#### **Original Paper**

# Wild sunflower and goat weed leaf meals composite-mix supplementation in broiler chickens: effects on performance, health status and meat

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Three hundred 1-day old Cobb 500 broiler chickens were randomly assigned to five experimental diets (60 birds/diet; 10 birds/ replicate) using a completely randomized design to assess the effects of wild sunflower and goat weed leaf meals composite mix (CLM) in broiler chickens. At the starter and finisher phases, a basic diet was formulated, divided into five equal parts and tagged diets 1 to 5. Diets 1 and 2 had 0% and 1.1% Oxytetracycline (Oxyt) supplementation; while the diets 3, 4 and 5 were supplemented with 0.4%, 0.8% and 1.2% CLM, respectively. In starter and finisher phases, the highest (P < 0.05) body weight gain (BWG) was recorded in the birds fed diet 5 and 4, respectively compared to other diets. During the overall phase, birds fed diet 5 had the highest BWG, which was similar to those fed diet 4 but higher (P < 0.05) than the birds fed the rest diets. The feed intake (FI) and feed conversion ratio (FCR) were influenced (P < 0.05) by the CLM supplementation at the starter phase. The FCR recorded in birds fed the 1.1% Oxyt, 0.8% and 1.2% composite leaf mix supplemented diets (diets 2, 4, and 5) were similar (P > 0.05) to those fed 0.4% CLM, but significantly better (P < 0.05) the birds fed the control diet. The dietary CLM supplementation caused increased (P < 0.05) serum catalase and glutathione peroxidase concentration. The meat cholesterol levels of the birds were significantly (P < 0.05) reduced by dietary CLM supplementation. Conclusively, the CLM supplementation at 0.8% and 1.2% enhanced the BWG. CLM supplementation at 0.4%, 0.8 and 1.2% increased the serum glutathione peroxidase and catalase activity and reduced the broiler's meat cholesterol.

**Keywords:** phytogens, avian, performance, health status, growth promoters

## 1 Introduction

Restriction on the use of direct-feed antibiotics as growth promoters as well as consumers increasing demand for animal products void of antibiotic residues has encouraged the search and utilization of phyto-additives as replacement alternatives in poultry production (Valenzuela-Grijalva et al., 2017). The use of these phytoadditives (e,g herbs, spices, and their extracts) in poultry diets are with several beneficial effects ranging from antioxidant, antimicrobial, immune-modulatory and antistress activities to improvement of gut health, nutrient absorption, consequently, enhanced performance characteristics (Oloruntola et al., 2020). Because of their abundant inherent aromatic compounds, the use of phyto-additives in broiler chicken diets was reported to improve taste, aroma, and palatability of feed and also the physicochemical qualities of the poultry (Valenzuela-Grijalva et al., 2017). Also, it was revealed the intake of diet fortified with phyto-additives can stimulate the production of digestive juices (saliva, gastric juices, pancreatic and intestinal secretion) and enhance appetite and digestion of poultry (Marcinčák et al., 2011).

Wild sunflower (*Tithonia diversifolia*), commonly known as Mexican sunflower, is a robust non-woody and bushy enduring plant which belong to family Asteraceae (Fasuyi et al., 2010). All parts of the plant including leaf, stem, root, and flower have been used in folk medicine for the treatment of different ailments and diseases (Omokhua et al., 2018). Reports on the in-vitro antioxidant analysis showed that sunflower leaf is very rich in bioactive compounds, flavonoid, and phenolic contents (Odedire and Oloidi, 2011). It's antioxidant and

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antimicrobial contents and activities have been reported (Gutierrez et al., 2015). More so, the nutritional content of wild sunflower leaf has been reported to range from 18–21% crude protein, 11–19% crude fibre, 13–14% ash, 4–5% ether extract, and 42–52% nitrogen-free extract (Oluwasola and Dairo, 2016). These nutrients have greatly encouraged its utilization as feed additives for poultry and pigs (Odunsi et al., 1996; Olayeni et al., 2006).

