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Growth Performance, Blood Profile, Liver Histology, Faecal Microbial Type and Count of Broiler Chicken Fed Diets Containing Fenugreek and Ginger Powder Blend

Okanlawon Eden Olusegun [1], Rafiu Tirimisiyu Adewale [2*], Hammed Opeyemi Oladipupo [3], Bamidele Rebecca Oluseyi [4], Adedeji Muiz Adebayo [5], Ohunmakin Olamide Tawa [6], Adegbile Adesola Semiloore [7], Salawu Taofiqah Kehinde [8]
[1-8] Department of Animal Production and Health, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

* Corresponding Author: **Rafiu Tirimisiyu Adewale**

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Abstract
A total of (160) unsexed day-old broiler chicks (Ross 308) were utilized to determine the impact of a combination of fenugreek and ginger powder on the performance, organs, carcass, and blood profile of broiler chickens**.** They were randomly allotted to four dietary treatments group which include T1- control diet, T2-2g/kg, T3-4g/kg and T4-6g/kg of 40 bird per treatment and (4) replicates of 10 birds and were arranged in a completely randomized design. Growth performance, organ, haematological, serum biochemistry, and carcass characteristics data were collected and subjected to ANOVA analysis. Significant ($p<0.05$) difference was recorded on growth performance, carcass, organ, blood profile. Higher final weight gain, total weight gain and average daily weight gain (2545.00g), (2509.70g) and (59.75g) were noticed with broiler chicken fed diet containing 4g/kg of fenugreek and ginger powder while the least value (2432.50g), (2397.10g) and (57.07g) with chicken fed diet containing 2g/kg of the blend respectively. Highest ($P<0.05$) pack cell volume (27.50%) was captured with broiler bird fed diet containing (4g/kg) of blend while broiler fed diet containing (2g/kg) had the least (24.00%).Highest ($P<0.05$) red blood cell (2.27×10^{12}/L) was recorded with broiler bird fed diet containing (6g/kg) of the test ingredient while broiler fed diet containing the blend (2g/kg) had the least ($1.92x10^{12}$/L). Highest ($P<0.05$) white blood cell ($141.5X10^{9}$/L) was recorded with broiler bird fed diet with the inclusion of (4g/kg) of the blend while broiler fed diet containing (2g/kg) had the least ($124.50X10^{9}$/L). Higher ($P<0.05$) cholesterol (3.40 mmol/l) was recorded with brother chicken fed control diet, while those fed diet that containing 2g/kg blend had the lowest (2.55 mmol/l). It was concluded that the use of 4g/kg of fenugreek and ginger powder in the diet improves growth performance, carcass yield, organ and health status and reduction in cost of production in broiler chicken enterprise.

Keywords: Fenugreek, Ginger, Broiler Chicken, Performance, Haemaotology and Serum

Introduction

Broiler chickens have a high feed conversion ratio and a quick development rate, producing them is one of the cheapest and simplest ways to close the gap between supply and demand for animal protein (Abdulsalam *et al.*, 2023) [2]. Furthermore, broilers have the relative advantages of easy management, a rapid return on capital investment, and widespread acceptance of their meat for human consumption when compared to their rapid growth rate. Poultry rations have included antibiotics as feed additives to improve feed efficiency and growth performance (Khan *et al*. 2012) [30]. Other compounds, such as organic acids, probiotics, prebiotics, natural herbs and spices (Alshelmani *et al*. 2021) [10], have now received considerable attention primarily because in 2006, the European Union prohibited the utilization of antibiotics as additives in animal diets (Israr *et al*. 2021) [25].

Phytobiotics, also known as NGPs, are advanced feed additives that support better immune function, growth, and digestive tract health (Panda and Raju, 2006) [43]. Feeding diets containing phytobiotic may prevent pathogenic microbes from growing and colonizing the digestive tract, promoting the ecology and balance of the gut microbiota and potentially treating a variety of oxidative stress-related illnesses (Harris *et al.*, 2001) [21]. Meanwhile, phytobiotics are limited in action due to plant species, type of soil, climate, location, cultivation, and storage conditions among others and may affect the composition of the plant and potency of the active ingredients (Vispute, 2019) [62]. Similarly, majority of the herbals are stable, but their various constituents are photo or thermo labile (Pullaiah, 2011) [46]. These therefore suggest the use of more than one combination of these natural growth promoters.

Phytogenics and plant derivative products attract a lot of attention as safe alternatives for AGPs in poultry. Recently research has shifted away from chemical-based feed additives to the use of phytogenics that exists naturally in the environment (Alloui *et al*., 2014) [8]. Phytogenic feed supplements can function as a substitute for antibiotics. Studies have indicated that herbal plants and their components may be beneficial in enhancing carcass yield and feed efficiency (Abudabos *et al*. 2016) [3]. The essential chemical compounds found in spices, herbs and essential oils may enhance blood circulation, stimulate digestive secretions, combat pathogens and bolster the immune system of birds (Ali *et al*. 2019) [6].

Ginger is rhizome of the plant known as *Zingiber officinale*. It is eaten as a spice, medicine, or delicacy and is native to southern Asia. Ginger's main active ingredients are gingerol, gingerdiol, and gingerdione; these substances can increase antioxidative activity, influence microbial activity, and stimulate digestive enzymes (Dieumou *et al*., 2009). When incorporated into diets, its supplementation enhances blood serum and antioxidant levels while also improving feed palatability. Additionally, it improves the way nutrients are utilized, increases appetite, increases gastric juice flow, and adds piquancy to bland food. Ginger is thus a potential rhizome with a wide range of medicinal effects that has been used in broiler and layer production in various forms, doses, and durations. The deficiency of ginger includes anti-platelet effect, and this capebale of preventing blood cloting.

