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Heamatologfical and Serum Biochemical Indices of Starter Broiler Chicks Fed Aqueous Extract of Balanites Aegyptiaca and Alchornea Cordifolia Bark Mixture

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ABSTRACT

A -28- day experiment was carried out to determine the hematological and some serum biochemical indices of starter broiler chicks fed aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* stem bark mixture (BACM). A total of two hundred and fifty (250) one-day old broiler chicks (Cobb) strain of mixed sex were randomly distributed into five treatments with 5 replicates, each replicates contained 10 birds each in a completely randomized design. Treatment 1 was fed basal diet + 0 % BACM (control), Treatment 2, 3, 4 and 5 were fed basal diet + 20, 40, 60 and 80 ml/liter BACM. Basal diet was formulated to meet the nutritional requirement of birds according to NRC (1994); feed and water was offered *ad libitum* and birds were vaccinated according to the prevailing disease condition in the environment. Result revealed that all the hematological parameters measured (PCV, ESR, Hb, RBC, WBC, leucocytes, monocytes, heterophils and eosinophils) were significantly ($P<0.05$) different among the treatments. Albumin, globulin, total protein, cholesterol, urea, calcium, phosphorus, potassium, bicarbonate, ALP, ALT and AST were significantly ($P<0.05$) influenced among the treatments. Glucose, creatinine, sodium and chloride were not significantly ($P>0.05$) affected by BACM. It was concluded that BACM is rich in several bioactive chemicals and could be used as an alternative to antibiotics without causing any deleterious effect on the health status of an animal once it is included up to 50 ml/liter.

1. Introduction

Due to the problems of antibiotic resistance bacteria and antibiotic residues in animal products and the danger posed to human health, there is a renewed and growing interest in quest for alternative to antibiotics for livestock medication (Olafadehan *et al*., 2020). Particularly the utilization of medicinal plants/herbs and spices due to the presence of phytochemicals or bioactive chemicals in them. Bioactive chemicals are found in an entire plant or in some specific parts confer them therapeutic effects or beneficial effects (Martins *et al*., 2000).

Medicinal plants are a reservoir of biologically active compounds with therapeutic properties used for the treatment of various ailments (Dilfuza *et al*., 2015). According to Mahima *et al*. (2012); Hashemi and Davoodi (2012) there are around 20, 000 species of medicinal plants in the world where about 800 plant species have been used by different medicinal communities for curing different diseases. Phytochemicals have two categories i.e. primary and secondary compounds. Primary compound contains chlorophyll, proteins, amino acids and sugar while a secondary constituent contains alkaloids, flavonoids, saponins, phenols, terpenoids, tannins etc. (Abdul *et al*., 2013). Phytochemicals have multiple beneficial advantages such as: anti-inflammatory, antifungal, antidiabetic, antimicrobial, antihelminthic, antioxidant, antiviral, neuroprotective, hypolipidemic, hepato-protective, hypotensive and antiaging (Alagbe *et al*., 2020; Dhan *et al*., 2012; Prakash *et al*., 2003; Scalbert *et al*., 2005; Kaul and Kapoor, 2001; Arts and Hollman, 2005).

Blood serves as a medium that transports of nutrients and other materials to different part of the body, therefore whatever imparts negatively on the blood, either nutrition or pathogenic organism will affect the animal's body (Etim, 2010). Haemato-biochemical indices are most commonly used in nutritional studies in chickens (Adeyemi *et al*., 2000). Variations in haemato-biochemical paramaters could be due to age variations, sex, changes in metabolic activities and nutrition (Ripon *et al*., 2013).

Therefore, an experiment was carried out to determine the hematological and some serum biochemical indices of starter broiler chicks fed aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* stem bark mixture (BACM).

2. Material and Methods

2.1 Site of the experiment

The experiment was carried at Sumitra Research Institute, Gujarat, India during the month of April to June, 2018.

