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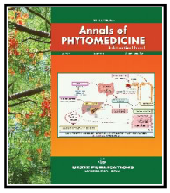

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Immunomodulatory and protective effects of polyherbal formulation against *Escherichia coli* infection in broiler birds

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Abstract

The present experiment was performed on a total 120 day-old broiler chicks, which were divided into 4 groups. *Escherichia coli* infection was experimentally induced in all groups except the environment control group. One group of infected birds was kept as infection control. Birds of two groups were treated with polyherbal formulation or standard antibiotic (Enrofloxacin). Mortality percentage, feed conversion ratio, blood and serum parameters as well as antibody titres against NDV and IBDV vaccines were measured. Feed intake data revealed no major difference among all treatment groups. Birds treated with standard antibiotic gained the highest weight. Moreover, no mortalities were observed in all three groups except the infection control group. Haematology on the 21st and 42nd day showed that all key parameters were improved in polyherbal and standard drug control group. The heterocyte/lymphocyte (H/L) ratio, the marker of stress indicator was found normal in all groups except increased in the infection control group on 42nd day of the experiment. In addition, uric acid, ALT and AST were found significantly improved in the polyherbal-treated group. The mild changes were found in the polyherbal and standard antibiotic-treated group whereas, marked pathological changes were observed in the infection control group. Polyherbal formulation can effectively act as an immunostimulant as highest antibody titre was found against NDV and IBDV antigens. In conclusion, polyherbal formulation containing *Allium sativum, Cinnamomum zeylanicum, Coriander sativum, Cuminum cyminum, Mentha piperita, Syzygium aromaticum*, and *Withania somnifera* was found effective to protect against *Escherichia coli* infected broiler birds as well as demonstrated immunostimulant property.

1. Introduction

The poultry business has remarkable contribution to the livestock industry. Backyard poultry farming faces limited problems but intensive cage farming produces high production stress and leads to more chances to get infection. *E. coli* cause many diseases in poultry and is reported to have a huge economic loss to poultry farmers through increased morbidity and mortality, decreasing bird's productivity, rejection of infected carcasses at slaughterhouse, as well as treatment expenditure (Lutful Kabir, 2010). *E. coli* causes diversified disease manifestations in poultry including colibacillosis, omphalitis, septicaemia, yolk sac infection, respiratory tract infection, swollen head syndrome, polyserositis, coligranuloma, enteritis, cellulitis, *etc*. (Baranwal *et al*., 2019). Many antibacterial drugs are used for the treatment of *E. coli* infection in poultry, which includes tetracycline, β-lactams, fluoroquinolones, aminoglycosides, and sulphonamides (Zakeri and Kashefi, 2012) and their frequent use led to the development of antibacterial resistance against *E. coli*. Moreover, some antibacterials are prohibited to use in food-producing animals. Therefore, to overcome the antibacterial resistance problem, and to improve growth performance, certain phytogenic supplementation should be used (Singh *et al*., 2023).

Herbal preparations and plants are used in birds as alternatives to antibacterial, antifungal, growth promoters, antiprotozoal, antioxidant, and anti-inflammatory as well as to improve immunity (Haniarti *et al*., 2019). Spices and herbs, *viz*., sage, clove, oregano, rosemary, cinnamon, *etc*., are rich in bioactive molecules, *viz*., phenolic compounds and are excellent sources of antioxidants. They also boost the immune system, antiviral and antibacterial effects, and hormone biotransformation as well as regulate gene expression in cell proliferation (Rani *et al*., 2023). Different chronic diseases and antibacterial drug-resistant bacteria are better managed by polyherbal formulation instead of a single herb due to synergism and lesser side effects (Sethumathi *et al*., 2021; Sundarrajan, 2023). Moreover, these, natural herbal combatants can be developed into frontline warriors against virus and other pathogens due to their various pharmacological potential and also to develop a strong foundation of the immunity to combat infection (Mehrotra, 2021). Looking at the great potential of

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antibacterial activity and easy availability of spices, a polyherbal formulation was formulated and evaluated for its protective and immunomodulatory effect on experimentally induced *E. coli* infection in broiler birds.

2. Materials and Methods

2.1 Experimental animals

A total of one hundred twenty (120), day-old broiler chicks of Vencobb-400 strain were procured from Bhavna hatchery, Dungri, Dist. Valsad (Gujarat) and used as experimental birds. The Institutional Animal Ethics Committee (IAEC) of Veterinary College, Kamdhenu University, Navsari, Gujarat, India has given necessary permission to carry out bird experimentation with protocol number 112-VCN-VPT-2022.

2.2 Housing, feeding, and management of birds

Birds were reared under the standard protocol of the deep litter system of management. All birds were provided with similar environmental and management conditions during the entire experimental period. An ideal floor space, standard ration, and potable drinking water were given to experimental birds. A photoperiod of 24 h duration was provided to all experimental groups during a brooding period and after that gradually dark period increased to 4 to 6 h (Kim *et al*., 2022). All the standard managemental practices were adopted to keep the birds stress free.

2.3 Experimental design

Experimental chicks were weighed individually and divided into 4 homologous groups (G1: Environment control; G2: Preventive test; G3: Standard drug control; G4: Infection control) based on their live body weight and it was considered as the initial body weight of chicks. All four groups were subdivided into six replicates with five birds in each replicate for easy management and data collection. All the birds were given the same standard diet formulated as per BIS, 2007 and supplements. The preventive test group was given the polyherbal formulation depicted in Table 1 from day 0 up to the end of experiment as extra supplement at the rate of 2.5 g/kg of feed. Enrofloxacin (100 mg/ml) as @ 1 ml/2 liter of water was administered through water in standard antibiotic control group. *E. coli* (9.0×10^8 CFU/ml) infection was given in all birds except the environment control group by oral route (0.5 ml) on the 8th day of experimentation (El-Sawah *et al*., 2018). One bird from each group was sacrificed to confirm *E. coli* infection five days post-infection. Samples from affected portion were also collected with sterilized swabs and were streaked on eosin methylene blue agar to confirm colonies of *E. coli* with a green metallic sheen.

