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FEED INTAKE, GROWTH PERFORMANCE, HEMATOLOGY AND SERUM BIOCHEMICAL INDICES OF SASSO CHICKEN RELIED ON INCLUSION OF NEEM (AZADIRACHTA INDICA) AND BITTER (VERNONIA AMYGDALINA) LEAF EXTRACTS

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ABSTRACT

The study evaluated the effectiveness of neem and bitter leaf extract as a natural feed additive for broiler chicks, focusing on feed intake, body weight gain, carcass parameters, and sensory evaluation of meat and its composition. A total of 122 unsexed Sasso chickens were randomly assigned to four treatments with three replications in a Completely Randomized Design (CRD). Feed intake, Average daily gain (ADG), and Feed Conversion Ratio (FCR) were analyzed for starter (1-21 days), finisher (22-42 days), and entire experiment periods (42 days), comparing daily offers and refusals. Four broilers were slaughtered and treated with bitter and neem leaf extract in water for different treatments (0 ml, 2 ml, 4 ml, and 6 ml per liter of water for T1, T2, T3 and T4 respectively), evaluating carcass evaluation at the end of the trial. The CP and ME content of neem leave powder (NLP) and bitter leave powder (BLP) were 22.90% and 2680.96 Kcal/Kg DM and 21.95% and 3020.85 Kcal/Kg DM respectively. The average daily feed intake (ADFI) during the entire period was 104.49,116.07,118.65,122.32 g/bird (SEM=2.03) for T1, T2, T3 and T4 respectively, and it was significantly higher ($p<0.05$) for T4 compared to other treatment groups. The body weight gain (BWG) during the entire experimental period was 45.95, 56.72, 57.92, and 61.95 g/bird/day (SEM=1.88) for T1, T2, T3 and T4 respectively, and significantly higher in T4. The Feed conversion ratio (FCR) during the entire period was 2.28, 2.04, 2.04, and 1.97 (SEM = 0.04) for T1, T2, T3 and T4, respectively and best for T4. Carcass's weight was 1331.96, 1622.01, 1674.27 and 1899.70gm (SEM=65.58) for T1, T2, T3 and T4, respectively, and it was higher in T4 than other treatments. The giblet organs and crop were significantly higher ($P<0.05$) for T4. The serum cholesterol (SC) and white blood cell (WBC) of broilers in the experiment period were significantly ($P<0.05$) lower for T4 as compared to other treatment. But Total protein (TP) was significantly ($P<0.05$) higher in T4 among treatment groups which showed that the addition of extract in drink water of broiler had positive effect on the chicks' blood. The study recommends a combination of neem and bitter leaf extract in 6ml/1lt of drink water as a beneficial natural feed additive for Sasso Chicken production.

Key words: Sasso chicks, Growth performance, Neem leaf, Feed intake, Serum profile

1.INTRODUCTION

Poultry farming bridges the protein gap in developing countries, providing protein for human nutrition (WHO, 2007). Broiler production accounts for 33% of global meat production, meeting WHO's requirement of 105 mg nitrogen/kg body weight per day (FAO, 2010). Feed additives, also known as growth promoters, are added to poultry diets to improve production efficiency, health, reduce morbidity, increase growth rate, and lower mortality rates (Singh and Panda, 1992; FAO, 1998).

Various feed additives are used in poultry to maximize net returns and carcass quality (Khan *et al*., 2012). Medicinal plant extracts are used in food as natural antimicrobials and as growth performance enhancers in poultry nutrition, replacing banned antibiotic growth promoters (AGP) (Hsieh and Mau, 2001; Jang *et al.*, 2008).

Neem and bitter leaves extract stimulate immune responses, enhancing future responses to disease organisms. Feeding immune-suppressed birds can boost cell-mediated immune responses, enhancing humoral effect (Sadekar *et al*., 1998; Vivian *et al*., 2015). Azadirachta indica and Vernonia amygdalina leaves offer diverse chemical, elemental, and macronutrient components, with Vernonia amygdalina leaf showing superior anti-oxidant properties, while containing minimal phytochemicals (Offor, 2014).

Large-scale antibiotic use has led to antibiotic resistance and residues in food and aquatic ecosystems, posing public health risks. Concerns over antibiotic growth promotion and human-to-animal resistance have led to drug discontinuation (Ratcliff, 2000; Patrick *et al*., 2003; Youcef *et al.,* 2018). The continuous use of antibiotics in farm animals has led to a significant increase in deaths and illnesses associated with antibiotic resistance (Newman, 2002; Muhammed *et al*., 2009).