Goat weed (Ageratum conyzoides) is an aromatic, annual medicinal plant, which is a part of the family of Asteraceae. The plant is native to tropical America but now widely found in Southwest Nigeria particularly on cultivated fields and other ecosystems such as pastures, grasslands, wastelands, and forest areas (Shailajan et al., 2013). Every part of the plant is widely utilised in customary medicine as an antiasthmatic, antispasmodic, anti-inflammatory, analgesic, anesthetic, and in treatment of bacterial infections (Santos et al., 2016). In Nigeria, goat weed is used in the treatment of skin diseases, wounds, diarrhea, and navel pains in children (Adetuyi et al., 2018). According to Santos et al. (2016), the plant possessed a vast variety of secondary metabolites, including mono and sesquiterpenes, triterpenes, steroids, flavonoids, coumarins, tannins, and alkaloids. Goat weed is rich in mineral, vitamins (Shailajan et al., 2013), and other chemical compounds: crude protein (16.03%) and ash (10%) (Agida et al., 2017). Despite the wide range of reports on its medicinal properties and chemical composition, hardly any feeding trials have been performed on their effect on broiler production.

Recently, the use of combinations of phyto-additives as composite meal mix have been disclosed to exert higher multidirectional effects on animals performance and physiological status than using it individually in diet, due to the advantage of the synergistic and additive effects of individual active ingredients in the mixtures (Oloruntola et al., 2018, Oloruntola et al., 2020). Therefore, the objective of the study was to examine the sequel of wild sunflower and goat weed leaf meal composite mix on growth performance, meat quality, and health standing of broiler chicken.

## 2 Materials and methods

#### 2.1 Phytogens collection, processing, and analysis

Leaves of wild sunflower and goat weed were collected within the vicinity of The Federal College of Agriculture, Akure, Nigeria. On the collection day, these leaves were washed off specks of dirt in running water, drained, spread on racks under a shed for an hour, chopped into small pieces with a stainless knife, spread lightly under the shed for 14 days for them to dry. Thereafter, the wild sunflower and goat weed leaves were ground with a hammer mill to about 100µm to produce wild sunflower leaf meal (WLM) and goat weed leaf meal (GLM), respectively. Equal portions (1:1) of WLM and GLM were mixed to form wild sunflower leaf and goat weed leaf composite mix (CLM). Thereafter the WLM, GLM, and CLM were analyzed for proximate composition (AOAC, 1995), phenol (Ignat et al., 2013), flavonoids (Bohm and Kocipal-Abyazan, 1994), saponin (Brunner, 1984), terpenoids (Sofowora, 1993), 2,

Composition	Wild sunflower leaf meal (WLM)	Goat weed leaf meal (GLM)	Composite Leaf meal mix (CLM*)					
Proximate composition (%)								
Crude protein	8.05 ±0.70	11.79 ±0.13	9.83 ±0.41					
Crude fibre	13.29 ±0.27	15.80 ±0.24	14.28 ±0.25					
Ether extract	6.02 ±0.01	4.97 ±0.20	5.59 ±0.13					
Ash	14.79 ±0.14	13.71 ±0.09	14.28 ±0.16					
Nitrogen free extract	48.54 ±0.63	45.87 ±0.51	47.14 ±0.47					
Phytochemicals (mg g <sup>-1</sup> )								
Phenol	6.27 ±0.01	5.87 ±0.16	6.07 ±0.09					
Flavonoid	10.25 ±0.04	8.52 ±0.13	9.56 ±0.16					
Saponin	60.11 ±0.15	81.15 ±0.06	68.91 ±1.75					
Terpenoid	46.56 ±0.05	47.17 ±0.27	46.87 ±0.14					
Antioxidant properties								
DPPH (%)	77.85 ±0.43	48.13 ±0.07	65.59 ±2.24					
FRAP (mg g <sup>-1</sup> )	1.44 ±0.01	2.01 ±0.15	1.70 ±0.09					

**Table 1**Composition of leaf meals and their composite mix

\*CLM – equal portion (1 : 1) of WLM and GLM; DPPH – 2, 2-diphenyl-1-picrylhydrazyl hydrate; FRAP – ferric-reducing antioxidant property

2-diphenyl-1-picrylhydrazy hydrate (Gyamfi et al., 1999) and ferric-reducing antioxidant property (Pulido et al., 2002) (Table 1).

