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Effect of supplementation of herbal growth promoters on the immunological response in broilers

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Abstract

A biological experiment was conducted to evaluate the efficacy of a herbal growth promoter (HGP) comprising equal proportions of *Andrographis paniculata*, *Boerhaavia diffusa*, *Achyranthes aspera*, and *Azadirachta indica* on the immunological response of commercial broiler chickens. A total of 420 day-old straight-run Cobb-400 broiler chicks were randomly allotted to seven dietary treatment groups (T₁-T₇), each consisting of four replicates with 15 chicks per replicate. The treatment groups included a basal diet control (T₁), basal diet supplemented with antibiotic growth promoter (Enramycin 10%) at 100 g/ton of feed (T₂), and basal diet supplemented with the herbal growth promoter at 200, 400, 600, 800, and 1000 g/ton of feed in T₃, T₄, T₅, T₆, and T₇, respectively. Immunological parameters were studied wherein the mean antibody titre for Newcastle disease virus (NDV) and Infectious bursal disease virus (IBDV) was significantly influenced ($p < 0.05$) by the addition of different levels of herbal growth promoters. The highest antibody titre for NDV was recorded in T₄ (1.80 ± 0.03), T₆ (1.78 ± 0.03), and T₇ (1.77 ± 0.02), showing no significant difference from the AGP group T₂ (1.76 ± 0.04). The highest antibody titre against IBDV was observed in T₅ (1489.00 ± 7.82) and T₃ (1478.75 ± 9.03), receiving HGP @ 600 and 200 g/ton of feed, respectively. Bursa of Fabricius weight was significantly influenced ($p < 0.05$), with the highest weight recorded in T₄ ($0.18 \pm 0.02\%$ of body weight). Thymus weight showed no significant difference among treatment groups. Overall, the results demonstrate that dietary supplementation of the polyherbal combination significantly enhanced immunity against both Newcastle disease and Infectious bursal disease in broiler chickens. HGP supplementation at 400 g/ton (T₄) produced NDV antibody titres comparable to the AGP group while also yielding the highest Bursa of Fabricius weight, indicating enhanced immune development. These findings suggest that this polyherbal combination is a safe, effective, and natural immunomodulatory alternative to antibiotic growth promoters in commercial broiler production.

Keywords: Broiler, immunity, herbal, antibiotic, antibody titre, lymphoid organs

Introduction

The total poultry population in India stands at 851.81 million, representing a 16.8% increase over the previous census (BAHS, 2019) [8], with annual growth rates in egg and broiler production of 5.57% and 11.44%, respectively. The poultry sector provides direct or indirect employment to 6.5 million people and contributes nearly 51.14% of the total meat production of the country (BAHS, 2019) [8]. Poultry feed comprises 70-80% of the total cost of poultry enterprises, making the optimization of feed utilization key to sustainable and profitable poultry production.

In poultry diets, antimicrobial compounds are frequently used as antibiotic growth promoters (AGPs) to stimulate growth, control diseases at sub-therapeutic doses, and treat sick conditions at therapeutic doses. However, the indiscriminate use of antibiotics has raised serious concerns over the development of drug resistance in human pathogenic bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococcus* (VRE), multi-drug-resistant *Mycobacterium tuberculosis* (MDR-TB), and Carbapenem-resistant *Enterobacteriaceae* (CRE). These concerns have led the European Union to ban feed-grade antibiotic growth promoters entirely. Beyond antimicrobial resistance, AGPs have been associated with immunosuppressive effects in poultry, compromising the birds' natural defense mechanisms and long-term flock health. Therefore, there is an urgent need for safe, effective, and natural alternatives that not only promote

growth but also strengthen the immunological response of broiler chickens.

