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PERFORMANCE AND HEAMATOLOGICAL PARAMETERS OF BROILER CHICKS GIVEN DIFFERENT LEVELS OF DRIED LEMON GRASS (*CYMBOPOGON CITRATUS*) AND GARLIC (*ALLIUM SATIVUM*) EXTRACT

J.O. Alagbe[1*], R.A. Oluwafemi[2]

[1]Department of Animal Nutrition, Sumitra Research Farm, Gujarat, India
[2]Department of Animal Science, Faculty of Agriculture, University of Abuja, Nigeria

Abstract. A 49 day feeding trial was carried out to determine the effect of lemon grass - garlic extracts (CLGE) on the performance and hematological parameters of broiler chickens. Two hundred and fifty (250), day old 308 broiler chicks of mixed sex was purchased and were randomly assigned to five treatment, each treatment was replicated 5 times consisting of 10 birds each in a completely randomized design. Birds in treatment 1 was given 0.025g/liter of Neomycin, Treatment 2, 3, 4 and 5 were given aqueous lemon grass-garlic extracts at 3.0ml, 6.0ml, 9.0ml and 12.0ml/liter respectively. Clean feed and water offered *ad libitum*. Results showed that CLGE had significant ($p<0.05$) effect on final body weight, body weight gain, daily weight gain and feed conversion ratio of the birds. Birds in T5 recorded significantly ($p<0.05$) higher body weight gain and superior feed conversion ratio than the control given 0.025 g/liter Neomycin. There was also a significant ($p<0.05$) difference in the mortality recorded, T1 had the highest mortality of 12%, none was recorded in T2, T3, T4 and T5. Data on hematological indices (PCV, Hb, RBC, MCV, MCH, MCHC and WBC) revealed that CLGE significantly ($p<0.05$) influenced all the parameters assessed. It was concluded that CLGE can be included up to 12ml/liters in broiler chicken since it resulted in improved weight, feed conversion ratio without any deleterious effect on the health and performance of the animal.

Keywords: *Performance, hematological parameters, lemon grass, garlic extract, broiler chicken.*

[*]***Corresponding Author:*** *Dr. J.O. Alagbe, Department of Animal Nutrition, Sumitra Research Farm, Gujarat, India, e-mail: demsonfarms@yahoo.com*

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1. Introduction

The side effects of synthetic drugs such as presence of antibiotic residues leads to antibiotic resistance in humans; residues in animal tissues and the byproducts of synthetic drug became a matter of concern in the long term usage of such drugs. This issues have prompted the search for herbal preparations (medicinal plants) which are safe, cheap, reduce mortality and are able to maintain the optimum growth of animals (Phondani *et al.*, 2010; Bentea *et al.*, 2010). Herbal medicines are being practiced in the form of therapy for livestock farmers because they contains phytochemicals or bioactive chemicals which have been reported to perform multiple biological activities such as antibacterial, antiviral, antifungal, antioxidant, antidiarrheal, anti-stress and anticancer (Hashemi *et al.*, 2009; Franco-Jimenez *et al.*, 2007).

The use of plant extract or phyobiotics has also increased because of the global awareness on the dangers of antibiotics and have been extensively used in feed as natural growth promoters, health protectants (Hashemi *et al.*, 2011), reduced microbial

load and feed palatability (Mountzouris & Paraskevas, 2009). Some of these plants of high therapeutic value includes; Azadirachtaindica, Allium sativum, Thymus vulgaris, Curcuma longa, Piper nigrum, Cymbopogon citratus and Allium sativum.

Cymbopogon citrates (Lemon grass) belongs to the family Poaceae, is a tall aromatic coarse grass (Burkill *et al*., 1996). It is found in most countries in Africa, Europe and Asia. The plants contains steroids, alkaloids, phenols, saponin, tannins, anthraquinines and flavonoids as phytochemical components which allows it to functions as antibacterial, antioxidants, antifungal and antidiarrheal. According to Thorat *et al.* (2017) Lemon grass leaves contains 71.03% moisture, 3.83% crude protein, 2.94% ash, 4.76% crude fat, 9.30% crude fibre and 20.73% carbohydrate. The leaves of the plant produces yellow or amber color and aromatic essential oil (such as citral α, citral β, nerol geraniol, citronellal, terpinolene, geranyl acetate, myrecene and terpinol) when squeezed (Adejuwon & Esther, 2007).

