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Effect of Dietary Supplementation of *Tinospora Cordifolia* and *Aloe Barbadensis* on the Immune Response of Commercial Broilers

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Abstract

Due to immunosuppressive diseases, the poultry industry faces challenges in maintaining bird health and productivity. Antibiotics, traditionally used to address these issues, have led to antibiotic resistance, prompting the search for natural alternatives. *Tinospora cordifolia* (Gurjo) and *Aloe barbadensis* (Aloe vera) are recognized for their immunomodulatory properties and potential as herbal feed additives to improve immunity and growth performance in poultry. A total of 180 Cobb500 broiler chicks were randomly divided into four treatment groups: T1 (control), T2 (1% *T. cordifolia*), T3 (1% *A. barbadensis*), and T4 (0.5% T. cordifolia + 0.5% A. barbadensis). Hematological parameters, including hemoglobin (Hb), total leukocyte count (TLC), packed cell volume (PCV), and differential leukocyte count (DLC), were measured at two-week intervals. Statistical analysis was performed using R software, with significance at $P < 0.05$. Herbal-treated groups showed significant improvements in hematological parameters compared to the control group. By the 4th week, the combination group (T4) recorded the highest TLC (18.5/cumm) and improved Hb (9.0 g/dl), while the control group (T1) consistently had the lowest values. Eosinophil counts, indicative of infection, were significantly reduced in T3 and T4. PCV values progressively improved in herbal-treated groups, with T4 exhibiting the highest recovery from anemia-like conditions. The significant improvements in hematological parameters observed in herbal-treated groups can be attributed to the immunostimulatory compounds in T. cordifolia and *A. barbadensis*. The combination group (T4) showed the greatest benefits, likely due to the synergistic effects of the two herbs, enhancing immunity and reducing stress-related hematological changes. Dietary supplementation with *T. cordifolia* and *A. barbadensis*, especially in combination (T4), significantly enhances immunity and growth performance in broilers. These findings support the use of herbal additives as a sustainable and natural alternative to antibiotics in poultry production. Further studies are recommended to optimize dosages and evaluate long-term effects.

Introduction

The poultry industry plays a pivotal role in addressing global food security, yet it faces persistent challenges in maintaining bird health and productivity amidst the rising risks of infectious diseases. Young chicks, in particular, are highly vulnerable to diseases such as Infectious Bursal Disease and Newcastle Disease due to their underdeveloped immune systems, often leading to significant economic losses for poultry producers (Gisavi et al., 2005). These diseases compromise poultry's growth, productivity, and survivability, underscoring the need for effective strategies to improve chick immunity during early growth stages.

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Historically, antibiotics have been extensively employed to prevent infections, boost growth performance, and enhance overall health in poultry. However, the indiscriminate and prolonged use of antibiotics has led to the emergence of antibiotic-resistant pathogens, creating global health concerns and prompting stringent regulatory actions to restrict antibiotic use in livestock production (Saeed et al., 2020). As consumer demand shifts toward antibiotic-free poultry products, the poultry industry is exploring alternative approaches to safeguard bird health and immunity without compromising productivity. Among these alternatives, herbal feed additives such as Aloe vera (Aloe barbadensis Miller) and Tinospora cordifolia (Gurjo) have gained increasing attention for their potential as natural immunomodulators.

Aloe vera, a drought-resistant tropical plant, is widely recognized for its diverse pharmacological properties, including antioxidant, antiviral, and immunomodulatory effects (Surjushe et al., 2008; Maan et al., 2018). The plant contains a variety of bioactive compounds, such as polysaccharides, vitamins, and enzymes, which contribute to its immunostimulatory properties. Studies have demonstrated that dietary supplementation with Aloe vera can enhance immune responses, improve hematological parameters, and mitigate the adverse effects of environmental and physiological stressors such as heat, cold, and malnutrition (Waihenya et al., 2002; Khan et al., 2014). Additionally, Aloe vera has been shown to enhance the production of white blood cells and antibodies, thereby boosting the body's natural defense mechanisms against pathogens.

