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Research Article

Effect of Dietary Supplementation of Cymbopogon Citratus Oil On The Haematology And Serum Biochemical Parameters Of Broiler Chicks

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Abstract

This study was carried out to investigate the effects of dietary supplementation of *Cymbopogoncitratus* oil (LGO) on the haematology and some serum biochemical parameters of broiler chicks. 250-1 day old broiler chicks of Ross 308 strain were randomly divided into five (5) dietary treatments of 5 replicates and each replicates had ten (10) birds. Birds in treatment 1 (T1) were fed basal diet with 0 % LGO, T2, T3, T4 and T5 were fed diet supplemented with LGO at 0.1 %, 0.2 %, 0.3 % and 0.4 % respectively in a completely randomized design. Basal diet was formulated to meet the nutritional requirement of birds according to NRC (1994), feed and water were given ad libitum, all necessary management practices were strictly observed throughout the experiment which lasted for 56 days. Data collected revealed that haemotological parameters (PCV, Hb, RBC, MCV, MCH, MCHC, WBC and its differentials) and serum biochemical analysis (total protein, albumin, globulin, cholesterol, creatinine, uric acid, LDL, HDL, triglycerides, ALT, AST and ALP)were significantly affected ($P<0.05$) by the dietary supplementation of LGO. Serum electrolytes (sodium, chloride, calcium and potassium) were not significantly ($P > 0.05$) influenced by the different inclusions of LGO. However, all values reported were within the normal range for healthy birds. It was concluded that LGO is loaded with various bioactive chemicals which possess significant biological and pharmacological activities, they are safe, effective and can be used to bridge the gap between food safety and production without causing any negative effect on the health and general performance of birds.

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Introduction

Due to challenges of antibiotic resistant bacteria, higher cost, anticipated toxicity and dangers posed to human health, there is growing awareness on the use of natural medicines which are more relatively effective, cheap and safe in prolonged use [1-3]. Among the potential natural alternatives is the use of essential oil (EOs); essential oil derived from plants have provided enough evidences to suggest as a tool for defending bacteria diseases, regulate feed intake and secrete digestive secretions [4]. They are also loaded with several bioactive chemicals or phytochemicals (alkaloids, flavonoids, saponins, terpenoids, phenols, tannins etc.) which performs a wide range of pharmacological activities such as: antimicrobial, antiviral, antioxidant, hypolipidemic, antifungal, hepato-protective, neuro-protective, antispasmodic, anti-allergic, immunomodulatory and hypotensive properties without showing any negative effects in especially oil from lemon grass (Cympobogoncitratus) [5-10]. According to EOs are volatile substances of a complex mixture of chemicals components which evaporate when in contact with air and are biosynthesized by plants.

Cymbopogon citrates (Stapf) commonly referred to as lemon grass belongs to the family Poaceae. The plant is a tropical grass resistant to different temperature and can grow in warm, semi-warm and temperate climates [11]. Cymbopogon is derived from twogreek words “Kymbe” meaning boat and “pogon” which means beard (arrangement of the spike of the flowers) while citratus is a latin word which means lemon-scented leaves [12,13].Lemon grass is rich in various phytochemicals and is used in traditional system and folk medicine to treat malaria, pneumonia, gastrointestinal infections, anxiety and diabetes [14,15].

Previous studies have shown that EOs have been used as phytogenic feed additives to ensure balanced micro flora (eubiosis), scavenge free radicals or prevent oxidative stress, immune modulatorand growth enhancer [16-19] there is also a correlation between nutrition (feeding) and immune system of an animal, therefore, proper feeding lessens the immune suppression associated with the stress response in the bird [20-21].

Therefore, in order to enhance food safety and explore other organic alternatives to antibiotics. This experiment was designed to examine the effect of dietary supplementation of *Cymbopogoncitratus* oil on the haematology and serum biochemical parameters of broiler chicks.

Materials and Methods

Study area

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

Collection, processing and extraction of lemon grass oil (LGO) Fresh, mature and healthy lemon grass (*Cymbopogoncitratus*) leaves were harvested within Sumitra Teaching and Research farm, Gujarat, India and identified by a plant taxonomist (Dr. Sharma Kumar), it was later washed with a running tap water to remove dirt's and air dried for 15 days to maintain the bioactive chemicals in the plant and to prevent the growth of microorganisms until a constant was obtained, thereafter powdered and kept in an air tight well labeled container. *Cymbopogoncitratus* essential oil (LGO) was obtained according to the methods outlined.