Table 1: Ingredients of polyherbal formulation

S. No.	Botanical name of plant	Common name	Parts used	Proportion
1.	*Allium sativum*	Garlic	Dried Fruit	¼ part
2.	*Cinnamomum zeylanicum*	Dalchini	Dried bark	1 part
3.	*Coriander sativum*	Coriander	Dried fruit	1 part
4.	*Cuminum cyminum*	Cumin	Dried fruit	1 part
5.	*Mentha piperita*	Pudina	Dried leaves	1 part
6.	*Syzygium aromaticum*	Clove	Dried fruit	1 part
7.	*Withania somnifera*	Ashwagandha	Dried roots	1 part

2.4 Parameters studied

2.4.1 Nutritional attributes

Feed was weighed and offered in each group followed by weighing leftover was measured on next day morning. The difference in offered feed and leftover was calculated at weekly intervals and considered as voluntary feed intake. An average feed intake in gram per bird per week was calculated by dividing the total feed consumed per week by the total number of birds in the respective group. Group wise individual body weight of the birds was recorded on the day of their procurement, and thereafter at every week during the entire experimental period up to 42nd day. The difference between the body weight of the current week and the previous week was calculated and was considered as body weight gain. In each group, replicate-wise average weekly body weight gain and cumulative body weight gain were calculated. Feed conversion efficiency was assessed as feed conversion ratio (FCR) and was calculated as amount of feed consumed to achieve unit weight gain as given by formula (Skinner-noble and Teeter, 2003): Feed conversion ratio (FCR) = Feed consumption/body weight gain.

2.4.2 Haemato-biochemical parameters

All birds were observed for clinical symptoms throughout the experimental period. Blood samples were collected randomly from twelve birds of each group (two birds per replicate) on the 21st and 42nd day of the experiment. Blood was collected early in the morning from the wing vein using a clean, sterilized syringe and needles into two separate and clean EDTA and clot activator tubes. Blood collected in EDTA containing tube was used for the estimation of hematology parameters whereas; a clot activator tube was used to separate serum and used to estimate biochemical parameters.

Evaluation of hematological parameters such as haemoglobin (Hb), total erythrocytes count (TEC), and total leucocyte counts (TLC) were completed manually on the same day of blood collection. The serum concentration of alanine transaminase (ALT/SGPT), aspartate transaminase (AST/SGOT) and uric acid were estimated by using their respective commercial analytical kits (Randox Laboratories Pvt. Ltd, Bangalore). The assay was carried out manually as per the manufacturer's instructions by using Merck Microlab 300 semi-automatic analyzer (Vital Scientific N.V., Netherland).

A total twenty four birds (6 from each group, 1 from each replicate) were used to study the histological changes in liver and intestine. Birds were euthanized using cervical dislocation and both organs were collected followed by fixation in neutral buffered formalin (10%) for 10 days. Organs were fixed in paraffin and blocks were prepared as standard method. Slides were prepared and the stain was carried out with hematoxylin and eosin (H & E) dye, followed by observation for histopathological lesions in the liver and intestine.

2.4.3 Immune status against NDV and IBDV

Hemagglutination inhibition (HI) test for NDV vaccine was performed according to the method described by Bansal (1996). Initially, a hemagglutination assay was performed in which, mat, lattice, shield, granular, or agglutination formation was considered a positive reaction whereas, button formation was a negative reaction. The last dilution having mat formation was considered as HA titer. Then after, 4 HA unit (4 HA = HA titre/4) of NDV antigen was prepared and a hemagglutination inhibition test was performed in which, observations were recorded for button formation as hemagglutination inhibition.

IBD virus generally does not give hemagglutination thereby passive hemagglutination was performed using the method described by Daodu *et al*. (2018). Human blood with blood group "O" was used in this assay and 1% sensitized RBC solution was prepared and used for the indirect hemagglutination test. Samples showing characteristic tent formation/reticulum setting of RBCs at the bottom of the microtiter plate were considered as positive while those with button formations were considered negative.

2.5 Statistical analysis

Data for nutritional and health attributes were expressed as Mean ± S.E. One-way ANOVA was used to analyze data at 1 and 5 per cent significance level using Duncan's New Multiple Range Test (DNMRT) through SPSS-20 software.

3. Results

3.1 Average feed intake

In the first week, the polyherbal treatment group and standard drug control group showed similar weekly individual feed intake of 169 g. Post-infection on the 7th day, the infection group showed a decrease in individual weekly feed intake of 325 g compared to the other three groups. The highest individual weekly feed intake was observed on the 4th week among all groups as 671 g (G1), 639 g (G2), 794 g (G3), and 794 g (G4). The overall feed intake was 2303 g, 2220 g, 2470 g, and 2474 g, respectively, from G1 to G4 (Table 2). Individual feed intake was identical among groups between the 4th to 6th weeks.

Table 2: Average weekly individual feed intake (g/bird) in broiler birds

Weeks	Treatment groups			
	G1 (Env. control)	G2 (Polyherbal)	G3 (Std. drug)	G4 (Inf. control)
First (Pre-starter)	162	169	169	158
Second (Starter)	336	338	360	325
Third (Starter)	470	446	517	491
Fourth (Starter)	671	639	794	794
Fifth (Finisher)	664	628	630	707
Sixth (Finisher)	657	647	629	673
Total	**2960**	**2867**	**3099**	**3148**

3.2 Average body weight gain and feed conversion ratio (FCR)

The average body weight of chicks on day 0 was 41 ± 0.42 g, 38 ± 1.02 g, 44 ± 0.72 g, and 41 ± 0.70 g for G1, G2, G3 and G4, respectively. Weekly body weight gain for each replicate of different studied groups (g/bird in Mean ± S.E.) was calculated and depicted in Table 3. All groups revealed similar weight gain except the standard drug control group which showed major improved body weight gain at 1899 g after end of six weeks. Throughout the experimental period, the environmental control group and polyherbal treated group remained similar in terms of body weight gain in each week. After the end of the experiment, group-wise total FCR was calculated. Feed conversion ratio was found as 1.67, 1.66, 1.63, and 1.84 for G1, G2, G3, and G4, respectively.

Table 3: Average weekly body weight gain (g/bird) (Mean ± S.E.) in broiler birds

Week	Treatment groups			
	G1 (Env. control)	G2 (Polyherbal)	G3 (Std. drug)	G4 (Inf. control)
First	86 ± 2.99^{a}	105 ± 6.73^{b}	115 ± 4.31^{b}	88 ± 3.71^{a}
Second	227 ± 9.51^{a}	231 ± 16.79^{a}	237 ± 12.22^{a}	214 ± 7.85^{a}
Third	324 ± 16.37^{a}	326 ± 37.63^{a}	363 ± 14.14^{a}	317 ± 23.25^{a}
Fourth	420 ± 20.99^{a}	416 ± 32.32^{a}	505 ± 19.03^{a}	424 ± 42.02^{a}
Fifth	344 ± 13.65^{a}	357 ± 19.15^{a}	342 ± 9.42^{a}	340 ± 31.70^{a}
Sixth	372 ± 15.92^{b}	294 ± 13.39^{a}	338 ± 23.53^{ab}	328 ± 26.75^{ab}
Total	**1773 ± 10.79^{b}**	**1728 ± 9.56^{a}**	**1899 ± 6.30^{c}**	**1710 ± 12.44^{a}**

The means with distinct superscripts between the treatment groups show a significant difference (p<0.05).

