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Effect of dietary supplementation with Ethiopian pepper (*Xylopia aethiopica*), cloves (*Syzygium aromaticum*), and their composite on growth performance, serum parameters, and haematological indices of broiler chickens

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SUMMARY

This study investigated the effect of dietary Ethiopian pepper (X*ylopia aethiopica*) and clove (S*yzygium aromaticum*) supplementation on the growth performance, serum parameters, and haematological indices of broiler chickens. A total of 360 unsexed Ross broilers were used for the study. Four experimental diets were formulated for the starter (0–28 days) and finisher phases (29–56 days). Diet 1 was the control, without phytogenic supplementation, Diet 2 was supplemented with 1% Ethiopian pepper (EP), Diet 3 was supplemented with 1% clove (CL), and Diet 4 was supplemented with 1% mixture of Ethiopian pepper and clove (EPCL) in equal quantities (0.5% each) (EPCL). Each treatment was replicated 6 times, with 15 birds per replicate. Growth response was measured weekly for the starter and finisher phases, and blood was collected for serum and haematological parameters after 4 and 8 weeks. Data were subjected to one-way analysis of variance using SAS 2000, and significant means were separated using Tukey's test in same software. At day 28, dietary supplementation with EPCL increased ($P < 0.05$) live weight (LW) (660.34 g) and weight gain (WG) (599.66 g) and improved the feed conversion ratio (FCR) (0.98). Total protein (TP) (48.00 g/L) and albumin (28.30 g/L) were higher ($P<0.05$) for broilers fed a diet supplemented with EPCL compared to the other treatments. Packed cell volume (PCV) (30.00%) was higher ($P < 0.05$) in broilers fed a diet with EPCL. Supplementation with CL

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and EPCL increased (P < 0.05) the red blood cell (RBC) count in broilers. Broilers fed a diet supplemented with EP had lower (P < 0.05) mean corpuscular volume (MCV) (97.77 fl), while those fed the control diet had higher (P < 0.05) mean corpuscular haemoglobin (MCH) (39.45 pg). Broilers fed the control diet had the highest (P < 0.05) mean corpuscular haemoglobin concentration (MCHC) (37.90%), while those fed diets supplemented with CL (33.29%) and EPCL (34.00%) had the lowest (P < 0.05) MCHC. At day 56, LW and WG were also increased (P < 0.05) by supplementation. Reduced (P < 0.05) cholesterol was observed for broilers fed a diet supplemented with EPCL. Haemoglobin (Hb) and RBC were higher (P < 0.05) in broilers fed a diet supplemented with EPCL. Supplementation with EP, CL and EPCL resulted in higher (P < 0.05) MCV and MCH. It was concluded that EPCL can be used to supplement the diet of broilers for increased performance and improved blood parameters.

KEY WORDS: Broilers, phytogenic plants, growth performance, serum parameters, haematological indices

INTRODUCTION

Broiler chickens are raised specially for meat production due to their fast growth rate and high feed conversion rate, with a low level of activity. Broilers often reach harvest weight of 4–5 pounds in just five weeks (Al-Homidan., 2005). Broiler production plays a significant role in human nutrition and serves as a good source of meat for the rapidly increasing population, but its production is challenged by diseases, which were initially combated with the use of antibiotics (Tollefson and Miller, 2000; Gaskins et al., 2002).

In response to concerns about antimicrobial-resistant bacteria in humans associated with the consumption of meat from antibiotic-treated broilers, there is strong advocacy against the use of antibiotics in the production of meat (Laxminarayan et al., 2015, Haque et al., 2020). Its consumption has been reported to adversely affect human health as a result of long residual properties and carcinogenic effects (Butaye et al., 2003). It is therefore imperative to find feed components associated with less risk than in-feed antibiotics in order to reduce mortality of animals and improve the quality of animal products. This has led to an intensive search for alternatives such as phytogenic plants (Shittu et al., 2002; Radzikowski and Milczarek, 2022). The search for substitutes for antibiotic growth promoters (AGP), which are currently banned by the European Union, has focused attention on alternative additives, including herbs (Barreto et al., 2008; Pałka et al., 2020).

Phytogenics are a group of natural non-antibiotic growth promoters derived from herbs, spices or other plants (Banerjee et al., 2011). Ethiopian pepper (*Xylopia aethiopica*) and cloves (*Syzygium aromaticum*) are plants that could serve as phytogenic feed additives due to their active constituents and nutritive value (Cortés-Rojas et al., 2014; Imo et al., 2018). Ethiopian pepper is a potential therapeutic weapon against various human and animal diseases, and this potential has made it valuable to human and animal lives (Ogbonnia et al., 2008). It has been widely used in traditional medicine, and beyond its therapeutic uses, it has been widely used as a food supplement (Evans, 2002; Okeke et al., 2008). Clove is known to have various therapeutic uses, including pain amelioration, improvement of digestion, protection against internal parasites, and antifungal activity (Burt and Reinders, 2003). Clove oils are known to stimulate and disinfect as they travel through the body, due to their antimicrobial properties (Shafi et al., 2002) and anti-inflammatory activity (Muruganadan *et al.*, 2001).