Pre-experimental operations

Before the commencement of the experiment deep litter pens were properly disinfected, wooden planks are been used to demarcate each treatments and replicates, properly labeled, feed and water troughs were properly washed and foot bath was put in place to ensure proper biosecurity.

Animals and their management

Two hundred and fifty (250) one day old broiler chicks (Ross 308) strain weighing 45 ± 0.05 g/bird (mean ± SD)were purchased from a commercial hatchery in India. The birds were randomly assigned into five (5) treatments of 50 birds; each group was further sub-divided into 5 replicates of 10 birds. The experiment lasted for 56 days, vaccines were administered according to the prevailing vaccination schedule in the environment, feed and water were provided ad libitum and all necessary management practices were strictly observed.

Formulation of experimental diet and design

Three experimental diets (Basal diet) were formulated consisting of starter diet fed 0-21 days, growers mash (22-35 days) and finisher diet (36-56 days). Diets were formulated to meet the nutrition requirement of birds according to NRC (1994) as presented in (Table 1). The experiment was carried out in a Completely Randomized Design (CRD) and the set up goes thus:

Treatment 1 (Control): Basal diet + 0 % LGO

Treatment 2: Basal diet + 0.1 % LGO

Treatment 3: Basal diet + 0.2 % LGO

Treatment 4: Basal diet + 0.3 % LGO

Treatment 5: Basal diet + 0.4 % LGO

Data collected

Feed consumed was recorded daily while body weight was recorded weekly. Mortality was recorded as it occurs.

Blood collection and analysis

On the 56th day, three birds were randomly selected for haematological and serum biochemical parameters. 5 ml of blood samples were collected early in the morning around 7 am via the wing web of the birds into sample bottles containing Ethylene Diaminetetraacetic Acid (EDTA) as anticoagulant for heamatologicalstudies while another sample of blood was emptied into bottles free from EDTA for serum metabolites analysis, all the bottles collected were properly labeled and transferred to the laboratory for further analysis. Pack Cell Volume (PCV), Red Blood Cell (RBC), Hemoglobin (Hb) were determined according to methods outlined. White Blood Cell (WBC) and its differentials were analyzed using an automated machine (Sysmex, Model KU-209 HPT, India).Values of Mean Corpuscular Hemoglobin (MCH) Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin concentration (MCHC) were calculated using the formulabelow:

Materials	Starter (1-21 days)	Grower (22-35 days)	Finisher (36-56 days)
Maize	50.00	56.00	60.50
Wheat offal	8.00	7.00	8.05
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
Determined analysis			
Dry matter (%)	90.10	93.63	92.04
Crude protein (%)	23.08	20.11	19.33
Ether extracts (%)	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

Table 1: Chemical composition of experimental dietsPremix 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.

MCV (fl) = PCV / RBC × 10

MCH (pg) = Hb/RBC × 10

MCHC (%) = Hb (100mg blood) / PCV × 100

Total protein, glucose levels, creatinine, uric acid, cholesterol, triacylglycerols (TG), high density lipoproteins, activities of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), serum ions (sodium, chloride, calcium and potassium) were analyzed using commercial diagonistic kits (Humburg, .Braunschweig, Germany, Model- 3401- UI-MN09-A). Low density lipoprotein (LDL) was estimated using

LDL (mmol/L) = cholesterol - HDL- (TG/2.2).

Caecal microbial population

At the end of the experiment (56 days), caeca microbial count was conducted using 5 birds per treatments. A 10-fold serial dilution

method, in which of 1% peptone solution was mixed with caeca samples and poured on Mac Conkey agar plates to determine the colony forming unit (cfu) of E. coli, lactobacilli and *Salmonella typhi* according to methods described [22].

Chemical analysis

Proximate analysis of the experimental diet was carried out according to [23].

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 [24]. Significant was declared if $P \leq 0.05$.

Results

Proximate composition of experimental diets

The proximate composition of experimental diet is presented in (Table 1). The feeding was done in three phases (starter, growers and finisher mash).Starter mash (0-21 days) contains dry matter (DM) 90.10 %, crude protein (CP) 23.08 %, ether extract (EE) 5.03 %, crude fibre (CF) 3.06 %, calcium 0.98 %, phosphorus 0.47 % and energy value 2936.0 (Kcal/kg). TheDM, CP, CF, EE, calcium, phosphorus and metabolisable energy of growers mash (22-35 days) contained 93.63 %, 20.11 %, 3.95 %, 4.28 %, 1.10 %, 0.51 % and 3000.8 Kcal/kg respectively. Finisher mash (36 -56 days) contained dry matter (92.04 %), CP (19.33 %), CF (3.42 %), EE (4.28 %), calcium (1.10 %), phosphorus (0.51 %) and metabolisable energy (3100.2 Kcal/kg).