Garlic (Allium sativum) belongs to the family lily, has been used as a spice and a native medicine for many years. It has possess excellent antibacterial, antifungal, antiviral, antioxidant,anti-cholesteremic, anti-cancerous, and vasodilator characteristics (Hanieh *et al.,* 2010; Kim *et al.,* 2009). The aqueous extract of Alliumsativum have been shown to inhibit E.coli and Salmonella (Kumar & Berwal, 1998; Singh & Shukla, 1984). The results attributed the efficacy of garlic due to the presence alliin, diallylsulphideand allicin (Amagase & Milner, 1993), which possess antimicrobial activity (Tsao & Yin, 2001).

Previous studies have shown that lemon grass positively influenced the performance and could be used as a botanical alternative in the diet of broilers (Mukhtar *et al.*, 2012; Thayalini *et al.*, 2011), quails (Sariözkan *et al.*, 2018). Similarly, Ozougwu *et al* (2011) reported a significant difference ($p<0.05$) in blood parameters white albino rats orally fed 300mg/kg garlic extract. These plant extracts have been found to be loaded with several bioactive chemicals, therefore a synergistic combination of this plants will give a positive result on the performance of animals, save cost and ensure total safety in the final products obtained.

Therefore, this experiment was designed to evaluate the performance and hematological parameters of broiler chicks fed different levels of dried lemon grass (*Cymbopogon citratus*) and garlic (*Allium sativum*) extract mixture.

2. Materials and methods

Site of the experiment

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

Collection and sample preparation

Fresh, mature and healthy leaves of Cymbopogon citratus were obtained within the farm premises,the leaves were thoroughly washed with running tap water to remove the debris and allowed to dry under shade for 11 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. 20.00 grams of Cymbopogon citratus powder was mixed with 100ml distilled water for 48 hours after which it was filtered using Whatmann filter paper to obtain Cymbopogon citratus extract (CCL).

Garlic cloves were purchased from a local market in Gutpa, the cloves were carefully peeled, cut into pieces and dried for 7 days. The dried garlic was blended into powder with an electric powder, extracts were prepared by soaking 100g dried garlic powder in one liter of water for 24 hours. The next morning, the extract (GLE) was obtained by filtering the infusion using a filter paper.

CCL and GLE were mixed in another clean bowl in the ratio of 1:1 to obtain lemon grass/garlic extract mixture (CLGE). The powdered samples and experimental diet were also subjected to proximate analysis to determine the crude protein, ether extract, crude fibre and ash according to AOAC (1990).

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

Pre-experimental activities

The house was cleaned and well disinfected before the commencement of the experiment, wood shaves were also spread on the floor of the pen.

Animal management and experimental diets

A total number of two hundred and fifty (250) one day 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 5 treatments of 250 chicks. The birds were kept under similar conditions of management throughout the experimental period. Chicks were bought 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 hours in a form of natural light during the day and artificial light during the night.

The chicks were fed on 5 dietary treatments. The first group A was given 0.025g/litre Neomycin in water. The other groups B, C, D and E were given CLGE at levels 3.0ml/litre, 6.0ml/litre, 9.0ml/litre and 12.0 ml/ litre of water respectively. The basal diet was formulated to meet the nutrients requirements of broiler chicks according to the (NRC, 1994).

Experimental design and parameters measured

Experimental design used was a completely randomized design. 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 were recorded daily and all management practices were strictly observed throughout the experimental period.

Blood sample analysis

At the 7^{th} week of the experiment, blood samples were collected from three randomly selected birds per replicate, the samples were collected via the branchial vein to aspire 5mls of blood from each bird into bottles containing Ethylene Diamine Tetra Acetate (EDTA) for haematological analysis. Some of the haematological parameters measured are Pack cell volume (PCV), Red blood cell (RBC), White blood cell (WBC),

Haemoglobin concentration (Hb) and absolute counts of neutrophils, lymphocytes, monocytes and eosinophils which were all computed according to standard techniques as reported by Jain (1986).

