



Evaluation of the Phytogetic potential of *Chromolaena odorata* Leaf Extract on Haematology, Serum Biochemistry, and Intestinal Bacterial Count of Broiler Chickens

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Abstract

This study investigated the effect of Chromolaena odorata leaf extract (COLE) on haematology, serum biochemistry, and intestinal bacterial load in broiler chickens. A total of 250 day-old Arbor Acre broiler chicks were brooded for two weeks then randomly assigned to five groups with varying COLE concentrations (0, 0.5, 1, 1.5, and 2 ml COLE per liter of water), each with five replicates of ten birds per replicate. The study lasted for four weeks, after which, phytochemical screening of COLE, haematological analysis, serum biochemical evaluation, and intestinal bacterial counts were assessed. Results revealed COLE contained various bioactive compounds, including saponins, glycosides, alkaloids, tannins, polyphenols, steroids, terpenoids, and flavonoids. Although haematological parameters remained unaffected, birds receiving 1.5 ml COLE/litre water showed significant reductions in LDL levels. Additionally, 1.5 ml COLE/litre water resulted in lower counts of total viable heterotrophic bacteria, Salmonella-Shigella, and Coliform bacteria, while 1.0 ml COLE/litre water reduced Staphylococcus species bacteria. This study demonstrates the potential of COLE as a natural additive in broiler production, reducing serum LDL and inhibiting the proliferation of pathogenic intestinal bacteria at a recommended dose of 1.5 ml COLE per liter of water.

Keywords: Broiler chickens, *Chromolaena odorata*, Phytogetic additive, Haematology,

Introduction

The challenges associated with poultry disease management have led to the failure of many poultry establishments, particularly in developing countries, resulting in a significant decline in protein intake (FAO, 2017). Poultry diseases not only negatively impact broiler growth and immunity but also cause substantial economic losses, necessitating the consumption of antibiotics and other drugs throughout the birds' lifespan, with the exception of the withdrawal period before slaughter (Lorençon *et al.*, 2007;

Adeyolanu *et al.*, 2019) Over time, antibiotic usage has become a common practice (Chattopadhyay, 2014), playing a crucial role in the profitability of the poultry and livestock industries (Sharifi *et al.*, 2013). Previous studies (Dibner and Richards, 2005; Attia and Al-Harthi, 2015) have suggested that antibiotic growth promoters enhance nutrient utilization and animal growth by modulating the intestinal microflora. However, the potential consequences of antibiotic use in farm animals on public health are a significant concern for

consumers (Sigolo *et al.*, 2021). The extensive usage of antibiotics has led to the emergence of antibiotic-resistant bacteria that can be transmitted from animals to humans (Attia *et al.*, 2018; Chokshi *et al.*, 2019), as well as drug residues in products of animal origin (Gonzalez-Ronquillo and Angeles-Hernandez, 2017). Furthermore, poultry waste containing antibiotic-resistant bacteria and active antibiotics may contaminate the environment (Wongsuvan *et al.*, 2018). This has resulted in the ban on antibiotic-growth-promoters usage for farm animals in certain countries (van der Aar *et al.*, 2017).

Poultry is highly susceptible to the emergence of antibiotic resistance due to its high antibiotic usage (van den Bogaard *et al.*, 2001; Graham *et al.*, 2017). As a result, research is increasingly focused on natural agents with similar benefits to antibiotics (Mehdi *et al.*, 2018). Phytogetic additives, such as herbs, spices, natural electrolytes, essential oils, and oleoresins, contain bioactive molecules with antioxidant and antimicrobial properties (Windisch *et al.*, 2008; Reisinger *et al.*, 2011; Diaz-Sanchez *et al.*, 2015; Attia *et al.*, 2017; Safiyu *et al.*, 2023). These additives have been revealed to improve performance (Giannenas *et al.*, 2005; Hong *et al.*, 2012; Attia and Al-Harathi, 2015; Hashemi *et al.*, 2018), nutrient digestibility (Hernandez *et al.*, 2004; Amad *et al.*, 2011; Mountzouris *et al.*, 2011), digestive enzyme activity (Lee *et al.*, 2003; Jang *et al.*, 2007), and antimicrobial activity (Cross *et al.*, 2007; Reisinger *et al.*, 2011) in broiler chickens. However, some studies have reported negligible responses to herbal additives on performance, gut morphology, and microbiota (Abildgaard *et al.*, 2010; Hafeez *et al.*, 2016; Ahsan *et al.*, 2018). This study focused on Siam weed

