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EFFECT OF LIMONIUM STOCKSII LEAF POWDER ON THE GROWTH PERFORMANCE AND INTESTINAL MICROBIAL POPULATION OF BROILER CHICKS

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Abstract: This study was carried out to investigate the effect of *Limonium stocksii* leaf powder on the growth performance and intestinal microbial population of broiler chicks. 500 -1-day old (Ross 308) broiler chicks were randomly distributed into five treatments with 5 replicates and 10 chicks in each replicate in a complete randomized design. Basal diet was adequate in all nutrients according to the requirements of birds. Birds in treatment one (T1) was fed basal diet without *Limonium stocksii* leaf powder while those in treatment two (T2), T3, T4 and T5 were fed same diet with *Limonium stocksii* leaf powder at 20 g, 40 g, 60 g and 80 g per kg diet. The experiment lasted for 28 days and chicks had unrestricted access to clean fresh water and feed. Result obtained showed that *Limonium stocksii* leaf powder contains several phyto-constituents with flavonoids having the highest concentration (851.4 mg/g) while coumarins had the lowest concentration of 82.63 mg/g. Average daily weight gain was higher in T5 (59.68 g/b), intermediate in T2 (54.63 g/b), T3 (54.74 g/b), T4 (54.80 g/b) and lowest in T1 (48.83 g/b) ($P<0.05$). Average daily feed intake and feed conversion ratio which varied from 74.18 – 78.95 g and 1.32 – 1.69 were significantly influenced by the treatment ($P<0.05$). Mortality was recorded only among birds fed T1 (1.21 %) ($P<0.05$). Intestinal microbial counts of *Streptococcus spp*, *Escherichia coli*, *Pseudomonas spp*, *Micrococcus spp* and *Salmonella spp* ranged from 3.85 – 6.55 (cfu/g), 4.92 – 8.13 cfu/g, 2.98 – 4.11 cfu/g, 2.01 – 2.93 cfu/g, 4.74 – 7.08 cfu/g and 4.16 – 6.14 cfu/g were higher in T1 relative to the other groups. Conversely, *Lactobacillus spp* was lowest in T1 relative to the other treatments. In conclusion, dietary supplementation of *Limonium stocksii* leaf powder up to 60 g/kg positively influenced the growth performance and gastrointestinal morphology of broiler chicks.
Keywords: Limonium stocksii; Growth; Microbes; Food safety; Broilers; Phyto-constituents

1 INTRODUCTION

Herbs and herbal preparations have been used to treat ailments since pre-historic times, and the treatment of various diseases with plant-based medicines have remained an integral part of many cultures across the globe. Such medicines were derived directly or indirectly from plants, constitute over 25 % of the pharmaceutical arsenal[1]. However, public concern over antimicrobial resistance risks related to human health has driven interests in animal nutrition and the adoption of antibiotic free feeding systems[2]. Antimicrobial resistance (AMR) is the ability of a microorganism to resist antimicrobial therapy or drug that was originally effective for the treatment of infections caused by it. It has become a major global issue, which threatens the health and well-being of all the people in the world, and therefore requires a global plan of action towards curtailing the severity it poses.

One of the alternatives that can be utilized for combating antimicrobial resistance is traditional herbal remedies from plants[3]. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body and these chemical substances are called phytochemicals[4-5]. These secondary metabolites (phytochemicals) from herbs that exert biological activities and can potentially be used to promote human health. These include alkaloids, amines, cyanogenic glycosides, diterpenes, flavonoids, glucosinolates, monoterpenes, non-protein amino acids, phenylpropanes, polyacetylenes, polyketides, sesquiterpenes, tetraterpenes, triterpenes, saponins and steroids. They have therapeutic properties and their concentration in the plant tissues is considered as the main factor to evaluate the therapeutic value and quality of a given herb. They contain numerous natural products with interesting pharmacology activities.