## 2.2 Diets, housing, and experimental design

A broiler chickens' basal diet each was produced for the starter phase and finisher phase (NRC, 1994) (Table 2). At each of the phases, the basal diet was divided into five equal parts and tagged diets 1 to 5. Diets 1 and 2 had 0 and 1.1% Oxytetracycline (Oxyt), while the diets 3, 4, and 5 were supplemented with 0.4%, 0.8%, and 1.2% CLM, respectively (Table 2).

Table 2Composition and nutrient contents of<br/>experimental basal diets

Ingredients (%)	Starter (1 to 21 days)	Grower/ finisher (22 to 42 days)						
Maize	42.66	48.56						
Wheat offal	12.10	10.10						
Soybean meal	38.69	34.79						
Vegetable oil	2.20	2.30						
Di-calcium phosphate	1.80	1.70						
Limestone	1.40	1.40						
Premix	0.30	0.30						
Methionine	0.30	0.30						
Lysine	0.25	0.25						
Salt	0.30	0.30						
Chemical analysis (% DM)								
Crude protein	22.00	20.56						
Crude fibre	4.53	4.38						
Calculated analysis (% DM)								
Metabolizable energy (kcal kg-1)	2,955.88	3,000.24						
Calcium	1.20	0.93						
Available Phosphorus	0.60	0.55						
Methionine	0.63	0.38						
Lysine	1.15	1.03						

Three hundred 1-day old Cobb 500 broiler chicks were randomly assigned to five experimental diets (60 birds/ diet; 10 birds/replicate) using a completely randomized design. A space of 200 × 100 cm was provided per replicate and was covered with wood shaving. The temperature of the experimental pen was maintained at 31 °C ±2 for the first 7 days and gradually reduced by 20C after each consecutive week until the experimental house temperature was 26 °C ±2. The light was provided for 23 hours.day<sup>-1</sup> and the birds were fed *ad libitum*.

## 2.3 Growth performance, Slaughtering procedures, Collection of blood samples and Carcass analysis

The birds' body weight gain (BWG) and feed intake (FI) were determined weekly. The feed conversion ratio (FCR) was estimated by dividing the total feed ingested by total weight gain. On day 42 of the experiment, the procedures earlier describe by Oloruntola et al. (2020) were used in the birds' slaughtering and blood collection for haematological, serum enzymes; antioxidant enzymes studies. The hematological indices were evaluated within 2 hours post-collection (Shastry, 1983). The blood in the plain sample bottle (5 ml) was spin and its serum decanted into another plain sample bottle and frozen at -20 °C before used for the serum enzyme and serum antioxidant enzyme analysis. The serum enzymes (alanine aminotransferase and creatinine) concentration were determined on a Reflectron ®Plus 8C79 (Roche Diagnostic, GombH Mannheim, Germany), using kits. The serum antioxidant enzymes i.e. superoxide dismutase (SOD) (Misra and Fridovich, 1972), glutathione peroxidase (GPx) (Rotruck et al., 1973) and catalase (CAT) (Aebi, 1974) were determined. The weights of the slaughtered and dressed birds were determined with a sensitive scale and the birds' dressed percentage was estimated as a percentage of the slaughtered weight. About 100g of the bird's breast meat was cut out the determination of the meat cholesterol (Allain et al., 1974).

## 2.4 Analysis of data

The model:  $Dmy = \mu + \alpha m + \beta my$ , was used in this experiment, where Tmy = any of the response variables; m = the overall mean;  $\alpha m =$  effect of the mth treatment (D = diets 1, 2, 3, 4 and 5); and  $\beta my =$  random error due to experimentation. Data were exposed to one-way ANOVA using SPSS version 20. The differences among the means were determined (P < 0.05) by Duncan multiple range test of SPSS.

# 3 Results and discussion

Plant leaf meals are generally considered to have an appreciable amount of nutrients and phytochemicals that can enhance growth and improve the physiological status of livestock (Oloruntola et al., 2018). The proximate composition, phytochemicals and antioxidant activities of leaf meals and their composite mix vary according to leaf meal types (Table 1). The phenol ( $6.27 \pm 0.01$  vs.  $5.87 \pm 0.16$  vs.  $6.07 \pm 0.09$ ), flavonoid ( $10.25 \pm 0.04$  vs.  $8.52 \pm 0.13$  vs.  $9.56 \pm 0.16$ ), saponin ( $60.11 \pm 0.15$  vs.  $81.15 \pm 0.06$  vs.  $68.91 \pm 1.75$ ), terpenoid ( $46.56 \pm 0.05$  vs.  $47.17 \pm 0.27$  vs.  $46.87 \pm 0.14$ ) contents of WLM vs. GLM vs. CLM shows that these phytogens contains essential phytochemicals of medicinal values. For instance, phenol, flavonoid, saponin, and terpenoid are essential