Tinospora cordifolia, a medicinal herb widely used in traditional Ayurvedic medicine, has also emerged as a promising herbal additive for improving immunity in poultry. Known as Guduchi or Gurjo, this climbing shrub is valued for its hepatoprotective, anti-inflammatory, and immunomodulatory properties (Alexander et al., 2008; Kumari et al., 2016). The bioactive compounds in Gurjo, such as alkaloids, diterpenoids, and polysaccharides, stimulate cellular and humoral immunity, increase lymphocyte proliferation, and enhance the activity of phagocytic cells (Mittal et al., 2014). Research indicates that dietary supplementation with Gurjo not only improves immune responses but also reduces the severity of infections, thereby promoting better health and survivability in poultry (Sharma et al., 2013).

Immunosuppression remains a major concern in poultry production, often caused by factors such as viral infections, environmental stress, and chemical drug usage. Immunosuppressed chicks are more susceptible to secondary infections and exhibit reduced vaccine efficacy, which compromises overall productivity and health outcomes (Chauhan, 2001). While vaccination programs provide essential protection, their success often depends on the birds' baseline immune competence. In this context, herbal feed additives such as Aloe vera and Gurjo can play a crucial role in bridging the gap by enhancing the immune system, reducing the risk of immunosuppression, and improving disease resistance in poultry flocks (Stanely et al., 1999).

This study aims to evaluate the immunomodulatory effects of dietary supplementation with Aloe vera and Gurjo in chicks. The research focuses on analyzing key immunological parameters, such as lymphocyte counts, total leukocyte counts, and other hematological markers, to determine the efficacy of these herbal additives in enhancing cellular immunity. Additionally, the study will assess the overall health, growth performance, and disease resistance of chicks fed varying proportions of Aloe vera and Gurjo. By providing empirical evidence on the immunomodulatory properties of these herbal feed additives, this research seeks to contribute to the development of sustainable and antibiotic-free poultry production systems. Ultimately, the findings may offer a robust framework for integrating natural immunostimulants into modern poultry management practices, promoting healthier birds, and addressing growing consumer demands for safer and more sustainable poultry products.

Methods

Site selection

The experiment was conducted from 3rd January 2021 to 13th February 2021 at AFU Livestock, Rampur, Chitwan. The site is situated 9.8 km Southwest of Bharatpur, the headquarters of Chitwan district. It is located at 27° 37'North latitude and 84° 25' East longitude.

Experimental birds

A total of 180-day-old Cobb500 broiler chicks were purchased from a private hatchery. The chicks were grouped and brooded in the deep litter for 10 days and were fed commercial broiler –prestarter ration (1-14 days, purchased from a commercial feed company). After 10 days, birds were shifted to a deep litter housing system for experimental trial and were given a starter (14-21 days) & finisher diet (21 days afterward) respectively.

Experimental design

A total of 180 unsexed, 10-day-old broiler chicks were randomly divided into four treatment groups, with five replicates each having nine chicks with similar body weight, in a Completely Randomized Design (CRD). The chicks were randomly assigned to 20 pens.

Procurement of test materials

Standard broiler B0, B1, and B2 rations were purchased from a commercial feed company at Bharatpur, Chitwan. The pre-starter ration was fed for 0-2 weeks, the starter ration for 2-3 weeks, and the finisher ration was fed thereafter. Herbal extract (Tinospora cordifolia(gurju)and Aloe vera (Aloe barbadensis) were purchased from a patanjali shop in Narayangadh, Chitwan.

Composition and mixing of the diet

Standard broiler starter and finisher feeds were used in the experiment. Herbal extracts composing Tinospora cordifolia (whole plant) and Aloe barbadensis (whole plant) were used in this study as herbal growth promoters, and immunomodulatory agents having anti-viral, anti-bacterial, and many other benefits. Then the extracts were mixed in the drinking water in the proportion of 1% for both gurju & aloe vera and 0.5%+0.5% gurju & aloe vera extract in combination as well per liter per day basis respectively for required treatment groups (Table 1).

Different dietary treatments used in the experiment were as follows.