There are various reports on phytogenic plants with the potential to replace antibiotic growth promoters (Mishra and Singh, 2000; Deepak et al., 2002: Jahan et al., 2008), but very few of them have been investigated. There is also a dearth of information about the potency and effectiveness of Ethiopian pepper and cloves as safe and sustainable feed additives in poultry production. Therefore this study seeks to determine the effect of dietary supplementation with Ethiopian pepper, cloves, and their composite on the growth performance, serum parameters, and haematological indices of broiler chickens.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Poultry unit of the Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta, Alabata, Ogun State, Nigeria. The site is situated in the derived savanna zone of south-western Nigeria at 7º9' 39N 3º20' 54E, 76 m above sea level. The mean annual rainfall is 1040 mm and occurs from March to October. The average temperature is 34ºC throughout the year.

Test ingredients and processing

The test ingredients, i.e. dried Ethiopian pepper (*Xylopia aethiopica*) and clove (*Syzgium aromaticum*), were purchased from Kuto market in Abeokuta Ogun State, Nigeria. The dried fruits of clove and Ethiopian pepper were milled to a particle size of 0.2 mm with a Kenwood blender and stored in plastic containers prior to diet formulation and commencement of the feeding trial.

Chemical composition of test ingredients

Ground samples (3 g) of the test ingredients were analysed for proximate composition (AOAC, 2005). Gross energy was estimated using an adiabatic bomb calorimeter (Model 1261; Parr Instrument Co., Moline, IL, USA). Mineral content was determined according to Kumari et al. (2017). Quantitative analysis was carried out by inductively coupled plasma atomic absorption emission spectroscopy (ICP-AES). Samples were also analysed for mineral contents using an atomic absorption spectrophotometer (Perkin Elmer Optima 4300DV ICP spectrophotometer, UK). The phytochemical constituents were analysed following standard procedures: total polyphenols (Wright et al., 2000), flavonoids (Arvouet-Grand et al., 1994), extractable tannins (Hoff and Singleton, 1977) and saponins (Edeoga et al., 2005).

Experimental birds and management

A total of 360 unsexed Ross broilers were purchased from Agric International technology and Trade Limited (AGRITED) hatchery, Ibadan, Nigeria. Essential biosecurity measures were observed prior to the arrival of the broiler chicks. The birds were reared in a deep litter system, with wood shavings provided as bedding. Feeding trays and water troughs were made available for the birds. External heat (electric bulb and charcoal) was provided in the poultry pen.

Experimental design and dietary treatment

The broilers were allotted on a weight equalization basis to four dietary treatments using a completely randomized design. Each treatment had six replicates, with 15 birds per replicate. The formulated diets were Diet 1 (control) without phytogenic supplementation, Diet 2 supplemented with 1% Ethiopian pepper (EP), Diet 3 supplemented with 1% clove (CL), and Diet 4 supplemented with 1% blend of Ethiopian pepper and clove in equal quantities (0.5% each) (EPCL). The phytogenic additive was added to the basal diet and thoroughly mixed to ensure uniform distribution. The diets were formulated (Table 1) for starter (0–28 days) and finisher (29–56 days) phases based on the nutrient requirement guide for Ross 308 broilers (Ross Broiler Nutrition Specifications 2016). The experiment lasted for 8 weeks. All birds were raised intensively on deep litter with spacing of 1 m^2 per bird. Feed and water were supplied ad libitum during the experimental period. No in-feed antimicrobials, anticoccidial drugs or antibiotics were administered to the birds for the duration of the study.

Table 1.
Composition of experimental diet

Ingredient (%)	**Starter (0-28 days)**	**Finisher (29-56 days)**
Maize	54.00	63.00
Soya meal	24.00	19.00
Wheat offal	13.00	10.00
Fish meal	3.00	2.00
Bone meal	3.00	3.00
Oyster shell	2.00	2.00
*Vitamin/Mineral Premix	0.25	0.25
Salt	0.25	0.25
Lysine	0.25	0.25
Methionine	0.25	0.25
Total	**100.00**	**100.00**
Determined nutrients (%)		
**Metabolized energy (kcal/kg)	2 827.80	3 078.80
Crude protein	23.41	20.41
Ether extract	3.62	3.32
Crude fibre	3.33	3.21
Calcium	1.34	1.25
Phosphorus	0.66	0.61

*Starter Premix: vit. A 10 000 000 IU, vit. D_3 2 000 000 IU, vit. E 23,000 mg, vit. K_3 2000 mg, vit. B_1, 1800 mg, vit. B_2 5 500 (mg), niacin 27 500 mg, pantothenic acid 750 mg, vit. B_6 3 000 mg, vit B_{12} 15 mg, folic acid 750 mg, biotin H_2 60 mg, chlorine chloride 300 000 mg, cobalt 200 mg, copper 300 mg, iodine 1000 mg, iron 20 000 mg, manganese 40 000 mg, selenium 200 mg, zinc 30 000 mg, antioxidant 1250 mg.