Haematological parameters of broiler chicken fed diets supplemented with LGO

Table 2 contains the haematological traits of broiler chicken fed diet supplemented with different levels of LGO. The Pack Cell Volume (PCV), Heemoglobin (Hb), Red Blood Cell (RBC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) values ranged between 25.62-33.80 %, 7.55-11.89 g/dL, 1.94 – 2.50 ($mm^3 \times 10^6$), 132.1-153.9 fl and 29.46 - 35.44 g/dL respectively. WBC (19.41-28.11 $mm^3 \times 10^6$), lymphocytes 12.73- 8.55 ($\times 10^3 \mu l$), monocytes 0.25-0.85 ($\times 10^3 \mu l$) and neutrophils 18.20-29.00 ($\times 10^3 \mu l$). All the parameters measured were significantly (P<0.05) different among the dietarytreatments and they follow similar trend. PCV, Hb, RBC, MCH, MCV, MCHC, WBC and its differentials (lymphocytes, monocytes and neutrophils) were highest in T3, T4 and T5, intermediate in T2 and lowest in T1 (P<0.05).

The reduction in the serum electrolytes were not below the normal physiologic range reported for healthy birds. Drastic decrease will lead to anorexia, pyrexia, loss of appetite and even death in critical stages.

Serum biochemical parameters of broiler chicken fed diets supplemented with LGO

Serum biochemical parameters of broiler chickens fed diets supplemented with different levels of LGO is presented in (Table 3). Total protein (TP), albumin, globulin, creatinine and glucose values ranged between 2.64 -3.98 g/dL, 1.09-1.98 g/dL, 1.55-2.00 g/dL, 0.12-0.41 Mg/dl and 105.9-200.8 Mg/dl respectively. The above values were significantly (*P*<0.05) affected by the dietary supplementation of LGO,

Parameters	T1	T2	T3	T4	T5	SEM
PCV (%)	25.62^c	30.02^b	31.93^b	32.80^a	33.80^a	0.44
Hb (g/dL)	7.55^c	9.81^b	10.80^a	11.63^a	11.89^a	0.71
RBC ($mm^3 \times 10^6$)	1.94^b	2.08^a	2.11^a	2.38^a	2.50^a	0.07
MCV (fl)	132.1^b	153.9^a	151.3^c	137.8^b	135.2^b	6.53
MCH (pg)	38.92^c	47.16^b	51.18^a	49.00^b	48.00^b	2.51
MCHC (g/dL)	29.46^b	32.68^a	33.82^a	35.44^a	35.18^a	0.88
WBC($mm^3 \times 10^6$)	19.41^b	21.57^a	23.89^a	25.07^a	28.11^a	0.12
Differentials ($10^3 \mu l$)						
Lymphocytes	12.73^c	13.00^c	14.56^b	15.70^b	18.55^a	1.56
Monocytes	0.25^c	0.29^c	0.30^b	0.33^b	0.85^a	0.04
Neuterophils	18.20^c	20.06^b	23.64^b	28.89^a	29.00^a	0.26

Table 2: Chemical composition of experimental dietsPremix supplied per kg diet: - vitHaematological parameters of broiler chicks fed different levels of LGO means in the same row with different superscripts differ significantly (*P*<0.05).

SEM: Standard Error of Mean

values recorded were highest in T4 and T5, in-between in T2 and T3 but lowest in T1. Cholesterol (110.4-191.7 Mg/dl), triglycerides (100.2-151.8 Mg/dl), HDL (108.3-140.9 Mg/dl), LDL (0.52-1.88 Mg/dl), uric acid (3.97-9.52 Mg/dl), ALT (4.07-7.16 u/L), AST (99.90-188.0 u/L) and ALP (40.40-89.50 u/L) respectively. The chlolesterol, LDL, HDL, triglycerides, ALT, AST and ALP values were elevated in T1, mid-way in T2 and T3 and lowest in T4 and T5 (*P*<0.05).