secondary metabolites found in plants that are very effective in scavenging oxidizing molecules such as singlet oxygen and other free radicals during production, thereby preventing oxidative damage in animal tissues (Tungmunnithum et al., 2018). Furthermore, the result of the antioxidant analysis showed that WLM had the highest DPPH free radical scavenging activity (77.85  $\pm 0.43\%$ ) compared to GLM and CLM at 48.13  $\pm 0.07\%$  and 65.59  $\pm 2.24\%$ , respectively. While GLM had the highest FRAP value (2.01  $\pm 0.15$  mg g<sup>-1</sup>) compared to WLM and CLM at 1.44  $\pm 0.01$  mg g<sup>-1</sup> and 1.70  $\pm 0.09$  mg g<sup>-1</sup>, respectively. This study showed that WLM, CLM, and their composite mix (WLM + CLM 1 : 1) can scavenge free radicals or prevent the initiation of free radicals when applied in a biological system.

Phyto-additives are now considered as a potential key panacea for antibiotic-free livestock nutrition because of their ability to improve growth performance and feed utilization of broiler chicken. Table 3 showed the effect of composite leaf mix supplementation on BWG, FI, and FCR. The BWG of the birds at both starter (1-21 day) and grower/finisher (22-42 day) phases, was significantly influenced (P < 0.05) by composite leaf meal mix. The birds on diet supplemented with 1.2% CLM had the highest BWG compared to other diets at the starter phase; while those on diets supplemented with 0.8 and 1.2% CLM had the highest BWG compared to other diets at the finisher phase. However, during the overall period (1-42 day), birds receiving 1.2% CLM supplemented diets had the highest BWG, which is comparable to BWG in birds fed 0.8% CLM but significantly (P < 0.05) higher than those birds fed the rest diets.

The highest BWG recorded in birds fed the 0.8% CLM and 1.2% CLM supplemented diets at 42 days of age could be attributed to combined mechanism of action of bioactive compounds (phenol, flavonoids, saponins, and terpenoids) resident in composite leaf meal mix in promoting broiler chickens' growth performance. Several studies have shown that phyto-additives containing rich bioactive compounds can stimulate feed intake, facilitate nutrient digestion and absorption in the gut through secretion of endogenous digestive enzymes and enlargement of villi diameters, thereby improving the broiler chickens' BWG (Mohiti-Asli and Ghanaatparast-Rashti, 2017, Oloruntola et al., 2018). Also, GLM and WLM are known to possess antibacterial, antifungal and antiprotozoal properties (Gutierrez et al., 2015; Osuntokun et al., 2018) which can suppress harmful microbes and promote intestinal health and stimulate growth in broiler chicken than the birds fed the control diet and antibiotic supplemented diet as seen in this current study.

The FI and FCR were only significantly influenced (P < 0.05) by the CLM supplementation at the starter phase. The highest FI recorded in the birds fed the control diet was similar to those fed 1.2% CLM supplemented diet and significantly (P < 0.05) higher than those birds fed the rest diets. However, the FCR recorded in birds fed the 1.1% Oxyt, 0.8%, and 1.2% composite leaf mix supplemented diets were comparable to those fed 0.4% CLM, but significantly better (P < 0.05) than the birds fed the control diet. This is suggesting that at the starter phase, the 1.1% Oxyt supplementation, and CLM supplementation at 0.4,

