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Growth Response and Bacteria Count of Broiler Starter given *Delonix regia* Leaf Extract as a Natural Alternative to Antibiotics

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ABSTRACT

This study was carried out to investigate the growth response and bacteria count of broiler starter given *Delonix regia* leaf extract (DRP) as a natural alternative to antibiotics. Two hundred (200) one-day-old commercial unsexed broilers of Ross-308 strain were randomly distributed into 4 dietary treatments of 5 replicates, each replicate had 10 birds. Treatment 1 (T1) was given Oxytertracycline at 0.30 g/l of water, T2, T3 and T4 were given DRP at 5 ml, 10 ml and 15 ml/l of water. Feed was formulated to meet the nutritional requirement of birds according to NRC and the experiment lasted for 4 weeks. Feed and water offered were offered *ad libitum* in a completely randomized design (CRD). Data on growth performance revealed that DRP had significant ($p<0.05$) effect on final body weight, body weight gain, daily weight gain and feed conversion ratio of the birds. Birds fed 15 ml/l DRP received significantly ($p<0.05$) higher body weight gain and superior feed conversion ratio than the control. The data on bacteria count indicated no significant difference ($p>0.05$) in the *E. coli* counts, a significant increase was observed in the Lactobacillus count ($p<0.05$). No mortality was recorded throughout the experimental period. Based on the results of this study, it can be concluded that DRP is an organic and effective herbal alternative to antibiotics because of its numerous bioactive chemicals and can be given to broilers up to 15 ml/l of water without any deleterious effect on the health and performance of the birds.

Keywords: *Delonix regia* leaf extract, Broiler starter, Growth, Bacteria count, Antibiotics

INTRODUCTION

The use of plant parts such as leaves, roots, fruits, seeds and flowers have been used as phytomedicines since the old civilization because plants are able to produce several bioactive chemicals or secondary metabolites called phytochemicals [1,2]. Phytochemicals or phytobiotics have been used for animal nutrition due to their biological functions such as anti-bacterial, antiviral, anti-inflammatory, antimicrobial, antidiarrheal and antioxidant effects [3,4].

Plant extract consists of various compounds characteristic to the plant (Lina Šernaite, 2017). Secondary metabolites secreted by plants depend on their age, soil type, plants species and environmental condition. Bioactive chemicals synthesized by plants are flavonoids, tannin, phenol, saponin, steroids, glycosides and phytate. Plant extract are cheap, safe and effective against bacteria, thus making them useful as growth promoters and health protectants [5]. For instance, phenols are strong antioxidants and protect animals against stress [6,7]. Flavonoids are known to possess anti-inflammatory, anti-allergic, antiviral and anti-proliferative activities [8,9].

Delonix regia is a flowering plant in the pea family found in tropical areas such as Taiwan, Malaysia, India, Vietnam and central region of South America. Its leaves are used informally to treat diseases in folk medicine to treat many diseases including constipation, arthritis, rheumatoid, diabetes, pneumonia, malaria and so on [10]. Several bioactive chemicals such as saponin, flavonoids, alkaloids, tannin, sterols, carotenoids and phenols have been reported to exist in leaves, flowers, barks and seeds of *Delonix regia* extracts [11,12].

Delonix regia extract (DRP) possess significant biological and pharmacological activities such as antioxidant [13], anti-inflammatory [14], anti-diabetic [15], hepatopretective [16], anti-microbial [17] and anti-diarrheal [18]. According to Shewale et al. [14]; Lung-Shuo Wang et al. [19], DRP was safe and non-toxic in the acute toxicity studies *in vivo* in mice at 400 mg/kg/d. The methanolic extract of leaf of *Delonix regia* have also been found to have beneficial effects in reducing the elevated blood glucose level of hyper-

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glycemic mice [15].

In view of the abundant potential in DRP, this research work was carried out to determine the growth response and bacteria count of broiler starter given *Delonix regia* leaf extract as a natural alternative to antibiotics.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at Division of Animal Nutrition, Sumitra Research Farm, Gujarat, India during the month of January to March, 2018.

Collection and sample preparation

Fresh mature and healthy leaves of *Delonix regia* were harvested within the farm premises. The leaves were thoroughly washed with running tap water to remove the debris and allowed to dry under shade for 7 days until a constant weight was obtained, the dried samples was blended into fine powder using an electric blender and stored in air tight container. The extract (DRP) was prepared by soaking 100 g of *Delonix regia* powder in 2 L of water, then boiled for 2 min, allowed to cool for 24 h sieved with a filter paper and stored in a refrigerator at 4°C.