Limonium stocksii (Boiss) Kuntze belonging to the family Plumbaginaceae and order Caryophyllales which primarily grows in the desert or dry shrubland biome possess antimicrobial capacities and can serve as potential candidates for combating antimicrobial resistance[6]. The plant is widely distributed in Arabian Peninsula, iran, Pakistan, india and other parts of Asia[7-8]. Limonium genus comprises 120 to 150 species of herbs and shrubs, perennial, low-branched, salt secreting, woody shrub found in different parts of the world[9-10]. According to Kanakiya et al. (2018)[11] Limonium stocksii possess phytochemicals such as oxygenated terpenes, tannins, alkaloids, aldehydes and cardiac glycosides, which are responsible for the plant's biological properties. Parts of the plant like the leaves, stem and roots are used for the treatment and management of a range of infections, and of disease conditions like dermatitis, bronchitis, tooth decay, malaria, stomach cramps and dysentery[12]. Furthermore, it was shown that a methanolic extract of *Limonium stocksii*

inhibited the growth of *Staphylococcus aureus, Pseudomonas aeruginosa*, *Salmonella typhi, Bacillus subtilis* and *Klebsiella pneumonia* amongst others[11].
The possible mechanisms of action of medicinal plants or herbs in the animals for growth promotion include changes in the intestinal microbiota, increased digestibility and nutrient absorption, improvement of the immune response and gastrointestinal tract[13-15]. There are also evidences that plant contents have digestion-stimulating properties that have intrinsic bioactivities on animal physiology and metabolism[16]. However, there is little or no information on the dietary supplementation of *Limonium stocksii* leaf powder on the performance of broiler chicks. This research is timely, as it will help to address food safety and provide optimum level for birds.

2 MATERIALS AND METHODS

2.1 Experimental Location and Ethical Approval

This experiment was carried out at the Poultry department, Sumitra Research Institute, Gujarat India situated between 28^{o} 18' N and 70^{o} 35' E India in the month of October 2022. The experiment with an approval number (FL/008C/2022) was carried out according to the ethical guidelines stipulated by the department of Animal management, Sumitra Research Institute, India.

2.2 Collection and Processing of Limonium Stocksii Leaf Powder

Limonium stocksii leaf was collected from Orathur village and sent to the department of crop protection of Sumitra Research Institute, Gujarat for proper identification and authentication, a voucher number 17/9A/2022 was assigned to the collected plant. Thereafter leaves were washed with deionized distilled water, air dried in a shed for 13 days to retain its phyto-constituents, and the dried leaves are powdered to uniform size particle by using an electric blender. Grinded powder of Limonium stocksii leaf was packed into an air tight container for further evaluation.

2.3 Quantification of Phyto-Constituents in Limonium Stocksii Leaf Powder

Quantification of tannins, flavonoids, glycosides, anthocyanins, saponins, steroids, phenolic compound, triterpenoids and coumarins was estimated at different optical densities using Midray GC-MS 6800 gas chromatography/mass spectrometer. The details of the procedure followed is as described earlier by Alagbe (2024)[17].

3 ANIMAL AND THEIR MANAGEMENT

Two hundred and fifty 1- day-old unsexed Ross 308 broiler chicks were used in the experiment. The birds were transferred to 5:00 Am from a reputable hatchery in Gujarat to the poultry unit of Sumitra Research Institute. The initial weights of birds were taken using an electronic weighing balance on arrival. Thereafter, chicks were unboxed and distributed into five treatments in a complete randomized design, with 5 replicates and 10 chicks in each replicate. Battery cage used for the experiment was equipped with nipple drinkers, feeders and 200 W bulbs in each cage to supply heat to birds. Cages were placed in a semi-housed pens which was properly disinfected two weeks before the arrival of chicks. Anti-stress was provided containing Glucomol® with Amivit® for two days administered according to the manufacturers recommendation. Basal diet containing Corn-soya based meal was adequate in all nutrient[43], fed unrestricted for 28 days and also allowed to drink water ad libitum. Light was provided for 24 hours per day throughout the experiment. Feed intake was measured by subtracting the difference in weight of leftovers from that offered per day and the total was divided by the total number of chickens per pen. The feed offered per day and leftovers were measured using the electronic weighing balance used. Average daily weight gains were calculated by subtracting the initial weight of the chicken from the final weight and the outcome was divided by the number of days. Average daily feed intake and average daily weight gain were used to calculate feed conversion ratio. Mortality recorded as encountered throughout the experimental period and mortality percentage was calculated as number of dead birds divide by number of total birds multiplied by 100.

