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**Research Article**

# Effect of Coneflower, Neem, and Thyme Extracts on Growth Performance, Blood Chemistry, Immunity and Intestinal Microbial Population of Broilers

Atif REHMAN [1,‡] Kashif HUSSAIN [1,‡ (*)] Muhammad Arfan ZAMAN [2]
Muhammad Ashraf Zaman FAURK [3] Asghar ABBAS [1 (*)] Wijdan Mohammad Salih MERO [4]
Rao Zahid ABBAS [5] Muhammad Umair WAQAS [1] Zurisha RANI [6] Junaid Ali KHAN [7]
Muhammad Asif RAZA [1,8,9] Muhammad NADEEM [10]

‡ *Both the authors have equal contribution*

[1] Department of Pathobiology, Faculty of Veterinary and Animal Sciences, Muhammad Nawaz Sharif University of Agriculture, Multan, 66000, PAKISTAN

[2] College of Veterinary and Animal Sciences Jhang, PAKISTAN

[3] College of Science, Nawroz University, Duhok, Kurdistan Region, IRAQ

[4] Department of LivestockServices, Ministry of Fisheries and Livestock, Government of the People's Republic, Dhaka, BANGLADESH

[5] Department of Parasitology, Faculty of Veterinary Sciences, University of Agriculture Faisalabad,38000, PAKISTAN

[6] Department of Zoology, Faculty of Science, University of Agriculture Faisalabad,38000, PAKISTAN

[7] Department of Pharmacology and Physiology, Faculty of Veterinary and Animal Sciences, Muhammad Nawaz Sharif University of Agriculture, Multan, 66000, PAKISTAN

[8] Department of Animal Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang, Central Java Indonesia, 50275, INDONESIA

[9] Xinjiang Agricultural Vocational Technical College, Changji, 830023, P. R. CHINA

[10] Department of Pathology, PMAS-Arid Agriculture University Rawalpindi Sub Campus Khushab, 41100, PAKISTAN

[(*)] **Corresponding authors:**
Kashif HUSSAIN & Asghar ABBAS
Phone: +92 335 6953686 (K. Hussain),
+92 336 1734029 (A. Abbas)
E-mail: kashif.hussain@mnsuam.edu.pk (K. Hussain), asghar.abbas@mnsuam.edu.pk (A. Abbas)

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## ABSTRACT

This study investigated the effects of herbal extracts on growth performance, organ development, immunity parameters and intestinal microbial population of broilers. A total of 840 (one day old, initial weight 45±2 g) broiler chicks were divided into 5 groups with 6 replicates having 28 chicks in each. The control group (G1) fed with basal diet, and the antibiotic group (G2) was added with 15 mg/kg virginiamycin, while herbal extracts were given coneflower 20 mL/L (G3), neem 20 mL/L (G4), and thyme 20 mL/L (G5), respectively, in drinking water. The results showed that G3 presented significant results ($P<0.05$) on parameters like feed intake, body weight, weight of bursa of fabricius, weight of the small intestine, blood parameters as compared to the G2 group. As for as intestinal microbiota population concerned G5 decreased the *Escherichia coli (E. coli)* contents while, G4 increased the *Lactobacillus* contents. In conclusion, coneflower improved feed conversion ratio compared to virginiamycin in broilers while neem and thyme were the most effective in the enhancement of immune parameters, serum chemistry and *E. coli* contents.

**Keywords:** Herbal Medicine, Broiler, Immunity, Intestinal microbe

## Introduction

In recent decades, antibiotics have received immense attention from scientists owing to their effectiveness against several diseases. However, these have been found to have various side effects like toxicity, drug residual problem and drug resistance. Thus, scientists are trying to find alternative protocols. For this, the use of additives and natural therapies like medicinal plants rich in anti-oxidents have become best choice to control the different poultry diseases and improve the weight gain and feed conversion ratio (FCR) [1,2]. Among many additives, herbal extracts have been used to replace the generation of antibiotics, which can promote animal health in intensive poultry production [3,4]. Herbal medicine, extracts or compounds can promote animal growth and improve the immune system of the animal which helps to fight against various diseases [5-8].