2.2 Collection and preparation of extract

Fresh stems of bark of *Balanites aegyptiaca* and *Alchornea cordifolia* were obtained within the Teaching and Research Farm, Gujarat, India. The plant materials were identified and authenticated by a plant taxonomist (Dr. Sharma Ram) thoroughly washed with running tap water to remove soil and other bound particles, air dried separately to ensure that the bioactive chemicals are intact until a constant weight was obtained and made into powder using a pulverizer. Samples were later stored in a well labeled air tight container and kept for further analysis. 100 g of each ground sample (*Balanites aegyptiaca* and *Alchornea cordifolia*) were mixed together (1:1) dissolved in 1000 ml water, stirred continuously and kept in the refrigerator for 48 hours. The extract was filtered using Whatman filter paper No. 1 to obtain filtrate (BACM).

2.3 Pre-experimental operations

Prior to the commencement of the experiment deep litter pens used for the experiment were properly cleaned and disinfected, feeders and drinkers were properly washed, electric fittings were properly fixed and foot bath was put in place to ensure biosecurity.

2.4 Experimental birds and design

A total of two hundred and fifty one-day old broiler chicks (Cobb) strain of mixed sex were randomly distributed into five treatments with 5 replicates, each replicates contained 10 birds each in a completely randomized design. Birds were weighed on arrival to the farm to determine their initial body weight and weekly thereafter. Wood shavings were used as litter material and lighting was continuous, vaccines were administered according to the prevailing disease condition in the environment and all necessary management practices were strictly adhered to, clean feed and water were offered *ad libitum* and the experiment lasted for 28 days.

2.5 Experimental diet

A standard starter's ration was formulated to meet the nutritional recommendation of birds by NRC (1994). It was made up of corn –soya meal based diet and it contained 23 % crude protein and 2900 Kcal/kg energy.

Treatment 1- Basal diet + 0 % BACM
Treatment 2 – Basal diet + 20 ml/liter BACM
Treatment 3 – Basal diet + 40 ml/liter BACM
Treatment 4 – Basal diet + 60 ml/liter BACM
Treatment 5 – Basal diet + 80 ml/liter BACM

2.6 Measured parameters

Feed intake (g/bird) = Feed offered (g) - Leftover (g)

2.6.1 Heamatological and serum biochemical analysis

At the end of the 28th day of the experiment, blood samples were collected via wing web of four randomly selected birds per treatment. The operation is done very early in the morning to reduce stress on the animals and prevent oxygenated blood from been de-oxygenated, 4ml each was collected from each animal. 2 ml of blood sample was transferred into a sterile EDTA bottle for hematological assay. The parameters (Pack cell volume, haemoglobin concentration, erthyrocyte sedimentation rate, red blood cell, mean corpuscular volume, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin concentration, white blood cell and its differentials) were analyzed using Diasis Diagnostic Systems ASI-671N, India (an automated digital analyzer).

The remaining 2 ml was collected into another bottle without anticoagulant (EDTA) for serum biochemical analysis. Albumin, globulin, cholesterol and total glucose, creatinine, calcium, sodium, bicarbonate and chlorine ions were analyzed using Randox ® commercial kits, USA (Model 2R-TR4). Activities of Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) were recorded according to (Doumas and Briggs, 1972).

Proximate compositions of test material and experiment diet were determined by using official method of analysis by AOAC (2000).

2.7 Statistical analysis

All data were subjected to one -way analysis of variance (ANOVA) using SPSS (23.0) and significant means were separated using Duncan multiple range tests (Duncan, 1955). Significant was declared if $P \leq 0.05$.