Parameters	T1	T2	T3	T4	T5	SEM
Total protein (g/dL)	2.64^b	3.51^a	3.83^a	3.89^a	3.98^a	0.63
Albumin (g/dL)	1.09^b	1.60^b	1.88^a	1.91^a	1.98^a	0.01
Globulin (g/dL)	1.55^c	1.91^b	1.95^b	1.98^a	2.00^a	0.02
Creatinine (Mg/dl)	0.12^c	0.28^b	0.30^a	0.37^a	0.41+	0.01
Glucose (Mg/dl)	105.9^c	134.5^b	151.6^a	191.0^a	200.8^a	10.6
Cholesterol (Mg/dl)	191.7^a	150.1^a	135.1^a	110.4^b	100.1^b	12.1
Triglycerides (Mg/dl)	151.8^a	134.2^a	120.7^b	100.2^b	99.76^c	5.84
HDL (Mg/dl)	140.9^a	121.6^a	116.1^b	108.3^b	101.2^b	4.93
LDL (Mg/dl)	1.88^a	0.95^b	0.73^b	0.52^b	0.35^b	0.12
Uric acid (Mg/dl)	9.52^a	5.04^b	4.60^b	3.97^c	3.00^c	0.02
ALT (u/L)	7.16^a	6.49^a	5.65^b	4.21^b	4.07^b	0.18
AST (u/L)	188.0^a	140.5^a	110.4^b	100.7^b	99.9^c	9.12
ALP (u/L)	89.5^a	73.7^a	65.4^b	54.3^b	40.4^c	3.56

Table 3: Serum analysis of broiler chicks fed different levels of ATSM Means in the same row with different superscripts differ significantly (P<0.05).

ALP: alanine phosphatase; AST: alanine serum transaminase; ALT: alanine amino transferase.

Serum electrolytes of broiler chicken fed diets supplemented with LGO

Table 4 reveals the serum electrolytes of broiler chicken fed diets supplemented with different levels of LGO. Sodium ion ranged between 130.2-136.8 Mmol/L, chloride (100.5-108.1 Mmol/L), calcium (71.11- 83.41Mmol/L) and potassium (5.00-6.84 Mmol/L). The parameters measured were not significantly affected (P>0.05) by the dietary supplementation of LGO.

The dry matter values obtained in this experiment are in agreement with the values reported by [25] who examined the effects of

Parameters	T1	T2	T3	T4	T5	SEM
Na (Mmol/L)	131.7	130.5	134.8	130.2	136.8	11.40
Cl (Mmol/L)	107.3	108.1	106.4	102.3	100.5	8.65
Ca (Mmol/L)	83.41	78.03	73.05	70.08	71.11	5.02
K (Mmol/L)	6.84	6.50	5.81	5.04	5.00	0.25

Table 4: Serum electrolytes of broiler chickens fed diet supplemented with LGO.

Na: sodium; Cl: chloride; Ca: calcium; K: potassium.

aqueous leaf extract on the performance of broiler chicks but contrary to the reports of when turmeric extract rhizome was supplemented in the diet of broiler chickens. Crude protein levels in this study were within the recommended ranges by [26,27]. Crude fiber and ether extract content reported were slightly higher than those reported but in conformity with the values [28,29] who evaluated the effects of *Albizia lebbeck* stem bark aqueous extract as alternative to antibiotic feed additives in broiler chicks diets. Calcium and phosphorus content were in accordance with the findings of [30,31] on the influence of two plant extracts on broilers performance, digestibility and digestive organ size. Energy values were in conformity with the findings obtained who determined the effects of black pepper, turmeric powder and coriander seed combination as feed additives on the growth performance of broiler chickens.

Blood act as a pathological reflector of the status of exposed animals to toxicants and other conditions [32]. It also plays a vital role in the transportation of nutrients, metabolic waste products and gases around the body [33]. Blood constituents change in relation to the physiological conditions of health and provide useful diagnosis and prognosis of disease in animals [34,35]. According to [36-38] haematological studies are can be used as a useful tool to determine the extent of blood damage. The result obtained in this study revealed that increase in PCV levels especially among birds in T4 and T5 had a direct effect with corresponding increase in RBC and Hb values (P<0.05). This result is in agreement with the findings of [39]. However, all values were within the normal physiological range for healthy birds reported [40-42] who reported a PCV range of 25-34 %, RBC 1.5 – 3.0 ($mm^3 \times 10^6$) and Hb (6.5-13.0 g/dL) respectively. Similarly, MCV, MCH, MCHC, WBC and its differentials also increased with increase dietary supplementation of LGO (P<0.05), this is evident especially among birds in T3, T4 and T5.PCV plays a vital role in oxygen, carbon (IV) oxide and nutrients in the body [43], this implies that animals in T4 and T5 will perform better compared to T1, due to sufficient nutrients and gases in the body. Poor nutrition as one of the cardinals of management could trigger low Hb and RBC levels or concentrations, thus making animal's susceptibility to diseases [44]. RBC 1.22-2.50 ($mm^3 \times 10^6$) and Hb (7.55-11.89 g/dL) range reported in this study are in agreement with the findings [45,46] also reported the lower range of RBC, MCH and MCHC in the blood could be an indication of anaemia. WBC and its differentials are responsible for fighting infections through the production of antibodies, thus, animals with high WBC count have high degree of resistance to diseases [47]. However, the WBC counts reported in this experiment were in conformity to the findings of [48,49] who carried out a comparative studies on the haematological parameters of different strains of broiler chicks (Ross, Arbo acre, Cobb and Arian). Leucocytes counts in this study was less than the values of neutrophils, this is an indication that the anti-nutrient content of LGO is below the lethal levels which agreed with the earlier report by [50,51].