3.3 Clinical observations and mortality percentage

Daily clinical observations were done throughout the experimental period. Birds of the environment control group were found healthy and had no any clinical signs of disease. Birds from the standard drug control group and polyherbal treated group were also found normal whereas, birds from the infection control group show diarrhoea, lethargy, dullness and depressed after establishment of infection. Two mortalities were seen in infection control group in 3rd week after the onset of infection, followed by a third mortality during 4th week and a fourth mortality was observed in 5th week in the infection control group. No mortalities were seen in the other three groups during the experimental period. Even though, the G2 and G3 groups were also infected with *E. coli*, no mortalities were seen during the whole experimental period showing a protective effect of polyherbal formulation against *E. coli*.

3.4 Haematology

Haematology parameters of treatment groups on the 21st and 42nd day with statistical analysis are shown in Tables 4 and 5, respectively. The concentration of Hb, TEC, TLC, and differential leucocyte count (DLC) showed considerable ($p<0.05$) changes among groups on the 21st day. Among all groups, the polyherbal group showed improved overall parameters. A significant increase in total erythrocyte count values in polyherbal and standard drug control groups whereas, the infection group showed a significantly ($p<0.05$) lower value. TLC was found significantly lower in polyherbal treated and standard drug control than infection control group showing potential effects of polyherbal formulation and antibiotic.

Table 4: Haematological parameters in various treatment groups on 21st day

Parameters	Treatment groups			
	G1 (Env. control)	G2 (Polyherbal)	G3 (Std. drug)	G4 (Inf. control)
Haemoglobin (g/dl)	8.03 ± 0.44^{ab}	8.93 ± 0.52^{b}	7.72 ± 0.40^{a}	7.28 ± 0.18^{a}
TEC ($\times 10^6$)	4.10 ± 0.05^{b}	4.67 ± 0.07^{c}	4.66 ± 0.09^{c}	3.28 ± 0.09^{a}
TLC ($\times 10^3$)	12.17 ± 0.47^{ab}	13.83 ± 0.59^{b}	11.92 ± 0.40^{a}	22.59 ± 0.91^{c}
Heterophils (%)	22.08 ± 1.34^{a}	25.33 ± 0.74^{b}	26.00 ± 1.15^{b}	30.17 ± 1.13^{c}
Lymphocytes (%)	66.17 ± 0.77^{b}	62.92 ± 0.93^{a}	62.75 ± 1.00^{a}	61.58 ± 0.38^{a}
Monocytes (%)	7.42 ± 0.51^{a}	6.92 ± 0.61^{a}	6.67 ± 0.28^{a}	6.08 ± 0.57^{b}
Eosinophils (%)	3.42 ± 0.61^{a}	4.17 ± 0.44^{a}	3.75 ± 0.39^{a}	1.75 ± 0.30^{b}
Basophils (%)	1.00 ± 0.34^{a}	1.00 ± 0.29^{a}	0.83 ± 0.27^{a}	0.42 ± 0.29^{a}
H/L	0.34 ± 0.03^{a}	0.40 ± 0.02^{b}	0.42 ± 0.03^{b}	0.49 ± 0.01^{c}

The means with distinct superscripts between the treatment groups show a significant difference ($p<0.05$).

On 42nd day, Hb was found significantly ($p<0.05$) higher in G2 and G3 as 9.07 ± 0.12 and 9.18 ± 0.18, respectively. G4 recorded lower Hb value than normal range (7-13 g/dl) as 6.856 ± 0.20. Polyherbal showed its effect in the treatment group and recorded highest TEC among all groups. Infection control group showed anaemia with leucopenia generally resulted from chronic infection.

Table 5: Haematological parameters in various treatment groups on 42nd day

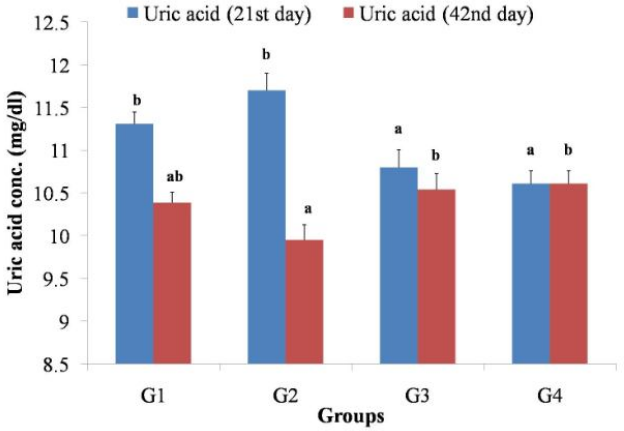
Parameters	Treatment groups			
	G1 (Env. control)	G2 (Polyherbal)	G3 (Std. drug)	G4 (Inf. control)
Haemoglobin (g/dl)	8.48 ± 0.07^{b}	9.07 ± 0.12^{c}	9.18 ± 0.18^{c}	6.86 ± 0.20^{a}
TEC ($\times 10^6$)	4.10 ± 0.05^{b}	4.67 ± 0.07^{c}	4.65 ± 0.09^{c}	3.28 ± 0.09^{a}
TLC ($\times 10^3$)	12.58 ± 0.47^{b}	14.58 ± 0.38^{c}	12.75 ± 0.49^{b}	9.58 ± 0.43^{a}
Heterophils (%)	17.50 ± 0.61^{a}	20.50 ± 0.26^{b}	21.67 ± 0.54^{b}	26.67 ± 0.77^{c}
Lymphocytes (%)	66.00 ± 0.70^{b}	66.17 ± 0.41^{b}	66.00 ± 0.67^{b}	62.83 ± 0.47^{a}
Monocytes (%)	8.83 ± 0.30^{c}	7.92 ± 0.31^{bc}	7.08 ± 0.38^{b}	6.00 ± 0.44^{a}
Eosinophils (%)	5.57 ± 0.31^{b}	4.33 ± 0.38^{a}	4.25 ± 0.25^{a}	3.58 ± 0.23^{a}
Basophils (%)	2.00 ± 0.35^{b}	1.08 ± 0.23^{a}	1.00 ± 0.17^{a}	0.92 ± 0.19^{a}
H/L	0.27 ± 0.01^{a}	0.31 ± 0.00^{b}	0.33 ± 0.01^{b}	0.42 ± 0.01^{c}

The means with distinct superscripts between the treatment groups show a significant difference ($p<0.05$).