T_1: Control diet (Basal diet)

T_2: Basal diet + 1% gurju extract/liter DW/day

T_3: Basal diet + 1% aloe vera extract/liter DW/day

T_4: Basal diet + 0.5% gurju extract + 0.5% aloe vera extract/liter DW/day

The composition of ingredients in herbal extracts and the calculated nutrient composition in the feed have been given in Table 2 and Table 3 respectively.

Ingredients	Compositions
Gurju extract	Each 100ml contains pure giloy juice 100ml with permitted class II preservatives (whole plant extract)
Aloe vera extract	Each 100ml contains pure aloe juice 100ml with permitted class II preservatives (whole plant extract)

Table 1: *Ingredients compositions of experimental herbal extracts used in the experiment*

Ingredients	Pre-Starter	Starter	Finisher
CP%	22%	20.6%	19.4%
ME (kcal/kg)	2973	3061	3140
D-Lysine %	1.18	1.4	0.91
D-Methionine %	0.44	0.48	0.39
D-Methionine + Cystine %	0.89	0.78	0.77
D-Tryptophan %	0.16	0.16	0.17
D-Threonine %	0.76	0.64	0.60
Valine %	0.87	0.74	0.71
Available Phosphorous %	0.46	0.39	0.39

Table 2: *Feed compositions of experimental pre-starter feeds, starter feeds, and finisher feeds.*

General Management

Housing and Feeding Management

All the interior surfaces like the floor, roof, walls, windows, and ventilation were dry cleaned with a brush, and then wet cleaned with detergent water. All the pens of the experimental site were cleaned with lime and disinfected by using Virkon-S. The concrete floor was sprinkled with lime at the rate of 1kg/m^2. Feeders and drinkers were washed with detergent and allowed to dry in the sun. A thin layer of lime was sprayed and then the rice husk 2 inches thick was placed on the floor to use as litter. The house was preheated to 95 °F using electric heaters and bulbs. The chicks were then placed in the house. Raking was done twice a week to prevent cake formation. Litters were removed and replaced with fresh litter every 14 days. A large bucket filled with lime was placed at the entrance of the house to disinfect the footwear every time the house was entered. Pens were constructed using bamboo poles and plastics and each pen was provided with an equal number of drinkers and feeders. Feed and water were provided from 6 am onwards and herbal extracts were given via drinking water (10ml/1L DW) at 6 am every day since the start of the experiment. The temperature of brooding was controlled by increasing or decreasing the height of the bulb. Experiment groups were separated using concrete partitioning. All groups were provided with individual feeders and drinkers. Manual turning and mixing of feed was done frequently 4-5 times a day. All the groups were provided with similar environmental and management conditions during the entire experimental period.

Vaccination

All the experimental birds including the control group were vaccinated with ND vaccine- F1 (Lentogenic strain) at the age of 7 days

through the intraocular route. Similarly, all 4 groups were vaccinated with a modified live IBDV vaccine containing an intermediate plus form of IBD hot strain virus intraocular IBDV (Intermediate plus) at 14 days, RDV Lasota vaccine on the 21st day in drinking water, and then a booster dose of IBD in 28th day in the drinking water.

Age	Vaccine	Route
5 – 7 days	ND Vaccine – F1	Intra-ocular, intra-nasal
14 days	Gumboroo intermediate (live)	Intra-ocular, intra-nasal
21 days	Gumboroo intermediate	Intra-ocular
28 days	ND booster (Lasota)	Intra-ocular

Table 3: *Vaccination schedule.*

Layout of experiment

One hundred & eighty chicks were equally and randomly divided and distributed into four dietary treatment groups T1, T2, T3, and T4 having five replications in each. Each dietary treatment group consists of 36 chicks distributed in five replicated pens (R1, R2, R3, R4 & R5) with 9 chicks in each.

Groups	Treatments	Replicates					Total chicks
		R_1	R_2	R_3	R_4	R_5	
T_1	Basal diet only (Control group)	9	9	9	9	9	45
T_2	A basal diet with TCE @1% / liter drinking water per Day (or per gallon DW/day)	9	9	9	9	9	45
T_3	A basal diet with Aloe vera extracts @1% / liter drinking water per day (or per gallon DW/day)	9	9	9	9	9	45
T_4	Basal diet with Giloy extract 0.5% + Aloe vera extract 0.5%) /liter drinking water	9	9	9	9	9	45
Total							180

Table 4: *Number of chicks assigned randomly assigned to four experimental treatments.*

Selection of Birds

Birds representing the average weight of the treatment group were selected. They were kept apart one day before slaughter. The selected birds were starved at least for 12 hours, before slaughter time. The live weight of each bird was recorded separately before slaughtering to know the carcass characteristics.