According to [52], serum protein may be used as an indirect measurement of dietary protein quality. Total protein is the summation of albumin and globulin concentrations, serum albumin are influenced by age, breed, environment, physiological state and antigen exposure [53]. Albumin and globulin values reported in this study were significantly (P<0.05) different among the dietary treatments. Birds in T3, T4 and T5 had the highest blood protein concentrations; it is also an indication that the protein levels in the diet were enough to support normal protein reserves across the treatments for growth and maintenance [54,55]. According to [56], globulin is an index to measure the immune system and antibody production in the blood. Lower total protein concentration in the blood could be a sign of hypo albuminemia and could advance to liver dysfunction during critical conditions [57,58]. The total protein, albumin and globulin contents observed in this study were within the normal ranges for birds reported [59]. The result obtained is in agreement with the findings of [60-62]. Birds in T4 and T5 had the concentration of glucose, low glucose level in the blood could be attributed to stress, anorexia disturbance in digestion and environment or housing [63] Glucose levels determined in this study were within the range of reference values reported for birds in previous studies [64,65]. Uric acid and creatinine are end products of nitrogen and muscle metabolism Nutrition and age are factors that influence the concentrations of urea in birds [66]. High creatinine is indicative of poor protein and amino acid metabolism that can lead to impaired renal function and cardiac infarction The creatinine and urea value obtained in this study were within 0.6-1.2 mg/dl and 5-20 mg/dl reported [67,68] for healthy birds. Cholesterol, triglycerides, HDL and LDL follow similar trends, the values reduced as the level of LGO increased at a significant level (P<0.05). This could be due to the presence of phytochemicals in LGO, capable of modulating the parameter, thus promoting food safety and preventing the incidence of cardiovascular diseases [69-71], high concentrations of cholesterol and LDL in blood serum are the major cause of cardiovascular diseases. The results obtained (LDL, HDL, cholesterol and triglycerides) were in agreement with the reports of [72,73] who examined the haematological and serum biochemical indices of necked neck and normally feathered Nigerian indigenous chickens. Serum enzymes ALT, AST and ALP are estimated to determine liver functions. However, the range of ALT and AST level obtained for birds in this study were similar to 6.94 -8.63 u/L and 147.95 – 192.56 u/L reported [74]. Serum enzyme values slightly decreases as the level of LGO increased at a significant level (P<0.05). This is a clear indication that LGO is non-toxic and the birds have the ability to tolerate the anti-nutrients in the diets; significant elevation of serum enzymes above normal is an indication of pathological disorders in the liver of animals. Result obtained agreeswith the findings of [75] but contrary to the reports of who evaluated the blood profile of broiler chickens fed three local sorghum varieties grown in Bauchi State.

Serum electrolytes follow similar trend, the values were not significantly (P>0.05) influenced by dietary supplementation of LGO. The result obtained is in conformity with the values obtained by [76,77] on the effect of dietary supplementation of hemp (Cannabis sativa) and dill seed on performance, serum biochemical's of broiler chicken but contrary to the reports of the normal values of serum electrolytes is an indication that the integrity of the liver and kidney were not compromised, thus ensuring health stability in the birds.

Conclusion

Antibiotic resistant strain of bacteria is an increasing threat to animal and human health, these necessitated the use of medicinal plants

and their Extract (EOs) which are found to be effective and safe because of their abundant composition of phytochemicals and other nutrients. LGO is found to perform multiple biological activities and its supplementation in the diet of broiler chicks at 0.4 % does not have any deleterious effect on the performance and health status of the animal.

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