A member of the Fabaceae family, fenugreek (*Trigonella foenum-graecum*) is an annual leguminous plant. A semiarid crop that is grown all across the world, it was previously acknowledged as a food and medicine homology in China. Fatty acids, minerals, dietary fiber, crude protein, and amino acids are all abundant in fenugreek seeds (Nasim *et al*., 2016). Fenugreek seed extract (FSE) is mostly composed of alkaloids, polysaccharides, flavones, and steroidal saponins. Studies have demonstrated the numerous health benefits of FSE, including its hypoglycemic (Lu *et al*., 2015) [33], hypocholesterolemic (Ramulu *et al*., 2011) [47], anti-oxidation antibacterial (Mozhdeh *et al*., 2019) [38], anti-inflammatory (Ahmed *et al*., 2017) [5], immuno-stimulating activities and improve growth performance (Covarrubias *et al*., 2018) [15].

The effects of fenugreek seed extract on the neurological system and intestinal flora increased the appetite of broilers. Three grams per kilogram of FSE could boost broiler feed intake, accelerate feed conversion, and raise live weight (Alloui *et al*., 2012) [9]. When broilers are fed fenugreek seed, their growth performance has been shown to improve. Fenugreek seed extract used at a rate of 30 ml/L aqueous extract speeds up broiler muscle growth, according to Khan *et al.* (2011) [29]. Toaha *et al*. (2016) [54] found that, in comparison to diets supplied with antibiotic growth boosters, diets supplemented with fenugreek enhanced productive performance, decreased feed costs, and boosted profit. This study is conducted in other to combine the two phytobiotic in order to form a complimentary action to improve all performance of broiler chicken and this necessitated this study.

Materials and Methods

Experimental Site

The experiment was carried out at the Poultry Unit of Teaching and Research Farm, Ladoke Akintola University of Technology Ogbomoso, Oyo State Nigeria. The area is in derived savannah zone of Nigeria. It lies on longitude 4.5^0 east of greenish meridian and latitude 8.5^0 North-East towards Ibadan the capital of Oyo State. The mean annual rainfall is 1247mm while relative humidity is between 75% and 95%. It is situated at about 300-600 matters above the sea level with a mean annual temperature of 27^0C (Ayinla and Odetoye, 2015) [12].

Source and preparation of test ingredients

The test ingredient fenugreek seed (*Trigonella foenum-graecum L*.) and ginger were purchased from an open market in Ogbomosho, Oyo State. Ginger only was washed using clean water to remove the dirt since they are rhizome. It was poured into clean basket to drain off water and was sliced into flakes in order to increase the surface area to aid drying. Thereafter, it was air dried until the weight remained constant. Ginger was reduced using a mortar and pestle to a lentil-size portion, then both the fenugreek seed and ginger were grounded using an electric blender to a fine powder. After that, it was sieved and kept until use in an airtight container as describe by (Okanlawon, *et al.*, 2020a) [39].

Experimental Birds and Management

One hundred and sixty (160) unsexed day-old Ross 308 chicks were purchased from a reputable hatchery and was used for the experiment. The birds were randomly allotted to four (4) dietary treatments of 40 birds per treatment with four (4) replicates each in a complete randomized design (CRD). Diets 1 serve as control while 2,3 and 4 will be supplemented with ratio 1:1 combination of fenugreek and ginger powder blend at 2g, 4g, and 6g per kilogram of feed respectively. T1 (control), T2 (1gFP and 1gGP), T3 (2gFP and 2gGP) and T4 (3g FP and 3g GP). All routine management practice was strictly adhered to; however, Feeds and water were given *ad-libitum* throughout the experimental period. The experiment lasted for six (6) weeks.

Table 1: Gross Composition of Experimental Diets for Broiler Chicken

	Starter Phase				Finisher Phase			
Diets	1	2	3	4	1	2	3	4
Inclusion Levels of Fenugreek Seed (g/kg)	0	2	4	6	0	2	4	6
Nutrient Composition of feed								
Crude Protein (%)		22.50				18.00		
Crude Fat (%)		4.50				5.00		
Crude fibre (%)		4.00				5.00		
Calcium (%)		1.00				0.85		
Phosphorus (%)		0.50				0.37		
Metabolization energy (kCal)		3000				3200		
Lysine (%)		1.35				1.10		
Methionine (%)		0.65				0.50		
Salt (%)		0.30				0.30		

Data Collection
Growth Performance
Weight Gain

Total weight gain (g) = Final body weight (g)−Initial body weight (g)

Feed Intake
Feed intake was recorded on weekly basis for each replicate per treatment. Leftover feed was subtracted from the total feed supplied to determine feed intake. Using the weighing scale of (Camry Q.C PASSED NO: 1, Model: EK 3250, Max: 5kg/ 11Lb, d=1g/0.050$_Z$).
feed intake=feed offer−left over feed numbers of birds

Feed Conversion Ratio (FCR)
This was calculated as ratio of feed intake per body weight gain

$$FCR = \frac{\text{Feed intake}}{\text{Weight gain}}$$

Organs and Carcass Characteristics
At the end of experimental period (6 weeks), four birds were randomly selected, were starved of feed for 12 hours with the presence of abundant water and slaughtered by severing the jugular veins. The birds were bled; defeathered after which the visceral organs such as liver, intestine, pancreas, spleen, kidney, proventriculus, and hearts were removed. The bled, defeathered and eviscerated weights were evaluated accordingly. The head and shanks were removed to determine the carcass weight.

$$\text{Proportional weight of the organs} = \frac{\text{Weight of the organ}}{\text{Live weight}} \times 100$$

The carcass was cut into various parts (thigh, breast, back, shank, drumstick, wings and head) and their weights were expressed in percentage relative to the carcass weight. The weights of the organs were also be expressed in relative values. The following calculations were evaluated:

$$\text{Relative cut parts weight} = \frac{\text{Weight of the cut}}{\text{Carcass weight}} \times 100$$