4 EXPERIMENTAL SET-UP

Treatment 1: A Corn soya meal basal diet without Limonium stocksii leaf powder
Treatment 2: The basal diet with 20 g Limonium stocksii leaf powder/100 kg
Treatment 3: The basal diet with 40 g Limonium stocksii leaf powder/100 kg
Treatment 4: The basal diet with 60 g Limonium stocksii leaf powder/100 kg
Treatment 5: The basal diet with 80 g Limonium stocksii leaf powder/100 kg

4.1 Proximate Analysis of Experimental Diet

Proximate composition of experimental diet (starter mash) was determined using diode array based near infra-red reflectance and trans reflectance analyzer. 150 g of sample is placed in the collection funnel after the machine was calibrated following the manufacturers lay down procedures. To maintain further precision, it was adjusted at an optical bandwidth (8.75 nm), spectral resolution (1.0 nm), absorbance ranges (up to 2 AU), wavelength accuracy (less than 0.05 nm), photometric noise (400 – 700 nm less 50 micro au; 700 – 2500 nm less than 20 micro au) before results on moisture content, crude protein, crude ash, ether extract and carbohydrate was generated via the svisual display unit in 1 minutes.

4.2 Intestinal Microbial Population

At the end of the experiment, samples were taken from six randomly selected birds per treatment for microbial analysis. Intestinal samples were collected into a labeled sample bottle and analyzed immediately using 60 IST Cards fully automated microbial ID/AST system with incubator and dispenser (model CHL-DL96A, China). Machine were used in enumerating the population of *Streptococcus spp*, *Escherichia coli, Pseudomonas spp, Micrococcus spp, Salmonella spp and Lactobacillus spp* at different orbit according to the manufacturers' recommendation.

4.3 Statistical Analysis

All the data were subjected to one-way ANOVA using Statistical Package of Social Science (version 25) The differences among the treatment means were determined ($P<0.05$) by Duncan multiple range test of same software.

Table 1 Ingredient and chemical composition (%) of starter diets (as fed basis)

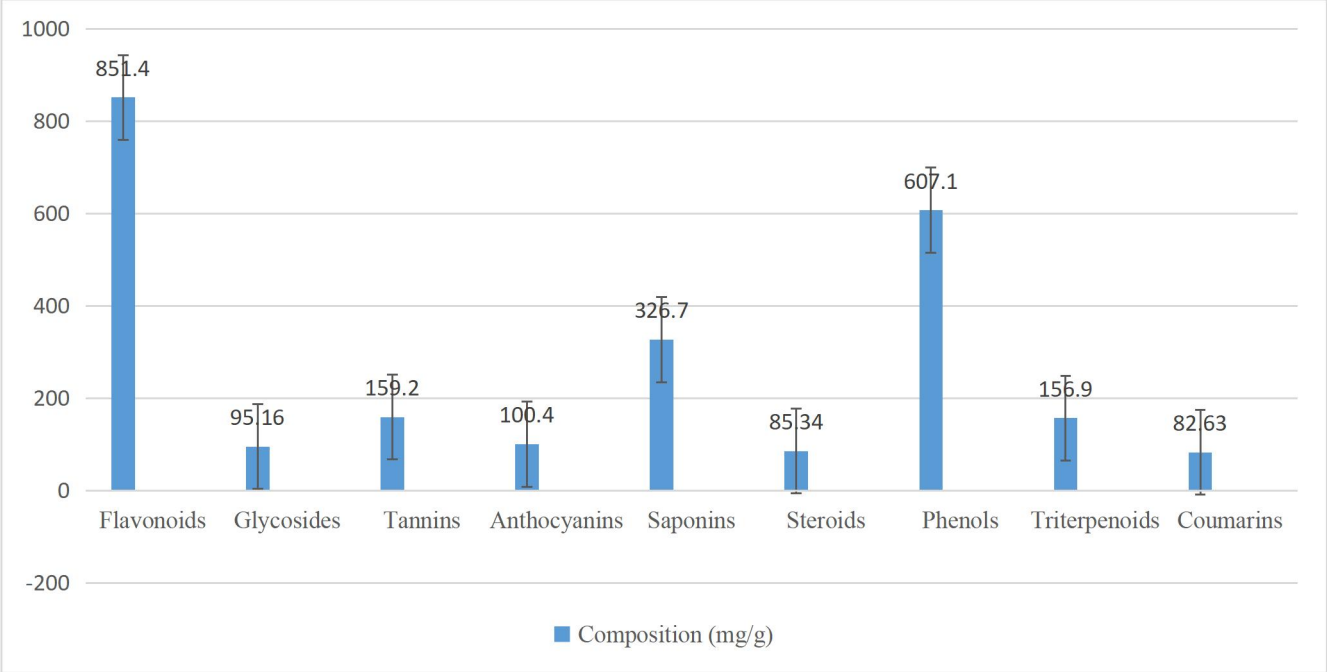
Ingredients	Quantity (%)
Yellow Corn	50.00
Soya bean meal	36.00
Fish meal	4.00
Limestone	1.40
Di-calcium phosphate	2.95
Lysine	0.20
Methionine	0.20
Palm oil	5.00
*Mineral – Vitamin premix	0.25
Salt	0.30
Total	100.0
Determined analysis	
Metabolizable energy (Kcal/kg)	2997.8
Dry matter (%)	90.83
Crude protein (%)	23.40
Crude fibre (%)	4.00
Ether extract (%)	3.92
Ash (%)	5.80
Calcium (%)	1.56
Phosphorus (%)	0.70
Methionine + Cysteine (%)	0.73
Lysine (%)	1.12

