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Albizia lebbeck Stem Bark Aqueous Extract as Alternative to Antibiotic Feed Additives in Broiler Chicks Diets: Haematology, Serum Indices and Oxidative Status

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ABSTRACT

A total of Three hundred and seventy five (375) one day old (Ross 308) broiler chicks with mixed sex were used to examine the effects of Albizia lebbeck stem bark (ATSM) aqueous extract as alternative to antibiotic feed additives in broiler chicks diets: haematology, serum biochemical indices and oxidative status. Birds were divided to five treatments with five replicates of fifteen (15) birds in a completely randomized design. Treatment 1 (basal diet + 0 % ATSM), treatment 2 (basal diet +1.2 grams Oxytetracycline per litre of water), treatment 3 (basal diet + 10 ml ATSM per liter of water), treatment 4 (basal diet + 20 ml ATSM per litre of water) and treatment 5 (basal diet + 30 ml ATSM per liter of water) and the trial lasted for 56 days. Results on some haematological parameters revealed that red blood cell (RBC), pack cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell (WBC) and its differentials were significantly (($P<0.05$) different among the treatments. Total protein, glucose, urea, cholesterol, creatinine, aspartate aminotransaminase (AST) and alanine aminotransferase (ALT) were significantly (($P<0.05$) affected by ATSM. Activities of superoxide dismutase (SDA), glutathione peroxidase (GPx), catalase (CAT) and malonyldialdehyde (MLA) were significantly influenced by ATSM ($P<0.05$). It was concluded that ATSM could be administered to broiler chicks at 30 ml/litre without any negative effect on the general performance of birds.

1. Introduction

Phytogenics are heterogeneous group of feed additives emanating from plants and consists of herbs, fruit, spices and other plant parts (Santi and Kim, 2017). According to Veerschari et al. (2011), there are over 100, 000 species of plants used globally for medicinal purposes, many have been used in the form of therapy for livestock among resource poor smallholder farmers to treat variety of conditions of animals (Mirazaei-Aghsaghali, 2012; Alagbe *et al*., 2020). Most medicinal plants have been found to be abundant in minerals, vitamins, amino acid and bioactive chemicals [phytochemicals] (Olafadehan *et al*., 2020). However, only a small percentage have been explored or studied for their pharmacological properties (WHO, 1992). Nutrients in plants have great influence on responses of animals to a disease challenge and it has a direct correlation to the immune system (Gary and Richard, 2002). One of the numerous plant used for therapeutic purposes is *Albizia lebbeck.*

Albizia lebbeck (Mimosaceae) is a perennial tree native to tropical and subtropical regions of Asia and Africa. The genus Albizia comprises of almost 150 species spread all over India, China, Nigeria, Senegal, Ghana, Togo, Congo, Benin, Angola, Uganda, Botswana among others (Ukpabi and Offor, 2018; Karuppannan, 2013). The plant parts (stem, leaf and seeds) have been found to be loaded with minerals (calcium, phosphorus, iron, copper, zinc, selenium, molybdenum and potassium), vitamins and amino acids (Alagbe and Soares, 2018; Uzoekwe and Mohammed, 2015; Mohammed et al., 2012). The leaf and stem has traditionally been used for the treatment of fever, tooth ache, wounds, leprosy, ulcer, cold, leprosy, sexually transmitted diseases and other respiratory infections (Labaran, 2016; Uwaya et al., 2017).

Several reports on the biological activity of *Albizia lebbeck* revealed that the plant performs antimicrobial (Labaran et al., 2016), anti-inflammatory (Gupta et al., 2004), antioxidant (Mc Donald et al., 2001), analgesic (Sharma et al., 2007), antihelminthic (Karuppannan, 2013), hepato-protective (Edeoga et al., 2005; Alagbe, 2019), antidiabetic (Kareru et al., 2007), immuno-modulatory (Sharma et al., 2007) and antihyperlipidemic properties (Ueda et al., 2003) due to the presence of several bioactive chemicals such as alkaloids, flavonoids, saponins, phenols, tannin etc (Labaran et al., 2016). In view of these abundant potential, administration of *Albizia lebbeck* stem bark to birds will possibly supply nutrients to meet all the body's need during a time of challenge.

Therefore, this experiment was designed to determine the effects of *Albizia lebbeck* stem bark aqueous extract as alternative to antibiotic feed additives in broiler chicks diets: haematology, serum biochemical indices and oxidative status.

2. Materials and Methods

2.1 Study Area

The experiment was carried out at Division of Animal Nutrition, Sumitra Research Institute, Gujarat, India during the month of April to June, 2019.