Blood Collection Analysis
Hematological Parameters
Four birds were randomly selected from each treatment, about 2.5 mL of blood were collected in tubes containing EDTA anticoagulant, The blood was slowly expressed into EDTA tubes to reduce the risk of haemolysis after removing the needles from syringes (Haen, 1995) and analyzed to determine the parameters (haemoglobin concentration, packed cell volume, red blood cells count, total white blood cells count, differential white blood cell count, platelets count, and red cell) indices as describe by Iranloye *et al.* (2002) and Venkatesan *et al.* (2006) [24, 61].
Serum parameters include, total protein was obtained by biuret method in the assay as described by Kohn and Allen (1995) [31]. The globulin concentration was obtained by subtracting albumin from the total protein. Albumin was determined using Bromocresol Green (BCG) method as described by Peter *et al.* (1982) [45]. Aspartate transferase (AST) activities were determined using spectrophotometric methods as described by Rej and Hoder (1983) [48]. Alanine transferase (ALT) activities were determined using spectrophotometric methods as described by Rej and Hoder (1983) [48].

Liver Histology
At the end of the feeding trial, samples of small cut from the liver of 2 randomly selected chickens per treatment were submerged inside 10% formalin, and slide preparation (Fixation of each tissue, Grossing, Processing, Infiltration and Embedding, follow by Sectioning using microtone, staining using eosin negrosin stains before mounting on the permanent slide with cover slide), was done for liver in order to observed the slide under AmScope USB enabled microscope at x100 magnification for histopathological alteration of liver.

Data Analysis
Data collected were analysed using ANOVA as contained in SAS (2002). Significant means were separated using Duncan Multiple Range Test of same software package.

Results
Table 2 shows the result of growth performance of broiler chickens fed fenugreek and ginger blend. Significant ($P<0.05$) difference was recorded on final weight, total weight, average daily feed weight gain, average daily feed intake, feed conversion ratio, cost per average daily feed intake and cost per kg body weight gain. Broiler chicken fed a diet containing 4g/kg of fenugreek and ginger powder showed the highest final weight gain, total weight gain, and average daily weight gain (2545.00g), 2509.70g, and 59.75g),

while chicken fed a diet containing 2g/kg of the blend showed the lowest values (2432.50g), 2397.10g, and 57.07g). The average daily feed intake and feed conversion ratio of broiler chickens fed a control diet were higher (P<0.05) at 106.88g and 1.83g, respectively, whilst the lowest were reported by those fed a diet supplemented with 4g/kg (95.71g and 1.60g). In terms of cost per average daily feed intake and feed conversion ratio, Higher (P<0.05) cost per average daily feed intake and cost per kg body weight gain value (N126.35) and (N2169.15) was noted with broiler chicken fed diet that contains 6g/kg of fenugreek and ginger powder blend while the least (N112.17) and (N1877.23) were recorded at T4 (4g/kg). No significant (P>0.05) difference was recorded on cost per kilogram diets.

Table 2: Effect of Fenugreek and ginger powder blend on growth performance of broiler chicken

Parameter	T1(0g/kg)	T2(2g/kg)	T3(4g/kg)	T4(6g/kg)	SEM
Initial weight (g)	35.5	35.40	35.50	35.50	0.21
Final weight(g)	2485.00^{ab}	2432.50^{b}	2545.00^{a}	2482.50^{ab}	17.65
Total weight gain (g)	2449.50^{ab}	2397.10^{b}	2509.70^{a}	2447.00^{ab}	17.68
ADWG (g)	58.32^{ab}	57.07^{a}	59.75^{a}	58.26^{ab}	0.42
ADFI (g)	106.88^{a}	100.04^{b}	95.71^{c}	103.81^{b}	1.25
FCR	1.83^{a}	1.75^{b}	1.60^{c}	1.79^{b}	0.03
Cost per/kg diet (#)	1100.00	1136.00	1172.00	1208.00	10.39
Cost per/ADFI (#)	117.57^{b}	113.65^{bc}	112.17^{c}	126.35^{a}	1.57
Cost per/kg body weight (#/g)	2017.08^{b}	1991.79^{b}	1877.23^{c}	2169.15^{a}	29.52

^{abc}: means in the same row by factor with different superscripts differ significantly (p<0.05)
ADWG- Average Daily Weight Gain, ADFI-Average Daily Feed Intake, FCR-Feed conversion ratio, SEM: Standard Error of Mean,

The impact of a combination of fenugreek and ginger powder on the carcass and relative cut-up portions of broiler chicken is shown in Table 3. Live weight, bled weight, defeathered weight, carcass weight, eviscerated weight, and dressed % all showed significant (p<0.05) differences. Broiler chicken birds fed diets containing 4g/kg of fenugreek and ginger powder blend had the highest (p<0.05) live weight, bled weight, defeathered weight, carcass weight, eviscerated weight, and dressed percentage (2648.50g) (2594.50g) (2506.00g) (2284.00g) (2024.00g) and (78.47%), respectively, while broiler fed diets containing 2g/kg had the lowest (2433.00g) (2343.50g) (2270.50g) (2094.50g) (1793.00g) and (73.69%), respectively. Shank weight was highest (p<0.05) in broiler fed diet containing 2g/kg of fenugreek and ginger powder blend (3.85%), while the lowest value (3.26%) was found in broiler fed diet containing 6g/kg.