Many countries are exploring the use of medical plant extract for alternative production due to concerns about antibiotic residue in poultry meat and demand for drug-free food products (Griggs and Jacob, 2005). This has led to the search for alternative natural growth enhancers such as plants and their extracts. However, no work has been conducted regarding the use of mixtures of neem and bitter leaf extracts and its effect on body weight, performance, and blood parameters. Therefore, this study was designed to investigate the potential of neem and bitter leaf extracts as natural feed additives for broiler chicks and assess their inclusion effects on feed intake, growth performance, carcass characteristics, blood parameters, chemical composition, and sensory evaluation of meat.

2. MATERIALS AND METHODS

2.1. Study Area

The map of the study site is indicated in Figure 1. The study was conducted in Wollega University main campus, Oromia Regional state, western parts of Ethiopia which is located at 328km away from Finfine city/Addis Ababa to the west direction on the main highway to Assosa. The area is located at 10^0 5` N latitude, $36^0$, 33` E longitude. The study area is located at an altitude of 2,088 meters above sea level (masl). The minimum and maximum temperature of the area is 8^0 C and 30^0 C. The mean temperature of the area was reported as 19^0 C. The mean annual rainfall of the study area was about 1998mm; relative humidity ranges from 31 to 110% (Nekemte Meteorology Agency).

2.2. Collection and Preparation of Leaves Extracts and Rations

Bitter and neem leaves were selected as a growth promoter on experimental chicks. Neem leaf was collected from Bako Tibe area farm and Bitter leaf was collected from Guto Gida Distitcs, Ethiopia. The samples were dried and ground thoroughly powdered at Wollega University feed processing plant. The obtained powder was packed in a polyethylene bag and preserved in the feed storage room until used with water for the broiler. Then 30 grams of each leaves powder was added to 1Lt of cold distilled water, shake thoroughly and placed overnight at room temperature (Mollah *et al.,* 2012). The starter and finisher broiler diets were purchased from the Bishoftu Alema farm. Formulated rations were purchased to meet the nutrient requirement for starter and finisher broiler chicks. The starter phase was 3 weeks of age. The finisher phase was offered from 3 weeks up to slaughtering (42 days).

2.3. Management of Experimental Animals

The experimental house, pens, watering troughs, and pens were thoroughly cleaned and disinfected two weeks before the experimental on the animal's arrival, using commercial disinfectant and sprayed against parasites.

Each pen was equipped with a 250-watt heat bulb. For the experiment, a total of unsexed one hundred ninety-two-day-old Sasso breed of chicks were purchased from Ethio Chicken farm. The chicks were vaccinated against Newcastle Disease on day 7 (HB1) by the ocular route and on day 21 (Lasota) was given through by drinking water. They were also vaccinated for Gumboro on day 14 and 28 through drinking water. Other health precautions and disease control measures were taken throughout the study period. Vitamins were given to chicks through drinking water to recover from the stress of transportation and early age acclimatization problems according to the manufacturer’s recommendation.

2.4. Experimental Design and Treatments

The completely randomized design was used, 192 broiler chicken were grouped into four treatments and three replicates, with 16 chicks per replicate. Treatment 1 was control group, and T2, T3, and T4 were given 2ml, 4ml, 6 ml of neem and bitter leaf infusion per liter of drinking water respectively. Similar commercial broilers' diet and water were offered ad libitum throughout the experimental period.

Table 1. Experimental Treatment Design

Treatment	No of replication	Starter phase	Finisher phase		
		Chicks/replications	Chicks/replication		
		R	R1	R2	R3
T1	3	16	15	14	15
T2	3	16	15	15	15
T3	3	16	15	15	16
T4	3	16	15	15	15

2.5. Parameters Evaluated and Data Collection Procedure

Data were collected on initial weight, final body weight, body weight gain, feed intake, daily feed intake, feed conversion ratio (FCR), carcass characteristics, hematology of chickens, and chemical composition of the meat, and mortality rate.

2.5.1. Feed intake

A weighed amount of feed was offered twice a day, and orts were collected every next morning and weighed after removing external contaminants by visual inspection. Feed intake was calculated as the difference between offered and leftover. Average total feed intake per was calculated by subtracting the amount of leftover collected from the amount of feed offered and divided by number of chicks for that day. Average daily feed intake was determined by dividing average total feed intake for a number of experimental days. Mean daily feed intake per bird was computed as:

$$\text{Mean daily feed intake} = \frac{Mean\ Total\ Feed\ Intake}{Number\ of\ experimental\ days}$$

The mean feed conversion ratio was determined by dividing the average daily feed intake (DFI) with a mean daily body weight gain (DBWG).