Phytogenic feed additives derived from medicinal plants and herbs have gained considerable attention as promising alternatives to AGPs due to their low cost, easy availability, and capacity to improve bird performance, growth rate, and immune function without adverse side effects. Plant-origin compounds incorporated into animal feed enhance livestock productivity through improvement of digestibility, nutrient absorption, and elimination of gut pathogens (Athanasiadou *et al.*)^[7]. Among these, *Andrographis paniculata*, *Boerhaavia diffusa*, *Achyranthes aspera*, and *Azadirachta indica* have emerged as candidates for enhancing both the productive performance and immunological competence of broiler chickens.

Andrographis paniculata, commonly known as the king of bitters, possesses well-documented anti-inflammatory, antibacterial, antipyretic, and immunostimulatory properties (Julaton *et al.*)^[12]. It has been widely used in China, India, and other Southeast Asian nations to treat various infectious disorders including fever, diarrhea, dysentery, malaria, laryngitis, and colds. Dietary supplementation with *A. paniculata* has been demonstrated to enhance immunological and antioxidant systems, protect against gastrointestinal disorders, and encourage growth in poultry (Arify *et al.* and Aneesh *et al.*)^[4, 3].

Boerhaavia diffusa, commonly known as Punarnava, exhibits a range of immunologically relevant properties including hepatoprotective, immunomodulatory, anti-inflammatory, and anti-convulsant effects. Antibacterial activity against gram-negative bacteria has been demonstrated by *B. diffusa*, and it contains alkaloids such as punarnavoside with documented anti-cancer, anti-estrogenic, anti-amoebic, and antifibrinolytic properties (Manu and Kuttan)^[18].

Achyranthes aspera possesses anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, and gastroprotective properties, and has long been used as a medicinal agent for wound healing, blood purification, and treatment of various dermatological and gastrointestinal conditions (Monika *et al.*)^[20]. Its immunostimulatory and anti-stress activities make it particularly relevant in the context of broiler production, where birds are frequently exposed to environmental and production stressors that compromise immune function.

Azadirachta indica (neem) has a wide range of medicinal traits including antibacterial, antiviral, hepatoprotective, antifungal, and antiprotozoal properties without known side effects (Kale *et al.*)^[13]. In poultry, dietary neem supplementation acts as an effective immunomodulator by enhancing both cellular and humoral immune responses (Sadekar *et al.*)^[22]. The antimicrobial and antiprotozoal properties of neem reduce the total microbial load in the gastrointestinal tract, thereby improving gut health and overall immune competence of broiler chickens.

In view of the above, the present study was undertaken to evaluate the effect of supplementation of a polyherbal growth promoter comprising equal proportions of *Andrographis paniculata*, *Boerhaavia diffusa*, *Achyranthes aspera*, and *Azadirachta indica* at different dietary doses on the immunological response in broiler chickens, with the aim of identifying a natural alternative to antibiotic growth promoters in commercial broiler production.

Materials and Methods

A total of 420 newly hatched day-old straight-run Cobb-400 broiler chicks were procured from a commercial hatchery. Chicks were wing-banded, weighed, and randomly assigned to seven treatment groups (T₁-T₇) with four replicates in each group, having 15 chicks per replicate (60 chicks per treatment). Birds were reared under a deep litter system with all standard managemental practices, and all broiler chicks were fed crumbled feed. All procedures followed during the trial were approved by the Institutional Animal Ethical Committee of Veterinary College, Bidar, Karnataka, under project proposal No. VCB/IAEC/LPM-09/2023-24.

Marek's disease vaccine (HVT strain), ND vaccine (Live B1 strain), and Infectious bursal disease (IBD) vaccine of intermediate strain were procured from Ventri Biologicals, Bengaluru. On day 7, chicks were vaccinated against Newcastle disease with the B1 strain, followed by a booster dose on day 21 through the intra-ocular route. On day 14, chicks were vaccinated against IBD with an intermediate vaccine, followed by a booster dose on day 28 through the intra-ocular route.