Phytochemical components of the extracts were determined according to methods described by Harbone (1973) and Odebiyi and Sofowora (1978).

Animals and their management

A total number of two hundred (200) one-day-old commercial unsexed broilers of Ross-308 strain were obtained from a commercial hatchery in India and transported to the farm. The chicks were weighed individually at the beginning of the experiment. They were wing – banded and distributed randomly into 4 dietary treatments of 5 replicates each. Each replicate had 10 birds. The chicks were kept under similar conditions of management throughout the experimental period. Birds were also vaccinated according to the prevailing disease condition in the environment. Water soluble multi-vitamin (Miavit Super) was given to the chicks before 3 days of vaccination and 3 days after vaccinations in order to guard stress. Each pen was equipped with feeders and drinkers to allow *ad libitum* consumption of feed and water. Light was provided approximately 24 h in a form of natural light during the day and artificial light during the night.

Experimental diets and design

The basal diet was formulated to meet the nutrients requirements of broiler chicks according to the NRC (1994).

Treatment 1 – Basal diet + 0.30 g/l of Oxytetracycline in water

Treatment 2 – Basal diet + 5 ml/l DRP

Treatment 3 – Basal diet + 10 ml/l DRP

Treatment 4 – Basal diet + 15 ml/l DRP

The experimental design was Completely Randomized design.

Data collected

Daily feed intake (g) was calculated by difference between feed offered and the left over, feed conversion ratio was determined as feed intake divided by body weight gain, water consumption and mortality were recorded daily throughout the experimental period. Mortality was recorded daily and all management practices were strictly observed throughout the experimental period which lasted for 4 weeks.

Bacteria count analysis

At the end of the 4th week, five birds were randomly selected per treatment; they were slaughtered. After evisceration, One gram of each sample of the jejunal content was collected and transferred into the test tube and was then diluted with 9 ml of 1% peptone broth and homogenized. Counts of bacteria and lactobacillus were determined.

Chemical analysis

The proximate components of *Delonix regia* leaf meal and experimental diet were determined by the method of AOAC (1990).

STATISTICAL ANALYSIS

All data collected were subjected to one-way Analysis of Variance (ANOVA) using the General linear model of SAS (1999) and significant means were separated by Duncan Multiple Range Test (Duncan 1955).

RESULTS AND DISCUSSION

Table 1 reveals the percentage composition of the experimental diet. The proximate components contain crude protein (23.23%), crude fibre (3.14%), ether extract (5.01%), dry matter (91.44%), calcium (1.13%), phosphorus (0.86%) and metabolizable energy (2901.9 Kcal/kg). The diets were formulated to meet the nutritional requirement of birds according to NRC (1994).

Table 1. Percentage composition of experimental diets (0-4 weeks).

Ingredients	Quantity (Kg)
Maize	52.00
Soya meal	38.60
Groundnut cake	3.00
Fish meal (72%)	1.00
Bone meal	3.00
Limestone	1.50
Lysine	0.15
Methionine	0.20
Toxin binder	0.01
Premix	0.25
Salt	0.30
Total	**100.0**
Determined analysis	
Dry matter (%)	91.44
ME (Kcal/kg)	2901.9
Crude protein (%)	23.23
Ether extract (%)	5.01
Crude fibre (%)	3.14
Calcium (%)	1.13
Phosphorus (%)	0.85

** Premix supplied per kg diet: Vit A, 10,000 I.U; Vit E, 5 mg; Vit D3, 3000 I.U, Vit K, 3 mg; Vit B2, 5.5 mg; Niacin, 25 mg; Vit B12, 16 mg; Choline chloride, 120 mg; Mn, 5.2 mg; Zn, 25 mg; Cu, 2.6g; Folic acid, 2 mg; Fe, 5 g; Pantothenic acid, 10 mg; Biotin, 30.5 g; Antioxidant, 56 mg*

The phytochemical analysis of *Delonix regia* extract (DRP) is presented in **Table 2**. The phytochemical components show that saponin (2.03%), flavonoids (3.10%), alkaloids (1.81%), tannin (2.08%), steroids (1.01%), phenol (2.43%) and glycosides (0.07%). Phytochemicals are secondary metabolites or bioactive chemicals found in plants. Secondary metabolites of plants play a vital role as defense mechanism against attack by microorganisms [20,21].