*Vitamins and minerals premix: (each 2.5 kg recommended for 1000 kg) contain Vit. A, 10000000 IU; Vit.D3 2000000 IU; Vit.E, 10000 mg; Vit.K3, 2000 mg; Vit.B1, 1000 mg; Vit.B2, 5000 mg; Vit. B6, 1500 mg; Vit.B12, 10mg; biotin, 50mg; pantothenic acid, 10000 mg; nicotinic acid, 30000 mg; folic acid, 1000 mg; choline chloride, 250000 mg; Mn, 60000 mg; Zn, 50000 mg; Fe, 30000 mg; Cu, 10000 mg; I, 1000 mg; Se, 100mg; Co, 100 mg and complete to 2.0 kg by calcium carbonate.

5 COMPOSITION OF PHYTO-CONSTITUENTS IN LIMONIUM STOCKSII LEAF POWDER

Composition of phyto-constituents in Limonium stocksii leaf powder is presented in Table 2. Flavonoids had the highest concentration of 851.4 mg/g followed by phenols (607.1 mg/g), saponins (325.7 mg/g), tannins (159.2 mg/g), triterpenoids (155.9 mg/g), anthocyanins (100.4 mg/g), glycosides (95.16 mg/g), steroids (85.34 mg/g) and coumarins (82.63 mg/g) respectively. The presence of these phyto-constituents suggests that the plants possess several medicinal or pharmacological properties such as; antimicrobial, antioxidant, immune-stimulatory, hypolipidemic, antibacterial, cytotoxic, antidiuretic, anti-cancer, anti-inflammatory, antiviral, anti-fungal amongst others. The result on phyto-constituents of Limonium stocksii leaf powder recorded in this study is in agreement with the findings of Kanakiya et al. (2018)[11]. Medicinal plants are regarded as the richest source of natural antimicrobial agents due to their medicinal properties[5]. They are widely used because of its easy availability, eco-friendly, cost effective and without a withdrawal period[18, 5]. The active principles of many drugs found in plants are secondary metabolites or phyto-constituents[19-20]. The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds[3, 21]. Factors like age of plant, harvesting method, processing technique, geographical location, species, amongst others have been reported to influence the phyto-constituents of medicinal plants[22-24]. Concentrations of flavonoid and phenol recorded in this study was higher than those recorded for Pterocarpus erinaceus leaves 112.61 mg/g and 106.39 mg/g recorded by Olujimi et al. (2024)[25]. Concentrations of tannins was higher than those reported for the leaves of Morinda lucida (2.94 mg/g), Parquetina nigrescens (0.64 mg/g), Magnifera indica (0.38 mg/g), Oscium gratissimum (2.92 mg/g), Chenopodium ambrosoides (0.40 mg/g) and Veronia amygaldalina (1.02 mg/g) by Abiodun et al. (2017)[26].