*Finisher premix: vit. A. 5 500 000 IU, vit. D_3 1500 000 IU, vit. E 10 000 mg, vit. K_3 1500 (mg), vit. B_1, 1600 (mg), vit. B_2 24 000 (mg), niacin 20 000 mg, pantothenic acid 5 000 mg, vit B_6 1500 mg, vit. B_{12} 10 mg, folic acid 500 mg, biotin H_2 750 mg, chlorine chloride 175 500 mg, cobalt 200 mg, copper 300 mg, iodine 1000 mg, iron 20 000 mg, manganese 40 000 mg, selenium 200 mg, zinc 30 000 mg, antioxidant 1250 mg.

**Estimated using the Nutrient Requirements of Poultry, NRC (1994) formulae, ME = 26.7 (%dry matter) + 77 (%ether extract) − 51.22 (%crude fibre)

Growth performance

The live weight of the birds per pen (n = 15 per treatment) was measured weekly, and the weight gain was computed. Daily feed intake was computed as the difference between the feed offered and feed left over, and the feed conversion ratio was calculated as the ratio of feed consumed to weight gain.

Measurement of blood parameters

Blood collection

On days 28 and 56 of the study, blood samples (2.5 ml each) were collected from the brachial wing vein of two birds per replicate into vials containing ethylene diamine tetra-acetate (EDTA) for determination of haematological indices. Additional blood samples were collected into plain bottles (without EDTA), centrifuged (2500 × *g* for 15 min), and analysed for serum chemistry.

Haematological indices

Haemoglobin concentration (Hb) was estimated using the cyanmethaemoglobin method (Cannan, 1958). Packed cell volume (PCV), red blood cell count (RBC), and leukogram were determined using a Wintrobe haematocrit tube according to Schalm et al. (1975). The standard ratios of the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated according to Jain (1986).

Serum chemistry

Plasma samples were harvested by centrifuging the whole blood samples at 3000 rpm for 15 min. Serum parameters were determined following standard procedures. Total serum protein, albumin and globulin were determined by the bromocresol purple method (Varley et al., 1980). Serum creatinine was determined according to Bousnes and Taussky (1945), and serum uric acid concentration according to Wootton (1964). Serum enzymes (alanine transaminase (ALT) and aspartate serum transaminase (AST) were analysed spectrophotometrically using the Randox® diagnostic cholesterol kit. Alkaline phosphatase (ALP) activity was measured colorimetrically using commercial clinical diagnostics kits (ELItech, France). Serum cholesterol was estimated using commercial kits (Qualigens India. Pvt. Ltd., Catalogue number 72201-04).

Statistical analysis

Data were subjected to one-way analysis of variance using SAS statistics software (SAS, 2000) to determine the effect of supplementation with phytogenic plants (Ethiopian pepper, clove, and Ethiopian pepper and clove composite). Differences between mean values were separated using Tukey's Test in SAS Software, and differences were considered significant at $P < 0.05$.

RESULTS

The proximate, gross energy, mineral and phytochemical composition of Ethiopian pepper (*Xylopia aethiopica*) and cloves (*Syzygium aromaticum*) is presented in Table 2.

Table 2.
Proximate, gross energy, mineral and phytochemical composition of Ethiopian pepper (*Xylopia aethiopica*) and cloves (*Syzygium aromaticum*)

Composition	**Ethiopian pepper**	**Cloves**
Proximate (%)		
Moisture	12.80	20.10
Crude Protein	16.54	6.69
Crude Fat	3.93	2.37
Crude Fibre	12.35	7.83
Total Ash	8.96	5.49
NFE	58.22	77.62
Gross energy (Kcal/kg)	4404.44	4368.88
Mineral (mg/g)		
Calcium	5.10	2.80
Magnesium	1.87	2.90
Potassium	45.05	25.03
Sodium	0.24	1.80
Manganese	0.20	0.47
Iron	0.24	0.26
Copper	0.041	0.052
Zinc	0.012	0.019
Phytochemical content		
Tannins (mg/100 g)	1435.00	890.00
Phenolics (GAE/g)	24.20	51.50
Alkaloids (mg/100 g)	980.00	1990.00
Flavonoids (mg/100 g)	0.07	0.23