Table 3: Effect of Fenugreek and ginger powder blend on carcass and relative cut-up parts of broiler chicken

Parameter	T1(0g/kg)	T2(2g/kg)	T3(4g/kg)	T4(6g/kg)	SEM
Live weight (g)	2590.00^{a}	2433.00^{b}	2648.50^{a}	2510.50^{ab}	20.77
Bled weight(g)	2514.50^{a}	2343.50^{b}	2594.50^{a}	2448.00^{a}	21.52
Defeathered weight (g)	2435.50^{a}	2270.50^{b}	2506.00^{a}	2363.00^{ab}	20.18
Carcass weight (g)	2269.00^{a}	2094.50^{b}	2284.00^{a}	2095.50^{b}	25.37
Eviscerated weight (g)	2001.50^{a}	1793.00^{c}	2024.00^{a}	1869.00^{bc}	23.95
Dressing percentage (%)	77.30^{a}	73.69^{b}	78.47^{a}	74.45^{b}	0.50
Cut-Part (%)					
Head	1.97	2.12	1.82	1.99	0.05
Neck	3.65	4.10	3.68	3.90	0.10
Back	14.81	14.86	14.85	14.33	0.26
Breast	29.75	29.22	30.32	31.35	0.55
Thigh	12.44	12.78	12.93	12.19	0.24
Drum stick	9.86	10.45	9.94	9.31	0.21
Wing	7.51	7.20	7.67	7.19	0.09
Shank	3.66^{ab}	3.85^{a}	3.29^{b}	3.26^{b}	0.01

^{ab} = Means on the same row bearing different superscript differed significantly (p<0.05)
SEM: Standard Error of Mean,

Table 4 illustrates the impact of fenugreek and ginger powder blend on organ characteristics of broiler chicken. Significant (P<0.05) difference was obtained on kidney, liver and Proventriculus. Broiler chickens fed the control food had the highest kidney weight (P<0.05) at 0.49%, while those fed the diet containing 2g/kg mix had the lowest kidney weight (0.33%). The liver weight of broiler chickens fed a diet containing 2g/kg was greater (P<0.05) at 1.98%, whereas the weight of those fed a control diet was the lowest at 1.69%. The control diets fed to broiler chicken had the higher (P<0.5) proventriculus (0.65%), while those fed with the diets containing 6g/kg had the least value (0.39%). There was no significant (P>0.05) influence of the blend on gizzard, lungs, spleen and heart. The outcomes of the various treatment groups had a substantial (p<0.05) impact on the relative organs (liver, kidney, and venticulus) of broiler chicken.

Table 4: Effect of Fenugreek and ginger powder blend on organ characteristics of broiler chicken

Parameter (%)	T1(0g/kg)	T2(2g/kg)	T3(4g/kg)	T4(6g/kg)	SEM
Kidney	0.49[a]	0.33[b]	0.34[b]	0.38[ab]	0.02
Liver	1.69[b]	1.98[a]	1.91[ab]	1.81[ab]	0.05
Whole gizzard	1.64	1.27	1.56	1.63	0.06
Empty gizzard	1.29	1.07	1.19	1.28	0.04
Lungs	0.59	0.42	0.51	0.53	0.03
Proventriculus	0.65[a]	0.44[b]	0.46[b]	0.39[b]	0.03
Spleen	0.09	0.09	0.10	0.10	0.004
Heart	0.46	0.41	0.37	0.32	0.02

[ab] = Means on the same row bearing different superscript differed significantly (p<0.05)
SEM: Standard Error of Mean.

Table 5 shows the hematological parameters of broiler chicken fed diet containing fenugreek and ginger power. Red blood cells, white blood cells, and packed cell volume are all significantly (p<0.5) impacted by the food intervention. The broiler chick fed a diet containing 4g/kg of fenugreek and ginger powder had the highest (P<0.05) pack cell volume (27.50%), while the broiler fed a diet containing 2g/kg had the lowest (24.00%). Highest (P<0.05) red blood cell (2.27×10^{12}/L) was recorded with broiler bird fed diet containing (6g/kg) of the test ingredient while broiler fed diet containing (2g/kg) had the least ($1.92x10^{12}$/L). White blood cell counts were highest (P<0.05) in broiler birds fed a meal containing a blend of fenugreek and ginger powder (4g/kg), and lowest (124.50X109/L) in broiler birds fed a diet containing 2g/kg.

Table 5: Effect of Fenugreek and ginger powder blend on hematological parameter of broiler chicken

Parameter	T1(0g/kg)	T2(2g/kg)	T3(4g/kg)	T4(6g/kg)	SEM
PCV(%)	24.50[ab]	24.00[b]	27.50[a]	26.00[ab]	0.59
RBC($\times10^{12}$/L)	2.09[ab]	1.92[b]	2.23[ab]	2.27[a]	0.06
Hb(g/l)	116.00	113.00	125.00	122.50	2.43
MCV (f/L)	120.50	124.50	124.50	121.50	1.52
MCH (pg)	55.50	59.50	56.50	54.00	0.69
MCHC (g/L)	472.50	478.00	475.50	473.50	3.01
WBC ($X10^9$/L)	126.00[b]	124.50[b]	141.50[a]	142.50[a]	2.89
Lymphocytes (%)	44.50	57.50	58.00	64.00	3.86
Neutrophils (%)	45.50	36.00	20.50	23.00	4.41
Monocytes (%)	4.50	6.00	4.50	5.50	0.51
Eosinophils (%)	6.50	7.00	8.50	7.00	0.42
Platelet($\times10^9$/L)	25.00	23.00	29.50	23.50	1.21

[ab]: means in the same row by factor with different superscripts differ significantly (p<0.05)
PCV- Pack Cell Volume, RBC- Red Blood Cell, Hb- Hemoglobin concentration, MCH- Mean Corpuscular Hemoglobin, MCV- Mean Corpuscular Volume, MCHC- Mean corpuscular hemoglobin concentration, WBC- White Blood Cell, SEM: Standard Error of Mean.

Table 6 shows the effect of fenugreek and ginger powder blend on serum biochemistry of broiler chicken. Significant (p<0.5) difference was recorded on cholesterol and low-density Lipoprotein. Broiler chickens fed the control food had higher (P<0.05) cholesterol levels (3.40 mmol/l), whereas those fed the feed containing 2g/kg mix had the lowest (2.55 mmol/l). Broiler chickens fed a control diet had higher (P<0.05) levels of low-density lipoprotein (2.50 mmol/l), whereas those fed a diet containing 4g/kg mix had the lowest (2.07 mmol/l).