2.5.2. Body weight gain and feed conversion ratio

Body weight gain was measured every week by weighing the chicks with sensitive balance initially ten in one; second week seven in one; third week four in one; fourth a week two in one. Finally, for the sixth weeks, body weight was measured by weighing the broilers individually. The body weight change per pen was summarized for a starter as well as finisher phases and the entire experiment period for analysis. The body weight gain of birds was computed by subtracting the mean initial weight from the mean final weight. Average daily gain (ADG) was determined as a difference in mean final and mean initial body weights divided by the number of experimental days.

$$\text{Average daily gain} = \frac{Total\ Weight\ gained}{Number\ of\ experimental\ days}$$

2.5.3. Chick mortality

Daily monitoring of the birds was followed as a routine activity to check the health status and to record deaths. Mortality was recorded as it occurred and was determined for each treatment as a percentage of the total mortality at the end of the whole experiment. Calculation of mortality percentage /MP/ is expressed as percentage as follows:

$$\text{Mortality Percentage} = \frac{Number\ of\ Dead\ chick}{Number\ of\ total\ chicks} * 100$$

2.5.4. Carcass evaluation

At the end of the experiment, four birds from each group were picked up randomly then starved for 12 hours, weighed immediately before being slaughtered and exsanguinated by severing the neck for complete bleeding by using an instrument bleeding cone and dressed. Birds were eviscerated, and carcass cuts and non-edible offal components were determined according to the procedure described by Kekeocha (1985). Dressed weight was measured after the removal of blood and feather, and the dressing percentage was calculated as the proportion of dressed carcass weight to slaughter weight. Eviscerated carcass weight was determined after removing blood, feather, shank, head, kidney, lungs, pancreas, crop, proventriculus, small intestine, large intestine, caeca and urogenital tracts. The eviscerated percentage was determined as the proportion of slaughter weight. Drumstick, thigh, breast meat, heart, gizzard, and liver were separated, weighed and calculated as a percentage of slaughter weight. Fat around the proventriculus, gizzard, against the abdominal wall and the cloacae were separated, weighed and expressed as a percentage of slaughter weight. Dressed and eviscerated weights were calculated following the method of FAO (2001) as:

Dressed weight=Thighs+Wings+Breast+Ribs+Back+Heart+Liver+Gizzard+neck+Feet+Head + Viscera (inedible offal)

Eviscerated Weight= Dressed weight- Viscera

$$\text{Dressing (\%)} = \frac{Dressed\ Weight}{Pre\ slaughter\ weight} * 100$$

$$\text{Eviscerated (\%)} = \frac{\text{Eviscerated } Weight}{Number\ of\ experimental\ days} * 100$$

2.5.5. Chemical composition of meat

Chemical composition of the meat samples was analyzed following the procedure of AOAC (1995). Samples of breast and thigh muscles were minced, dried and ground with 1mm size then analyzed for the content of dry matter, crude

protein, ether extract and ash. Dry matter was determined by drying 6 g of ground meat samples in a draft oven at 105°C for 24 hrs. Nitrogen (N) was determined according to Kjeldahl procedure and crude protein content of the sample was calculated as N*6.25 (AOAC, 1995). Total lipid (ether extract) content of the muscles determined following the standard procedure (AOAC, 1995). Total mineral content was determined by burning 6 g of the samples in a muffle furnace at 550°C for 3 hours.

2.5.6 Hematological parameters and serum biochemical analysis

At the end of the experimental period, four broilers were randomly selected from each replicate of each treatment group and blood samples were taken from the brachial vein with a syringe on a tube containing anticoagulant namely, Ethylene Diamine Tetra acetic acid (EDTA) for analysis of hematology parameters (Hemoglobin, Packed Cell Volume, Total white blood cells and red blood cells counts). Hemoglobin (Hb) was determined from samples before spinning in a centrifuge by the Acid hematin method via the procedure followed by Davice and Lewis (1991). Packed cell volume (PCV) was determined by spinning blood-filled capillary tubes in a centrifuge (Hitachi, EBA 20, Germany) at 1200 rpm x g for 5 minutes and recorded on hematocrit reader according to the method described by Campbell (1995). Total white blood cell (WBC) and red blood cell (RBC) counts were determined by using improved neubauer hemocytometer following the procedure described by Campbell (1980). Blood for serum biochemical analysis was collected from the same birds slaughtered for carcass measurements. Each blood sample was collected without anticoagulant for serum biochemical analysis. Serum was separated after centrifugation at 3,000 rpm x g for 15 min and stored at 20 ^{0}C until analyzed. Total serum protein was determined by a refractometer (George, 2001).