Blood sample collection

Randomly selected two birds from each treatment were sacrificed and samples will be collected for the experimental data in 2nd week and 4th week respectively i.e., at 2-week intervals after the birds have been placed to carry out the experimental trial. Blood samples will then be collected via wing veins and were differentiated for hematological analysis. Different hematological parameters viz., packed cell volume (PCV), hemoglobin %, total leucocyte count (TLC) count, percentage lymphocyte count (PLC), and Leukogram, percentage were estimated.

Estimation of hemoglobin

Hemoglobin (Hb) concentration was measured by using Sahli's hemoglobinometer as per Schalm et al. (1975). For that, the graduated tube of the hemoglobinometer was filled with a decinormal (N/10) solution of hydrochloric acid up to mark 10. By using a clean and dry Hb pipette, the blood was sucked from the vial containing anticoagulant up to mark 20. The blood was expelled directly into the graduated tube containing the hydrochloric acid solution. The last trace of blood was removed by drawing the solution up in the pipette and was expelled several times. The contents were mixed thoroughly with the stirrer after every addition and the color was matched with the standard. Adding was continued until the color in the tube corresponded exactly to the color of the standard. The corresponding reading was taken to which the solution has risen in Sahil's tube and it was recorded the concentration of Hb as g/dl of blood.

Estimation of Total Leucocyte Count (TLC)

Hematological analyses for total leukocyte count were done according to the method of Natt and Herrick (1952). The Natt-Herricks-Tic® method performed well concerning staining quality and countability of the granulocytes by the hemocytometer. This method showed acceptable precision for a manual method and demonstrated good agreement with the reference method. It can be recommended as a reliable and suitable method for determining white blood cell counts in avian EDTA blood if non-statistical quality control measures are used in the daily routine. The application of individual reference intervals for the interpretation of white

blood cell counts in birds may improve the diagnostic performance of this important analyte in a clinical setting.

Estimation of Differential Leucocyte Count (DLC)

This differential blood count is based on the staining of the nucleus and cytoplasm of the white blood cells. For differential count, generally, the combination of polychrome methylene blue and eosin stains (Wright's Stain) was used because of their selective staining properties; methylene blue stains the nucleus while eosin stains the cytoplasm. Whole blood was used using EDTA as an anticoagulant. Dried blood smears from each of the two chicks of different groups were fixed in absolute methanol for 5 minutes, airdried, flooded with a staining solution, and allowed to stand for 15 minutes. Slides were then rinsed in distilled water, dried, and observed under an oil-immersion microscope and DLC was calculated. Neutrophils, Lymphocytes, Eosinophils, Monocytes, and Basophils were calculated in this category.

Estimation of Packed Cell Volume (PCV)

Packed cell volumes (PCV) / hematocrit values were determined using the microhematocrit capillary tube method (Campbell, 1995). Whole blood from two chicks of each of the four groups was collected separately in 2 ml microcentrifuge tubes containing 100 µl of EDTA solution (@2mg/ml final concentration). Blood was then filled into microcapillary tubes up to its 3/4th length, the rear end closed by melting, and centrifuged at 12,000g for 5 minutes in a microcentrifuge machine. Packed cell volume was assessed for chicks of each group by comparing them with the calibrated plate.

Estimation of Total Platelet count (TPC)

TPC was calculated by the Rees-Ecker Direct Method for Platelet Count. In the direct method, the number of platelets in diluted blood is counted in a specific volume on the hemocytometer slide. From this number, the platelets in undiluted blood are calculated and reported as several platelets/mm^3 of blood.