The H/L ratio is generally considered as stress indicator (Gross and Siegel, 1983). In present study, H/L ratio found significantly higher on 21st day in all groups except environment control group after *E. coli* infection whereas, on 42nd day, only infection control group observed a higher H/L ratio compared to other groups. It indicates a positive effect of polyherbal and standard drug on *E. coli* infection model.

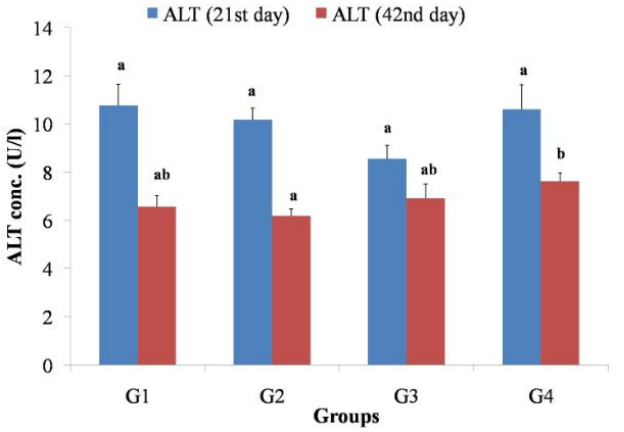
3.5 Serum biochemistry

AST, ALT, and serum uric acid levels were measured in the present study to understand the effect of *E. coli* infection on vital organs and amelioration by polyherbal formulation. Biochemical profile at 21st day and 42nd day of experiment containing data of uric acid (mg/dl), ALT (U/l) and AST (U/l) are depicted in the form of graphs (Figures 1, 2 and 3).



The means with distinct superscripts between the treatment groups show a significant difference ($p<0.05$).

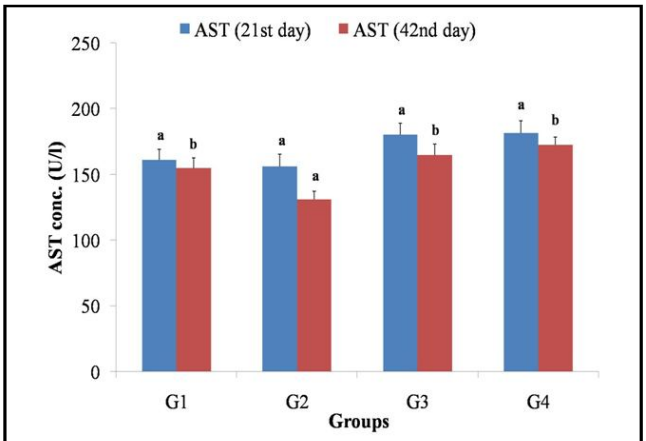
Figure 1: Uric acid concentrations at 21st day and 42nd day of experiment.



The means with distinct superscripts between the treatment groups show a significant difference ($p<0.05$).

Figure 2: ALT concentrations at 21st day and 42nd day of experiment.

On the 21st day, the result revealed no significant ($p<0.05$) difference in ALT and AST whereas uric acid (UA) was found remarkably ($p<0.05$) improved in G3 and G4. Results of the 42nd day revealed actual effect of drug and polyherbal formulation. All three parameters showed significant ($p<0.05$) difference among all groups. UA values were found significantly ($p<0.05$) low in polyherbal treated group as 9.95 ± 0.18 compared to other groups and were found highest in infection control group as 10.61 ± 0.15. ALT and AST data revealed significantly ($p<0.05$) improvements in the polyherbal and standard drug treated group compared to the other two groups.



The means with distinct superscripts between the treatment groups show a significant difference ($p<0.05$).

Figure 3: AST concentrations at 21st day and 42nd day of experiment.

3.6 Gross and histopathology

3.6.1 Gross morphology

Gross changes in the liver and intestine were documented following 42 day of experimentation (Figure 4). On gross examination, the liver from the environmental control group showed normal structure while the liver from the infection control group was enlarged, friable, and showed rounded borders. The liver from the polyherbal treatment group was apparently normal and comparable with environment control group. The liver from standard drug control group revealed mild paleness with rounding of borders was noted. Intestine from all studied groups were found apparently normal on gross examination.

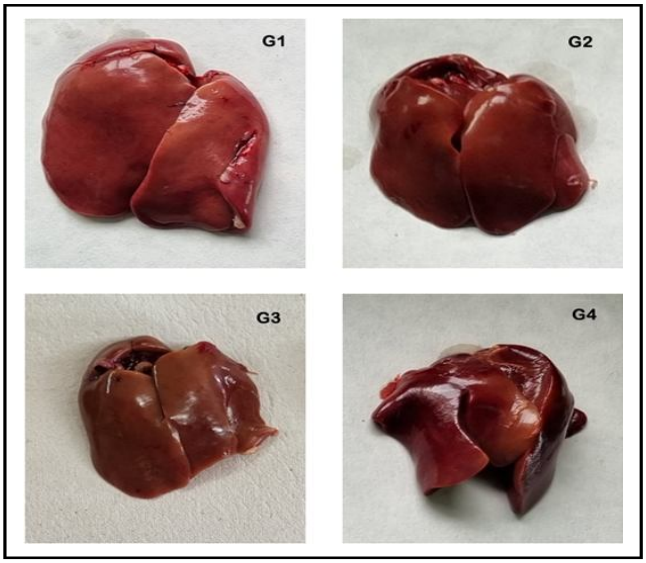


Figure 4: Gross photographs of the liver of different groups [G1 (Environment control) - normal; G2 (Polyherbal treated) - normal; G3 (Standard drug control) - slightly pale and rounded edges; G4 (Infection control) - enlarged, friable and rounded edges].

3.6.2 Histopathological examination

Upon microscopic examination, the liver of the environment control group showed well-organized hepatic cords and normal hepatocytes. The liver from infection control group showed dilation of central veins, severe diffuse hydropic degeneration of hepatocytes, and focal congestion in the portal triad, central veins, and sinusoids at a few places with disorganization of hepatic cords. Polyherbal treated group showed normal hepatocytes with minimal congestion of sinusoids and central veins at places. In the standard drug control group, the liver revealed mild sinusoidal congestion and diffuse hepatic degeneration. In comparison with the infection control group, the liver from polyherbal and standard drug control group revealed improved hepatic structures suggesting a repairing process (Figure 5).