3. Results and Discussion

3.1 Proximate composition of experimental diet

Table 1 reveals the proximate composition of experimental diet. The basal diet was analyzed according to procedures outlined by AOAC (2000). The proximate components contained crude protein, crude fibre, ether extract, calcium, phosphorus and energy at 23.11 %, 3.09 %, 5.12 %, 0.97 %, 0.46 % and 2990.7 Kcal/kg respectively. This conforms to the findings of NRC (1994); Aduku and Olukosi (1990). The crude protein value obtained in this study is in conformity with the values obtained by Mohammed *et al*. (2016); Alabi *et al*. (2017) who evaluated the effect of feeding aqueous *Moringa olifera* leaf extract on the performance of Hubbard broiler chicken. The crude ether extract recorded in this experiment was slightly higher than the values recorded by Yakhkeshi *et al*. (2011) when herbal extracts, antibiotics and probiotics were compared on the growth performance of broiler chicks (Cobb). The values for crude fibre and energy were in close agreement with the findings of Olafadehan *et al*. (2020) who examined the effect of feeding aqueous *Daniellia oliveri* leaf extract on the performance and physiological response of broiler chicks.

Table 1: Composition of experimental diet

Ingredients	Quantity (kg)
Maize	52.00
Wheat offal	5.24
Soya meal	38.00
Fish meal (72%)	3.00
Bone meal	0.50
Limestone	0.25
Lysine	0.20
Methionine	0.25
*Premix	0.25
Salt	0.30
Toxin binder	0.01
Total	100.00
Analyzed nutrient (%)	
Crude protein	23.11
Crude fibre	3.09
Ether extract	5.12
Calcium	0.97
Phosphorus	0.46
Energy (kcal/kg)	2990.7

* Premix supplied per kg diet :- Vit A, 10,000 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

3.2 Hematological parameters of broiler chicks fed BACM

Haematological parameter of broiler chicks fed different levels of BACM is presented in Table 2. PCV values ranged between (27.13 – 32.01 %), ESR (3.18 – 5.03 mm/h), Hb (9.85 – 13.04 g/dl), RBC 1.94 – 3.00 (10^6/µl), MCHC (36.3 – 40.7 g/dl) were higher (P<0.05) in T5 than in other treatments. MCV (106.7 – 139.0 fl), MCH (41.7 – 50.7 pg) were lower (P<0.05) for T4, T5 and T2 than for the other treatments. WBC 19.23 – 23.70 (10^3/µl), lymphocytes 8.11 – 11.4 (10^3/µl), monocytes 0.34 – 1.01 (10^3/µl), heterophils 5.11 – 8.66(10^3/µl) and eosinophils 0.55 – 1.05 (10^3/µl) were highest in T5 (P<0.05) compared to other treatments. The reference values for all the examined haematological parameters were within the reference range of broilers (Talebi *et al*., 2005; Elagib and Ahmed, 2011; Islam *et al*. (2004); Alagbe, 2019; Livingston *et al*., 2020). PCV, RBC and Hb values increased with an increase in BACM level relative to ESR which decreased as the level of BACM increased. According to Obikaonu *et al*. (2011) ESR rates are increased in cases of acute general infection and pregnancy. PCV and Hb are indices for evaluating circulatory erythrocytes and are useful in diagnosis of anaemia (Chineke *et al*., 2006; Alagbe, 2017). High RBC especially among birds in T5 is an indication that oxygen is well circulated within the body, hence better nutrient transport and performance (Johnson and Morris, 1996). WBC and its differentials follow similar pattern, the rate was rather increasing with an increase in BACM. WBC play a role in the formation of antibodies and prevention of diseases, thus animals with low WBC stands a risk of disease infection (Nse Abasi *et al*., 2014). High WBC counts have been reported during period of stress and unfavourable conditions (Alagbe *et al*., 2020). Butterworth (1999) described basophils and eosinophils as important effector cells in allergy and host defense responses particularly against parasitic infections.