Table 2 Composition of phyto-constituents in Limonium stocksii leaf powder



Effect of *Limonium stocksii* leaf powder on growth performance of broiler chicks is presented in Table 3. Weight gain was highest in T_5 (1670.98 g), followed by T_4 (1534.54 g), T_3 (1532.59 g) and T_2 (1529.66 g), and lowest in T_1 (1227.22 g) ($P<0.05$). Also, total feed intake was higher ($P<0.05$) in T_5 (2210.7 g), T_4 (2208.6 g), T_3 (2200.5 g) and T_2 (2209.3 g) which had similar values compared to T_1 (2077.1 g) which had a lower value. Feed conversion ratio were significantly difference ($P<0.05$) across treatment groups. The result obtained showed that dietary supplementation of *Limonium stocksii* leaf powder in T_2 (20 g/kg), T_3 (40 g/kg), T_4 (60 g/kg) and T_5 (80 g/kg) could improve the activities of endogenous enzymes and improve nutrient digestion and absorption due to improved permeability of the gut wall of birds. A significant increase in weight gain of birds fed T5 relative to T2, T3 and T4 could also suggest an increase in bile flow in their gut due to the presence of phytochemicals, especially saponins which facilitates the digestion of fat in birds and brush border secretion as well as feed conversion ratio[27]. The result obtained is in agreement with the findings of Alipour et al. (2015)[14] who recorded a significant increase in weight gain of broilers fed plant extracts derived from thyme. Increase in feed intake among chicks fed *Limonium stocksii* leaf powder suggests that it is capable of improving palatability due to its good flavor[28]. Similarly, no mortality was recorded in T2, T3, T4 and T5 indicating that the test ingredient contains antimicrobial properties[29-30]. The antimicrobial activities of phytochemicals can vary, for instance due to tannins act by deprivation of iron; binding with hydrogen or via interactions non-specifically with proteins of the virus or bacteria such as

enzymes[31-32]. The antiviral activity of some medical plant is due to its potent interaction with viral protein[33] and inhibition of the viral ribonucleic acid synthesis[34].

Table 3 Effect of Limonium stocksii leaf powder on growth performance of broiler chicks

Variables	T_1	T_2	T_3	T_4	T_5	SEM
Initial body weight (g/bird)	52.18	52.44	52.01	51.96	51.92	0.01
Final body weight (g/bird)	1279.4^{c}	1582.1^{b}	1584.6^{b}	1586.5^{b}	1722.9^{a}	11.19
Weight gain (g/bird)	1227.22^{c}	1529.66^{b}	1532.59^{b}	1534.54^{b}	1670.98^{a}	9.86
Average daily weight gain (g/bird)	43.83^{c}	54.63^{b}	54.74^{b}	54.80^{b}	59.68^{a}	0.03
Total feed intake (g/bird)	2077.1^{b}	2209.3^{a}	2200.5^{a}	2208.6^{a}	2210.7^{a}	20.09
Average daily feed intake (g/bird)	74.18^{b}	78.90^{a}	78.59^{a}	78.88^{a}	78.95^{a}	0.05
Feed conversion ratio	1.69^{a}	1.44^{b}	1.44^{b}	1.44^{b}	1.32^{c}	0.02
Mortality (%)	1.21^{a}	0	0		0	0.01

Means on the same row having different superscripts are significantly different ($P<0.05$); SEM: standard error of mean; T_1: basal diet without *Limonium stocksii* leaf powder; T_2: basal diet with 20 g *Limonium stocksii* leaf powder/kg; T_3: basal diet with 40 g *Limonium stocksii* leaf powder/kg; T_4: basal diet with 60 g *Limonium stocksii* leaf powder/kg; T_5: basal diet with 80 g *Limonium stocksii* leaf powder/kg