Table 6: Effect of Fenugreek and ginger powder blend on Serum biochemistry of broiler chicken

Parameter	T1(0g/kg)	T2(2g/kg)	T3(4g/kg)	T4(6g/kg)	SEM
Total protein (g/dL)	30.50	30.50	29.50	27.50	1.02
Albumin (g/dL)	15.50	17.50	18.50	15.00	0.53
Globulin (g/dL)	15.00	13.00	12.50	12.50	0.63
Alkaline Phosphate (IU/L)	198.00	183.50	150.50	220.50	12.09
AST (IU/L)	122.50	120.50	131.50	113.00	3.89
ALT (IU/L)	2.50	2.00	2.50	3.00	0.25
Cholesterol (mmol/l)	3.40[a]	2.55[b]	2.70[ab]	2.95[ab]	0.20
Triglyceride (mmol/l)	0.20	0.30	0.35	0.20	0.03
HDL (mmol/l)	1.15	1.40	1.20	1.18	0.08
LDL (mmol/l)	2.50[a]	2.05[ab]	2.07[ab]	1.85[b]	0.16
Creatinine (µmol/l)	52.50	61.00	59.00	51.00	2.78

[ab]: means in the same row by factor with different superscripts differ significantly (p<0.05)
AST - Aspartate Aminotransferase, ALT - Alanine Aminotransferase, HDL - High Density Lipoprotein, LDL- Low density lipoprotein, SEM: Standard Error of Mean.

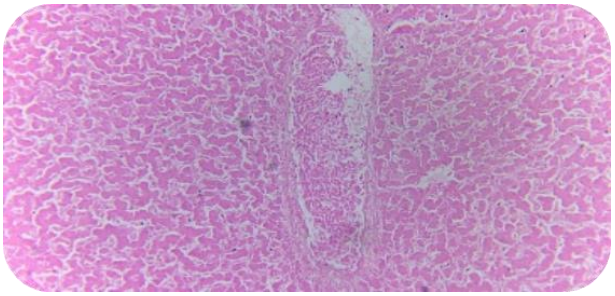
Table 7 shows the effect of fenugreek and ginger powder blend on faecal microbial type and count of broiler chicken. Significant (p<0.5) difference was recorded on Faecal microbial Count($x10^3$cfu/ml). Broiler chickens fed control diets had higher (P<0.05) Faecal microbial Count($28.50x10^3$cfu/ml) cholesterol levels (28.50

x10^{3}cfu/ml), whereas those fed diets containing g/kg blend had the lowest (24.00 x10^{3}cfu/ml).

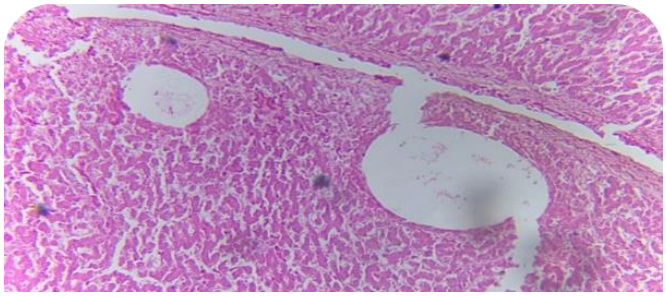
Table 7: Effect of Fenugreek and ginger powder blend on faecal microbial type and count of broiler chicken

Parameter	T1(0g/kg)	T2(2g/kg)	T3(4g/kg)	T4(6g/kg)	SEM
E. coli	0.50	1.00	1.00	0.50	0.16
Proteus	1.00	1.00	0.50	0.50	0.19
Faecal microbial Count(x10^{3}cfu/ml)	28.50^a	25.00^{ab}	24.00^b	24.50^{ab}	0.78

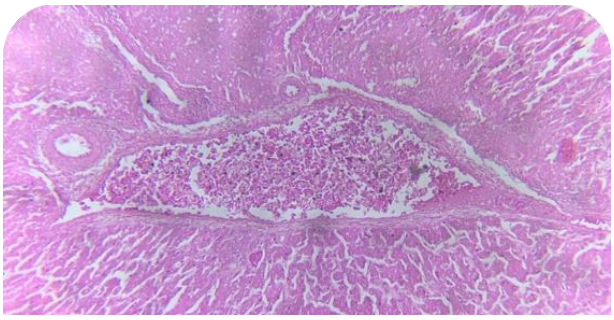
^{ab}: means in the same row by factor with different superscripts differ significantly ($p<0.05$)
SEM: Standard Error of Mean