MCV, MCH and MCHC were calculated according to Jain (1986) method as shown below:

MCV (fl) = PCV (%) ×10/RBC (10^6/μL)

MCH (pg) = Hb (g/dl) × 10/RBC (10^6/μL)

MCHC (%) = Hb (g/dl) × 100/PCV (%)

Statistical analysis

All data generated were subjected to a one way analysis of variance (ANOVA) and treatment means were compared using Steel and Torrie (1986). Differences among treatment means were separated by least significance difference test.

Table 1. Composition of experimental diets

Ingredients	**0-4 weeks (Starter)Kg**	**4-7weeks (Finisher)Kg**
Maize	52.00	60.99
Soya meal	38.60	30.60
Groundnut cake	3.00	3.00
Fish meal (72%)	1.00	0.00
Bone meal	3.00	3.00
Limestone	1.50	1.50
Lysine	0.15	0.15
Methionine	0.20	0.20
Toxin binder	0.01	0.01
Premix	0.25	0.25
Salt	0.30	0.30
Total	100.0	100.0
Determined analysis		
ME (Kcal/kg)	2990.9	3110.4
Crude protein (%)	23.23	20.51
Ether extract (%)	3.67	3.01
Crude fibre (%)	3.79	4.08
Calcium (%)	1.11	1.08
Phosphorus (%)	0.87	0.95

* 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

Table 2. Proximate composition of dried lemon grass leaf meal (CCL) and Garlic powder (GRE)

Parameters	% lemon grass powder	% Garlic powder
Dry matter	90.45	93.11
Crude protein	13.71	4.60
Crude fibre	9.76	6.11
Ether extract	2.51	0.66
Ash	8.77	5.11
Nitrogen free extract (NFE)	65.25	83.52

Table 3. Phytochemical components of CCL and GRE

Components (mg)	CCL	GRE	Safe recommended levels (mg)
Tannins	0.650	7.00	15.0
Alkaloids	0.520	4.59	3.50
Saponins	0.700	-	7.02
Phytate	0.001	27.80	23.40
Flavonoids	0.532	33.03	-
Oxalate	-	1.02	1.3-1.8
Phenol	0.400	0.041	

Table 4. Effect of feeding different levels CLGE on the performance of broiler chicken

Parameters	**Treatments**					
Breed (Ross 308)	**1**	**2**	**3**	**4**	**5**	**SEM**
No of birds	50	50	50	50	50	-
Live body weight (g)						
Initial weight	42.10	40.01	40.03	40.08	40.71	0.08*
7^{th} day	110.5	110.2	110.0	110.4	111.0	3.51*
28^{th} day	889.3	845.1	850.6	856.8	860.0	7.20*
49^{th} day	1775.0^{c}	2001.7^{b}	2088.2^{a}	2113.0^{a}	2190.6^{a}	30.21*
Body weight gain (g)						
0-7 days	71.22	70.07	70.01	69.98	71.33	1.58*
7-28 days	779.0	735.0	742.1	740.7	743.5	5.02*
0-49 days	1534.7^{c}	1961.7^{b}	2048.2^{b}	2072.9^{a}	2081.8^{a}	33.18*
Feed intake (g/bird)						
0-7 days	1413.00	1409.31	1401.11	1400.23	1400.08	12.34*
7-28 days	2140.22	2100.90	2100.08	2100.03	2100.00	22.56*
0-49 days	3640.88	3633.07	3624.01	3602.08	3600.01	35.05*
Feed conversion ratio (feed/gain)						
7-28 days	2.41^{a}	2.49^{a}	2.47^{b}	2.46^{b}	2.46^{c}	0.07*
0-49 days	1.99^{a}	1.78^{a}	1.73^{b}	1.71^{b}	1.70^{c}	0.04*
Mortality						
	6/50	0/50	0/50	0/50	0/50	-
% Mortality	12.00	-	-	-	-	-

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

*: Significant difference

Table 5. The effect of feeding different levels CLGE on the haematological traits of broiler chickens