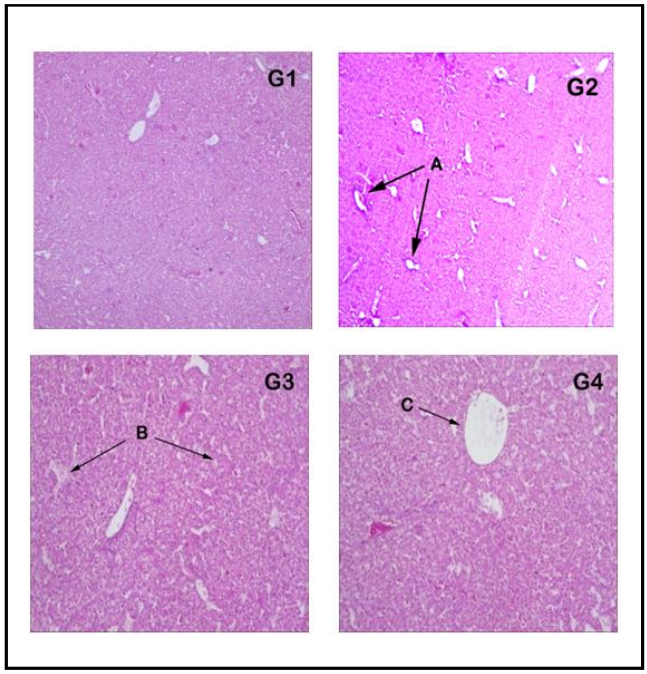


Figure 5: 10x liver (A. mild sinusoidal congestion; B. mild diffused hepatic degeneration; C. dilated central vein) [G1- Environment control; G2 - Polyherbal treated; G3 - Standard drug control; G4 - Infection control].

Microscopic examination of the intestine of the environment control group revealed a normal and well-organized structure with slightly atrophied glands (Figure 6). The infection control group documented thinning of epithelium lining, leucocytic infiltration, and congestion in lamina propria at a few places, shortening, fusion, and sloughing of villi with atrophied glands. Polyherbal revealed comparatively normal histoarchitecture. The standard drug control group also showed comparable histoarchitecture with an environmental control group.

3.7 HA and HI test

HA and HI test was performed to determine titre of NDV whereas indirect or passive hemagglutination was performed on the 42nd day of experimentation to determine the titre of IBDV as it doesn't give hemagglutination. Data containing hemagglutination average inhibition titre in a different group of birds are mentioned in Table 6 and Figure 7.

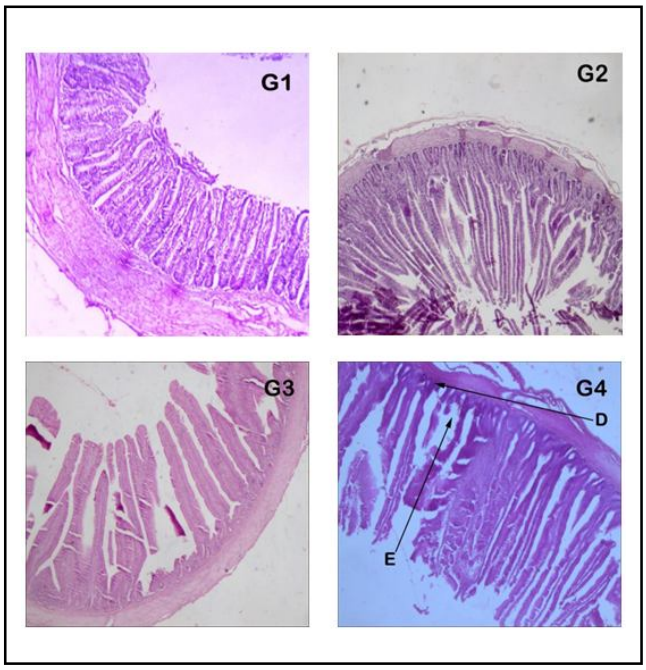


Figure 6: 4x intestine [(G1- Environment control) – normal villi; (G2 - Polyherbal treated) and (G3 - Standard drug control) - comparatively normal histoarchitecture; (G4 - Infection control) - atrophied glands (D) and sloughing of villi (E)].

Table 6: Mean titre value against NDV and IBDV in treatment groups

Titre	Treatment groups			
	G1 (EC)	G2 (PH)	G3 (SD)	G4 (IC)
NDV	128.00 ± 28.62^{a}	362.67 ± 133.91^{b}	181.33 ± 34.73^{ab}	85.33 ± 13.50^{a}
IBDV	234.67 ± 21.33^{a}	469.33 ± 122.18^{b}	341.33 ± 53.97^{ab}	160.00 ± 32.00^{a}

No significant difference was observed among the environmental and infection control group against NDV and IBDV. Polyherbal treated group significantly (p<0.05) highest titre against both of these antigens as 362.67 ± 133.91 and 469.33 ± 122.18 against NDV and IBDV, respectively. The standard drug control group showed mixed results but not better than polyherbal treated group showing better efficacy of polyherbal formulation as an immunomodulator.

