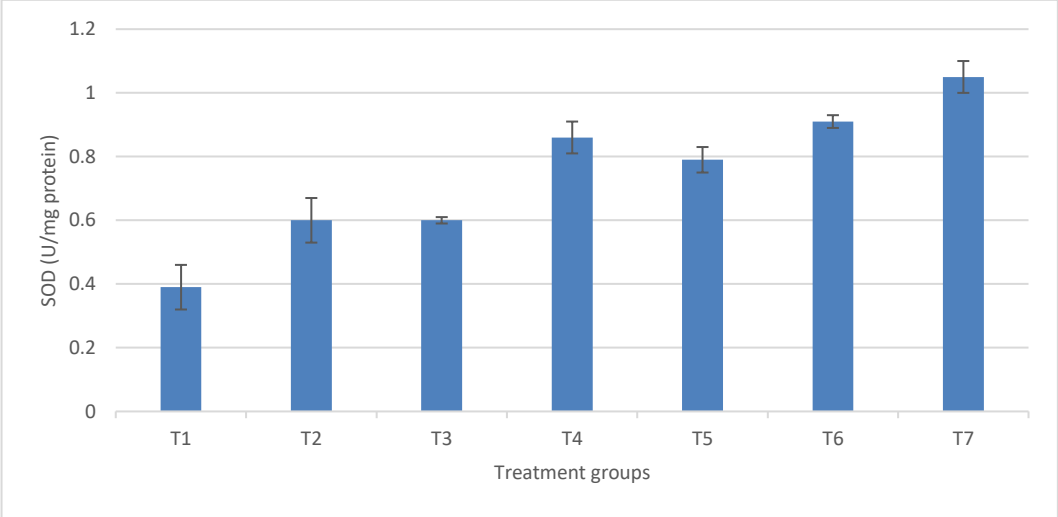

Figure 7. Effect of Triphala supplementation on Superoxide dismutase (SOD) enzyme activity (mean ± SE) in layer cockerels

DISCUSSION

The phytochemical screening of Triphala revealed the presence of several bioactive compounds, including alkaloids, flavonoids, coumarins, tannins, phenols, saponins, quinones carbohydrates and Vitamin C [16]. The occurrence of these diverse classes of phytochemicals justifies the wide range of biological activities attributed to Triphala in traditional and modern medicine.

Alkaloids are well recognized for their antimicrobial, analgesic, and anti-inflammatory effects, which may contribute to the immune-modulating potential of Triphala [17]. Flavonoids are potent antioxidants that scavenge free radicals, reduce oxidative stress, and protect cellular integrity, thereby playing a significant role in enhancing growth performance and immunity in poultry [18]. Coumarins have anticoagulant, anti-inflammatory and hepatoprotective activities, suggesting a role in maintaining metabolic balance and liver health [19].

The presence of tannins and phenols further strengthens the therapeutic profile of Triphala, as these compounds are known for their antioxidant, antimicrobial and astringent properties. Carbohydrates provide not only an energy source but also act as supportive molecules in fermentation and gut microbiota balance [16] which improve gut health by modulating microbial populations and enhancing nutrient absorption. Saponins, apart from their antioxidant activity, are reported to have immunostimulatory and cholesterol-lowering effects, which may benefit both growth and health [20]. Quinones, although in smaller quantities, act as electron carriers and contribute to antimicrobial activity [21]. Overall, the presence of these bioactive constituents demonstrates the multi-functional nature of Triphala.

The significant improvement in body weight and body weight gain observed with Triphala powder supplemented group (1:2:3 ratio at 0.5 % inclusion), could be attributed to the synergistic effects of acceptable levels of its bioactive compounds such as tannins, vitamin C, gallic acid and flavonoids [22]. These phytoconstituents of Triphala powder are known to enhance digestive enzyme secretion, improve intestinal morphology and promote balanced gut microbiota, thereby facilitating superior nutrient utilization and growth performance. Similar findings were reported who recorded improved feed conversion efficiency and body weight gain in broilers supplemented with 0.1 per cent level of inclusion of Triphala powder at 1:1:1 ratio of ingredients [5].