Statistical analysis

Data collected during the experiment were analyzed using R statistical software (version 4.0.3). All hematological parameters, including hemoglobin (Hb), total leukocyte count (TLC), packed cell volume (PCV), and differential leukocyte counts (DLC), were analyzed using a two-way Analysis of Variance (ANOVA) with treatment and time as fixed factors. Interaction effects between treatment groups and time (2nd week and 4th week) were tested. Post-hoc comparisons were performed using Duncan's Multiple Range Test (DMRT) to detect significant differences between treatment means. Statistical significance was set at $P < 0.05$. Results are presented as mean ± standard deviation (SD) unless otherwise stated. Graphs and tables were generated using RStudio and Microsoft Excel for visual representation.

Results

Hematological evaluation is useful for the assessment of many disease conditions. Primary alterations in hematological parameters result from disorders within the hemic system itself. Perhaps more importantly, secondary hematological alterations commonly occur as the result of abnormalities in other body systems, so hematological evaluation can provide important diagnostic clues to many diverse conditions. Because similar hematological abnormalities may occur in response to widely different processes, the determination of hematological parameters alone rarely provides a definitive diagnosis. However, when hematological alterations are interpreted in conjunction with other patient information, such as the clinical history, physical examination findings, and other relevant laboratory data, appropriate diagnostic decisions can often be made. Hence, this study aimed to record the physiological blood parameters in cobb500 broilers supplemented with herbal extracts against the control group.

Hemoglobin

The level of hemoglobin of chicks in all four groups remained in the normal range (7-13 g/dL) throughout the study except in the control group in which the level was a bit less than the normal range. The hemoglobin (g/dl) was found to be significant ($p<0.5$) in terms of treatment groups over time. Treatment group T1 (control) had the lowest Hb (5.40 g/dl) and T3 (Aloe Vera 1%) had the highest Hb (9.35g/dl) at the end of 2nd week whereas other values lie in between the two treatment groups. Likewise, at the end of the 4th week of treatment group T4(0.5% Gurju +0.5% aloe vera) had the highest Hb (9.0 g/dl) whereas T1 still recorded the lowest (6.5g/dl) amongst all other treatments. Within the treatment groups, the hemoglobin level values of the chicks remained on the higher side in herbal-treated groups.

Parameters	2nd wk.				4th wk.				Sd (avg.)	F-value			P-value		Trt: Time
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4		Trt	Time	Trt: Time	Trt	Time	
Hb (g/dl)	5.40^{c}	8.10^{abc}	9.35^{ab}	7.70^{abc}	6.50^{bc}	10.65^{a}	8.40^{ab}	9.00^{ab}	0.96	6.467	2.809	1.476	0.0156*	0.1322	0.292
TLC (/cumm)	6.45^{cd}	7.45^{d}	7.20^{cd}	8.30^{d}	9.10^{bc}	16.95^{ab}	15.30^{a}	18.50^{a}	1.06	1.180	1.620	2.956	0.3766	<0.0001***	0.097
Neutrophils %	67.00^{a}	58.00^{a}	57.00^{a}	57.50^{a}	37.55^{a}	47.00^{a}	71.00^{a}	52.55^{a}	14.58	0.386	0.786	1.019	0.766	0.401	0.434
lymphocytes %	23.50^{a}	37.56^{a}	37.57^{a}	37.55^{a}	57.50^{a}	53.00^{a}	35.50^{a}	47.00^{a}	12.90	0.386	0.786	1.019	0.766	0.401	0.434
eosinophils %	3.55^{a}	2.00^{ab}	1.00^{ab}	2.00^{ab}	1.00^{ab}	0.00^{b}	0.50^{ab}	0.00^{ab}	0.70	1.259	8.000	1.630	0.3516	0.0222*	0.257
monocytes %	6.00^{a}	2.57^{a}	4.54^{a}	3.00^{a}	4.00^{a}	0.00^{a}	2.00^{a}	0.55^{a}	1.85	1.532	3.034	0.008	0.279	0.120	0.999
basophils %	0	0	0	0	0	0	0	0	-	-	-	-	-	-	-
PCV %	16.40^{c}	24.45^{abc}	28.55^{a}	24.05^{abc}	19.45^{bc}	32.05^{a}	24.85^{abc}	27.00^{ab}	2.72	6.779	1.973	1.743	0.0137*	0.1977	0.235
TPC(/cumm)	5.31^{a}	5.75^{a}	5.40^{a}	6.30^{a}	5.26^{a}	4.00^{a}	5.60^{a}	3.90^{a}	0.85	0.288	3.819	1.611	0.8327	0.0864	0.261