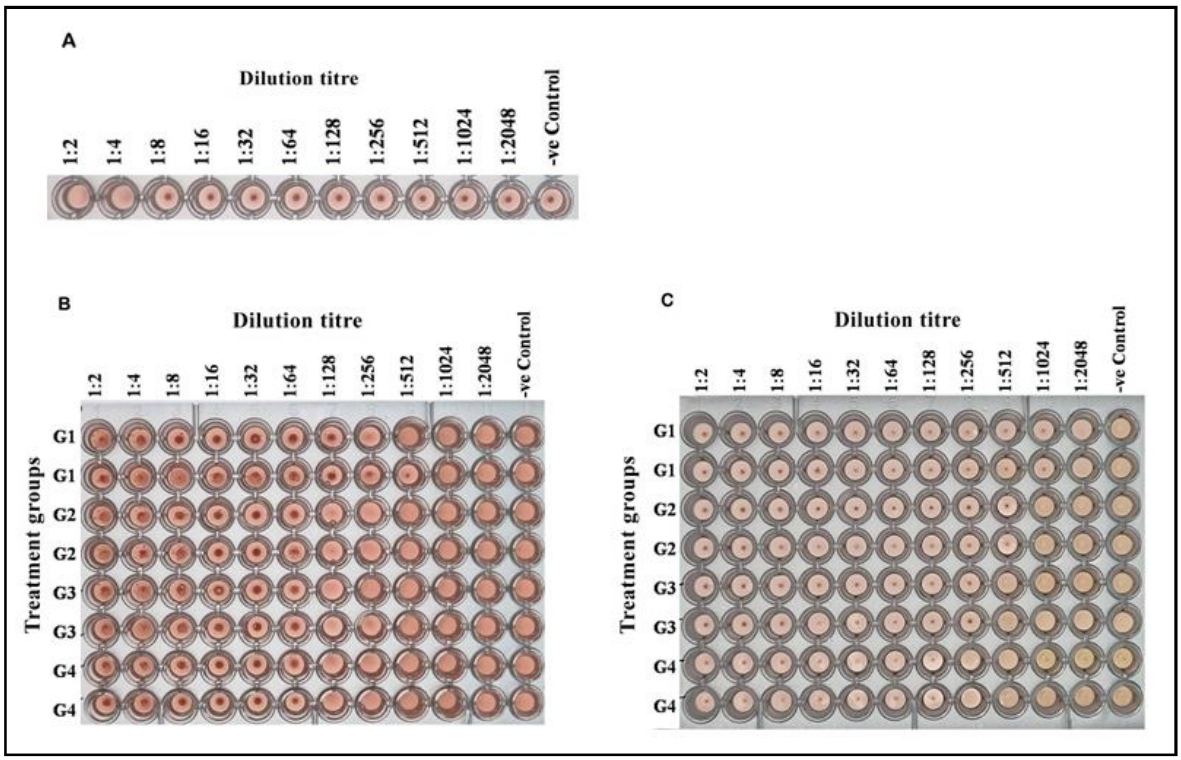


Figure 7: Representative photographs of HA and HI (A- HA for NDV; B- HI for NDV; C- IHA for IBDV) [G1- Environment control; G2 – Polyherbal treated; G3 - Standard drug control; G4 - Infection control].

4. Discussion

Polyherbal formulation containing garlic, dalchini, coriander, cumin, pudina, clove and ashwagandha is used in present study to evaluate its protective effect on *E. coli* infection poultry model. These spices might improve digestibility of feed but due to less palatability, feed intake in polyherbal treated group was decreased. This phenomenan was also observed by various researchers (Sharma *et al*., 2008; Singh *et al*., 2009; Alam *et al*., 2014; Qamar *et al*., 2015). Many contrast studies were reported higher feed consumption in the polyherbal formulation supplemented group (Molla *et al*., 2012; Nath *et al*., 2012; Elagib *et al*., 2013). Feed intake and energy homeostasis are influenced by several hormones, ghrelin, and cholecystokinin through the neuronal hypothalamic axis (Awad *et al.*, 2020). Addition of polyherbal formulation in feed is likely to produce effect on palatability of feed, may resulted in decreased feed intake in polyherbal treated group.

There was no much difference in body weight gain amongst groups. Results of the present study supported findings of earlier workers (Ogbe and Affiku, 2012; Nkukwana *et al*., 2014; Lonkar *et al*., 2023). Contrary to present study, Bhushan *et al*. (2008); Sharma *et al*. (2008); Kadam *et al*. (2009); Gatne *et al*. (2010); Molla *et al*. (2012) and Naresh *et al*. (2017) revealed significantly higher bodyweight in the herbal treatment group than basal diet group. Although, FCR was shown improved due to polyherbal formulation indicates improved birds' feed utilization capacity. In current study, observations are found in accordance with the findings of other researchers (Sharma *et al*., 2008; Singh *et al*., 2008; Alam *et al*., 2014; Rindhe *et al*., 2016; Singh *et al*., 2023) whereas, Nkukwana *et al*. (2014) as well as Rao and Gurram (2021) found no major alterations in FCR among treatment and control groups. Improved FCR in the current experiment was due to decreased feed consumption in the polyherbal treated group compared with others and there was no major change in body weight gain amongst all groups. This can be concluded that the polyherbal mixture used in the present experiment has better feed utilization capacity even in the presence of infection. The reason might be due to the protective effect of formulation against *E. coli* infection.

Clinically, there was no mortality observed in polyherbal treated group and symptoms of *E. coli* infection were also decreased. Total leukocyte count was within normal range in polyherbal treated group inspite of *E. coli* infection as compared to infection control group. Hartati *et al*. (2021) revealed similar findings of decreased TLC compared with birds treated with herbal supplements whereas, Krishan *et al*. (2015) found increased TLC in polyherbal treated group vs. infection control group. Decreased TLC indicates protective effect of herbal supplements against bacterial infection. Normal levels of Hb and leukocytes for chickens are: 7-13 g/dl and 7.0 - 32.0 × 10^3/ mL, respectively (Li *et al*., 2013). Increased levels of Hb and TEC in the present study are in line with many findings (Ansari *et al*., 2012; Kamal *et al*., 2015; Krishan *et al*., 2015). Opposite to present study, Hartati *et al*. (2021) revealed slightly decreased Hb (9.92 g/dl) compared to infection group (10.15 g/dl) in layer birds. Al-Shammari *et al*. (2017) evaluated a decreased H/L ratio in the herbal treated group compared to the control which supports the data of the present study. H/L ratio is one the stress indicator in poultry. This protective effect might be due to antibacterial and antioxidant properties of phytochemicals present in polyherbal formulation. Similarly, uric acid, ALT and AST values were also found improved in polyherbal treated group compared to infection control group. These parameters are lined with Krishan *et al*. (2015); Sharma *et al*. (2015); Al-

Shammari *et al*. (2017) and Kumari *et al*. (2020) who evaluated effect of herbal powder supplement and found lowered values of ALT, AST, and UA in polyherbal treated groups compared with infection or environment control groups. Present data suggestive of protective effect of polyherbal formulation to vital organs, *viz*., liver, kidney and heart. Improved ALT value may be due to less fatty deposition or infiltration in hepatocytes. Similarly, decreased serum UA indicates improved filtration capacity of kidney and protection of glomeruli against bacterial infection.

Gross and histopathological observations of liver and intestine also exhibited protective effect of polyherbal formulation used in current experiment. Bhushan *et al*. (2012) revealed degenerative changes in hepatocytes as well as congestion and dilation of central veins in *E. coli* infected broilers. Present findings supported the results of earlier worker (Kilany *et al*., 2018) who also found desquamation of epithelium of intestinal villi, necrosis of muscularis mucosa, and leucocytic infiltration.

Ashwagandha was one of the ingredient of formulation has proven immunostimulant property and that was proved in the form of increased NDV and IBDV vaccine titers. Present findings supported the results of various workers (Kadam *et al*., 2009; Gatne *et al*., 2010; Krishan *et al*., 2015; Qasem *et al*., 2015; Mohanambal *et al*., 2018; Eladl *et al*., 2020). *Withania somnifera* increases levels of immunoglobulins, interferons, T-helper cells, CD3+, CD4+/CD8+, and cytokines (Tharakan *et al*., 2021).