Broiler performance

Table 3 shows the growth performance of broilers fed diets supplemented with phytogenic additives. At day 28, live weight (LW) was highest ($P < 0.05$) in broilers fed the diet supplemented with EPCL and lowest ($P < 0.05$) in those fed the diet supplemented with EP, while broilers fed the control diet and those fed the diet supplemented with CL had intermediate weight. Weight gain (WG) was highest ($P < 0.05$) for broilers fed the diet supplemented with EPCL and lowest ($P < 0.05$) for broilers fed the diet supplemented with EP. Dietary supplementation with EPCL resulted in the best ($P < 0.05$) feed conversion ratio (FCR), while broilers fed the diet supplemented with EP had the worst ($P < 0.05$) FCR, and those fed the control diet and those fed the diet supplemented with CL had intermediate FCR. Mortality was lowest ($P < 0.05$) in broilers fed the diet supplemented with EPCL and highest ($P < 0.05$) in those fed the control diet and those fed the diet supplemented with EP. Feed intake (FI) was not significantly ($P > 0.05$) affected by phytogenic supplementation.

At day 56, LW and WG was highest ($P < 0.05$) in broilers receiving EPCL supplementation and lowest in those fed the diet supplemented with CL ($P < 0.05$). Broilers fed the control diet and those fed the diet supplemented with EP had intermediate weight. FI was highest (P < 0.05) for broilers fed the diet supplemented with EPCL and lowest in those fed the control diet and those fed the diet supplemented with EP (P < 0.05). Broilers fed the CL-supplemented diet had intermediate weight. Broilers fed the diet supplemented with CL had a poor (P < 0.05) FCR compared to the other treatments. At day 56, no mortality was recorded for broilers fed the diet supplemented with EPCL. Broilers fed the control diet had the highest (P < 0.05) mortality, while the mortality of those fed the diet supplemented with EP and those fed the CL-supplemented diet was intermediate.

Table 3.
Performance of broilers fed diets supplemented with Ethiopian pepper, cloves, and their composite

Parameter (g)	Phytogenic supplementation				SEM	P-value
	Control	**EP (1%)**	**CL (1%)**	**EPCL (1%)**		
Initial weight	60.98	61.54	62.50	60.68	2.20	0.36
Day 28						
Live weight	576.12^{b}	480.21^{c}	560.32^{b}	660.34^{a}	35.70	0.03
Weight gain	515.14^{ab}	418.67^{b}	497.82^{ab}	599.66^{a}	38.80	0.04
Feed intake	601.23	595.10	589.50	586.50	42.11	0.20
FCR (g/g)	1.17^{ab}	1.42^{a}	1.18^{ab}	0.98^{b}	0.09	0.02
Mortality (%)	6.25^{a}	5.89^{a}	4.17^{b}	2.08^{c}	2.30	0.02
Day 56						
Live weight	2150.23^{b}	2144.34^{b}	1933.26^{c}	2396.42^{a}	77.55	0.01
Weight gain	1574.11^{b}	1664.13^{b}	1372.94^{c}	1736.08^{a}	70.20	0.02
Feed intake	2489.23^{b}	2525.35^{b}	2586.98^{ab}	2605.88^{a}	149.40	0.03
FCR (g/g)	1.58^{b}	1.52^{b}	1.88^{a}	1.50^{b}	0.10	0.01
Mortality (%)	5.21^{a}	3.20^{b}	3.50^{b}	0.00^{c}	1.71	0.04

^{ab}Means in the same row with different superscripts are significantly different (P < 0.05). EP = Ethiopian pepper, CL= cloves, EPCL = blend of Ethiopian pepper and cloves, FCR = Feed conversion ratio
SEM = Pooled standard error of mean

Serum parameters of broilers

The serum parameters of broiler chickens fed diets supplemented with phytogenic additives are shown in Table 4. At day 28, total protein (TP) was highest (P < 0.05) in the serum of broilers fed the diet supplemented with EPCL, lowest (P < 0.05) in those fed the diet supplemented with EP, and intermediate in those fed the control diet and those fed the diet supplemented with CL. Supplementation with ECPL resulted in higher (P < 0.05) serum albumin compared to the other treatments. Uric acid was lowest (P < 0.05) for broilers fed the diet supplemented with CL and highest (P < 0.05) for broilers fed the control diet. The broilers fed the control diet and those fed the diet supplemented with EP had the highest (P < 0.05) serum glutamic oxaloacetic transaminase (SGOT), while those fed the diet supplemented with CL had the lowest (P < 0.05) SGOT. Globulin, cholesterol, serum glutamic pyruvic transaminase (SGPT), and creatinine levels were not significantly (P > 0.05) affected by phytogenic supplementation. At day 56, only cholesterol and SGOT were significantly (P < 0.05) affected by phytogenic supplementation. Cholesterol was lower (P < 0.05) in broilers fed the diet supplemented with EPCL compared to the other treatments. SGOT was higher (P < 0.05) in broilers fed the diet supplemented with EP than in the other treatments.