2.2 Sources, collection and preparation of *Albizia lebbeck* stem bark extract

The stem of *Albizia lebbeck* stems were obtained from different plants in Gujarat, India and authenticated by a botanist Sharma Xing. The stem bark were cut into pieces and thoroughly washed with distilled water, air dried under the shade to maintain the bioactive chemicals in the test material. The dried samples were pulverized into powder using pestle and mortar, thereafter 250 grams of the sample was soaked into 1000 litres of water, sample was continuously stirred and kept in the refrigerator at 4^{o}**C** for 72 hours. All mixtures were filtered using Whatman filter paper and the filterates (ATSM) were collected into a clean labelled container.

2.3 Experimental animals and management

Three hundred and seventy five one day old (Ross 308) broiler chicks with mixed sex were used for the experiment. The birds were purchased from a commercial hatchery in India and weighed on arrival on the farm to obtain their initial body weight and thereafter weekly. A deep litter housing system was used, it was fumigated two weeks prior to the commencement of the study, and the surrounding environment was also cleaned daily to ensure proper hygiene. Birds were divided to five treatments with five replicates of fifteen (15) birds in a completely randomized design. Electric brooders were used and wood shavings serve as the litter material. Daily feed intake (g) was calculated as a difference between feed offered and left-over.Vaccines were administered according to the prevailing disease condition in the environment and all other management practices were strictly adhered to throughout the experiment which lasted for 56 days.

2.4 Ration formulation

Three (3) basal diets were formulated at different stages of production to meet up with the requirements of birds according to NRC (1994). Broiler starter's mash (0-21 days), growers mash (22-35 days) and finishers mash (36-56 days).

Treatment 1 (basal diet + 0 % ATSM), treatment 2 (basal diet +1.2 grams Oxytetracycline per litre of water), treatment 3 (basal diet + 10 ml ATSM per liter of water), treatment 4 (basal diet + 20 ml ATSM per litre of water) and treatment 5 (basal diet + 30 ml ATSM per liter of water).

2.5 Parameters measured

Proximate compositions of experiment diet were determined by using official method of analysis by AOAC (2000). Amino acid analysis was carried out using amino acid analyzer with ion exchange chromatographic method (Model NH-09b) India.

2.6 Haematological and serum biochemical analysis

Blood samples were collected very early in the morning from the wing vein from three (3) randomly selected birds per replicate into a 5 ml sterile syringe using 23 gauge needles and transferred into an ethylene diamine tetra acetic acid (EDTA) bottle. Haematological parameters: pack cell volume (PCV), red blood cell (RBC), haemoglobin (Hb), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), white blood cell (WBC) and its differentials were analyzed using an automated machine (Sysmex, Model KU-30 HG, India).

Serum analysis was carried out using bottles free from EDTA, blood were analyzed for total protein, albumin, globulin, glucose, cholesterol, creatinine, alanine transaminase (ALT) and aspartate transaminase (AST) were assayed using diagnostic kit manufactured by Merck India Ltd (Model PS-09R) as described by Olubukola *et al*. (2015).

2.7 Antioxidant status

Activity of superoxide dismutase (SDA), glutathione peroxidase (GPx), catalase (CAT) and malonyldialdehyde (MLA) were carried out using method outlined by Mahipal *et al*. (2015).

2.8 Statistical analysis

All data were subjected to one -way analysis of variance (ANOVA) using SPSS (18.0) and significant means were separated using Duncan multiple range tests (Duncan, 1955). Significant was declared if P ≤ 0.05.

Table 1: Chemical composition of experimental diets

Materials	Starter (1-21 days)	Grower (22-35 days)	Finisher (36-56 days)
Maize	50.00	56.00	60.50
	8.00	7.00	8.05
Wheat offal			
Soya meal	28.55	22.00	21.00
Groundnut cake	10.00	11.55	6.05
Fish meal	2.00	2.00	2.00
Bone meal	0.35	0.40	0.40
Limestone	0.20	0.20	0.20
Lysine	0.15	0.15	0.15
Methionine	0.20	0.20	0.20
Premix	0.25	0.25	0.25
Salt	0.30	0.30	0.30
TOTAL	100.0	100.0	100.0
Calculated analysis			
Crude protein	23.08	20.11	19.33
Ether extract	5.03	4.87	4.28
Crude fibre	3.06	3.95	3.42
Calcium	0.98	1.00	1.10
Phosphorus	0.47	0.40	0.51
Lysine	1.17	1.29	1.60
Meth +Cyst	0.87	0.82	0.51
ME (Kcal/kg)	2936	3000.8	3100.2

*****Premix supplied per kg diet: - vit A, 13,000 I.U; vit E, 5mg; vit D3, 3000I.U, vit K, 3mg; vit B2, 5.5mg; Niacin, 25mg; vit B12, 16mg; choline chloride, 120mg; Mn, 5.2mg; Zn, 25mg; Cu, 2.6g; folic acid, 2mg; Fe, 5g; pantothenic acid, 10mg; biotin, 30.5g; antioxidant, 56mg.