Parameters	Diet 1 Control	Diet 2 1.1% Oxyt	Diet 3 0.4% CLM	Diet 4 0.8% CLM	Diet 5 1.2% CLM	SEM	P value		
Starter phase (1 to 21d)									
IBW (g bird <sup>-1</sup> )	50.86	52.17	51.97	51.14	51.88	0.92	0.99		
BWG (g bird-1)	741.38 <sup>b</sup>	787.62 <sup>ab</sup>	720.78 <sup>b</sup>	754.72 <sup>ь</sup>	839.29ª	13.67	0.02		
FI (g bird <sup>-1</sup> )	1,697.57ª	1,442.64 <sup>bc</sup>	1,455.85 <sup>bc</sup>	1,380.56 <sup>c</sup>	1,611.66ªb	38.69	0.02		
FCR	2.30ª	1.83 <sup>b</sup>	2.02 <sup>ab</sup>	1.83 <sup>b</sup>	1.92 <sup>b</sup>	0.05	0.04		
Finisher phase (22 to 42d)									
BWG (g bird-1)	1,567.06 <sup>b</sup>	1,591.45ªb	1,585.40ªb	1,772.90ª	1,766.62ª	32.57	0.05		
FI (g bird <sup>-1</sup> )	2,538.25	2,626.25	2,510.93	2,587.95	2,512.40	76.74	0.99		
FCR	1.61	1.64	1.58	1.45	1.42	0.04	0.49		
Overall (1 to 42d)									
BWG (g bird-1)	2,308.45°	2,379.07 <sup>bc</sup>	2,306.18 <sup>c</sup>	2,527.62ªb	2,605.91ª	39.63	0.01		
FI (g bird <sup>-1</sup> )	4,235.82	4,068.89	3,966.78	3,968.51	4,124.06	91.46	0.91		
FCR	1.83	1.70	1.72	1.57	1.58	0.04	0.29		

 Table 3
 Effects of composite leaf mix supplementation on performance of broiler chickens

means within a row with different superscripts are significantly different (P < 0.05); Oxyt – oxytetracycline; CLM – composite leaf mix (WLM + GLM 1 : 1); IBW – initial body weight; BWG – body weight gain; FI – feed intake; FCR – feed conversion ratio; SEM – standard error of the mean

0.8 and 1.2% promotes better utilization of diets than the birds on the control diet and this, in turn, may have contributed to the highest BWG recorded for those birds fed the Oxyt and CLM supplemented diets, compared to the birds on the control (Valenzuela-Griljalva et al., 2017).

There were no significant effects (P > 0.05) of CLM supplementation on the carcass traits and relative weights of the internal organ of the experimental birds across the treatments (Table 4). This is suggesting the addition of CLM in the broiler diet does not have any negative effects on the carcass and organ weight of the broiler chicken. However, the present result is in contract

with the reports of Glamoclijaet al. (2016) and Oloruntola et al. (2018) who found that addition phyto-additives in broiler chickens significantly increase their dressing percentage and relative internal organ weights.

Blood indices are major tools for assessing the physiological, pathological, and nutritional status of animals. The result of haematological indices and serum metabolites of broiler chickens fed the supplemented diets is presented in Table 5. The CLM supplementation did not influence (P > 0.05) the haematological indices of the experimental birds across the various dietary treatments. This result suggests the various dietary supplementations

Table 4Effects of composite leaf mix supplementation on carcass traits and relative internal organ weights (% body<br/>weight) of broiler chickens

Parameters	Diet 1 Control	Diet 2 1.1% Oxyt	Diet 3 0.4% CLM	Diet 4 0.8% CLM	Diet 5 1.2% CLM	SEM	P value
Slaughter weight (g bird-1)	2,566.66	2,533.33	2,466.67	2,400.00	2,500.00	44.14	0.84
Dressed weight (g bird-1)	2,133.33	2,016.67	1,919.70	2,040.63	2,074.63	33.67	0.39
Dressing (%)	83.42	79.58	77.91	85.07	83.19	1.20	0.32
Liver	2.33	2.24	2.56	2.44	2.75	0.12	0.79
Heart	0.43	0.32	0.42	0.37	0.39	0.02	0.47
Lung	0.57	0.61	0.62	0.60	0.54	0.02	0.81
Proventriculus	0.36	0.29	0.40	0.39	0.35	0.01	0.25
Gizzard	2.28	1.85	2.35	2.09	2.01	0.07	0.35
Spleen	0.11	0.07	0.11	0.08	0.11	0.01	0.54

Oxyt – oxytetracycline; WLM – wild sunflower leaf meal; GLM – goat weed leaf meal; CLM – composite leaf mix (WLM + GLM 1 : 1); SEM – standard error of the mean