Table 2 Hematological parameters of broiler chicks fed BACM

Parameters	T1	T2	T3	T4	T5	SEM
PCV (%)	27.13^{b}	30.11^{a}	31.94^{a}	31.59^{a}	32.01^{a}	0.79
ESR (mm/h)	5.03^{a}	3.65^{b}	3.33^{b}	3.27^{b}	3.18^{b}	0.33
Hb (g/dl)	9.85^{c}	10.22^{b}	12.02^{a}	12.47^{a}	13.04^{a}	0.21
RBC (× 10^6/µL)	1.94^{b}	2.45^{a}	2.66^{a}	2.93^{a}	3.00^{a}	0.01
MCV (fl)	139.0^{a}	123.0^{b}	120.0^{b}	107.0^{c}	106.7^{c}	0.66
MCH (pg)	50.7^{a}	41.7^{b}	45.2^{b}	42.6^{b}	43.4^{b}	0.48
MCHC (g/dl)	36.3^{b}	33.9^{b}	37.6^{b}	39.5^{b}	40.7^{a}	0.79
WBC (× 10^3/µL)	19.23^{c}	22.45^{b}	23.11^{a}	23.53^{a}	23.70^{a}	0.06
Lymphocytes(×10^3/µL)	8.11^{b}	9.56^{b}	10.2^{a}	10.9^{a}	11.4^{a}	0.44
Monocytes (× 10^3/µL)	0.34^{c}	0.81^{b}	0.87^{b}	0.94^{b}	1.01^{a}	0.01
Heterophils (× 10^3/µL)	5.11^{b}	6.56^{b}	8.01^{a}	8.32^{a}	8.66^{a}	0.05
Eosinophils (× 10^3/µL)	0.55^{b}	0.61^{b}	0.87^{b}	1.00^{a}	1.05^{a}	0.02

Means in the same row with different superscripts differ significantly ($P<0.05$)
PCV: Pack cell volume; ESR: Erythrocyte sedimentation rate; Hb: Haemoglobin; RBC: Red blood cell; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; WBC: White blood cell.

3.3 Serum biochemical indices of broiler chicks fed different levels of BACM

Table 3 reveals the serum biochemical parameters of broiler chicks fed different levels of BACM. Total protein ranged between (3.93 – 4.90 g/dl), albumin (1.93 – 2.40 g/dl) and globulin (2.00 – 2.50 g/dl) were lowest ($P<0.05$) for T1. Cholesterol (71.0 – 120.1 mg/dl), urea (21.0 – 32.1 mg/dl), Alanine transaminase (30.4 – 53.1 iu/l), Aspartate transaminase (23.8 – 35.6 iu/l) and Alkaline phosphatase (138.2 – 288.1 iu/l) were highest ($P<0.05$) for T1 than the other treatments. Glucose (202.4 – 220.4 mg/dl) and creatinine (0.70 – 0.77 mg/dl) were not affected by treatments ($P>0.05$). Total serum protein increased as the level of BACM increases, this is an indication that the test material is loaded with nutrient and other bioactive chemicals that are capable of supporting the growth of an animal (Musa *et al*., 2020). Globulin play a significant role in fighting infections, hormone carrier as well as blood clotting process because of the presence of antibodies and enzymes in them (Vivian *et al*., 2015). However, the total proteins were within the physiological reference range reported by Ibrahim (2012). Alikwe *et al*. (2010) reported that serum protein may be used as an indirect measurement for dietary protein quality. The result on glucose is in agreement with the findings of Liukkonen (2001) who reported that animals maintain a high and relatively constant blood sugar even in low feed intake. The stability in the value of creatinine and urea are indications that the integrity of the animal's kidney is not compromised (Olafadehan *et al*., 2020; Alagbe and Oluwafemi, 2019). The urea level is within the limit (3.7 – 5.2 mg/dl) reported by Ibrahim (2012). BACM has also proven to be a cholesterol lowering agent because the rates of cholesterol in the blood reduces as BACM is increased, this could be due to the presence of phytochemicals in the test material especially saponin which have been reported to have a beneficial effect on blood cholesterol levels and stimulation of the immune system (Cheeke, 2000). ALP, AST and ALP follow similar pattern, the values decrease with an increase in BACM. This is an indication that BACM is non-toxic and will not adversely affect the animal's health; it also reveals that the phytochemicals in the test material are within the threshold for the animal. This conforms to the findings of Kwiecień *et al*. (2015); Olabanji *et al*. (2007) when rabbits were fed *Tithonia diversifolia* leaf – blood meal mixture.