Table 4.
Serum parameters of broilers fed diets supplemented with Ethiopian pepper, cloves, and their composite

Parameter	Phytogenic supplementation				Reference value¶	SEM	P-value
	Control	EP (1%)	CL (1%)	EPCL (1%)			
Day 28							
Total protein (g/L)	43.97[b]	42.50[c]	44.30b[b]	48.00[a]	25.00-45.00	9.33	0.01
Albumin (g/L)	22.54[b]	21.11[b]	23.20[b]	28.30[a]	11.70-27.40	5.77	0.04
Globulin (g/L)	20.90	21.39	21.10	19.70	20.80-21.00	2.01	0.06
Cholesterol (mmol/L)	6.72	6.75	6.80	6.70	6.93-11.10	1.10	0.09
SGOT (iU/L)	135.43[a]	136.10[a]	103.40[c]	111.20[b]	37.80-311.00	13.22	0.03
SGPT (iU/L)	27.96	28.10	28.30	25.70	16.87-23.00	9.10	0.15
ALP (iU/L)	201.33	204.20	200.30	198.50	150.00-280.00	12.7	0.76
Uric acid (mmol/L)	0.22[a]	0.17[b]	0.12[c]	0.18[b]	0.11-0.69	0.24	0.01
Creatinine (mmol/L)	0.06	0.06	0.07	0.06	0.02-0.09	0.04	0.21
Day 56							
Total protein (g/L)	53.21	51.50	52.78	51.45	25.00-45.00	9.03	0.06
Albumin (g/L)	31.22	31.20	30.07	29.35	11.70-27.40	5.27	0.22
Globulin (g/L)	20.40	20.30	21.71	21.10	20.80-21.00	1.00	0.15
Cholesterol (mmol/L)	11.77[a]	11.88[a]	11.93[a]	11.12[b]	6.93-11.10	3.25	0.01
SGOT (iU/L)	198.78[b]	215.00[a]	204.00[b]	202.60[b]	37.80-311	17.22	0.03
SGPT (iU/L)	17.86	17.57	18.00	17.30	16.87-23.00	5.10	0.09
ALP (iU/L)	221.00	231.00	210.00	224.00	150.00-280.00	47.7	0.32
Uric acid (mmol/L)	0.15	0.16	0.16	0.15	0.11-0.69	0.12	0.18
Creatinine (mmol/L)	0.06	0.06	0.07	0.06	0.02-0.09	0.02	0.12

[ab]Means in the same row with different superscripts are significantly different (P < 0.05).

SEM = Pooled standard error of mean, SGOT = serum glutamic oxaloacetic transaminase, SGPT = serum glutamic pyruvic transaminase, ALP = alkaline phosphatase, EP = Ethiopian pepper, CL = cloves, EPCL = blend of Ethiopian pepper and cloves, ¶ Mitruka and Rawnsley (1997), Clinical Diagnostic Division (1990)

Haematological indices of broilers

The haematological indices of broilers fed diets supplemented with phytogenic additives are presented in Table 5. The results at day 28 show significant ($P < 0.05$) effects of phytogenic additive supplementation on PCV, Hb, WBC, RBC and lymphocytes, while other parameters were not significantly ($P > 0.05$) affected. PCV was the highest ($P < 0.05$) in broilers fed the diet supplemented with EPCL and lowest ($P < 0.05$) in those fed the diet supplemented with EP. Broilers fed the control diet and those fed the diet supplemented with EPCL had higher ($P < 0.05$) Hb than in the other treatments. Dietary supplementation of CL resulted in increased ($P < 0.05$) WBC compared to the other treatments. RBC was highest ($P < 0.05$) for broilers fed the diet supplemented with CL and those fed the diet supplemented EPCL compared to the other treatments. Lymphocyte count increased ($P < 0.05$) with EPCL supplementation but was reduced ($P < 0.05$) by EP supplementation. Broilers fed the diet supplemented with EP had lower ($P < 0.05$) MCV compared to the other treatments. Broilers fed the control diet had higher ($P < 0.05$) MCH compared to the other treatments. MCHC was highest ($P < 0.05$) in broilers fed the control diet and lowest ($P < 0.05$) in those fed the diet supplemented with CL, while those fed the diet supplemented with EP had an intermediate MCHC count.

At day 56, PCV was highest ($P < 0.05$) in broilers fed the diet supplemented with EPCL and lowest ($P < 0.05$) in those fed the control diet. Dietary supplementation with EPCL resulted in the highest ($P < 0.05$) Hb and RBC count compared to the other treatments. WBC was highest ($P < 0.05$) in broilers fed the diet supplemented with CL and lowest ($P < 0.05$) in those fed the diet supplemented with EP. Broilers fed the control diet and those fed the diet supplemented with EPCL had intermediate WBC counts. Broilers belonging to the control group had lower ($P < 0.05$) MCV and MCH compared to the other groups.

Neutrophil, lymphocyte, basophil, eosinophil and MCHC counts were not significantly ($P > 0.05$) affected by phytogenic supplementation.