(*Chromolaena odorata*), a plant widely distributed in Nigeria and known by various names, including "Obiarakara", "ewe Akintola", "Awolowo", "Independence", "Diochie", trifid weed, and bitter bush (Jiwuba *et al.*, 2017), has been extensively utilized in traditional medicine for its diverse therapeutic properties. The plant possess antimicrobial, antifungal, anti-diarrheal, astringent, anti-inflammatory, antispasmodic, diuretic, tonic, antihypertensive, antipyretic, and heart tonic properties (Vital and Windell, 2009; Moses *et al.*, 2010), making it a valuable resource in the prevention and treatment of various diseases.

Phytochemical analysis has revealed the prevalence of a wide range of bioactive compounds in *C. odorata*, including alkaloids, flavonoids, essential oils, phenolics, saponins, tannins, terpenoids, and other essential constituents such as chromomoric acid, quercetagenin, and quercetin (Sirinthipaporn and Jiraungkoorskul, 2017). These compounds contribute to the plant's therapeutic properties and make it a promising candidate for use in poultry production. Previous studies have investigated the use of *C. odorata* in poultry and livestock nutrition, including its nutrient composition and feeding value for laying birds (Fasuyi *et al.*, 2005; Ekenyem *et al.*, 2009), broilers (Jiwuba *et al.*, 2017; 2018; Safiyu *et al.*, 2024), and rabbits (Akinmutimi and Akufo, 2006). These studies demonstrated the prospects of *C. odorata* as a natural additive for improving animal performance and health. However, despite its potential, there is a lack of information on the impact of *C. odorata* leaf extract on blood profile, and gut microbial population in broiler chickens. This study investigated these effects and provided valuable insights into the potential of Siam weed as

a natural agent in poultry production. By exploring the effects of *C. odorata* leaf extract on haematology, serum biochemistry, and intestinal bacteria count of broiler chickens, this study seeks to contribute to the development of sustainable and antibiotic-free poultry production practices.

Materials and Methods

Location of Study

The experiment was conducted at the Poultry Unit of the Teaching and Research Farms, Michael Okpara University of Agriculture, Umudike. COLE preparation was carried out in the Animal Nutrition Laboratory while blood analyses were performed at the Veterinary Pathology Laboratory, of the same institution.

Preparation of *Chromolaena odorata* leaf Extract (COLE)

Fresh *Chromolaena odorata* leaves were collected from the surroundings of Michael Okpara University of Agriculture, Umudike. The plant was identified by Dr. I. A. Ewetola, a Forage and Grassland Agronomist and co-author of this study. Although no voucher specimen was deposited, the plant was fully validated on (http://mpns.kew.org/mpns-portal/?_ga=1.111763972.1427522246.1459077346). The leaves were carefully separated from the stems, and approximately 1 kg of *C. odorata* leaves were immersed in 4 litres of water and boiled for 15 minutes as enunciated by Sogunle *et al.* (2021). The mixture was then filtered, and the extract was stored in PET bottles under appropriate conditions to maintain its stability prior to administration to the experimental birds according to predetermined treatment guidelines.

Experimental Birds and Management

A total of 250 day-old Arbor Acre broiler chicks were obtained from a reputable source in Umuahia, Abia State, and were brooded for two weeks before being evenly divided into five groups, carefully balanced for body weight. The chicks were distributed randomly into five treatment (0, 0.5, 1, 1.5, and 2 ml COLE per liter of water), each comprising fifty chicks, for a study duration of four weeks. Each treatment had five replicates comprising ten birds per replicate. To assess the efficacy of COLE, it was added via drinking water provided daily for each group of birds at 0, 0.5, 1, 1.5, and 2 ml per liter of water, respectively, for 28 days. Chicks assigned to each treatment group were individually housed in pens with deep litter, and received the same basal diet (Table 1) *ad libitum*. All experimental birds were vaccinated against Newcastle disease on days 5 and 24, and infectious bursal disease on days 10 and 20.

Data Collection

Phytochemical Screening of *Chromolaena odorata* leaf Extract (COLE)

The phytochemical screening of COLE was conducted according to standard protocols described by Manjulika *et al.* (2015). The extract was screened for the following bioactive substances: saponins, glycosides, alkaloids, tannins, steroids, polyphenols, terpenoids and flavonoids.