Table 1: Description of experimental design from 0-42 days period

T ₁	Basal diet (Without Antibiotic)
T ₂	Basal diet + AGP (Enramycin 10%) at 100g/ton of feed
T ₃	Basal diet + Herbal Growth Promoter (200g/ton of feed)
T ₄	Basal diet + Herbal Growth Promoter (400 g/ton of feed)
T ₅	Basal diet + Herbal Growth Promoter (600g/ton of feed)
T ₆	Basal diet + Herbal Growth Promoter (800g/ton of feed)
T ₇	Basal diet + Herbal Growth Promoter (1000g/ton of feed)

Herbal growth promoter are *Andrographis paniculata*, *Boerhaavia diffusa*, *Achyranthus aspera* and *Azadirachta indica* at equal proportion.

Immunological Parameters

1. Antibody Titres against Newcastle Disease and Infectious Bursal Disease

Blood samples were collected from two birds per replicate at the end of the experiment (42nd day). Serum was separated, and antibody titres against Newcastle disease virus (NDV) and Infectious bursal disease virus (IBDV) were estimated by the Haemagglutination Inhibition (HI) test and indirect ELISA, respectively.

(a) Newcastle Disease (HI Test)

The antibody titre against Newcastle disease was carried out using the HA followed by HI test. The micro-test method described by Allan and Gough^[1] was used for detection of HI titres from serum samples. The HI test was performed manually by the B-procedure in 'U' bottom micro-plates using diluters and droppers with 4 HA units of ND viral antigen. Serial two-fold dilutions of serum in normal saline were prepared, and 25 µl/well of 4 HA units of antigen was added. Plates were incubated for 45 min at room temperature. Subsequently, 50 µl of 0.8% chicken erythrocytes was added to each well and the plates were incubated for one hour at room temperature before reading the results. Titres (log₁₀ values) were expressed as the reciprocal of the highest dilution of serum showing haemagglutination inhibition or button formation.

(b) Infectious Bursal Disease (ELISA)

The serum antibodies against IBDV were titrated by using Poultry Diagnostic and Research Centre (PDRC) indirect ELISA Kit. Each of the steps was followed as per the manufacturer's instructions. Each of the wells of antigen pre-coated plate provided in the kit was used for the test. 100 dl each of the positive control serum and the negative control serum was added in duplicates to the respective control wells. Then, 100 dl of each test serum sample diluted in the sample buffer was added in duplicates to corresponding wells of the plate (apart from the control wells) and was incubated at 37 °C for one hour. The plate was washed using the wash buffer provided in the kit. 100 dl of mouse anti-chicken IgG conjugated with Horse Raddish Peroxidase (HRP) in wash buffer was added to each of the wells and incubated at 37 °C for one hour. The plate was washed. 100 dl of freshly prepared chromogen-substrate solution containing OPD (ornithine phenylene diamine) and 3% H₂O: as substrate (4 dl/ml of chromogen) was added to each of the wells and the plate was kept at room temperature for 15 min. Finally, 50 dl of 2.5 N HCl was added to each of the wells to stop enzyme-substrate reaction. Absorbance values were read using the ELISA reader (Bio Rad) with an interference filter at 402 nm. Readings were taken after the wells with only substrate-chromogen and HCl were blanked to "zero" at 492 nm.

2. Lymphoid Organ Weights

The weights of lymphoid organs — Bursa of Fabricius and thymus — were recorded on the 42nd day of the experiment during slaughter of the birds. Organ weights were expressed as a percentage of live body weight.

$$\text{Organ weight (\%)} = \frac{\text{Weight of organ (g)}}{\text{Live weight before slaughter (g)}} \times 100$$

3. Statistical Analysis

Data obtained from the present study were analyzed using Two-way ANOVA with Bonferroni post-test utilizing Graph Pad Prism version 5.0 software. Group comparisons were made at $p < 0.05$ level of significance.

Results and Discussion

1. Antibody Titre against Newcastle Disease and Infectious Bursal Disease

The results of the efficacy of supplementation of different levels of herbal growth promoters on antibody titres against IBDV and NDV are presented in Table 2.