Table 5.
Haematological indices of broilers fed diets supplemented with Ethiopian pepper, cloves, and their composite

Parameter	Control	Phytogenic supplementation EP (1%)	CL (1%)	EPCL (1%)	Reference values	SEM	P-value
Day 28							
Packed cell volume (%)	26.65^b	25.62^c	27.34^b	30.00^a	#22.00-35.00	9.22	0.00
Haemoglobin (g/dl)	10.10^a	9.00^b	9.10^b	10.20^a	#7.00-13.00	6.02	0.02
WBC (x10^9/L)	19.65^b	21.50^b	24.00^a	22.00^b	#21.18-22.17	7.55	0.01
RBC (x10^{12}/L)	2.56^b	2.62^b	2.60^a	2.89^a	#2.50- 3.50	0.99	0.03
Neutrophil (%)	19.87	20.00	21.00	21.00	NR	5.90	0.24
Lymphocyte (%)	68.94^b	65.00^c	68.00^b	70.00^a	#45.00-70.00	19.70	0.53
Basophil (%)	1.00	0.00	0.00	0.00	NR	0.01	0.12
Eosinophil (%)	0.10	0.00	2.00	0.00	ǂ1.50- 6.00	0.01	0.23
MCV (fl)	104.11^a	97.77^b	105.14^a	103.81^a	ǂ104.00-135.00	10.34	0.00
MCH (pg)	39.45^a	34.35^b	35.00^b	35.29^b	ǂ32.00-43.90	3.55	0.01
MCHC (%)	37.90^a	35.12^{ab}	33.29^b	34.00^b	ǂ30.20-36.20	2.43	0.02
Day 56							
Packed cell volume (%)	29.26^c	32.20^{ab}	31.02^b	34.23^a	#22.00-35.00	10.20	0.01
Haemoglobin (g/dl)	9.86^b	10.50^b	10.30^b	11.20^a	#7.00-13.00	5.02	0.03
WBC (x10^9/L)	19.56^b	18.20^c	20.00^a	19.70^b	#21.18-22.17	7.25	0.02
RBC (x10^{12}/L)	2.93^b	2.90^b	2.87^b	3.15^a	#2.50- 3.50	0.79	0.00
Neutrophil (%)	19.89	22.01	19.64	20.12	NR	6.90	0.00
Lymphocyte (%)	62.21	61.65	60.01	61.23	#45.00-70.00	20.70	0.87
Basophil (%)	1.00	0.00	2.00	0.00	NR	0.01	0.14
Eosinophil (%)	0.00	1.00	2.01	1.05	ǂ1.50- 6.00	0.01	0.38
MCV (fl)	99.76^b	110.83^a	108.08^a	108.67^a	ǂ104.00-135.00	9.42	0.00
MCH (pg)	33.65^b	36.31^a	35.89^a	35.66^a	ǂ32.00- 43.90	2.34	0.03
MCHC (%)	33.52	32.61	33.21	32.62	ǂ30.20- 36.20	1.35	0.65

^{ab}Means in the same row with different superscripts are significantly different (P < 0.05).

SEM = Pooled standard error of mean, EP = Ethiopian pepper, CL= cloves, EPCL = blend of Ethiopian pepper and cloves, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, MCV = Mean corpuscular volume, # Abdulazeez et al. (2016), ǂ Harrison and Lightfoot (2005), NR = Not reported

DISCUSSION

Growth performance at day 28 shows that broilers fed a diet supplemented with EPCL had increased LW. This is similar to the improved performance obtained when different concentrations of *Xylopia aethiopica* dried fruits were given in drinking water to broilers, which was similar to the effect of an antibiotic growth promoter (Gendox) (Isikwenu et al., 2014). This indicates that phytogenic plants have growth-promoting potential similar to that of antibiotics. Supplementation with EPCL increased WG and improved FCR compared to the other treatments. This is in agreement with the reports of Pourali et al. (2010) and Aji et al. (2011), who found that phytogenic additives such as garlic (powder or aqueous extract) as an additive for broiler chickens improved body weight gain, daily feed intake, and feed conversion ratio. In the present study, however, FI was not significantly affected at day 28. The increase in WG observed for broilers fed the diet supplemented with EPCL could be attributed to the synergistic growth-promoting properties of the phytogenic additives. Improved WG and FCR following the use of a mixture of phytogenic additives have been reported in previous literature (Jamroz et al., 2005; Brenes and Roura, 2010). Giannenas et al. (2003) also reported significant improvements in the WG and FCR of birds fed diets supplemented with oregano essential oil compared to the control. Ciftci et al. (2005) observed better WG and FCR for broilers fed a diet supplemented with anise oil. Mortality was significantly reduced with EPCL supplementation in the diet of broilers, which demonstrates the health-promoting effect of the constituents of the phytogenic mixture. Phytogenic preparations administered even at small doses can improve the health and productivity of animals (Wojcikowski and Gobe, 2014; Upadhaya and Kim, 2017).