5. Conclusion

There was no remarkable change in average feed intake found in all studied groups except a slight decrease in polyherbal treated group. Average bodyweight gain was significantly decreased in the polyherbal treated and infection control group with highest bodyweight gain observed in the standard drug control group. Moreover, improved FCR was observed in the polyherbal treated group. No mortality was observed in all groups except in the infection control group. Haematology and serum biochemistry parameters were found to be significantly improved in polyherbal treated group compared to infection control group. No remarkable changes were found in gross and histopathological lesions in polyherbal, environmental, and standard drug control groups whereas, pathological changes were found in infection control group. Significant increase in antibody titres against NDV and IBDV antigens were observed in the polyherbal-treated group. In conclusion, the polyherbal formulation studied in the present research was found effective in amelioration of adverse effect due to *E. coli* infection in broiler birds as well as improve immunity.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Alam, M. N.; Uddin, J.; Akter, T. D.; Moni, I. Z.; Alom, F.; Rahman, A. and Al Noman, M. A. (2014). Broiler production by using polyherbal medication (neem, nishyinda, tulsi and turmeric extract). International Journal of Innovation and Applied Studies, **9**(3):1161-1175.

Al-Shammari, K. I. A.; Batkowska, J. and Gryziñska, M. M. (2017). Effect of various concentrations of an anise seed powder (*Pimpinella anisum* L.) supplement on selected hematological and biochemical parameters of broiler chickens. Brazilian Journal of Poultry Science, **19**:41-46.

Ansari, J.; Khan, S. H.; Ul Haq, A. and Yousaf, M. (2012). Effect of the levels of *Azadirachta indica* dried leaf meal as phytogenic feed additive on the growth performance and haemato-biochemical parameters in broiler chicks. Journal of Applied Animal Research, **40**(4):336-345.

Awad, E. A.; Najaa, M.; Zulaikha, Z. A.; Zulkifli, I. and Soleimani, A. F. (2020). Effects of heat stress on growth performance, selected physiological and immunological parameters, caecal microflora, and meat quality in two broiler strains. Asian-Australasian Journal of Animal Sciences, **33**(5):778-787.

Bansal, R.P. (1996). Techniques in viral diagnosis in "Technology in diagnostic virology" published by ICAR, New Delhi, pp:76-82.

Baranwal, A.; Singh, N. P.; Kumar, A.; Kumar, A.; Upadhyaya, P. K. and Srivastava, A. K. (2019). Colibacillosis in Broilers: A case report.

Bhushan, B.; Choudhury, S.; Garg, S. K. and Ram, R. (2012). Pathomorphological studies on the effect of polyherbal feed supplement on certain vital organs of healthy and *E. coli* challenged broiler chicks. Indian Journal of Poultry Science, **47**(3):326-329.

Bhushan, B.; Garg, S.K. and Kumar, J. (2008). Effect of a polyherbal feed supplement on production performance and nutrient utilization in *Escherichia coli* challenged broiler chicks. Indian Journal of Veterinary Research, **17**(1):27-36.

Daodu, O. B.; Oludairo, O. O.; Aiyedun, J. O.; Ambali, H. M.; Kadir, R. A.; Daodu, O. C. and Adah, A. D. (2018). Assessment of antibody assay methods in determination of prevalence of infectious bursal disease among local chickens and guinea fowls in Kwara state, North Central Nigeria. Veterinary World, **11**(8):1183-1187.

Eladl, A. H.; Mosad, S. M.; El-Shafei, R. A.; Saleh, R. M.; Ali, H. S.; Badawy, B. M. and Elshal, M. F. (2020). Immunostimulant effect of a mixed herbal extract on infectious bursal disease virus (IBDV) vaccinated chickens in the context of a co-infection model of avian influenza virus H9N2 and IBDV. Comparative Immunology, Microbiology and Infectious Diseases, **72**:101505.

Elagib, H. A.; El-Amin, W. I. A. and Malik, H. E. (2013). Effect of dietary garlic (*Allium sativum*) supplementation as feed additive on broiler performance and blood profile. Journal of Animal Science Advances, **3**(2):58-64.

EL-Sawah A. A.; Dahshan A. H. M.; El-Nahass E. and El-Mawgoud A.I.A (2018). Pathogenicity of *Escherichia coli* O157 in commercial broiler chickens. Beni-Suef University Journal of Basic and Applied Sciences, **7**(4):620-625.

Gatne, M. M.; Patil, R.; Ravikanth, K.; Shivi, M. and Rekhe, D. S. (2010). Evaluation of immunomodulatory effect of stresroak premix in broiler chick. Veterinary World, **3**(3):122-125.

Gross, W. B. and Siegel, H. S. (1983). Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian diseases, **27**(4):972-979.

Haniarti, H.; Munir, M.; Akib, M.A.; Ambar, A.; Rusman, A.D.P. and Abdullah, A. (2019). Herbal for Increasing Immunity and Weight of Poultry. 1st International Conference of Animal Science and Technology (ICAST) 2018. IOP Conference Series: Earth and Environmental Science. pp:1-7.

Hartati, S.; Untari, T.; Sutrisno, B. and Fitriana, I. (2021). Hematology profile and liver histopathology in *Escherichia coli* infected layers treated with combination of phyllanthus (*Phyllanthus niruri* L.) and turmeric (*Curcuma domestica*). Jurnal Sain Veteriner, **39**(1):55-61.

Kadam, A. S.; Nikam, M. G.; Patodkar, V. R.; Muglikar, D. M.; Lonkar, V. D.; Yadav, G. B. and Meshram, M. D. (2009). Influence of herbal early chick nutritional supplement on the growth performance, serum biochemicals and immune response of broiler chicken. International Journal of Poultry Science, **8**(4):349-354.

Kamal, A. B.; Boby, F.; Shahjada, Z.; Lutfuzzaman, A. K.; Motin, A. A.; Alam, K. J. and Mostofa, M. (2015). Effects of Neem, Nishyinda and Papaya leaves as growth promoters in broiler chicks. International Journal of Natural and Social Sciences, **2**(2):49-55.

Kilany, O.; Youssef, F.; Mabrouk, M. and Fares, I. (2018). Clinicopathological studies on the effect of some antibacterial medicinal plants in broilers. Journal of Clinical Pathology Forecast, **1**(1):1003.

Kim, H. J.; Son, J.; Jeon, J. J.; Kim, H. S.; Yun, Y. S.; Kang, H. K. and Kim, J. H. (2022). Effects of photoperiod on the performance, blood profile, welfare parameters, and carcass characteristics in broiler chickens. Animals, **12**(17):22-90.

Krishan, G.; Shukla, S. K.; Prakash, B.; Rajesh, K.; Ruchi, T.; Malik, Y. S. and Kuldeep, D. (2015). Immunomodulatory and protective effects of a polyherbal formulation (Immon) against infectious anemia virus infection in broiler. International Journal of Pharmacology, **11**(5):470-476.

Kumari, M.; Gupta, R. P.; Lather, D. and Bagri, P. (2020). Ameliorating effect of *Withania somnifera* root extract in *Escherichia coli* infected broilers. Poultry Science, **99**(4):1875-1887.