The main effects of herbal extracts on animals health include to promote appetite, increased feed intake, secretion of endogenous digestive enzymes, activation of the immune system, antibacterial, antiinflammatory and antioxidant properties [9,10]. Like other compounds medicinal plants are also rich in flavonoids, glucosinolates and their intermediates affect the physical structure and chemical properties of the digestive tract [11]. There are many research reports on herbal medicines to cure human and animal diseases [12]. For example, the volatile oil extracted from Incense has antibacterial and antiviral effects [13,14]. Similarly, allicin is widespread on the earth and has many biological functions, such as lowering serum cholesterol, inhibiting bacterial growth and reducing oxidative stress [15,16]. In animals, the effect of applied herbal medicine is not obvious, but it can significantly improve nutrient digestibility and the reduction of *E.coli* bacteria population in intestine and also reduce the number of *clostridia* [17]. The use of feed antibiotics was once considered for the livestock industry in the 20$^{th}$ century and the revolution in feed industry has promoted rapid economic development [18]. However, with the long-term and large-scale abuse of antibiotics residues in the product directly threaten the animal and human health[19]. Antibiotics are helpful to screen resistant strains, leading to identify drug resistance problem which is ultimately threatening to poultry industry all over the world [20]. After the emergence of these problems, countries around the world quickly introduced relevant policies or alternatives to antibiotics. Switzerland banned the use of feed antibiotics in 1992 while, South Korea completely banned addition of antibiotics in animal feed in 2011. Even if there is no prohibition on the use of antibiotics as growth promoters, countries such as Japan and the United States have also legislated to strictly restrict the use and stipulated withdrawal period. Similarly, European countries have also implemented the newly revised feed reforms in May 2012 and feed additives management regulations with the prohibition of antibiotics for growth-enhancing purposes [21,22]. Thus, the choice of alternative especially the use of plants and their herbal extracts has been highly appreciated with no side effects or harmful residues in poultry.

Virginiamycin is an antibiotic produced through mutation of *Streptococcus virginiae* which is found to be effective against gram-positive bacteria and resistance development found minimal also againt gram-negative bacteria like *Enterobacteriaceae*. It inhibits *Enterobacteriaceae* growth, improves digestive system and tissue residues are rare [23]. Supplementing broiler diets with virginiamycin enhanced energy metabolism, growth and FCR. When virginiamycin was added to diets containing insufficient levels of protein, broilers metabolised protein more effectively [24]. It is also discovered that pullets given diets containing virginiamycin improved weight gain [25].

Latest studies have investigated the addition of herbals extract as replacement to antibiotics as growth promoters [26,27]. These herbal extracts have unique mechanism of action based on the alteration of intestinal microbiota, enhancement of enzyme secretion, improvement of immune response, morpho-histological maintenance of the gastrointestinal tract and anti-oxidant activity [28,29]. Several research studies have demonstrated their *in vitro* effect against many pathogens, with antimicrobial, antifungal and anthelmintic activity in addition to antioxidant effects [30,31]. Furthermore, some studies showed the the positive effects of neem and thyme leaf extracts on growth, FCR, intestine microflora, body weight and immune system of birds [32,33]. Therefore, on the basis of importance of above mentioned medicinal plants, the current study was planned to evaluate the effects of three commercial herbal extracts including coneflower, neem, and thyme as a substitute to antibiotics on growth performance, blood chemistry, immune system and intestinal microflora in broiler chickens. Moreover, the antidiabetic potential of these herbal extracts was also studied.

## Material and Methods

### Ethical Statement

The research was conducted with approval from ethical committee of MNS University of Agriculture Multan under the Ref. No. 144/IPFP-II (Batch-I) /SRGP/NAHE/HEC/2020/93.

### Plant Material

Herbal extracts of three plants including coneflower,

neem, and thyme were purchased from Multan Herbal Pharma® Company.

## Experimental Design

A total of 840 (one-day-old, intial weight 45±2 g) commercial cob broilers of Jadeed Group® were purchased and randomly divided into 5 groups, each group was further divided into 6 replicates and each replicate contained 28 birds. The G1 served as Normal control group while G2 served as as antibiotic treated Positive control group (+ Control) and other three groups G3, G4 and G5 were treated with herbal extracts of coneflower, neem, and thyme. Positive control group was treated with virginiamycin at 15 mg/kg with a basal diet, and G3, G4, and G5 were given coneflower at 20 mL/L, neem at 20 mL/L, and thyme at 20 mL/L, respectively, in a drinking water. During the experiment, simple water and commercial feeds (starter, grower, and finisher ration) were offered to the birds. The experiment was conducted in a closed system farm with a 21 hours light and 3 hours dark cycle. *Table 1* shows the diet composition and nutritional level in feed.

## Growth Performance

During the experiment, body weight was measured on weekly basis until 42 days and daily feed intake was measured.