At day 56, LW and WG increased with EPCL supplementation, as observed at day 28. This improvement suggests enhanced nutrient utilization due to the active constituents of phytogenic additives. This is in agreement with the report of Oso et al. (2019), who observed increased body weight gain in broilers fed a diet supplemented with a 1% phytogenic blend (*Cynodon dactylon*, *Aerva lanata* and *Piper nigrum*). In the present study, FI was significantly affected at day 56, when broilers fed a diet supplemented with EPCL had the highest ($P < 0.05$) feed intake, followed by those fed the diet supplemented with CL. The differences observed at these stages of growth are in agreement with previous studies by Giannenas et al. (2003) and Hernandez et al. (2004), who observed that the growth response of broilers receiving phytogenic additives acts in an age-dependent manner, with different responses in different age groups. The increased feed intake observed for broilers fed these diets suggests that the phytogenic additive improves digestion to encourage feed intake. The bioactive compounds contained in phytogenic additives encourage secretion of endogenous digestive enzymes and improve liver function (Al-Kassie, 2009) and nutrient digestion (Lee et al., 2004). Cloves are known to contain the active constituent eugenol, which exhibits digestive stimulatory properties, in addition to their antimicrobial activity against bacteria found in the intestine (Cabuk et al., 2003). This indicates that the increased feed intake observed for broilers fed the diet supplemented with EPCL may be influenced by CL as a component of the phytogenic blend. FCR was poor for broilers fed the diet supplemented with CL compared to the other treatments. This indicates that the increased feed intake did not result in better weight gain, which suggests reduced protein utilization affecting tissue accretion. No mortality was observed for broilers fed the diet supplemented with EPCL. This confirms the effectiveness of the active biological components (alkaloids, saponins, flavonoids, tannins, pectins and organic acids) in herbal additives, which exert antioxidant and antibacterial effects and support immunity in animals (Magi et al., 2006; Kostadinović and Lević, 2018).

Supplementation with EPCL increased serum TP and albumin, while EP supplementation reduced serum TP at day 28. The TP and albumin values obtained for broilers fed a diet supplemented with EPCL was slightly higher than the upper limit of the reference value, and this slight increase may be attributed to increased nutrient availability and absorptive capacity, due to the effect of phytogenic activity through increased pancreatic enzyme stimulation by bioactive compounds (Duwa et al., 2020). The increased serum TP and albumin concentrations indicate improved utilization of dietary protein as a result of improved feed intake, which in turn resulted from increased digestion (Corzo et al., 2009; Liu et al., 2015). Increased SGOT was observed in the blood of broilers fed the control diet and those fed the diet supplemented with EP. An increased SGOT level in the liver may be due to the phytochemicals contained in the plant, which on ingestion are detoxified and metabolized in the liver, leading to an increase in this blood chemistry index due to phytogenic inclusion. However, the values were within the normal range, and there was also an increase in the SGOT of broilers fed the control diet. Therefore the increase in SGOT may not necessarily be due to damage to the liver or other organs. An increase in serum enzymes such as AST may signal damage to the liver, and other organs as well (Lu, 2017), but this was not the case in this study, as the values were within the normal range. The highest serum uric acid was obtained for broilers fed the control diet. High serum uric acid levels have been linked to inefficient protein utilization and consequent increased deamination. When energy or protein utilization is inefficient, the serum uric acid concentration is normally high as a consequence of increased deamination (Oduguwa and Ogunmodede, 1995). This was not the case in this study, as the values across treatments were within the normal range, which suggests that phytogenic supplementation has a non-antagonistic effect on protein utilization.

At day 56, reduced cholesterol was observed for broilers fed the diet supplemented with EPCL. The reduced cholesterol level is due to the ability of bioactive components such as saponins to form insoluble complexes with cholesterol and inhibit intestinal absorption of endogenous and exogenous cholesterol (Oakenfull and Sidhu, 1990). Saponins also inhibit key enzymes in the cholesterol and lipid biosynthetic pathways (Konjufca et al., 1997). As observed at the end of starter phase, dietary supplementation with EP also increased SGOT, possibly due to the phytochemicals contained in the phytogenic plant, which, as mentioned above, are detoxified during metabolism, causing an increase in this blood index. Supplementation with phytogenic additives in the starter and finisher phases did not significantly influence SGPT or ALP, and the values were within the normal range. This is an indication of normal liver function and the absence of damage to liver cells and myocardial tissues, as enzymes are rarely found in the serum unless there is damage to one or more organs or tissues (Oloyede and Sunmonu, 2008).