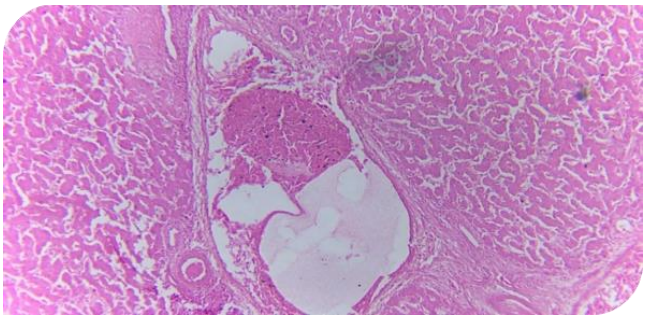
PLATES 1a



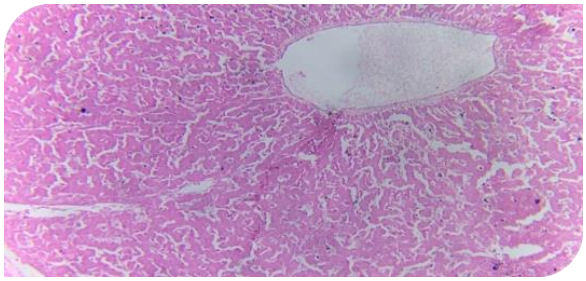
PLATES 1b



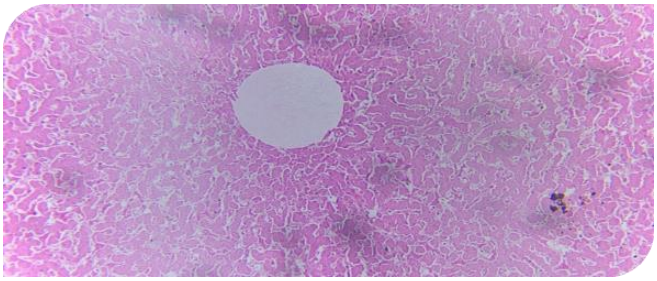
PLATES 2a



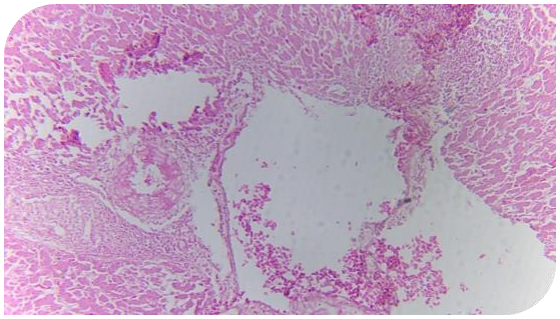
PLATES 2b



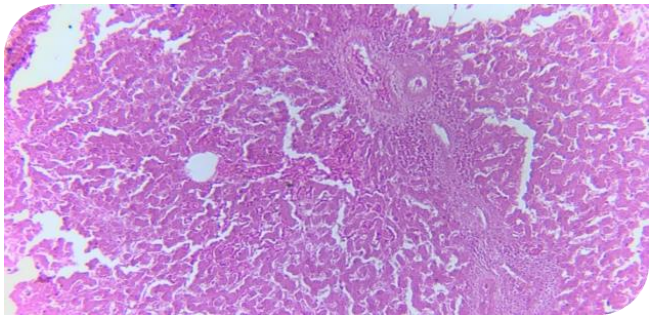
PLATES 3a



PLATES 3b



PLATES 4a



PLATES 4b

Fig 1: Shows Histological changes/responses of the liver of broiler chickens fed of diet containing graded level of fenugreek and ginger powder blend.

PLATES 1a and 1b: Photomicrograph of the liver tissue of broiler chickens fed diet without the inclusion of fenugreek and ginger blend (T1) showed Portal Vein (PV) with associated bile duct (BD) was observed to contain fat aggregates. The Sinusoids (S) appear relatively clear with some containing scattered Kupffer cells (KC). The dominant feature is the expansive liver parenchyma traversed by a significantly expanded portal tract with bile duct. The presence of a significant fibrous band dissecting the liver parenchyma is a notable finding. This suggests fibrosis, which could be part of a septal fibrosis extending from portal tracts, bridging fibrosis, or even early cirrhosis.

PLATES 2a and 2b: Photomicrograph of the liver tissues of broiler chickens fed diets containing 2g/kg of fenugreek and ginger powder blend (T2) reveals sinusoids are clearly visible throughout the parenchyma. The general architecture away from the central abnormality appears relatively preserved. Also, observed are markedly dilated bile ducts. There is an increased amount of fibrous connective tissue surrounding these dilated bile ducts, indicating some degree of fibrosis or expansion of the portal tract stroma. Scattered inflammatory cells are interspersed within expanded fibrotic stroma, especially around the ducts.

PLATES 3a and 3b: Photomicrograph of the liver tissues of broiler chickens fed diets containing 4g/kg of fenugreek and ginger powder blend (T3) reveals liver parenchyma with well-preserved lobular architecture. A prominent, clear, circular space is visible centrally, which is characteristic of a central vein. The background appears homogeneous with scattered dark specks, which are artifacts or pigment.

PLATES 4a and 4b: Photomicrograph of the liver tissues of broiler chickens fed diets containing 6g/kg of fenugreek and ginger powder blend (T4) reveals normal liver histological features. Sinusoidal capillaries are lined by flattened endothelial cells, and occasional Kupffer cells. The hepatic cords, sinusoids, central veins, and portal tracts all appear healthy and within expected morphological limits for typical liver parenchyma. There is no evidence of significant inflammation, necrosis, steatosis, fibrosis, or architectural remodeling (such as nodule formation) that would indicate liver disease.

Discussion

The weight increases of broiler chickens fed diets containing 3 g/kg of fenugreek and ginger powder were consistent with the findings of (Ademola *et al.*, 2009; Yasin *et al.*, 2020; Olayeni *et al.*, 2024) [4, 36, 40]. This increase may be attributed to the beneficial effects of fenugreek and ginger powder as spices, which are known to increase appetite and enhance the secretion of digestive enzymes that improve the digestion of protein and carbohydrates and the absorption of these digestive products, leading to better growth performance. The results also suggest the positive beneficial growth enhancing synergy effects of these herbs.

The growth and colonization of many pathogenic and non-pathogenic bacterial species in the intestine may have been inhibited by a combination of these herbs, improving the conversion of feed to meat. Furthermore, fenugreek and ginger powder has been found to contain essential fatty acids and high-quality protein (Zuk-Crolaszewska *et al.*, 2017 and Jacob *et al.*, 2018) [64, 26], and it has been reported to stimulate the villus height of the digestive system. The observed significant improvement in body weight gain and final weight gain of broiler chickens with the addition of fenugreek and ginger powder and this might be possible just because they also contain phytochemical (tannins) which help to improve feed palatability as reported by (Lawal *et al.*, 2018, and Shaimaa *et al.*, 2023) [32, 50].