## Immune Performance and Relative Organ Weight

The immune organ index was calculated through the following formula:

Immune organ index (mg/g) = immune organ weight/live weight

At the end of experiment, 3 birds from each replicate were randomly selected for slaughtering. Carcasses and different organs weight were calculated including abdominal fat, liver, pancreas, proventriculus, gizzard, heart and small intestine. On the 28$^{th}$, 35$^{th}$, and 42$^{nd}$ days of age, the thymus, spleen, and bursa of fabricius were taken for organ weight.

## Hematological Parameters and Intestinal Bacterial Population

At the end of the experiment, 5 mL blood samples were taken from 6 birds and stored in an ETDA coated tube for haemoglobin concentration (Hb) and packed cell volume (PCV) analysis [4]. Hb and PCV were determined using Blood Chemistry Analyzer (Sysmax KX-21). Other blood parameters such as red blood cells (RBC) and white

***Table 1.*** *The composition and nutritional level of the basal diet for broilers at each stage*

| **Raw Material Composition** | **1～21 d** | **22～35 d** | **36～42 d** |
|---|---|---|---|
| Corn (%) | 58.5 | 65.00 | 61.50 |
| Soybean meal (%) | 28.00 | 20.00 | 16.50 |
| Wheat (%) | 4.40 | 6.50 | 14.00 |
| Fish meal (%) | 4.60 | 2.80 | 2.80 |
| Canola meal (%) | 1.80 | 1.90 | 3.30 |
| Stone powder (%) | 0.90 | 0.95 | 0.93 |
| Soybean oil (%) | 0.90 | 1.90 | — |
| Salt (%) | 0.21 | 0.25 | 0.25 |
| DL-methionine (%) | 0.10 | 0.10 | 0.13 |
| L-lysine (%) | 0.09 | 0.10 | 0.09 |
| Compound multi-ore (%) | 0.25 | 0.25 | 0.25 |
| **Nutritional Level** | | | |
| Metabolizable energy (Mcal/kg) | 2.85 | 2.95 | 3.05 |
| Crude protein (%) | 21.50 | 19.50 | 17.50 |
| Calcium (%) | 1.00 | 0.95 | 0.90 |
| Available phosphorus (%) | 0.50 | 0.45 | 0.42 |
| Lysine (%) | 1.20 | 1.15 | 1.00 |
| Methionine (%) | 0.55 | 0.48 | 0.45 |
| Methionine + cysteine (%) | 0.95 | 0.85 | 0.75 |
| Sodium (%) | 0.18 | 0.16 | 0.14 |

blood cells (WBC) were also calculated. Meanwhile, diabetes parameters such as cholesterol level and blood glucose were also calculated following the method of [4]. A compound microscope was used to obtain differential leukocyte count of blood samples. The contents of *Escherichia coli* and *Lactobacillus* in the ileum were also determined by agar plate method.

### Statistical Analysis

Using SPSS Software, one-way analysis of variance was done to test the data, and the Tukey test was performed for comparison of the means. In comparison, P≤0.05 was used for significant difference.

## Results

The results on growth performance and organ weight of herbal extracts on broilers are given in *Table 2*. The results showed that the addition of herbal extracts to the diet affected the daily feed intake, feed conversion ratio, body weight and organ weight (%) of broilers. G3 showed significantly higher results (P≤0.05) as compared to G2 group.

The results of the Immune Index and Performance of herbal extracts are given in *Table 3*. The results showed that the addition of herbal extracts to the diet affected the Thymus, Spleen and Bursa weight of broilers. G3 showed the results which were significantly higher (P≤0.05) as compared to control group G2.

The results of herbal extracts on blood chemistry are shown in *Table 4*. The results showed that the addition of herbal extracts positively affected on blood sugar leval, packed cell volume, blood cells, hemoglobin and serum chemistry of broiler. G3 showed better results than G4 and