The mean log HI titre values for Newcastle disease virus for T₁, T₂, T₃, T₄, T₅, T₆, and T₇ groups were 1.52±0.04, 1.76±0.04, 1.75±0.03, 1.80±0.03, 1.66±0.03, 1.78±0.03, and 1.77±0.02, respectively. Significantly highest ($p < 0.05$) HI titre for NDV was observed in T₂, T₄, T₆, and T₇, whereas significantly lowest values were observed in T₁ and T₅ groups. Among T₃, T₄, T₆, and T₇, the influence of herbal growth promoter on the HI titre for Newcastle disease virus was similar, and differences were found to be non-significant compared to T₁ (control).

The mean antibody titre values for IBDV for T₁, T₂, T₃, T₄, T₅, T₆, and T₇ groups were 1445.50±11.97, 1449.25±06.16, 1478.75±09.03, 1420.25±04.44, 1489.00±07.82, 1444.50±08.51, and 1448.50±06.41, respectively. Significantly highest ($p < 0.05$) antibody titres for IBDV were observed in T₃ and T₅, whereas significantly lowest values were observed in T₄. Among T₂, T₆, and T₇, the influence of herbal growth promoter on the IBDV antibody

titre was similar and non-significant compared to T₁ (control).

Table 2: Effect of herbal growth promoters and their combination on antibody titre against Infectious bursal disease and New castles disease virus

Treatment	NDV	IBDV
T ₁	1.52±0.04 ^b	1445.50±11.97 ^b
T ₂	1.76±0.04 ^a	1449.25±06.16 ^b
T ₃	1.75±0.03 ^{ab}	1478.75±09.03 ^a
T ₄	1.80±0.03 ^a	1420.25±04.44 ^c
T ₅	1.66±0.03 ^b	1489.00±07.82 ^a
T ₆	1.78±0.03 ^a	1444.50±08.51 ^b
T ₇	1.77±0.02 ^a	1448.50±06.41 ^b

Values bearing different superscripts (a, b, c, d) differ significantly ($p < 0.05$) between groups

These results are in agreement with earlier reports (Sadekar *et al.*; Mathivanan and Kalairasi; Jawad *et al.*; Tomar *et al.*; Arify *et al.*; Astuti *et al.*; Fayed *et al.*; Lonkar *et al.*; Omidwura *et al.*) [22, 19, 11, 23, 4, 6, 9, 17, 21].

The higher antibody titre against NDV and IBDV observed in HGP-supplemented broilers can be attributed to the synergistic immunostimulatory action of the polyherbal combination. When *Andrographis paniculata*, *Boerhaavia diffusa*, *Achyranthus aspera*, and *Azadirachta indica* are used in combination, they enhance biological efficacy by increasing antibody titres against NDV and IBDV viruses. *Andrographis paniculata* has been reported to possess as an immunostimulant (Kumar *et al.*) [15]. The neem component of the combination has anti-protozoal and immunostimulatory characteristics (Kharde and Soujanya) [14].

2. Lymphoid Organ Weights

The results of the efficacy of supplementation of different levels of herbal growth promoters on lymphoid organ weights on the 42nd day of the experimental period are presented in Table 3.

The mean relative weight of Bursa of Fabricius for T₁, T₂, T₃, T₄, T₅, T₆, and T₇ groups was 0.11±0.01, 0.13±0.01, 0.10±0.01, 0.18±0.02, 0.12±0.02, 0.13±0.02, and 0.10±0.02, respectively. Significantly higher ($p < 0.05$) bursal weight was observed in T₄, while significantly lowest weight was observed in T₃ and T₇. Among T₂, T₅, and T₆, the influence of herbal growth promoter on bursal weight was similar and non-significant compared to T₁ (control).