Table 2: Amino acid compositions of *Albizia lebbeck* stem bark

Amino acids	Composition (%)	*Reference level
Lysine	0.86	5.50
Arginine	1.65	1.00
Aspartic acid	2.00	-
Threonine	1.12	0.65
Histidine	3.45	0.30
Serine	0.78	-
Glycine	1.00	1.20

Alanine	3.00	-
Cystine	4.11	0.35
Valine	0.65	0.82
Leucine	1.00	1.20
Phenylalanine	0.34	0.50
Tyrosine	0.10	0.45
Isoleucine	2.00	0.60
Proline	0.03	0.20
Methionine	0.02	0.35

*NRC (1994)

Table 3: Haematological parameters of broiler chicks fed different levels ATSM

Parameters	T1	T2	T3	T4	T5	SEM
PCV (%)	26.50[c]	29.31[b]	31.02[b]	33.56[a]	34.00[a]	0.37
Hb (g/dl)	9.12[c]	10.21[b]	11.93[b]	12.11[a]	12.50[a]	0.64
RBC $\times 10^6$µl	1.88[c]	2.00[b]	2.10[b]	2.60[a]	2.97[a]	0.07
MCV (fl)	111.2[b]	119.8[a]	120.5[a]	123.6[a]	130.4[a]	8.10
MCH (pg)	34.51[c]	50.43[b]	56.11[b]	57.67[a]	59.00[a]	2.51
MCHC (g/dl)	29.80[b]	35.60[a]	39.00[b]	39.10[a]	40.03[a]	0.88
WBC$\times 10^3$µl	20.41[b]	20.62[b]	22.74[b]	22.88[b]	30.04[a]	0.12
Differentials (10^3µl)						
Lymphocytes	10.45[c]	14.08[b]	15.44[b]	18.71[b]	20.04[a]	1.96
Monocytes	0.07[c]	1.11[b]	1.20[b]	1.26[b]	1.72[a]	0.01
Heterophils	4.23[b]	5.06[a]	5.40[a]	5.89[a]	6.11[a]	0.41
Eosinophils	0.88[b]	1.02[a]	1.09[a]	1.21[a]	1.27[a]	0.02

Means in the same row with different superscript are significantly different (*P*<0.05)

Table 5: Serum analysis of broiler chicks fed different levels of ATSM

Parameters	T1	T2	T3	T4	T5	SEM
Total protein (g/dl)	2.57[b]	3.22[a]	3.69[a]	3.88[a]	3.97[a]	0.67
Albumin (g/dl)	1.34[b]	1.55[b]	1.91[b]	2.00[a]	2.03[a]	0.02
Globulin (g/dl)	1.23[c]	1.67[b]	1.78[b]	1.88[a]	1.94[a]	0.15
Creatinine (mg/dl)	0.15[c]	0.45[b]	0.81[a]	0.87[a]	0.91[a]	0.01
Glucose (mg/dl)	196.1[c]	204.3[a]	234.1[a]	241.5[a]	250.6[a]	4.33
Cholesterol (mg/dl)	101.4[a]	98.6[b]	90.4[a]	89.4[a]	87.5[a]	2.87
Uric acid (mg/dl)	7.33[a]	4.89[b]	4.22[b]	4.00[b]	3.88[b]	0.05
ALT (u/l)	74.1[a]	70.5[a]	61.6[b]	58.1[b]	50.7[b]	1.45
AST (u/l)	300.7a	288.5[b]	230.4[b]	218.0[b]	200.9[b]	9.45

Means in the same row with different superscripts differ significantly (*P*<0.05)

Table 6: Antioxidant status of broiler chicks fed different levels of ATSM

Parameters	T1	T2	T3	T4	T5	SEM

MLA (U/mg Hb)	1.85^c	2.77^b	2.93^b	3.04^a	3.11^a	0.03
SDA (U/mg Hb)	35.7^b	39.8^b	40.7^a	43.5^a	45.3^a	1.21
GPx (U/mg Hb)	27.1^b	29.4^b	33.8^a	34.7^a	38.3^a	1.96
CAT (U/mg Hb)	54.2^a	45.7^b	42.5^b	41.6^b	40.1^b	0.52