***Table 2.*** *Effect of Coneflower, Neem and Thyme extracts supplementation on growth performance and carcass characteristics of broilers*

| Parameters | Control (G1) | Virginiamycin (G2) | Coneflower (G3) | Neem (G4) | Thyme (G5) |
|---|---|---|---|---|---|
| Initial body weight (g) | 188±1.250 | 191±2.123 | 189±1.740 | 185±2.175 | 186±2.132 |
| Final body weight (g) | 1826±1.433$^{c}$ | 1924±2.121$^{ab}$ | 2024±2.145$^{a}$ | 1981±1.631$^{b}$ | 1962±1.231$^{ab}$ |
| Weight gain (g) | 1635±2.092$^{b}$ | 1760±2.030$^{ab}$ | 1878±1.241$^{a}$ | 1795±1.302$^{ab}$ | 1775±1.432$^{ab}$ |
| Feed conversion ratio | 1.85±0.302$^{b}$ | 1.69±0.241$^{a}$ | 1.74±0.213$^{a}$ | 1.87±1.012$^{b}$ | 1.78±1.081$^{b}$ |
| Feed intake (g) | 3389±1.451$^{c}$ | 3331±2.456$^{a}$ | 3467±1.922$^{a}$ | 3421±1.132$^{b}$ | 3424±2.383$^{b}$ |
| Dressing (%) | 65.81±1.425$^{c}$ | 66.31±2.$^{21ab}$ | 67.69±2.901$^{ab}$ | 65.01±1.892$^{c}$ | 66.76±2.931$^{ab}$ |
| Liver (g/100 g body weight) | 2.41±1.751$^{b}$ | 2.49±1.021$^{a}$ | 2.89±1.012$^{a}$ | 2.79±1.081$^{ab}$ | 2.39±1.021$^{c}$ |
| Heart (g/100 g body weight) | 0.44±1.009$^{c}$ | 0.56±1.021$^{ab}$ | 0.57±0.518$^{ab}$ | 0.58±0.065$^{ab}$ | 0.59±0.069$^{b}$ |
| Abdominal fat (g/100 g body weight) | 3.21±0.731$^{b}$ | 3.09±0.531$^{a}$ | 3.19±0.077$^{a}$ | 3.10±0.312$^{ab}$ | 2.89±0.941$^{b}$ |
| Breast (g/100 g body weight) | 20.98±1.075$^{c}$ | 22.96±0.921$^{b}$ | 24.31±1.025$^{b}$ | 22.08±1.028$^{ab}$ | 21.38±1.213$^{b}$ |
| Thigh (g/100 g body weight) | 19.98±1.061 | 20.98±1.511 | 21.89±0.189 | 18.97±1.031 | 19.89±1.0251 |
| Gizzard (g/100 g body weight) | 1.51±0.415$^{c}$ | 1.61±0.221$^{b}$ | 1.68±0.074$^{a}$ | 1.69±0.314$^{a}$ | 1.58±0.104$^{ab}$ |

$^{abc}$ *means with different superscripts in a row are significantly different (P<0.05)*

***Table 3.*** *Effect of Coneflower, Neem and Thyme extracts supplementation on immune performance of broilers*

| Parameters | | Control G1 | Virginiamycin G2 | Coneflower (G3) | Neem (G4) | Thyme (G5) |
|---|---|---|---|---|---|---|
| Days | Organs | | | | | |
| (7-14 Days) Week 1 | Thymus | 0.221±0.107$^{c}$ | 0.269±0.243$^{ab}$ | 0.259±0.102$^{ab}$ | 0.269±0.097$^{ab}$ | 0.261±0.215$^{b}$ |
| | Spleen | 0.112±0.129$^{c}$ | 0.141+0.078$^{b}$ | 0.169±0.092$^{b}$ | 0.156+0.079$^{ab}$ | 0.151+0.068$^{b}$ |
| | Bursa | 0.149±0.081$^{bc}$ | 0.161±0.089$^{ab}$ | 0.185±0.096$^{ab}$ | 0.157±0.086$^{b}$ | 0.173±0.046$^{ab}$ |
| (15-21 Days) Week 2 | Thymus | 0.599±0.125$^{c}$ | 0.609±0.206$^{ab}$ | 0.641±0.276$^{ab}$ | 0.624±0.198$^{ab}$ | 0.632±0.356$^{ab}$ |
| | Spleen | 0.239±0.098$^{c}$ | 0.253±0.059$^{a}$ | 0.291±0.056$^{a}$ | 0.266±0.079$^{ab}$ | 0.271±0.083$^{ab}$ |
| | Bursa | 0.448±0.076$^{c}$ | 0.469±0.214$^{ab}$ | 0.498±0.201$^{ab}$ | 0.472±0.247$^{ab}$ | 0.479±0.524$^{ab}$ |
| (22-28 Days) Week 3 | Thymus | 1.039±0.461$^{c}$ | 1.052±0.151$^{a}$ | 1.190±0.283$^{a}$ | 1.172±0.149$^{b}$ | 1.68±0.098$^{ab}$ |
| | Spleen | 0.441±0.215$^{c}$ | 0.489±0.212$^{ab}$ | 0.519±0.142$^{ab}$ | 0.508±0.214$^{b}$ | 0.491±0.134$^{ab}$ |
| | Bursa | 0.719±0.182$^{bc}$ | 0.726±0.218$^{a}$ | 0.768±0.412$^{a}$ | 0.762±0.241$^{b}$ | 0.761±0.316$^{ab}$ |
| (29-35 Days) Week 4 | Thymus | 1.834±0.922$^{c}$ | 2.042±0.461$^{ab}$ | 2.214±0.204$^{ab}$ | 2.195±0.704$^{a}$ | 2.185±0.682$^{ab}$ |
| | Spleen | 0.649±0.203$^{c}$ | 0.741±0.184$^{a}$ | 0.780±0.215$^{a}$ | 0.772±0.161$^{b}$ | 0.769±0.189$^{a}$ |
| | Bursa | 0.321±0.210$^{bc}$ | 0.374±0.182$^{a}$ | 0.424±0.950$^{a}$ | 0.415±0.213$^{b}$ | 0.408±0.271$^{ab}$ |