Li, C.; Dong, Y.; Hou, H.; Li, Q.; Zhang, R.; Qin, R. and Shi, W. (2013). Effects of traditional Chinese herbal medicines on blood cell count and immunity in chickens. African Journal of Pharmacy and Pharmacology, **7**(29):2081-2086.

Lonkar, V. D.; Kadam, A. S.; Srivastava, A. A.; Chaudhari, F. N.; Dhaygude, V. S.; Tumlam, U. M. and Shinde, A. K. (2023). Effect of polyherbal liver stimulant (GenoLiv) on production performance, carcass characteristics, serum biochemistry and immune response in commercial broiler chickens. Indian Journal of Veterinary Sciences and Biotechnology, **19**(3):49-52.

Lutful Kabir, S. M. (2010). Avian colibacillosis and salmonellosis: A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. International Journal of Environmental Research and Public Health, **7**(1):89-114.

Mehrotra N. (2021). Herbs that heal: Nature's pharmacy endowed remedies for better health. Ann. Phytomed., **10**(1):6-22.

Mohanambal, K.; Selvaraju, G.; Palanivel, K. M. and Rajeswar, J. J. (2018). Evaluation of immune potentiating effect of medicinal plant products in commercial layer flock vaccinated against Newcastle disease. Indian Journal of Animal Research, **52**(8):1343-1346.

Molla, M. R.; Rahman, M. M.; Akter, F. and Mostofa, M. (2012). Effects of Nishyinda, black pepper and cinnamon extract as growth promoter in broilers. Bangladesh Veterinarian, **29**(2):69-77.

Naresh, P.; Usha, R. M.; Vijay, K. M.; Ravindra, N. S.; Shivi, M. and Reddy, A. G. (2017). Efficacy evaluation of polyherbal antistress formulation and synthetic vitamin C on broiler birds' performance under physiological heat stress. The Pharma Innovation, **6**(2a):18-21.

Nath, D. D.; Rahman, M. M.; Akter, F. and Mostofa, M. (2012). Effects of tulsi, black pepper and cloves extract as a growth promoter in broiler. Bangladesh Journal of Veterinary Medicine, **10**(1-2):33-39.

Nkukwana, T. T.; Muchenje, V.; Pieterse, E.; Masika, P. J.; Mabusela, T. P.; Hoffman, L. C.; and Dzama, K. (2014). Effect of *Moringa oleifera* leaf meal on growth performance, apparent digestibility, digestive organ size and carcass yield in broiler chickens. Livestock Science, **161**:139-146.

Ogbe, A. O. and Affiku, J. P. (2012). Effect of polyherbal aqueous extracts (*Moringa oleifera*, gum arabic and wild *Ganoderma lucidum*) in comparison with antibiotic on growth performance and haematological parameters of broiler chickens. Res. J. Recent Sci., **1**(7):10-18.

Qamar, S. H.; Haq, A. U.; Asghar, N.; Rehman, S. U.; Akhtar, P. and Abbas, G. (2015). Effect of herbal medicine supplementations (Arsilvon Super, Bedgen40 and Hepa-cure Herbal Medicines) on growth performance, immunity and haematological profile in broilers. Advances in Zoology and Botany, **3**(2):17-23.

Qasem, M.; Alhajj, M.; El Nabi, G. and Al-Mufarrej, S. (2015). Effects of turmeric powder as a dietary supplement on performance indicators and immune responses in broiler chickens. Journal of Animal and Veterinary Advances, **14**(2):30-35.

Rani, J.; Kaur, P and Chuwa, C. (2023). Nutritional benefits of herbs and spices to the human beings. Ann. Phytomed., **12**(1):187-197.

Rao, H. and Gurram, S. (2021). Effect of herbal feed additives on performance, immunity, serum parameters and *E. coli* counts of broilers under heat stress. Indian Journal of Animal Nutrition, **38**(1):61-67.

Rindhe, S.; Suryavanshi, S. U.; Khose, K. K.; Ravikanth, K. and Reothia, A. (2016). Effect of supplementation of herbal antistress product and synthetic vitamin C on performance of broiler birds under physiological heat stress. World Journal of Pharmaceutical Sciences, **4**(1):54-59.

Sethumathi, P. P.; Manjuparkavi, K.; Lalitha, V.; Sivakumar, T.; Menaka, M.; Jayanthi, A. and Ashok Kumar B. (2021). Evaluation of *in vitro* antioxidant and antimicrobial activity of polyherbal formulation of Thirikadugu chooranam and Parangipattai chooranam. Ann. Phytomed., **10**(2):169-174.

Sharma, R. K.; Maini, S. and Ravikanth, K. (2008). Beneficial effects of Superliv DS and Xlivpro on growth promotion and carcass quality traits in broilers. Veterinary World, **1**(12):363-365.

Sharma, V.; Jakhar, K. K.; Nehra, V. and Kumar, S. (2015). Biochemical studies in experimentally *Escherichia coli* infected broiler chicken supplemented with neem (*Azadirachta indica*) leaf extract. Veterinary World, **8**(11):1340-1345.

Singh, P.; Neeraj; Pandey, R. and Singh A. K. (2023). Effect of supplementation of different levels of phytogenic mixture on growthperformance and carcass characteristics of broiler chickens. Ann. Phytomed. **12**(1):687-694.

Singh, R.; Shushni, M. A. and Belkheir, A. (2015). Antibacterial and antioxidant activities of *Mentha piperita* L. Arabian Journal of Chemistry, **8**(3):322-328.

Singh, V. K.; Chauhan, S. S.; Ravikanth, K.; Maini, S. and Rekhe, D. S. (2009). Effect of dietary supplementation of polyherbal liver stimulant on growth performance and nutrient utilization in broiler chicken. Veterinary World, **2**(9):350-352.

Skinner-Noble, D. O. and Teeter, R. G. (2003). Components of feed efficiency in broiler breeding stock: energetics, performance, carcass composition, metabolism, and body temperature. Poultry Science, **82**(7): 1080-1090.

Sundarrajan, P. (2023). Unveiling the hidden treasures: Underexploited herbs and spices beneficial tomankind. Ann. Phytomed., **12**(2):1-4.

Tharakan, A.; Shukla, H.; Benny, I. R.; Tharakan, M.; George, L. and Koshy, S. (2021). Immunomodulatory effect of *Withania somnifera* (Ashwagandha) extract - a randomized, double-blind, placebo-controlled trial with an open label extension on healthy participants. Journal of Clinical Medicine, **10**(16):3644.

Zakeri, A. and Kashefi, P. (2012). Antimicrobial susceptibilities of avian *Escherichia coli* isolates in Tabriz, Iran. African Journal of Biotechnology, **11**(19):4467-4470.

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