Blood Sampling and Analysis

At the end of the trial (42-day-old broilers), two birds from each replicate were fasted overnight and blood samples (4 ml) were collected from the wing web vein of each bird. The blood samples were then divided into two portions: one for haematological analysis (collected in EDTA tubes) and the other for serum biochemical analysis (collected in dry centrifuged glass tubes). The blood

samples were properly labeled, stored in a sample transport box with ice packs (2-8 °C), and conveyed to the laboratory for analysis. Haematological parameters (Packed Cell Volume, Haemoglobin Concentration, Red Blood Cell, White Blood Cell, Mean Cell Volume (MCV), Mean Cells Haemoglobin (MCH), and Mean Cell Haemoglobin Concentrations (MCHC)) were determined using standard procedures (Jain, 1993). Serum biochemical parameters were measured using various methods: glucose (hexokinase method) (Sonagra *et al.*, 2024), total protein and albumin (biuret method) (Bianchi-Bosisio, 2005), globulin (calculated by total protein minus albumin), urea (urease method) (Fawcett and Scott, 1960), liver functions (ALT, AST, and ALP, optimized IFCC methods, (Schumann and Klauke, 2003), cholesterol profile (RANDOX laboratories kits, Model BT294QY), and serum electrolytes (Na⁺, K⁺, and Cl⁻, Caretium Electrolyte Analyzer, Model XI-921C).

Intestinal Bacterial load

Two birds (42-day-old broilers) from each replicate were humanely euthanized by cervical dislocation, and then eviscerated. The duodenum was removed, and the content was collected and serially diluted. The duodenal content was cultured on MacConkey agar plates and incubated at 37 °C for 24hrs. Lactose and non-lactose fermenters colonies were counted. Microbiological analysis was done by homogenizing 1 g of each sample into 9 ml of normal saline, followed by ten-fold serial dilution. Enumeration and isolation of total viable heterotrophic bacteria, *Coliform* bacteria, *Staphylococcus species*, and *Salmonella Shigella* were carried out as enunciated by Oso *et al.* (2017).

Statistical Analysis

Data were subjected to one-way Analysis of Variance using General Linear Model procedure of Minitab® software version 21.1.0. Statistical analyses were conducted at a significance level of 0.05, and significant differences among treatment means were separated using Tukey's HSD test.

Results

Phytochemical screening

The phytochemical screening of COLE, as presented in Table 2, yielded a diverse array of bioactive compounds, including Saponins (42.88 mg/100g), Glycosides (56.90 mg/100g), Alkaloids (16.72 mg/100g), Tannins (68.26 mg/100g), Steroids (31.86 mg/100g), Polyphenols (72.87 mg/100g), Terpenoids (69.11 mg/100g), and Flavonoids (46.59 mg/100g).

Haematological Parameters

Table 3 shows the haematological parameters of broiler chickens administered varying levels of COLE. The experimental treatments had no significant ($p > 0.05$) influence on all parameters measured.

Serum Biochemical Parameters

The serum biochemical parameters of broiler chickens administered varying levels of COLE are presented in Table 4. Results showed that most parameters measured, except LDL, were not significantly ($p > 0.05$) affected. The LDL levels significantly ($p = 0.016$) ranged from 31.10 g/dl in birds administered 1.5 ml COLE/litre of water to 56.95 g/dl in their counterparts in the control.

Intestinal Bacteria Count

Intestinal bacterial counts of broiler chickens administered varying levels of *Chromolaena odorata* leaf extract are illustrated in Figure 1. Total Viable Heterotrophic Bacteria Count (TVHBC),

Total Viable *Salmonella Shigella* Bacteria Count (TVSSBC), and Total Viable *Coliform* Bacteria Count (TVCBC) were lowest in birds administered 1.5 ml of COLE per litre of water and highest in birds from the control. However, Total Viable *Staphylococcus Species* Bacterial Counts (TVSSBC) was lowest in birds administered 1.0 ml of COLE per litre of water and highest in birds with access to 1.5 ml of COLE per litre of water.