$^{abc}$ *means with different superscripts in a row are significantly different (P<0.05)*

*Table 4. Effect of Coneflower, Neem and Thyme extracts supplementation on broilers blood chemistry*

| Parameters | Control (G1) | Virginiamycin (G2) | Coneflower (G3) | Neem (G4) | Thyme (G5) |
|---|---|---|---|---|---|
| Cholesterol (mg/dL) | 134.12±0.201$^{c}$ | 129.91±0.082$^{a}$ | 120.67± 0.842$^{a}$ | 119.00±0.715$^{a}$ | 126.00±0.961$^{ab}$ |
| Blood sugar (mg/dL) | 212.74±0.981$^{c}$ | 201.41±0.120$^{b}$ | 189.41±0.197$^{b}$ | 196.42±1.40$^{ab}$ | 201.02±0.270$^{b}$ |
| Packed cell volume (%) | 25.98±0.416 | 26.83±2.091 | 28.94±2.913 | 26.93±2.191 | 27.92±6.021 |
| White blood cell (%) | 203.42±0.532 | 209.45±0.294 | 229.00±0.963 | 217.15±1.510 | 219.41±0.214 |
| Red blood cell (%) | 2.09±0.098$^{c}$ | 2.15±1.721$^{b}$ | 2.31±2.015$^{a}$ | 2.21±1.352$^{ab}$ | 2.24±2.104$^{a}$ |
| Hemoglobin (g/dL) | 9.49±0.561 | 9.79±0.691 | 10.35±0.582 | 9.97±1.141 | 10.26±0.516 |
| Triglyceride mmol/L | 93.9±24.7$^{bc}$ | 98.8±10.4$^{a}$ | 100.7±38.7$^{a}$ | 67.4±18.0$^{c}$ | 93.5 ± 35.5$^{ab}$ |
| HDL (mg/100 mL) | 36.00±0.50 | 39.00±1.87 | 38.00±1.67 | 40.00±1.22 | 38.00±1.14 |
| LDL (mg/100 mL) | 94.00±5.60$^{bc}$ | 87.75±3.10$^{a}$ | 86.75±2.22$^{a}$ | 85.00±1.83$^{b}$ | 89.50±1.91$^{bc}$ |
| Blood lipoprotein mmol/L | 3.04±0.05$^{b}$ | 2.96±0.04 $^{b}$ | 3.31±0.10$^{ab}$ | 3.22±0.12$^{ab}$ | 3.30±0.10 $^{ab}$ |

$^{abc}$ *means with different superscripts in a row are significantly different (P<0.05)*

*Table 5. Effect of coneflower, neem and thyme extracts supplementation on intestinal microbial population of broilers*

| Bacteria | Control (G1) | Virginiamycin (G2) | Coneflower (G3) | Neem (G4) | Thyme (G5) |
|---|---|---|---|---|---|
| *E. coli* | 7.10$^{a}$ | 4.42$^{b}$ | 4.82$^{b}$ | 5.20$^{ab}$ | 4.32$^{b}$ |
| *Lactobacillus* | 4.52$^{c}$ | 4.92$^{b}$ | 6.20$^{a}$ | 5.37$^{ab}$ | 5.82$^{ab}$ |

$^{a,b,c}$ *with in row, value with a similar superscript were not different with probability P≥0.05*

G5 groups which were significantly higher (P≤0.05).