Table 2. Phytochemical components of DRP.

Parameters	Composition (%)	Recommended safe
Saponin	2.03	7.02
Flavonoids	3.10	6.11
Alkaloids	1.81	3.50
Tannin	2.08	31.20
Steroids	1.01	1.30
Phenol	2.43	1.00
Glycosides	0.07	0.02

Phenols are found in many plants and they function as antioxidants, free radicals scavengers [22,23], anti-inflammatory, anti-aging and ant carcinogen. Saponin performs antimicrobial and anti-inflammatory roles [24], there are 11 classes of saponins: dammaranes, tirucallanes, lupanes, hopanes, olananes, taraastrranes, ursanes, cycloartanes, lanostanes, cucurbitanes and steroids [25,26].

Flavonoids have protective effects including anti-inflammatory, antioxidant, antiviral, anti-diabetic and anti-carcinogenic properties [27-29]. Alkaloids are heterogeneous group of naturally occurring compounds found in the leaves, roots and barks of some plants, they are found to have antimicrobial properties due to their ability to intercalate DNA of microorganisms [30]. However, all the DRP values obtained were within the safe recommended range of phytochemical for animals reported by Kumar and Amit [31] and Alagbe [32].

Performance traits of broiler chicks given DRP as alternative to antibiotics is presented on **Table 3**. The final weight range 1001.7-1130.6 g while the final weight gain is 959.0, 965.3, 1081.8 and 1088.5 g for treatment 1, 2, 3 and 4, respectively. There was a significant difference ($p<0.05$) among the treatment in terms of the final weight gain. Birds in T5 had the highest weight gain (1088.5 g) followed by T4 (1081.8 g), T3 (965.27 g) and T1 (959.0 g), respectively. The result obtained in this experiment is in agreement with the finding of Dalkiliĉ et al. [33] when clove extract was supplemented at 400 ppm in the diet of broilers. Similarly, Hassan et al. [34] reported a significant difference in weight gain of broiler given 2% Tulsi leaf extract when compared with the control group but contrary with the reports of some researchers when lemon grass was supplemented in the diet of quails at 3%. A significant difference was also recorded ($P<0.05$) in the feed intake among the treatment, this is parallel with the finding of Karangiya et al. [35] when garlic and ginger was supplemented at 1% in the diet of broiler chicken which is inconsistent with the finding of Imasuen et al. [36]. Feed conversion ratio was significantly ($P<0.05$) influenced in all the treatment groups and no mortality was recorded throughout the experimental period. According to some researchers the growth rate of an animal provides information that helps to ascertain the level of feeding and management for economic advantage.

Table 3. Performance traits of broiler chicks given DRP as alternative to antibiotics.

Parameters	T1 (0.3 g/l Ox)	T2 (5 ml/l DRP)	T3 (10 ml/l DRP)	T4 (15 ml/l DRP)	SEM
No of birds	50	50	50	50	-
IBW (g)	42.70	42.63	42.00	42.10	0.03
FW (g)	1001.7	1007.9	1123.8	1130.6	11.3
BWG (g)	959.0^{c}	965.27^{c}	1081.8^{b}	1088.5^{a}	10.4*
FI (g/bird)	1670.1^{a}	1645.8^{a}	1599.0^{b}	1590.1^{c}	17.5*
FCR	1.70^{b}	1.71^{c}	1.49^{a}	1.46^{a}	0.02*
MORT	0/50	0/50	0/50	0/50	-

^{abc} *means different superscript along rows differs significantly at $p<0.05$*

**: Significant difference*

IBW: Initial Body Weight; FW: Final Weight; BWG: Body Weight Gain; FI: Feed Intake; FCR: Feed Conversion Ratio; MORT: Mortality