Discussion

Phytochemical analysis of *Chromolaena odorata* leaf extract

Understanding the impact of *Chromolaena odorata* extract on immune response and gut microbiota in broiler chickens is fundamental to the development of strategies that utilize alternatives to antibiotics in poultry production. The detection of saponins, glycosides, alkaloids, tannins, polyphenols, steroids, terpenoids, and flavonoids in COLE suggests that the *C. odorata* plant possesses potential bioactive substances of therapeutic and prophylactic importance for treating various illnesses. This is consistent with the protective function and physical and chemical characteristics of the *C. odorata* plant, as previously reported by Oklo *et al.* (2019). Our findings confirm earlier studies (Anyasor *et al.*, 2011; King *et al.*, 2017) that observed the presence of compounds such as alkaloids, saponins, glycosides, flavonoids, tannins, phlobatannins, steroids, and terpenoids in different *C. odorata* plant parts. Our results align with those of Igboh *et al.* (2009), who previously reported a high saponins content in *C. odorata*, as we also found a substantial amount of saponins in COLE. However, Anyadoh-Nwadike *et al.* (2019) reported the absence of terpenoids in *C. odorata* extract, which is contradicted with our findings. Notably, the doses used in this study were non-

toxic and safe for both poultry and human consumption, as they fell below the lethal dose of 2000 mg/kg reported by Elebiyo *et al.* (2023) for crude *C. odorata* extract.

Haematological parameters of broiler chickens administered varying levels of *Chromolaena odorata* leaf extract

Common hematological indices are useful for assessing the physiological and health status of animals (Khan and Zafar, 2005; Etim *et al.*, 2014). The PCV, Hb, and MCH are important indices for evaluating circulatory erythrocytes, anemia diagnosis (Peters *et al.*, 2011), and bone marrow's capability for red blood cell production (Olafadehan *et al.*, 2020). In this study, the statistical similarities in hematological parameters across COLE-treated groups suggest that *C. odorata* extract had no detrimental effect on the health status of experimental birds. *Chromolaena odorata* leaves have anti-anemic properties, as reported by Adedapo *et al.* (2016). This was evident in this study, as RBC and PCV values were slightly higher than the ranges reported by Adeyolanu *et al.* (2019), while MCH and MCHC fell within the normal physiological range reported by Jiwuba *et al.* (2017) for healthy broilers. This indicates the absence of anemia in birds supplemented with *C. odorata* extract. Our findings are consistent with previous reports by Qamar *et al.* (2015) and Jiwuba *et al.* (2017), who found that herbal medicine supplementation did not influence blood hemoglobin levels, packed cell volume, and white blood cell count in broiler chickens. However, Jiwuba *et al.* (2017) detected significantly higher WBC in birds fed *C. odorata* leaf-meal-based diets, which slightly deviates from our findings. This variation may be due to differences in the mode of administration and dosages of *C. odorata* leaf extract used.

Serum Biochemical parameters of broiler chickens administered varying levels of *Chromolaena odorata* leaf extract

The assessment of serum parameters in poultry species provides valuable data about their health status and overall performance (Islam *et al.*, 2019). This study demonstrated that COLE supplementation had no negative influence on metabolic responses in broiler chickens. Total protein values obtained fell within the normal range (5.45-6.82 g/dl) reported by Ragab *et al.* (2012) and (5.00-6.64 g/dl) reported by Jiwuba *et al.* (2017). The non-significance of serum glucose levels indicates that COLE supplementation did not affect glucose intake and metabolism by the cells in experimental birds. However, the non-significance in albumin and globulin levels in this study contradicts reports by Ekenyem *et al.* (2009) and Jiwuba *et al.* (2017), where significant differences were observed in total protein, albumin, and globulin of layers and broilers, respectively. Low-density lipoprotein, commonly referred to as 'bad' cholesterol, is a significant contributor to sudden death syndrome in fast-growing animal species, including broilers, as elevated levels can lead to plaque formation, blood vessel blockage or narrowing, and impeded blood circulation (Johnson *et al.*, 2023). Therefore, significantly reduced serum LDL in COLE-treated groups confirms the hypoglycemic and hypolipidemic properties of *Chromolaena odorata* leaf extract, earlier reported by Adedapo *et al.* (2016). This is consistent with the findings of Uhegbu *et al.* (2016), where reductions in cholesterol levels were observed in rats supplemented with *Chromolaena odorata* leaf extract. Similarly, Ikewuchi *et al.* (2011) and Jiwuba *et al.* (2017) reported significant reductions in serum cholesterol levels in birds fed *Chromolaena odorata* leaf-