*Table 5* shows the results of the intestinal microbiota population. The results showed that the population of *E. coli* was decreased in all treatment groups except the control group suggesting the reduction of spoilage microorganisms which were significantly higher (P≤0.05) in the antibiotic group followed by G5 and G4 when compared with the control. Moreover, the concentration of *Lactobacillus* was increased in all treatment groups except control groups. The highest concentration was found in G3 suggesting improvement of probiotics bacterial community which ultimately enhanced the immune parameters.

## Discussion

The screening of natural plants and their derivatives for health-promoting activities has been a key priority owing to their least side effect and long-term benefits [34,35]. This study found that the coneflower group significantly improved the body weight gain of broilers during the whole trial period. Thyme and vigemycin had no significant effect on the daily weight gain of broilers. Some other studies reported that the supplementation of herbs like thyme extract improved the immunity and FCR of chickens [36]. The supplementation of 2% thyme extract in drinking water or feed of poultry has already been reported to have positive effects [37,38]. In this study improved growth performance and better carcass results of coneflower and neem treated group were also observed. Previous studies reported that supplementation of herbal extract showed improvement in weight of digestive tract. Similarly, addition of 2% thyme powder to broiler diets showed significant effect on relative weight of digestive tract and have no residual effect on poultry meat [39]. Likewise, another study showed that supplementation of herbs increased body weight, breast muscle weight and improved feed conversion ratio [40].

Blood glucose and cholesterol levels are the indicators of diabetes and generally low blood glucose levels along with low cholesterol are assumed for a healthy life in animals including poultry. High blood sugar influences hyperglycemia, thus should be controlled in order to control diabetes. In this study, it is observed that the supplementation of coneflower and neem lowered the blood glucose level which indicates the antidiabetic potential of these herbs. This might be attributable to the fact that extracts contain more phenolic compounds, resulting reduction in blood glucose and cholesterol levels and also help to boost the immune status and growth performance of the birds [41]. Supplementation of Chinese herbs in drinking water improved the weight of the thymus, spleen and bursa which represent better immunity in birds and also improved the production

of antibodies and immune organs [42]. According to a study, any kind of diseases in the body will reduce the growth of broilers and FCR [43]. Study showed that plant extracts improved immune response by activating cytokines, interleukin-1/6, interferon-α which are the earliest mediator secreted by the body used to respond to antigens [44]. The findings of the another study showed that RBC, WBC and hemoglobin levels were increased with the addition of herbal extracts which indicates the improvement of the blood and immunity parameters [45]. The results of this study indicated that herbal extracts (having polyphenols and antioxidants) have the potential to provoke erythropoietic release from the kidney, which acts as a hormonal regulator in order to produce RBC. Another study showed that herbal extracts raised the WBC and RBC levels, which have the ability to raise the oxygen-carrying capability and the transportation of nutrients [46].

The results of this study found that the addition of coneflower extract to the diet significantly reduced low-density lipoprotein content and significantly increased high-density lipoprotein protein content. Another study showed that allicin treated group reduced the serum triglycerides, cholesterol, and low-density lipoprotein content [47]. The inhibitory mechanism of allicin on bacteria is still unknown, but it can be concluded that the chemical components in allicin can affect the bacterial cell membrane and its cell permeability [48,49]. This study found that thyme extract increased the content of *Lactobacillus* in intestinal contents and it may be due to the oligosaccharide content, because oligosaccharides can increase the production of lactic acid, promote the reproduction of beneficial bacteria in the intestine and reduce the generation of gram-negative bacteria. The supplementation of chinese herbs in drinking water enhanced the weight of the thymus, spleen, and bursa weight, which represents better immunity in birds and also showed better growth performance [50].

The study provides meaningful insights regarding the immune enhancing potential and the growth promoting activities of herbal extracts to be used as an alternative to antibiotics.

### Availability of Data and Materials

Research and supporting data will be available from the author (A. Rehman) on request

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### Competing Interest

The author declared that there is no conflict of interest

### Ethical Statement

The research is conducted by the approval from ethical committee of MNS University of Agriculture Multan under the Ref. No. 144/IPFP-II (Batch-I) /SRGP/NAHE/HEC/2020/93.

### Author Contributions

AR conceived and designed the experiments; KH, AZ, MAZF, AA analyzed the data, drafted and the manuscript; WMSM, RZA and MUW, ZR, JAK, MAR, MN done additional changes in manuscript ;All authors read and approved the final manuscript.

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