Feed intake of the broiler chicken fed diets that includes fenugreek and ginger decreased, This The results is in agreement with the report of Herawati, (2010) [22] who stated that broilers fed dried supplementary red ginger meal had significantly lower feed intake than those on the control diet, This observation can be explained by the fact that fenugreek contains essential fatty acids and high-quality proteins, along with tannin and phenol steroid saponins that activate the hypothalamus gland and accelerate the digestive tract (Toaha *et al.*, 2016; Covarrubias *et al.*, 2018) [54, 15]. The feed intake among groups showed similar patterns, which aligns partially with the results of Zomrawi *et al*. (2013) [63], indicating that birds fed diets containing powdered ginger root exhibited reduced feed consumption. The current study's findings are consistent with those reported by Barazesh *et al*. (2013) [13] and Rafiee *et al*. (2013), who observed a significant (p<0.05) decrease in feed intake during the second week following administration of ginger powder to broiler chickens compared to the control group.

Feed conversion ratio significantly differs among all groups, this result supports the findings of Moorthy *et al*. (2009) and Onimisi *et al*. (2005) [34, 42] who reported significantly better feed conversion ratio in ginger fed groups of broilers compared to control. Consequently, feed conversion ratio was significantly improved in ginger supplemented broilers for the starter, finisher and whole periods. The results of the present study were consistent with the results of Tekeli *et al.* (2011) [53] showed that broilers fed with ginger (*Z. Officinal*) improved body weight gain. Also, Herawati *et al.*, (2010) [22] reported that the use of red ginger in the ration of broilers improved the body weight gain. (Onu *et al.*, 2010) [41] stated that the dietary ginger addition increased body weight in broilers. According to Kamel *et al*. (2001) [28], these additives inhibit the growth of harmful bacteria including E. coli in the intestinal tract due to antimicrobial activity. Thus, when the number of harmful bacteria in the intestinal is low, promotes the nutrient assimilation and sustains performance in broilers. In the other hand, the majority of the researchers ascribed the broiler chicks' improved performance to the natural product's increased palatability and rapid digestive impact. The findings of the cost per 25 kg of food, cost per kilogram of diet, cost per kilogram of body weight growth, and cost per day feed intake were consistent with the findings of Durrani *et al*. (2006) [17], who observed that the cost of feed for chicks at the 0.5% inclusion level was significantly more favorable than that of other treatments.

Among the chickens fed diets containing 4g/kg of fenugreek and ginger mix powder, the overall live weight, defethering weight, evisceration weight, and carcass weight were all proportionately greater. The combination of fenugreek and ginger powder in the current study resulted in a linear improvement in carcass development; this could be because the diets' protein was appropriately utilized, as described by Ademola *et al.* (2009) and Elbushra (2012) [4, 20]. Additionally, the results showed that the birds receiving the supplemental feed had greater dressing percentages (eviscerated), which is in line with the conclusions of Yesuf *et al*. (2017) [59]. This could be because the protein from the phytobiotics has a higher digestion, which promotes stronger muscle growth. The fibula's ideal development, which shows appropriate use of the calcium in the diet, may also be the cause of the high breast weight (Abbas, 2010). The chicken on the supplemental feed also had correspondingly larger weights for their wings and back, which could be because of improved skeletal growth brought on by greater utilization. Higher weights of the drumsticks and breast muscles have higher economic values because they are the most significant carcass cuts (Mamoun, 2014). The results of this study closely match those of Abbas (2010), who found that broilers fed 1% and 2% fenugreek seed powder supplementation had

higher dressing percentages than the unsupplemented group. This could be because the group given fenugreek powder had better muscle growth. According to Delimaris (2013) [16], broilers treated with fenugreek seed powder up to 3% of their food showed an improvement in dressing percentage as a result of consuming more dry matter and crude protein.

Yesuf *et al*. (2017) [59] and Medina *et al*. (2020) [36] discovered a substantial ($p<0.05$) impact on certain organs of broiler chickens fed diets containing fenugreek seeds due to the phytochemical's stated ability to enhance organ functions. However, dietary inclusion of fenugreek and ginger powder blend helps to improve the proventriculus, gizzard and the liver of the broiler chickens and this observation agreed with the findings of Olayeni *et al*. (2024) [40] and Medina *et al*. (2020) [36] that addition of fenugreek seed powder improves the organ characteristics of the broiler chickens. The improvement in the internal organ could be attributed to the presence of essential fatty acids and high-quality protein in and stimulating effect on the digestive system (Hind *et al*., 2013). Despite the level of significant recorded all the organ parameter weight still falls within normal range as reported by (Ademola *et al.*, 2009; Medina *et al*. 2020; and Olayeni *et al*. 2024) [4, 36, 40].

In line with the findings of Yatoo *et al*. (2012) [58], who found that all blood parameters were significantly ($P>0.05$) higher in treatment groups given fenugreek seed powder than in the control group, the addition of fenugreek and ginger powder increased the PCV, RBC, hemoglobin, and WBC. Additionally, feeding fenugreek seeds to broiler breeder chickens improved the packed cell volume percentage, red blood count, and Hb concentration significantly ($p<0.05$). Abdul-Rahman (2012) attributed this improvement in erythropoiesis to the increased antioxidant activity in fenugreek and ginger, which reduces the production of free radicals that destroy hemoglobin and cause hemolysis of RBCs. Thus, the broiler chickens' numerical improvements in pack cell volume RBC and WBC counts at the time indicate that the blood's ability to carry oxygen was improved. Additionally, it has been suggested that a high PCV reading (polycythemia) indicates either a decrease in the volume of circulating plasma or an increase in red blood cells, which may be the result of a pathological reaction to chronic respiratory or circulatory disease or a physiological adaptation to high altitudes (Chineke *et al*., 2006) [14]. In the present study, however, none of the PCV value of broiler chickens fed fenugreek and ginger was above 35%, and the values obtained were within the reference ranges for broilers of 22-35 percent (Merck 2012) [37] and 25-45 percent (Al-Nedawi, 2018). The primary functions of white blood cells are to fight infectious illnesses, protect the body from foreign substances entering it, and disseminate antibodies and immunological response. Paneru *et al*. (2022) [44] asserted that the increases in the WBC might be possible because of inflammation and damage to the jejunum that was characterized by infiltrations of White blood cell. Increasing the amount of white blood cells in the blood after they have increased can be crucial for boosting an animal's immune system (Al-Kassi, 2009) [7]. Ginger and fenugreek powder contain alkaloid and phenol as reported by (Jacob *et al.*, 2018 and Shaimaa *et al*., 2023) [26, 50] which helps to fight any foreign body or help to build immune system which has been shown in the study that inclusion of fenugreek seed in the diets helps suppress the multiplication of bacteria in the body. The result agrees with the results obtained by (Herms *et al*., 2010) who reported increase in value with no statistical difference. As a result, birds with low white blood cell counts are more likely to contract infections, whereas those with moderate levels can produce antibodies during phagocytosis and are more disease-resistant (Soetan and Ajibade, 2013) [52].