meal-based diets. In contrast, Ekenyem *et al.* (2009) observed no significant effect of *Chromolaena odorata* leaf meal on cholesterol concentration in laying birds. Thus, conflicting results may be due to differences in the mode of administration, dosages of *C. odorata* leaf extracts used, and the species or type of poultry. Varying COLE levels did not pose a detrimental threat to osmotic balance, as serum Na⁺ and K⁺ levels observed fell within the reference values (135–145 mEq/L for Na⁺ and 3.5–5.0 mEq/L for K⁺) for chickens (Jain, 1993). Furthermore, the similarities in liver enzymes between COLE-treated groups and the control indicate that *C. odorata* leaves extract did not adversely alter liver function and integrity, as these enzymes (AST and ALT) are hepatotoxicity biomarkers (Mitruka and Rawsley, 1977; Amer *et al.*, 2022).

Intestinal bacterial count of broiler chickens administered varying levels of *Chromolaena odorata* leaf extract

The gut microbial population in poultry plays a significant role in digestive processes and health maintenance (Wood *et al.*, 1999). Therefore, the results of this study suggest that *C. odorata* leaf extract possess the ability to mitigate pathogenic bacteria in the gut of broiler chickens, as *Salmonella-Shigella*, *Coliform*, *Staphylococcus*, and Heterotrophic bacteria populations were reduced in COLE-based treatments. This may be due to the presence of alkaloids, saponins, and flavonoids, which can modulate the composition of gut microbiota. According to Peng *et al.* (2014), phytochemicals like isoflavones, saponins, alkaloids, and lignocellulose effectively modulate gut microflora, improve immune function, and stimulate both absorption and digestion of nutrients in poultry and livestock. Our findings were consistent with the work of Jiwuba *et al.* (2017), where the inclusion of *C. odorata* leaf

meal in broiler diets controlled pathogenic microorganisms and improved the proliferation of beneficial microflora in the gut. Other studies (Awaad *et al.*, 2014; Wlodarska *et al.*, 2015; Zeng *et al.*, 2015; Stevanovic *et al.*, 2018) also reported that plant extract supplementation improved chicken growth via modifications of intestinal microbiota (reducing pathogenic and/or increasing the abundance of beneficial microorganisms).

Conclusion

This study shows that COLE supplementation at 1.5 ml/L significantly reduced serum LDL levels in broiler chickens, indicating a potential benefit for

cardiovascular health. Additionally, COLE's bioactive compounds exhibit antimicrobial properties against pathogenic intestinal bacteria. Therefore, these findings suggest that COLE can be a valuable natural additive in broiler production, supporting the development of sustainable and antibiotic-free practices.

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Table 1: Composition (%) of basal diet

Ingredients	Quantity (%)
Maize	58.60
Soybean Meal (48% CP)	36.10
Vegetable Oil	1.65
Bone Meal	1.75
Limestone	1.00
Salt (NaCl)	0.35
Lysine	0.10
DL-Methionine	0.20
*Vitamin-Mineral Premix	0.25
Total	100.0
Calculated Composition	
Metabolizable Energy (kCal.kg ⁻¹)	3036.26
Crude Protein (%)	20.49
Crude Fiber (%)	2.98
Available p (%)	0.38
Total Calcium (%)	0.88
Ether Extract (%)	3.43
Total Lysine	1.29
Total Methionine	0.52

*Vitamins and minerals premix contains vitamin A, 10,000,000 IU; vitamin D3, 2,000,000 IU; vitamin E, 12,500 IU; vitamin K, 1.30 g; vitamin B1, 1.30 g; vitamin B2, 4 g; D-calcium pantothenate, 1.3 g; vitamin B6, 1.3 g; vitamin B12, 0.01 g; nicotinic acid, 15 g; folic acid, 0.05 g; biotin, 0.02 g; copper, 0.05 g; cobalt, 0.20; iron, 25 g; iodine, 0.06 g; manganese, 48 g; selenium, 0.10 g; zinc, 45 g; choline chloride, 200 g; butylated hydroxytoluene, 50 g

Table 2: Phytochemical analysis of *Chromolaena odorata* leaf extract

Bioactive compound	Concentration (mg/100g)
Saponins	42.88
Glycosides	56.90
Alkaloids	16.72
Tannins	68.26
Steroids	31.86
Polyphenols	72.87
Terpenoids	69.11
Flavonoids	46.59

Table 3: Haematological parameters of broiler chickens administered varying levels of *Chromolaena odorata* leaf extract