Table 3 Serum biochemical indices of broiler chicks fed different levels of BACM

Parameters	T1	T2	T3	T4	T5	SEM
Albumin (g/dl)	1.93^b	2.00^a	2.21^a	2.31^a	2.40^a	0.12
Globulin (g/dl)	2.00^b	2.13^b	2.20^b	2.40^a	2.50^a	0.08
Total protein (g/dl)	3.93^b	4.13^a	4.40^a	4.70^a	4.90^a	0.25
Cholesterol (mg/dl)	120.1^a	99.3^b	87.4^b	81.6^b	71.0^c	1.04
Glucose (mg/dl)	202.4	209.3	211.3	219.7	220.4	5.53
Urea (mg/dl)	32.1^a	28.2^b	25.9^b	22.8^a	21.0^a	1.20
Creatinine (mg/dl)	0.77	0.75	0.73	0.71	0.70	0.01
ALP (iu/l)	288.1^a	194.3^b	188.4^b	144.3^c	138.2^c	2.55
ALT (iu/l)	53.1^a	40.6^b	38.5^c	35.1^c	30.4^c	0.45
AST (iu/l)	35.6^a	33.6^a	29.2^b	25.7^c	23.8^c	0.06

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

ALP: Alkaline phosphatase; AST: Aspartate transaminase; ALT: Alanine transaminase

3.4 Serum ions of broiler chicks fed different levels of BACM

The serum enzymes and electrolytes as influenced by BACM are presented in Table 4. Calcium values ranged between 6.7 – 9.84 mg/dl, phosphorus (2.97 – 3.49 mg/dl), potassium (3.61 – 6.67 Mmol/l) and bicarbonate (10.51 – 19.95 Mmol/l) were lowest (*P*<0.05) in T5 than in other treatments. Sodium (121.8 – 129.3 Mmol/l) and chloride (70.9 – 74.2 Mmol/l) were not (*P*>0.05) affected by BACM. This conforms to the findings of Ogbuewu *et al*. (2008); Nworgu *et al*. (2007); Alagbe (2019). The high calcium and phosphorus level is an indication that BACM is rich in minerals and other bioactive compounds as reported by Musa et al. (2020); Ngaha *et al*. (2016); Agyare *et al*. (2014). The sodium values obtained in this study were in conformity with the values reported by Obikaonu et al. (2011). A normal sodium and chloride ion prevents the risk of Hypernatremia and kidney damage.

Table 4 Serum ions of broiler chicks fed different levels of BACM

Parameters	T1	T2	T3	T4	T5	SEM
Calcium (mg/dl)	6.70^c	7.42^b	9.06^a	9.54^a	9.84^a	0.22
Phosphorus (mg/dl)	2.97^b	3.05^a	3.14^a	3.42^a	3.49^a	0.10
Sodium (Mmol/l)	129.3	121.8	125.5	121.8	123.6	7.11
Chloride (Mmol/l)	73.2	70.9	72.1	74.2	72.8	3.21
Potassium (Mmol/l)	3.61^c	5.95^b	6.21^a	6.60^a	6.67^a	0.05
Bicarbonate (Mmol/l)	10.51^c	12.78^c	16.78^b	19.75^a	19.95^a	1.62

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

4. Conclusion

It was concluded that BACM is rich in various phytochemicals and nutrients and could be used as an alternative to antibiotics in order to bridge the gap between food safety and livestock production. It has a potential medicinal plant with high pharmacological relevance and when administered to birds at 80 ml/liter does not have any deleterious effect on the health and general performance of the animals.

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