Parameters	Diet 1 Control	Diet 2 1.1% Oxyt	Diet 3 0.4% CLM	Diet 4 0.8% CLM	Diet 5 1.2% CLM	SEM	P value	
Haematological indices								
Red blood cells (× 10 <sup>12</sup> l <sup>-1</sup> )	1.70	1.90	1.66	1.76	1.67	0.07	0.87	
Haemoglobin conc. (g dl-1)	13.00	14.16	14.86	13.66	13.33	0.24	0.10	
Packed cell volume (%)	39.00	42.50	44.50	41.00	40.00	0.72	0.10	
Mean cell volume (fl)	231.90	233.46	276.46	249.26	247.13	10.84	0.76	
Mean cell haemoglobin (pg)	77.26	77.76	92.20	82.96	82.46	3.61	0.75	
Mean cell haemoglobin conc. (g dl <sup>-1</sup> )	33.26	33.30	33.30	33.30	33.31	0.01	0.45	
White blood cells (× 10 <sup>9</sup> l <sup>-1</sup> )	4.00	3.40	4.80	3.20	6.96	0.49	0.08	
Granulocytes (× 10 <sup>9</sup> l <sup>-1</sup> )	1.30	0.90	1.36	0.90	2.30	0.19	0.10	
Lymphocytes (× 10 <sup>9</sup> l <sup>-1</sup> )	2.56	1.96	3.46	2.26	4.30	0.30	0.06	
Monocytes (× 10 <sup>9</sup> l <sup>-1</sup> )	0.09	0.11	0.08	0.07	0.12	0.01	0.60	
Serum metabolites								
Alanine aminotransferase (U/L)	98.15	96.45	101.55	101.30	99.00	0.79	0.20	
Creatinine (mmol I <sup>-1</sup> )	21.85	21.84	28.05	34.30	32.75	2.24	0.25	

Oxyt – oxytetracycline; WLM – wild sunflower leaf meal; GLM – goat weed leaf meal; CLM – composite leaf mix (WLM + GLM 1 : 1); SEM – standard error of the mean

Diet 1 Control	Diet 2 1.1% Oxyt	Diet 3 0.4% CLM	Diet 4 0.8% CLM	Diet 5 1.2% CLM	SEM	P value		
Serum antioxidant enzyme status								
57.77	60.57	67.22	60.10	65.14	2.29	0.74		
100.49 <sup>b</sup>	97.91 <sup>b</sup>	121.24 <sup>ab</sup>	143.65ª	147.38ª	6.75	0.01		
56.87°	50.73°	79.62 <sup>b</sup>	83.77 <sup>b</sup>	130.45ª	7.61	0.00		
Meat analysis indices								
123.71ª	60.10 <sup>b</sup>	40.16 <sup>c</sup>	10.66 <sup>e</sup>	30.59 <sup>d</sup>	10.37	0.00		
	Diet 1 Control 57.77 100.49 <sup>b</sup> 56.87 <sup>c</sup> 123.71 <sup>a</sup>	Diet 1 Control         Diet 2 1.1% Oxyt           57.77         60.57           100.49 <sup>b</sup> 97.91 <sup>b</sup> 56.87 <sup>c</sup> 50.73 <sup>c</sup> 123.71 <sup>a</sup> 60.10 <sup>b</sup>	Diet 1 Control         Diet 2 1.1% Oxyt         Diet 3 0.4% CLM           57.77         60.57         67.22           100.49 <sup>b</sup> 97.91 <sup>b</sup> 121.24 <sup>ab</sup> 56.87 <sup>c</sup> 50.73 <sup>c</sup> 79.62 <sup>b</sup> 123.71 <sup>a</sup> 60.10 <sup>b</sup> 40.16 <sup>c</sup>	Diet 1 ControlDiet 2 1.1% OxytDiet 3 0.4% CLMDiet 4 0.8% CLM57.7760.5767.2260.10100.49b97.91b121.24ab143.65a56.87c50.73c79.62b83.77b123.71a60.10b40.16c10.66e	Diet 1 ControlDiet 2 1.1% OxytDiet 3 0.4% CLMDiet 4 0.8% CLMDiet 5 1.2% CLM57.7760.5767.2260.1065.14100.49b97.91b121.24ab143.65a147.38a56.87c50.73c79.62b83.77b130.45a123.71a60.10b40.16c10.66e30.59d	Diet 1 Control         Diet 2 1.1% Oxyt         Diet 3 0.4% CLM         Diet 4 0.8% CLM         Diet 5 1.2% CLM         SEM           57.77         60.57         67.22         60.10         65.14         2.29           100.49 <sup>b</sup> 97.91 <sup>b</sup> 121.24 <sup>ab</sup> 143.65 <sup>a</sup> 147.38 <sup>a</sup> 6.75           56.87 <sup>c</sup> 50.73 <sup>c</sup> 79.62 <sup>b</sup> 83.77 <sup>b</sup> 130.45 <sup>a</sup> 7.61           123.71 <sup>a</sup> 60.10 <sup>b</sup> 40.16 <sup>c</sup> 10.66 <sup>e</sup> 30.59 <sup>d</sup> 10.37		