Parameters	COLE levels (ml/litre of water)					SEM	P value
	0	0.5	1.0	1.5	2.0		
Packed cell volume (%)	32.00	35.50	30.50	37.00	38.50	2.99	0.393
Haemoglobin concentration (g/dl)	11.70	12.40	11.50	12.45	12.30	0.96	0.163
Red Blood Cell ($\times 10^6 \mu\text{l}$)	3.45	3.73	3.66	3.52	3.93	0.34	0.865
White blood cell ($\times 10^3 \mu\text{l}$)	32.60	35.80	36.10	35.30	34.90	3.75	0.653
MCV (fL)	92.75	94.92	83.33	105.11	97.96	13.80	0.383
MCH (pg)	33.90	33.24	31.42	35.37	31.30	1.61	0.745
MCHC (g/dl)	36.52	34.93	37.70	33.65	31.95	1.19	0.163

MCV=Mean Corpuscular Volume, MCH=Mean Corpuscular Haemoglobin, MCHC=Mean Corpuscular Haemoglobin Concentration

Table 4: Serum Biochemical parameters of broiler chickens administered varying levels of *Chromolaena odorata* leaf extract

Parameters	COLE levels (ml/litre of water)					SEM	P value
	0	0.5	1.0	1.5	2.0		
Glucose (mg/dl)	234.50	221.00	235.00	248.50	214.00	10.50	0.297
Total protein (g/dl)	6.06	6.28	6.38	5.89	6.91	0.76	0.889
Albumin (g/dl)	3.38	3.27	3.22	2.93	3.70	0.36	0.682
Globulin (g/dl)	2.68	3.01	3.16	2.96	3.21	0.51	0.948
Urea (mg/dl)	50.25	39.35	32.40	41.35	43.80	6.92	0.525
ALT (U/L)	71.30	68.90	70.90	83.20	99.00	28.30	0.927
AST (U/L)	107.10	123.60	130.20	114.70	143.30	30.50	0.922
ALP (U/L)	59.10	82.80	84.20	64.10	87.20	20.30	0.802
Total Cholesterol (mg/dl)	120.75	99.10	95.25	87.80	98.00	5.88	0.068
Triglycerides (mg/dl)	91.00	74.25	71.25	68.75	71.00	4.40	0.074
HDL (mg/dl)	45.60	44.40	45.35	42.95	46.95	3.89	0.957
LDL(mg/dl)	56.95 ^a	39.85 ^{ab}	35.65 ^b	31.10 ^b	36.85 ^b	3.26	0.016
VLDL (mg/dl)	18.200	14.850	14.250	13.750	14.200	0.879	0.074
Na ⁺ (mmol/L)	135.70	134.85	134.20	135.75	135.20	0.74	0.593
K ⁺ (mmol/L)	3.82	3.73	3.57	3.73	3.72	0.15	0.836
Cl ⁻ (mmol/L)	95.45	90.50	92.60	103.90	94.10	6.22	0.633

HDL = High-density-lipoprotein, LDL = Low-density-lipoprotein, VLDL = Very-low-density-lipoprotein, AST = Aspartate transaminase, ALT = Alanine transaminase, ALP = Alkaline Phosphatase

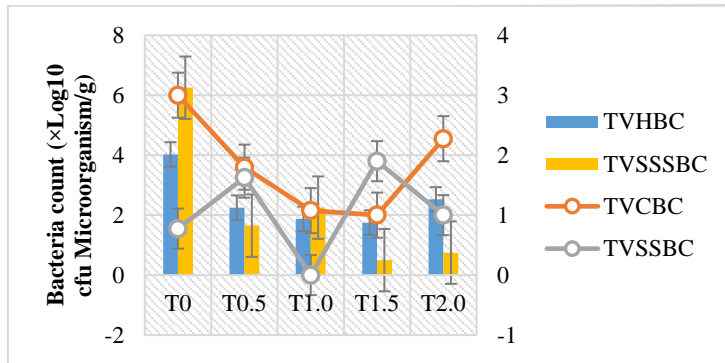


Figure 1: Intestinal bacterial count of broiler chickens administered varying levels of *Chromolaena odorata* leaf extract

TVHBC: Total Viable Heterotrophic Bacteria Counts
 TVSSBC: Total Viable *Salmonella Shigella Species* Bacterial Count
 TVCBC: Total Viable *Coliform* Bacteria Counts
 TVSSBC: Total Viable *Staphylococcus Species* Bacterial Counts