The increased PCV observed for broilers fed a diet supplemented with EPCL at day 28 suggests improved nutrient utilization, as blood haematological indices are important indicators of the body’s physiological, pathological, and nutritional status and alterations in the constituent compounds of blood. When compared to normal values, they can be used to interpret metabolic activity in animals, as well as the quality of feed the animal has consumed (Ologhobo et al., 2008). The increase in PCV suggests improved protein intake and tissue synthesis, as a decline in PCV has been associated with inhibition of protein synthesis (Denli et al., 2009). The values obtained for PCV were within the normal range reported by Abdulazeez et al. (2016), which suggests good erythrocyte condition and unimpeded blood flow. Improved Hb content was obtained for broilers fed the control diet and those fed the diet supplemented with EPCL. The increase in Hb concentration suggests high bioavailability of nutrients, particularly protein. Importantly, the haemoglobin values were within the normal range given by Abdulazeez et al. (2016). Blood composition is a reflection of the constituents of ingested feed, which has measurable effects on blood parameters (Maxwell et al., 1990). Haemoglobin reacts with oxygen carried in the blood to form oxyhaemoglobin during respiration, which is essential for metabolism (Chineke et al., 2006). Dietary supplementation with CL increased the WBC count of broilers, which indicates an increase in active protective mechanisms. An increased WBC count does not necessarily reflect impaired health status. A high WBC count in animals could be the initiation of a protective system for a well-adapted immune defence (Tambuwal et al., 2002). Supplementation with CL and EPCL in the diet of broilers increased the RBC count. This indicates improved oxygen-carrying capacity of the cells, which translated to better nutrient bioavailability. Red blood cells are also actively involved in the transport of oxygen and carbon dioxide in the animal body (Isaac et al., 2013). The RBC values obtained in this study are within the normal range reported by Abdulazeez et al. (2016). The increased lymphocyte count obtained for broilers fed the diet supplemented with EPCL is a sign of good health status, as an increase in the concentration of blood leukocytes indicates a response to invading foreign organisms (Alzawqari et al., 2011). Supplementation with CL and EPCL in the diet of boilers resulted in MCV similar to that obtained in the control group, with values within the normal range reported by Harrison and Lightfoot (2005). This indicates the absence of macrocytic and hypochromic anaemia, since a deviation from normal values has been associated with negative effects in some haematopoietic factors responsible for the production of red blood cells in bone marrow (Awodi et al., 2005). MCV values for broilers fed the diet supplemented with EP were below the lower limit of the normal range, which may be due to the phytogenic supplementation. However, this does not necessarily indicate impaired health, as PCV and RBC were within the normal range. The broiler group fed the control diet had higher MCH than the other groups, but the values across treatments were within the normal range. This indicates the absence of hypochromasia, in which MCH is lower than normal (Olafadehan, 2011). Broilers fed diets supplemented with EP, CL and EPCL had lower MCHC compared to the control group, and the values were within the normal range. The value in the control group was slightly above the upper limit of the normal range, but this slight change does not necessarily indicate challenged health status.

At day 56, supplementation with EPCL increased PCV, Hb and RBC, and the values were within the normal range. The increase in these blood parameters suggests good health status of the birds. According to Amusa et al. (2015), haematological indices are crucial in monitoring feed toxicity, particularly those constituents that influence the blood, as well as the health status of farm animals. The increased RBC count indicates that an adequate amount of oxygen is carried to the cells/tissues (Soetan et al., 2013), while increased PCV suggests adequate constituent components of the blood (Emenalum et al., 2009). The reduced WBC count observed for broilers fed the diet supplemented with EP indicates good health status of the birds. Elevated WBC counts have been recorded in the case of disease, infection, or immune system disorders (Maroufyan et al., 2010). The increased WBC count observed for broilers fed the diet supplemented with CL could be due to increase in lymphocyte count, as an element of the WBC differential, in response to incidence of foreign agents. A rise in WBC count is associated with an increase in lymphocytes, which are known to increase greatly in response to infection, as one of the body's first lines of defence (Masoudi et al., 2011). Broilers fed the control diet had reduced MCV compared to the other groups, and the value fell below the lower limit of the normal range reported by Harrison and Lightfoot (2005). This reduction in MCV could be linked to the low level of RBC. Mean corpuscular volume is the average volume of individual red blood cells, and the reduced MCV could be due to the lower red blood cell count in the control group. Groups of broilers fed diets supplemented with EP, CL and EPCL had higher MCH compared to the control group; however, the values across treatments were within the normal range. This suggests that the diets were adequate in terms of quality and quantity, as haematological parameters are response indicators to important factors, especially feeds and feeding (Adekunle and Omoh, 2014).

CONCLUSION

The synergistic effect of the combination of supplementation with Ethiopian pepper and cloves (EPCL) increased weight gain, improved FCR, and reduced mortality in broilers. Haematological indices and serum parameters improved following supplementation with EPCL in the diet of broilers. Therefore dietary supplementation with EPCL can be a suitable alternative to antibiotics.

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