**Table 6**Effects of composite leaf mix on serum and meat anti-oxidant enzymes status of broiler chickens

means within a row with different superscripts are significantly different (P <0.05); Oxyt – oxytetracycline; WLM – wild sunflower leaf meal; GLM – goat weed leaf meal; CLM – composite leaf mix (WLM + GLM); SEM – standard error of the mean

did not alter the normal haematopoiesis in these birds and is in contrast with the findings of Tijani et al. (2015) and Oloruntola et al. (2018) who reported that addition of phyto-additives significantly influences the haematological indices of broiler chickens.

The result revealed no significant effects (P > 0.05) in serum alanine aminotransferase and creatinine levels among dietary treatments. The stability of the serum alanine aminotransferase and creatinine of the birds across the dietary treatments indicate the normal functioning of the liver and kidneys, respectively (Lording and Friend, 1999).

The serum antioxidant enzymes status and meat cholesterol level of broiler chickens fed diets supplemented with CLM supplemented diets are presented in Table 6. The dietary CLM supplementation revealed a significant increase (P >0.05) on the concentration of total serum GPx and CAT with exception of SOD, compared to the control and Oxyt supplemented diet. The CAT, GsPx, and SOD have been reported as main antioxidant enzymes that inactivate, scavenge, and remove free radical and other reactive species in tissue to protect the body against oxidative stress. Specifically, CAT is known to directly react with radical species while GPx regenerates oxidized antioxidants and reduces hydrogen peroxide to water, lipid peroxides, and alcohols in the tissue (Delles et al., 2014). The observed increased level of catalase and glutathione peroxidase recorded in birds supplemented with CLM compared to control and antibiotic treatments suggest the ability of the phyto-additive to produce higher antioxidant enzymes to scavenge and remove free radicals and other reactive species, thereby enhancing the antioxidant status of the animal (Oloruntola et al., 2018). This result is in line with the report of Kostadinović et al. (2015) who found that the addition of phyto-additive in broiler diet significantly enhanced the concentration of GPx and CAT compared to control group.

The amount of total cholesterol in the meat samples was significantly (P >0.05) reduced by dietary CLM supplementation. The broiler chickens on CLM supplemented diets had lower cholesterol levels compared with the control and antibiotic group. This is of public health benefits because, the intake of animal products with high cholesterol content can lead to hypercholesterolemia which has been implicated in causing atherosclerosis and coronary heart diseases (Shen et al., 2019). This result is expected as the addition of phyto-additives in broiler diets has been reported to inhibit hypercholesterolemia and lower cholesterol levels in muscle food due to the inherent bioactive compounds (Onyimonyi, et al., 2012; Puvaca et al., 2015). A similar result has been reported by Puvaca et al. (2015) who found that the addition of phyto-additive in broiler diet significantly lowered the cholesterol concentrations in the meat and liver compared to control.

## 4 Conclusions

In conclusion, 0.8% and 1.2% CLM dietary supplementations enhanced the broiler chickens' body weight gain. The CLM dietary supplementation has no deleterious effects on the carcass traits, relative weights of internal organs, haematological indices, serum alanine aminotransferase, and creatinine of the experimental birds. Besides the CLM supplementation at 0.4%, 0.8 and 1.2% improves the serum catalase and glutathione peroxidase activity and reduced the broiler chick's meat cholesterol level.

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