Bacteria and Lactobacillus count of broiler given Oxytetracycline and DRP is presented in **Table 4**. *E. coli* count values obtained are 18.92, 18.77, 18.72 and 18.70 (cfu/g) for T1, T2, T3 and T4, respectively. The *E. coli* values slightly reduced from T1 to T4 though not at a significant rate ($P>0.05$). This is similar with the finding of Alagbe [32] who noted that there was no significant difference ($P>0.05$) in the *E. coli* count of broilers given 20 ml/l *Luffa aegyptiaca* leaf extract (LUF) and 1.25 g/l of Neomycin. The lactobacillus count values obtained are 9.44, 13.12, 17.02 and 20.44 (cfu/g) for T1, T2, T3 and T4, respectively. The values were significantly influenced ($P<0.05$) in all the treatment groups. This result clearly shows that DRP functioned as a probiotics, thus reducing the number of pathogenic bacteria making it possible for the animals gut to utilize feed effectively. According to Parker [37] prebiotics are microorganisms or substances that contribute to intestinal microbial balance. Similarly, Jemigan et al. [38] defined probiotics as culture of specific living microorganism, which implant in the animal to which it is given and ensures effective establishment of intestinal microbial population. They are capable of exerting a beneficial effect on host animal (increased growth and production). Others reported that probiotics supplementation in broilers increases intestinal length and weight. Improved digestibility of nutrients and metabolizable energy of diets has been reported with the administration of probiotics in diet [39,40] **(Figure 1)**.

Table 4. Bacteria and Lactobacillus count of broiler given Oxytetracycline and DRP.

Parameters	T1 (0.3 g/l Ox)	T2 (5 ml/l DRP)	T3 (10 ml/l DRP)	T4 (15 ml/l DRP)	SEM
E. coli (cfu/g)	18.92	18.77	18.72	18.70	0.24
Lactobacillus	9.44^{c}	13.12^{b}	17.02^{a}	20.44^{a}	0.45*

^{abc} *means different superscript along rows differs significantly at p<0.05*
**: Significant difference*

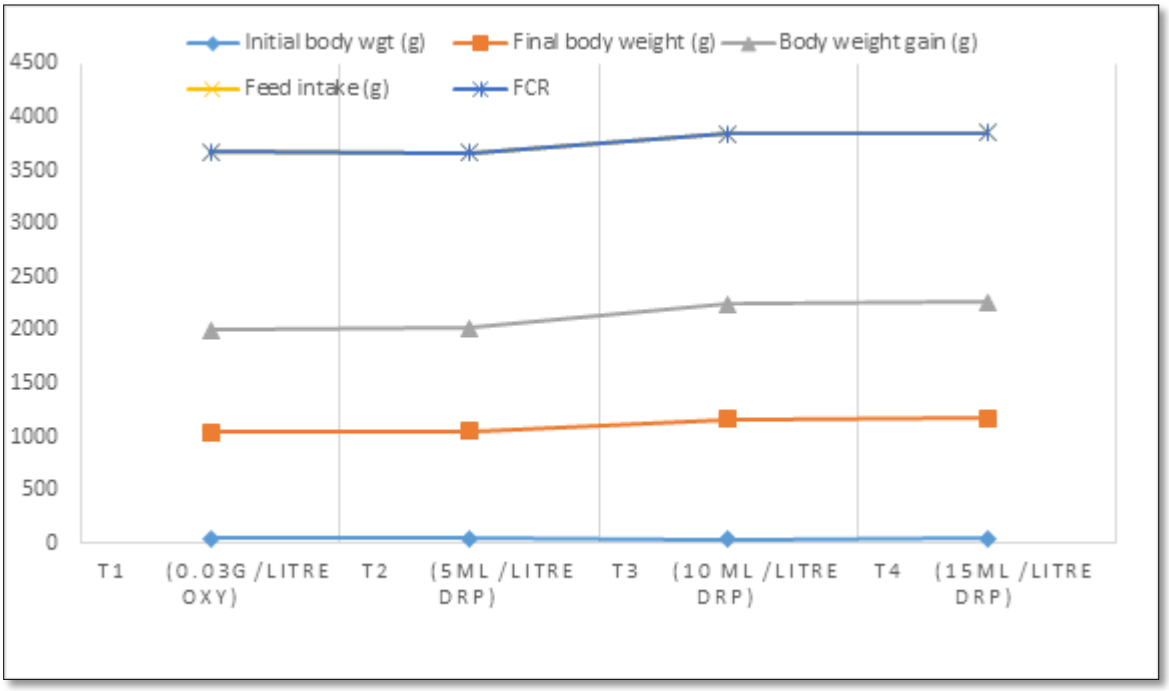

Figure 1. Performance traits of broiler chicks given Oxytetracycline and DRP.

CONCLUSION AND RECOMMENDATION

It can be concluded that DRP can be used as an alternative herbal drug because it contains several bioactive constituents such as saponin, flavonoids, alkaloids, tannin, phenol and steroids which are relatively safe, improves performance and efficient in controlling mortality and therefore can be included at 15 ml/l in the water of birds without any deleterious effect on the performance and health status of the animal.

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