The mean relative weight of thymus for T₁, T₂, T₃, T₄, T₅, T₆, and T₇ groups was 0.13±0.03, 0.10±0.01, 0.09±0.01, 0.14±0.05, 0.16±0.02, 0.11±0.02, and 0.10±0.00, respectively. There was no significant ($p > 0.05$) difference in thymus weight among the various treatment groups when compared with the control group.

Table 3: Effect of herbal growth promoters and their combination on lymphoid organ weight (percent of live body weight).

Treatment	Bursa	Thymus
T ₁	0.11±0.01 ^{ab}	0.13±0.03
T ₂	0.13±0.01 ^{ab}	0.10±0.01
T ₃	0.10±0.01 ^b	0.09±0.01
T ₄	0.18±0.02 ^a	0.14±0.05
T ₅	0.12±0.02 ^{ab}	0.16±0.02
T ₆	0.13±0.02 ^{ab}	0.11±0.02
T ₇	0.10±0.02 ^b	0.10±0.00

Values bearing different superscripts (a, b, c, d) differ significantly ($p < 0.05$) between groups

These results are in agreement with immunomodulating activity of herbal growth promoters reported by Lonkar *et al.* [17], who reported that the weight of bursa of fabricius was increased by inclusion of garlic powder, neem seed cake, and their combination in broiler ration. Tomar *et al.* [23] observed that Punarnava (*Boerhaavia diffusa*) at 1% and 1.5% supplementation had a maximum weight of bursa, spleen, and thymus. Tomar *et al.* [24] observed that treatment groups receiving *Boerhaavia diffusa* had higher lymphoid organ weight than the control group.

The relative increase in Bursa of Fabricius weight in T₄ could be attributed to the inhibitory effects of the polyherbal combination against pathogenic microorganisms throughout the gastrointestinal tract, providing scope for improved nutrient absorption. The higher immunity due to equal combination of *Andrographis paniculata*, *Boerhaavia diffusa*, *Achyranthus aspera*, and *Azadirachta indica* supplementation at 400 g/ton of feed (T₄) could also be attributed to higher nutrient utilization efficiency, which in turn might have triggered immunogenic cells to record a higher immune response. The Bursa of Fabricius, containing various bursal-derived peptides, is a unique humoral immune central organ in poultry (Feng *et al.*) [10]. The thymus is the main site of T cell production, and its immune function plays an important role in anti-infection, antitumour, and autoimmune effects (Amirghofran *et al.*) [2]. Immune organ indices are recognized measures of immune function in poultry (Zhang *et al.*) [25].

Notably, the group supplemented with herbal growth promoters at 1000 g/ton of feed (T₇) recorded the lowest weight of bursa and thymus among all study groups. This is in agreement with Assuncao *et al.* [5], who reported that neem had no influence on lymphoid organ weight and concluded that birds were not severely challenged, thus there was no need for greater immune system activity to contain infection, as bacterial counts were relatively low. This finding further suggests that excessively high doses of HGP may not confer additional immunological benefits and could potentially be counterproductive.

Conclusion

The present study demonstrated that the polyherbal combination comprising *Andrographis paniculata*, *Boerhaavia diffusa*, *Achyranthus aspera*, and *Azadirachta indica* possesses significant immunomodulatory potential in commercial broiler chickens. Supplementation at 400 g/ton of feed (T₄) emerged as the most effective dose, producing NDV antibody titres statistically comparable to the antibiotic growth promoter group (Enramycin 10%) while also yielding the highest Bursa of Fabricius weight, indicating enhanced humoral immune development. High antibody titres against IBDV were achieved at 200 g/ton (T₃) and 600 g/ton (T₅) supplementation levels. These findings collectively suggest that this natural polyherbal formulation can serve as a safe, effective, and economically viable alternative to antibiotic growth promoters in commercial broiler production, contributing to the reduction of antibiotic resistance and improvement of flock health and immune competence. Further studies are recommended to evaluate the long-term effects and mechanisms of action of this polyherbal formulation under field conditions.

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