From the observation of this study can suggest that total protein observed in broiler chickens fed diets containing of fenugreek and ginger powder blend may be due to nutritional potential effect of the treated diets and an increased body weight gain. Blood levels of the enzyme alanine aminotransferase (ALT) are bioindicators of liver injury and function (Yildirim *et al.*, 2011) [60]. Muscle injury brought on by the body's reaction to stress is linked to elevated levels of these enzymes (Yildirim *et al*., 2011) [60]. The values of these enzyme showed that broiler chickens fed diets with the inclusion of fenugreek and ginger powder blend elevated ALT than other treatment groups. In fenugreek and ginger Saponin, vitamins A, C, B1, nicotinic acid, and alkaloids are active ingredient that can act as immunomodulators and liver tonic ingredients. Alkaloids, including trigonelline, carpine, and gentianine compounds are the most important alkaloids of fenugreek and ginger (Lawal *et al.*, 2018, and Shaimaa *et al.*, 2023) [32, 50]. The current serum analysis supports the findings of Azouz (2001) [11], who discovered that feeding Hubbard broiler chicks diets supplemented with fenugreek seeds resulted in a considerable increase in protein and globulin. Similarly, Khard and Abdel-Fattah (2007) found that adding fenugreek to the broiler feed changed the cholesterol profile because the meat contains saponin, which lowers blood cholesterol and is beneficial for human diets. Weerasingh and Atapattu (2013) [56] and Mamoun *et al*. (2014) reported that serum cholesterol levels were reduced when fenugreek was added in diet of broiler. However, the presence of saponins and resins in the fenugreek and ginger blend (such as hemicelluloses, mucilage, tannin, and pectin) may be the cause of the drop-in serum cholesterol levels. These substances inhibit bile acid, lower LDL cholesterol, and inhibit intestinal cholesterol absorptions, which in turn lowers blood cholesterol levels (Jacob *et al.*, 2018 and Shaimaa *et al*., 2023) [26, 50]. The content of total cholesterol and triglycerides in blood can be utilized as indicators of lipid metabolism, and serum biochemical indicators can reflect the body's metabolism and certain illness states as a useful indication for monitoring the health of an animal's body. The anti-hypercholesterolemia properties of ginger may be the cause of this effect. This is in line with ginger's widely documented ability to decrease blood cholesterol levels (Ademola *et al.*,2009) [4]. The results show that the all the value reported still fall s within the normal range of healthy chicken as reported by Merck (2012) [37].

Most of these bacteria produced are beneficial bacteria that serve as probiotics that provide health benefit which produce lactic acid in gut in digestive tract that aid digestion, help in improving growth, healthy living of animal and they are also used to know the state of health of broiler according to (Elisa *et al.*, 2021) [19] which stated that microbes modulation helps to improve health, welfare and prevent gastrointestinal diseases. Also, the inclusion of the blend in the diet help to reduce the population of the microbes since the contain phytochemical such as alkaloid and phenol which act as antibacterial and anti-virus. They also help to produce proteins that prevent pathogenic ones from growing to the level where they can cause infections. The bacteria that can

be of significant or cause infection and intestinal disorder according to (Joerg, 2022) [27] are Staphylococcus, E.coli, Salmonella, Klebsiella and Pseudomonas and they can be multi drug resistant if there is shift in the pH balance or disturbance in the micro biota favouring any of the mentioned bacteria that overgrown others can lead to infection.

The plates showed histological responses of the liver of broiler chickens fed graded levels of ginger and fenugreek powder blend. Histological study on the liver showed a normal structure and no alteration in the livers of the treated broiler chickens fed phytobiotic blend. The histological states of the livers observed agrees with the other authors used phytoadditives as report by Uhegbu *et al.* (2015) [55] when aqueous extract of *Piper guinesse* seeds was observed on some liver enzymes, antioxidant enzymes and some haematological parameters in albino rats. The seeds and rhizome of fenugreek and ginger contains phytochemical constituents that possess hepato-protective effect according to (Ademola *et al.,* 2009 and Olayeni *et al.,* 2024) [4, 40]. Normal hepatocytes observed help to stress the usefulness of the two selected phytobiotics in the diet of broiler chickens. The gut-liver axis plays a significant role in liver health and changes in gut microbiota composition and intestinal permeability can impact liver function and the hepatocytes health. The result is also in line with the report of Shittu *et al.,* (2024) [51] who reported that *the* inclusion of Negro pepper, garlic and ginger in broilers diet improved gut ecosystem, intestine histomophometry without fear of toxicity.

Conclusion and recommendation

A combination of ginger and fenugreek powder was found to improve growth performance, carcass yield, organ features, cost-benefit analysis, haematology, and serum biochemistry when added to broiler chicken diets. It is therefore recommended that the inclusion of fenugreek and ginger powder blend at 4g/kg in the diets of broiler chicken can be used to improved performance, health status, liver function, reduce cost of production and reduce faecal microbes.

Conflicts of interest

No conflict of Interest

Acknowledgement

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