Table 5: *The various hematological profiles of birds used in this experiment.*

Total Leucocyte Count (TLC)

The TLC was found highly significant ($p<0.001$) over the time interval. The total WBC count of chicks in all four groups was below the normal range (15000-30000/µl of blood) for the first two weeks of age (indicating leukopenia). Treatment group T1 (control) showed the lowest TLC (6.45/cumm) and treatment T4(0.5% Gurju +0.5% aloe vera) showed the highest (8.30/cumm) at the end of 2nd week and other treatment groups T2 (1% Gurju) and T3 (1% aloe vera) lied in between them.

Interestingly, all the groups treated with herbs progressed towards faster recovery from leukopenia from 4th week of age. At the end of the 4th week of treatment, T4 showed the highest TLC (18.5/cumm) and T1 showed the lowest (9.10/cumm) while other treatment groups T2 & T3 lie between them. The chicks from the herbal groups showed progressive improvements along with age.

Differential Leucocyte Count (DLC)

Amongst the DLC only eosinophils were found to be significant ($p<0.5$) while the other hematological parameters viz. neutrophils, lymphocytes, and monocytes were found to be non-significant while basophils were not recorded. Treatment T1 (control) recorded the highest eosinophils count (3.5%), an indication of infection while the lowest was found to be in treatment T3 (1% aloe) at the end of the 2nd week at the end of the 4th week of treatment group T1 had the high count (1%) while T4 recorded no eosinophil count (indication of low infection rates). Other treatment groups lie between them.

Packed Cell Volume (PCV)

The packed cell volume/hematocrit values of chicks in all four groups assessed at two-week intervals are presented in Table 13. PCV% was found significant ($p<0.5$) in terms of treatment groups

rather than the time duration. T3 recorded the high PCV value (28.55%) and T1 showed the lowest (16.40%) at the end of 2nd week. Similarly, T2 (1% Gurju) showed the highest PCV % (32.05%) while T1 showed the lowest (19.45%). Other treatment groups lie between the two at the end of 4th week time.

The highest decrease in PCV values (indicative of anemia, the clinical disease of CIA) was observed in 2nd week, but this decrease was comparatively lesser in herbal-treated groups as compared to the control group which showed marked depression in PCV values. As per the 4th week of age, the chicks of herbal treated groups showed a progressive improvement in PCV values week-wise with highly significant improvements observed with all the herbal preparations as the age advanced.

Total Platelet Count (TPC)

Lastly, TPC(/cumm) was found to be non-significant in terms of treatment: time interaction.

Discussion

There are various infectious diseases capable of causing immunosuppression which results in economic threats to poultry producers. Herbal immunomodulation represents a new frontier area of research for achieving efficient and healthy poultry production as continuous genetic selection and intensive production systems have made the birds highly susceptible to various pathogens (Spelman et al., 2006; Verma and Singh, 2008; Aghsaghali, 2012; Hashemi and Davoodi, 2012; Mahima et al., 2012). The findings in this experiment are in correlation to Rege et al. (1989) and Bishavi et al. (2002) who have proved the hepatoprotective activities of T. cordifolia. In the present study, total lymphocyte count was increased in groups 1 & 2 significantly (i.e., the body is dealing with an infection or other inflammatory condition) as compared to the group fed combined with the extracts of gurju & aloe vera which have slightly lower lymphocyte count meaning that the chicks in this group have to deal less with such conditions hence increment in the immunity status. Manjrekar et al. (1999) also found that aqueous extract of T. cordifolia is capable of increasing leukocyte count in mice which is quite similar to the present findings.

Treatment 1, representing the immunomodulatory effects of the chicks in the control group, depicted the characteristic clinical changes of CIA with significant reduction observed in all the hematological parameters assessed viz., PCV, hemoglobin level, TLC, and PLC counts. This is to the findings of earlier workers (Dhama, 2002; Dhama et al., 2002, 2008a; Schat, 2009; Oluwayelu, 2010) whose findings are in correlation to this experiment.