Means in the same row with different superscripts differ significantly ($P<0.05$)

3. Results and Discussion

The proximate composition of experimental diet (Table 1) revealed that it contains crude protein of 23.08 %, 20.11 % and 19.33 %; energy of 2936.0 kcal, 3000.8 kcal and 3100.2 kcal for starter, growers and finisher mash. The ether extract range between (4.28 – 5.03 %) and crude fibre range between (3.06 – 3.95 %). The proximate components meet the nutritional needs of birds according to NRC (1994). The crude fibre and ether extract range also conforms to the report of Teodora *et al*. (2020) in feeding broilers *Hermetia illucens* meal. The calcium (0.98 – 1.10 %) and phosphorus (0.47 – 0.51 %) range in the experimental diet is in line with the reports of Fascina et al. (2007); Aduku (2004). Proper feeding is one of the key cardinals of management in livestock production, for animals to perform at their optimum, there is need to furnish them with proper balanced diet which contains all the necessary nutrients (Alagbe and Oluwafemi, 2019).

The amino acid composition of *Albizia lebbeck* stem bark is presented in Table 2. Results revealed the presence of threonine (1.12%), leucine (1.00 %), lysine (0.86 %), valine (0.65 %), glycine (1.00 %), phenylalanine (0.34 %), histidine (3.45 %), methionine (0.02 %), alanine (3.00 %), serine (0.78 %), proline (0.03 %), aspartic acid (2.00 %), arginine (1.65 %), tyrosine (0.10 %), isoleucine (2.00 %), aspartic acid (2.00 %) and cysteine (4.11 %). The sample contains high concentration of histidine and tyrosine has the lowest concentration. Amino acids are building blocks of protein which are necessary for gene expression and cell signal transduction regulation (Chzmruspollert et al., 2004). Phenylalanine plays a vital role in insulin secretion and fat oxidation (Ma *et al*., 2010). Lysine ensures effective production of hormones, enzymes and energy (Bazer et al., 2009). Alanine and glutamic acid enables a healthy skeletal system and energy production for the body Marc and Wu (2009); Kimura (2010). Regulation of blood sugar is been assisted by isoleucine (Tan et al., 2010; Yin et al., 2010). Adequate arginine ensures healthy immune system and maintains the visceral organs in the body (Brosnan and brosnan, 2010; Wu et al., 2010). Serine and cysteine play a key role as neuromodulator and antioxidant respectively (Wu *et al*., 2010; Wu *et al.*, 2010; Baker, 2009). Methionine maintains the integrity of the liver, feather formation and egg size or production in birds (McKnight *et al*., 2010; Palii *et al.*, 2009).

Haematological parameters of broiler chicks fed different levels of ATSM are presented in Table 4. PCV values ranged between (26.50 – 34.00 %), Hb (9.12 – 12.50 g/dl), RBC 1.88 – 2.97 (10^6/µl), MCV (111.2 – 130.4 fl), MCH (34.01 – 59.00 pg) and MCHC (29.80 – 40.03 g/dl). RBC, PCV, Hb, MCV, MCH and MCHC values were higher ($P<0.05$) in T3, T4 and T5 than for T2 and T1. WBC 20.41 – 30.04 (10^3/µl) were highest in T4 and T5 ($P<0.05$) compared to other treatments. Monocytes (0.07 – 1.72%), lymphocytes 10.45 – 20.04 (10^3/µl), heterophils 1.23 – 6.11 (10^3/µl) and eosinophils 0.88 – 1.27 (10^3/µl) were lowest ($P<0.05$) in T1 relative to other treatments. The haematological parameters measured follow similar pattern as it significantly (($P<0.05$) increased from T1 to T5. However, all values are within the physiological range for normal birds (Talebi *et al*., 2005; Ibrahim, 2012; Subhadarsini and Silpa, 2020). Islam *et al*. (2004); Abdi-Hachesoo *et al*. (2011) reported a RBC range (2.9 – 3.5 10^6/µl), this variation could simply be as a result of differences in age, sex, breed, environment, hormones and nutrition (Fudge, 2000). Haematological indices are used to in disease diagnosis as well as extent of damage to the blood (Nse Abasi *et al*., 2014; Omokore and Alagbe 2019). PCV and MCH are useful indices for the diagnosis of anaemia Nse Abasi *et al*. (2014); Alagbe (2019). A higher RBC level is an indication of adequate oxygen in the blood which gives room for effective nutrient transportation in the body (Isaac *et al*., 2013; Ugwuene, 2011). WBC plays a major role in the immune system by the production of antibodies, animals with low WBC stands a high risk of infection (Iwuji and Herbert, 2012; Isaac *et al*., 2013).