The enhanced humoral immune response, reflected by higher HI titres against Newcastle disease virus and HA titres against SRBC in Triphala-fed groups, indicates antibody mediated immunostimulatory potentials of Triphala. The group receiving Triphala at 1:2:3 ratio (0.5%) exhibited the strongest antibody response than at 1 % inclusion level, suggesting that moderate inclusion levels may be more effective than higher doses. Similar findings were also recorded a moderate immunomostimulant activity at 0.075 per cent level of inclusion of

Triphala powder at 1:1:1 ratio of ingredients [23]. In this study, humoral immunity was assessed by HI test against Newcastle disease virus (NDV) and HA test against sheep red blood cells (SRBC). Since HI titre may rise due to field exposure or subclinical infection with NDV, the HA test using SRBC was included to rule out such interference.

Cell-mediated immunity, as indicated by the DNCB- induced cutaneous delayed hypersensitivity test, was significantly stronger in T4 (1:2:3 @ 0.5%) and T7 (1:2:4 @ 1%) groups. This suggests that Triphala not only supports humoral immunity but also augments T-cell mediated immune mechanisms. Polyphenolic compounds in Triphala have been reported to activate macrophages and promote cytokine release, which are crucial for cell-mediated responses [24]. The observed trend that lower doses (0.5%) sometimes out-performed higher doses (1%) may be explained by the concept of immune homeostasis, where excessive antioxidant or immunostimulant intake can suppress the immune system rather than stimulate it [25].

The antioxidant enzyme activities (SOD and catalase) were significantly elevated in all treatment groups, with maximal effects observed in T7 (1:2:4 @ 1%) and T6 (1:2:4 @ 0.5%). This may be attributed to the higher proportion of *Emblica officinalis* (Amalaki) in this ratio, which is particularly rich in vitamin C, polyphenols and ellagic acid-potent free radical scavengers that enhance endogenous antioxidant enzyme activity. Additionally, Amla's high ascorbic acid content is known to directly enhance catalase and SOD activity, thereby reducing oxidative stress and maintaining cellular integrity under stressful conditions [26].

Taken together, the present study confirms that Triphala supplementation exerts a dual role in poultry by enhancing growth performance and strengthening both humoral and cell-mediated immunity, while simultaneously improving antioxidant status. Notably, the results suggest that lower inclusion levels (0.5%) may be more effective for immune stimulation, whereas higher inclusion levels (1%) are more beneficial for antioxidant defense. Further, more inclusion of *Emblica officinalis* is particularly important to the antioxidant activity and their synergistic effects likely underpin the adaptogenic, immunomodulatory, and antioxidant activities as reported in experimental and clinical studies thereby supporting its use as a natural growth promoter and health enhancer in poultry and other animals.

Overall, the findings of the present study provide a strong rationale for the use of Triphala as a safe, natural alternative to synthetic immunomodulators and antioxidants in poultry nutrition

CONCLUSION

Triphala supplementation, notably at 1:2:3 ratio at 0.5% inclusion, is highly effective as a natural feed additive for layer cockerels, delivering better growth, robust immunity and enhanced antioxidant defense, and can safely substitute the synthetic immunomodulators or antioxidants in modern poultry diets. It was also suggested that triphala supplementation at 1:2:4 ratio at 1% inclusion level can be used in poultry feed during the summer months, to mitigate the heat stress, as it has very good antioxidant property.

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Conflict of interest

The authors declared no conflict of interest.

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ORCID ID

Ahash T: https://orcid.org/0009-0004-7137-4226

Jagadeeswaran A: https://orcid.org/0000-0002-7024-4794

Sakthi Priya M: https://orcid.org/0000-0001-6425-1069

Sukumar K: https://orcid.org/0000-0002-2621-2787

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