Parameters	**Treatment**					
	1	**2**	**3**	**4**	**5**	**SEM**
PCV (%)	30.10^{c}	37.43^{b}	39.56^{b}	39.71^{a}	39.96^{a}	0.04*
Hb (g/dl)	9.88^{c}	11.01^{c}	12.06^{b}	13.71^{b}	13.80^{a}	0.27*
RBC $\times 10^{6}/\mu L$	2.01^{c}	3.61^{b}	3.68^{b}	3.71^{a}	3.95^{a}	0.01*
MCV (fl)	149.8^{a}	103.7^{c}	107.5^{b}	107.0^{b}	101.2^{c}	4.03*
MCH (pg)	49.15^{a}	30.50^{c}	32.77^{c}	36.95^{b}	34.94^{b}	0.07*
MCHC (%)	32.82^{c}	29.41^{b}	30.49^{b}	34.53^{a}	34.51^{a}	0.03*
WBC $\times 10^{6}/\mu L$	19.89^{c}	22.56^{b}	25.35^{b}	28.05^{a}	31.22^{a}	0.12*

Differentials (10^6/µL)						
Lymphocytes	14.18[c]	15.90[c]	16.56[b]	17.08[a]	18.22[a]	0.20*
Monocytes	0.99[c]	1.44[b]	1.33[b]	1.35[b]	1.38[a]	0.05*
Heterophils	5.09[c]	6.06[b]	6.17[c]	6.45[b]	7.58[a]	0.22*
Basophils	1.04[c]	2.10[c]	2.17[b]	2.23[b]	2.90[a]	0.05*
Eosinophils	1.03[c]	1.41[b]	1.51[a]	1.58[a]	1.74[a]	0.02*

[abc] means different superscript along rows differs significantly at $p<0.05$
*: Significant difference

3. Result and discussion

Table 1 shows the chemical composition of the experimental diets. The starter diet contained 23.23% crude protein, 3.67% ether extract, 3.79% crude fibre, 0.87% phosphorus, 1.11% calcium and 3000.9 MEkcal/kg energy while the finisher diet contained 20.51% crude protein, 3.01% ether extract, 4.08% crude fibre, 1.08% calcium, 0.95% phosphorus and 3110.4 MEkcal/kg energy. The basal diet was formulated to meet the nutrients requirements of broiler chicks according to the (NRC, 1994).

The proximate components of lemon grass leaf meal and garlic powder is revealed in Table 2. The proximate components of lemon grass leaf meal are 90.45%, 13.71%, 9.76%, 2.51%, 8.77% and 65.25% for dry matter, crude protein, crude fibre, ether extract, ash and nitrogen free extract. The values obtained is parallel with the findings of Sariözkan *et al.* (2018). Garlic powder contained dry matter (93.11%), crude protein (4.60%), crude fibre (6.11%), ether extract (0.66%), ash (5.11%) and nitrogen free extract (83.52%). The result obtained during this study is in agreement with the findings of Ayoola *et al.* (2016).

Results on the phytochemical analysis of Lemon grass leaf extract and garlic extract revealed the presence of tannin (0.650mg/100g), alkaloids (0.520mg/100g), saponins (0.700mg/100g), phytate (0.001mg/100g), flavonoids (0.532mg/100g) and phenol (0.400mg/100g) while those of garlic powder are tannin (7.00mg), alkaloid (4.59mg), phytate (27.80mg), flavonoids (30.03mg), phenol (0.041mg) and oxalate (1.02mg) respectively. Lemon grass leaf extract had a higher level of saponin followed by tannin, flavonoids, alkaloids, phenol and phytate respectively as presented in Table 3. This is consistent with the finding of Asaolu *et al.* (2009), though a slightly lower value was recorded for tannin (0.600mg), this may be attributed to differences in the variety, environmental condition and stage of growth of the plant. However, all values are below the lethal dose reported by Kumar et al. (2010); European Food Safety Authority (2014) on scientific opinion on the safety and efficacy of tannic acid when used in feed for all animal species. According to EFSA (2010), the use of tannic acid as feed additive up to 15mg/kg is safe for all animal species. Garlic extract had a higher level of flavonoid (30.03mg) when compared with the other parameters. Similar observation was made by Vaghasiya *et al.* (2011) who reported that Aristolochia bracteolate contained a higher percentage of flavonoid during the phytochemical analysis of some medicinal plants from Western region of India.