The serum biochemical indices of the experimental birds are presented in Table 5. Total protein ranges (2.57 – 3.97 g/dl), globulin (1.23 – 1.97 g/dl), albumin (1.34 – 2.03g/dl), creatinine (0.51 - 0.91 mg/dl) and glucose (196.1 – 250.6 mg/dl) were lowest ($P<0.05$) for T1 compared to the other treatments while cholesterol (87.5 – 101.4 mg/dl), uric acid (50.7 – 74.1 mg/dl), ALT (50.7 – 74.1 u/l) and AST (200.9 – 300.7 u/l) was higher ($P<0.05$) for T1 and T2 than for the rest of the treatments. Total protein value in T4 and T5 were significantly higher ($P<0.05$) compared to the other treatment, this could be attributed to the presence of some relevant nutrients in ATSM. (Alagbe *et al*., 2020). Albumin content in the blood are generally influenced by protein shortages, however, the values reported fall within the range reported by (Subhadarsini and Silpa, 2020). Ibrahim (2012); Obajuluwa *et al*. (2020); Olafadehan *et al*. (2020); Livingston et al. (2020) reported a globulin and uric acid range of (1.6 – 1.9 g/dl) and (3.7-5.2 mg/dl) respectively. This result is also in agreement with the findings of Obikaonu *et al*. (2011) and Simaraks *et al*. (2004). Cholesterol, creatinine, uric acid, ALT and AST values follow similar pattern as it significantly ($P<0.05$) decreased from T1 to T5. However, all the values were within the range reported by Olafadehan *et al*. (2020). Lower Creatinine and uric acid level is a sign that the kidney is not damage by feeding ATSM to the birds. According to Alagbe (2020), ATSM is loaded with several minerals, vitamins and bioactive chemicals or secondary metabolites (tannin, saponin, flavonoids, alkaloids, phenol etc.) which are within the lethal dose for broiler chicks. Urea levels is also reported to be influenced by dietary protein quality, quantity, bleeding time and are sensitive biomarkers employed in the diagnosis of renal damage (Akande and Odunsi, 2012). ATSM can also be serves as a hypolipidemic substance because of its ability to lower blood cholesterol, thus preventing heart diseases (Alagbe, 2020). ALT and AST are serum enzymes triggered due to the presence of a toxic substance in feed (Olabanji *et al*., 2007; Oluwafemi *et al*., 2020). The result obtain revealed that ATSM did not contain antinutrients or toxic substance which could hinder the general performance of birds, this result confirms the earlier report of Abdel *et al*. (2014); Cho *et al*. (2014); Alagbe (2017) on the effects phytogenic feed additive in broiler chicks.

The oxidative status as influenced by ATSM is presented in Table 6. Superoxide dismutase [SDA; 35.7 – 45.3 U/mg Hb], glutathione peroxidase [GPx; 27.1 – 28.3 U/mg Hb], catalase [CAT; 40.1 – 54.2 U/mg Hb] and malonyldialdehyde [MLA; 1.85 – 3.11 U/mg Hb] values were lowest ($P<0.05$) in T1 than in other treatments. According to Alagbe *et al*. (2019), ATSM contains antioxidants which are capable of scavenging free radicals, thereby giving total protection to animals. The presence of phenol and flavonoids prevent oxidative damage to biomolecules, superoxide anions and lipid peroxy radicals (Hollman, 2001; Ojewuyi *et al*., 2014). The same results were reported by Lin *et al.* (2003) who observed that intake of phytogenic feed additives resulted in the increase in serum antioxidant enzyme activities and a decrease in MDA concentration. Conversely, Lan *et al.* (2013) reported that the concentration of blood glutathione was not affected by phytogenic feed additives.

4. Conclusion

Feed additives (plants extracts) have been reported to perform multiple biological activities including antibacterial, antifungal, antiviral, antihelminthic, antioxidant and immune modulator because they contain phytochemicals such as phenols, flavonoids, alkaloids, tannins, saponins, terpenoids etc. They are relatively cheap, safe and effective without any side effect on continuous use. The use of ATSM at 30 ml/litre of water have shown to be able to give total protection to the body and its metabolism against free radicals due to the presence of antioxidants and have no deleterious effect on the blood profile of broiler chicks.

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