In the present study, the treated groups of broiler birds showed significantly higher Hb% similar to the findings of Mathew et al. (1999). Throughout the present study, no significant difference was observed among the T. cordifolia stem extract-fed group. The findings in the present study were those of Kolte et al. (2007). Higher Hb concentration in birds fed with the combination of gurju & aloe vera at 0.5 % inclusion level may result in allowing better absorption of the food particles in the intestinal lumen.

Amongst the herbal-treated chicks, T4 showed significant improvement from leukopenia much faster than the control group(T1) which has been attributed to the presence of G14A, an arabinogalactan polysaccharide present in its stem. Other researchers like Rege et al. (1999), Nair et al. (2006), Desai et al. (2007), and Ganguly and Prasad (2011) also reported its action on the immune system by stimulating macrophages through TLR6 signaling and NF kappa B translocation, leading to cytokine production. It was reported that T. cordifolia can stimulate the production of cytokines like IL1 and TNF (Dhanukar et al., 2000) which have an important role in hematopoiesis (Singh et al., 2006) which explains its action in improving the hematological parameters of the chicks treated with the combination of the herbal extracts whose results are quite similar to the findings of above experiment. The potential of medicinal herbs as valuable sources of therapeutic aids has gained a significant role in safeguarding the health system of humans, animals as well as birds (Okitoi et al., 2007; Ansari et al., 2012; Mahima et al., 2012).

Similarly, an important property of Aloe vera that has been the subject of many in vivo and in vitro experiments is improvement in immune response, probably due to the acemannan contained in Aloe vera (Harlev et al., 2012; Djeraba and Quere, 2000; Zhang and Tizard, 1996; Karaca et al., 1995). Acemannan contained in Aloe vera gel is a β (1-4)-linked acetylated mannan containing mannose that can attach to mannose receptors in macrophages (Karaca et al., 1995) and activate these macrophages. In addition, acemannan can stimulate the production of cytokines and, the release of nitric oxide (Zhang and Tizard, 1996; Karaca et al., 1995). Experiments on chickens suggest promoted macrophage activities in broilers caused by the acemannan contained in Aloe vera (Djeraba and Quere, 2000; Karaca et al., 1995). In another study, Alemi et al. (2012) added Aloe vera gel powder (at 0.5%, 0.75%, and 1%)

to broiler feeds and reported an increase in antibody titer against NDV which was similar to the above findings.

On the other hand, assessment of blood parameters showed an increase in total white blood cell and lymphocyte counts on days 37 and 52 for broilers that received 2% Aloe vera gel (mixed with drinking water) compared to the control group (Valle Paraiso et al., 2005). In addition, Darabighane et al. (2011 a) reported an increase in the total white blood cell count of broilers as a result of adding Aloe vera gel to broiler feeds. In another study that used Aloe vera gel powder in broiler feeds, a significant increase was observed in total white blood cell count, red blood cell count, and hemoglobin in groups treated with Aloe vera gel powder compared to the control group, with 0.5% -1% Aloe vera gel powder group showing the highest hemoglobin, red blood cell, and white blood cell count (Mahdavi et al., 2012) which was quite similar to the above findings.

Conclusion

This study highlights the immunomodulatory potential of Tinospora cordifolia and Aloe barbadensis as herbal feed additives in broiler production. Supplementation with these herbs, particularly the combination group (T4: 0.5% T. cordifolia + 0.5% A. barbadensis), resulted in significant improvements in key hematological parameters such as hemoglobin levels, total leukocyte counts, and packed cell volume. The reduction in eosinophil counts further demonstrated the herbs' effectiveness in minimizing infections and enhancing overall health. These findings underscore the efficacy of T. cordifolia and A. barbadensis as natural immunostimulants, offering a viable and sustainable alternative to antibiotics in poultry farming. Incorporating such herbal additives into modern poultry management practices can support healthier broiler flocks and address consumer demand for antibiotic-free poultry products. Future research should explore long-term effects and optimize dosages for various production systems.

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