Effect of *Limonium stocksii* leaf powder on intestinal microbial count of broiler chicks is presented in Table 4. *Streptococcus spp* count varied from 3.85 – 6.55 cfu/g, *Escherichia coli* (4.92 – 8.13 cfu/g), *Pseudomonas spp* (2.98 – 4.11 cfu/g), *Micrococcus spp* (2.01 – 2.93 cfu/g) and *Salmonella spp* (4.74 – 7.08 cfu/g) were highest ($P<0.05$) in T_1, followed by T_2, T_3 and T_4, and lowest in T_1. *Lactobacillus spp* population (4.16 – 6.14 cfu/g) were similar ($P>0.05$) for T_2, T_3 and T_4, lowest in T_1 and highest in T_5. Results indicated that the presence of phyto-constituents in *Limonium stocksii* leaf powder could increase the intestinal microbial count of *Lactobacillus spp* as well as decrease *Streptococcus spp*, *Escherichia coli*, *Pseudomonas spp*, *Micrococcus spp* and *Salmonella spp* in broiler chicks. This suggests that *Limonium stocksii* leaf powder contain bioactive compounds with antimicrobial properties[35-36]. For instance, phenols and flavonoids develop their action against bacteria by interacting with the cell membrane. These interaction causes conformational changes in the membrane structure, leading to the leakage of ions across the cell membrane[37-38]. A balanced population of microbes in the gut and improved pH causes the proliferation of beneficial bacteria (*Lactobacillus spp*)[39]. The result obtained is in agreement with the findings of Kanduri et al. (2013)[40] when natural growth promoter (AV/AGP/10) was supplemented in the diet of broiler chickens but contrary to the reports of Oloruntola et al. (2021)[41] who recorded a non-significant difference in the microbial population count of broiler chicken fed diets supplemented with Irvingia gabonensis kernel powder and Ocimum gratissimum leaf powder. This variation could be attributed to herbal combinations, quantity supplemented, species as well as processing method adopted[42].

Table 4 Effect of Limonium stocksii leaf powder on intestinal microbial count of broiler chicks

Parameters (Cfu/g)	T_1	T_2	T_3	T_4	T_5	SEM
Streptococcus spp	6.55^{a}	4.06^{b}	4.01^{b}	4.00^{b}	3.85^{c}	0.01
Escherichia coli	8.13^{a}	5.77^{b}	5.06^{b}	5.01^{b}	4.92^{c}	0.03
Pseudomonas spp	4.11^{a}	3.61^{b}	3.52^{b}	3.11^{b}	2.98^{c}	0.01
Micrococcus spp	2.93^{a}	2.52^{b}	2.41^{b}	2.38^{b}	2.01^{c}	0.01
Salmonella spp	7.08^{a}	5.56^{b}	5.12^{b}	5.03^{b}	4.74^{c}	0.02
Lactobacillus spp	4.16^{c}	5.22^{b}	5.73^{b}	5.77^{b}	6.14^{a}	0.03

Means on the same row having different superscripts are significantly different ($P<0.05$); SEM: standard error of mean; T_1: basal diet without *Limonium stocksii* leaf powder; T_2: basal diet with 20 g *Limonium stocksii* leaf powder/kg; T_3: basal diet with 40 g *Limonium stocksii* leaf powder/kg; T_4: basal diet with 60 g *Limonium stocksii* leaf powder/kg; T_5: basal diet with 80 g *Limonium stocksii* leaf powder/kg

6 CONCLUSION

In conclusion, *Limonium stocksii* leaf powder contains several phyto-constituents of beneficial effects on chicks, from antimicrobial, antioxidants, anti-inflammatory, immune-stimulatory, hepato-protective, antiviral, antifungal and sensorial

stimulators properties. Dietary supplementation of *Limonium stocksii* leaf powder up to 60 g/kg can positively influence the gastrointestinal morphology and improved digestion absorption of nutrients in chicks without causing any negative effect on their health status.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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