Saponin are play an active role as anti-inflammatory and immune stimulating remedies (Cheeke *et al.,* 2006). Tannins are been reported to perform antibacterial and

antiviral activity (Enzo *et al*., 2007). Phenol are antioxidants which prevents the oxidative damage of molecules such as DNA and protein (Ojewuyi *et al.*, 2014).

The performance of broiler fed different levels of CLGE is presented in Table 4. The average initial body weight was similar along treatments. However, the final average weight was higher (P<0.05) for birds in T5 (2190.6g), T4 (2113.0g) and T3 (2088.2g) than those in T2 (2001.7g) and T1(1775.0g). According to Preston & Leng (1987), the growth rate of an animal is determined by the feed intake and digestibility with feed intake being determined by nutrients. The result of the current study was also consistent with those reported for broilers, when supplemented with 1250 ppm *Citrus sinensis* peel extract (Ebrahimi *et al.*, 2014). Similarly, Alabi *et al.* (2017) reported that broilers fed 90ml/liter aqueous Moringa leaf extract had the highest body weight and growth rate (2392.00g and 53.61g respectively). Conversely, Hassan *et al*. (2014) reported a higher feed intake and lower body weight gain in broiler chicken fed diets without microbials.

Feed conversion ratio was significantly different (P<0.05) among the treatments. The best feed conversion ratio (FCR) was observed in birds fed 40ml/liter of CLGE. This was similar with the finding of Nurani *et al.* (2016) who noted that addition of 15ppm marigold leaf extract in the diet of quails increased their feed conversion ratio. Mortality rate was also affected by the inclusion of CLGE at 40ml/liter in T5. 12% mortality was recorded in birds given 10ml/liter (T1), none was recorded in T2, T3, T4 and T5 respectively.

Table 5 shows the effect of feeding different levels CLGE on the haematological traits of broiler chicken. The PCV, Hb, RBC, MCV, MCH and MCHC values ranged 31.10 – 39.96%,9.88-13.80g/dl, 2.01 – 3.95 10^6/μL, 101.2 – 149.8 fl, 34.94 – 49.15 pg, 29.41 – 34.53 % respectively while the WBC values ranged 19.89 - 31.22 10^6/μL. There was a significant (P<0.05) among the treatments. This was similar with the finding of Ozougwu (2011) who noted that administration of garlic extract at 300mg/kg showed positive haematological activities in rats but contrary to the reports of Oleforuh-Okoleh *et al.* (2015) when banana leaf was used as a phytoadditive in the diets of broiler chicks. Similarly, Alagbe (2019) also reported that dried *Centella asiatica* leaf meal did not (P>0.05) significantly affect the haematological parameters of broilers when fed at 6%.However, all the values of the haematological parameters fall within the ranges for broilers as reported by Talebi *et al.* (2005); Albokhadaim (2012); Al-Nedawi (2018). Hematological studies have been found useful for disease prognosis and for therapeutic and stress monitoring (Braun *et al*, 2010) but can vary due to age, gender, environment, infection and poisoning (Khan & Zafar, 2005). Red blood cell is involved in the transport of oxygen and carbon dioxide in the body (Isaac *et al*, 2013). This is a clear indication that birds in T5 will have a have enough oxygen especially in situation of oxygen starvation. Onu & Aniebo (2011) reported that hematocrit or PCV is an index of toxicity, lower value could be a sign of anemia. WBC helps to fight against infections and provide resistance against diseases (Soetan *et al.*, 2013).

4. Conclusion

The growth and hematological parameters measured showed significant differences. It could therefore be concluded that CLGE could be efficiently utilized and tolerated by broiler chickens up to 40ml/liter inclusion level without any negative effect

on the performance and health status of broiler chickens. CLGE it is safe, efficient and should be encouraged among small and medium scale farmers.

Recommendation

Further research needs to be carried to determine the effect of CLGE on bacteria load count, to ascertain whether it can reduce the load of pathogenic or opportunistic bacteria in the gut of birds.

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