2.6. Statistical Analysis

The experimental design employed was Completely Randomized Design (CRD). Data were subjected to analysis of variance (ANOVA) using a General Linear Model procedure of statistical analysis system (SAS) version 9.1 (SAS, 2008). Least significant difference (LSD) test was used to determine mean differences at $P \leq 0.05$.

The model used was: $Xij = \mu + Ti + Eij$, Xij = any observation made in the experiment, μ = Overall mean, Ti = Effect of treatments, Eij = random error

3. RESULT AND DISCUSSION

3.1. Chemical Composition of Feed Additives and Experimental Diets

Laboratory analysis for the experimental feed additives and chemical composition of commercial feeds is indicated in Table 2. Laboratory analysis revealed that neem leaves powder (NLP) and bitter leaves powder (BLP) have a Metabolizable energy content of 2680.96 and 3020.85 kcal/kg DM, respectively. The proximate composition of neem leaves powder was 91.3% dry matter, 22.90% crude protein, 7.8% crude fiber, 3.20% ether extract, and 9.2% ash, respectively. This result is comparable with Singh *et al*. (2015) and (Ahmed *et al*., 2020). Proximate composition of bitter leaves powder 86.00 % DM, 21.95 % CP, 12.90 % CF, 6.90% EE, 10.85% ash which was in line with Owen *et al.* (2011) and (Ahmed *et al*., 2020) report.

Table 2. Chemical composition of neem (Azadirachta indica), bitter (Vernonia amygdalina) leaves and commercial feeds.

Feed	DM (%)	Ash(%DM)	EE(%DM)	CF(%DM)	CP(%DM	ME(Kcal/kg DM)
NLP	91.3%	9.2	3.20	7.80	22.90	2680.96
BLP	86.00	10.86	6.90	12.90	21.95	3020.85
Starter diet	90.00	4.47	6.80	5.70	22.80	3080.00
Finisher diet	90.00	4.55	8.10	5.70	18.30	3275.0

DM = Dry Mater; EE = Ether Extract; CF = Crude Fiber; CP = Crude Protein; ME= Metabolizable Energy; NLP = neem leaves powder; BLP= bitter leaves powder

3.2. Feed Intake

Table 3 shows the impact of neem and bitter leaf extract mixtures on broiler feed intake during starter, finisher, and growth phases. The result showed that treatment of neem and bitter leaves extracts significantly improved ($P < 0.05$) feed intake during the starter and finisher phases, with varying levels of inclusion affecting average daily and total feed intake. The addition of neem and bitter leaves extract mixtures at varying levels significantly enhanced feed intake during the starter phase compared to the control. Feed intake increased with neem and bitter leaf extract mixtures, with higher levels in T3 and T4 during the entire experiment, with T1 intake significantly lower than supplemented group ($P<0.05$).

The results obtained for feed intake were in agreement with Singh *et al* (2015), who reported that group of broilers that feeds neem leaf powder was provided as 1, 2 and 3 g per kg of feed, were higher feed intake with change in weeks. The study contradicts Vivian *et al* (2015) findings, which found no significant difference in daily feed intake among four treatment groups (25ml, 50ml, and 75ml per liter of drinking). High feed intake of neem and bitter leaf infusion extracts may improve digestion and aid in intestinal parasite digestion, potentially decreasing feed utilization. (Ezeonu *et al*., 2012; Vivian *et al*., 2015).

Table 3. Effect of administering mixtures of neem and bitter leaves extract on feed intake of broilers during the starter and finisher phases as well as the entire growth period.

Parameters	Treatments				SEM	P-value
	T1	T2	T3	T4		
Starter phase						
Feed Intake (g)	1152.28 ^{c}	1286.78 ^{b}	1297.06 ^{ab}	1317.21 ^{a}	19.92	.0001
Daily Feed Intake (g/bird/day)	54.60 ^{c}	61.00 ^{b}	61.48 ^{ab}	62.45 ^{a}	0.94	.0001
Finisher phase						
Feed Intake (g)	3130.98 ^{c}	3471.16 ^{b}	3566.56 ^{b}	3696.95 ^{a}	64.64	.0008
Daily Feed Intake (g/bird/day)	156.64 ^{c}	173.65 ^{b}	178.42 ^{b}	184.97 ^{a}	3.23	.0009
Entire period						
Feed Intake (g)	4284.46 ^{c}	4759.14 ^{b}	4864.64 ^{ab}	5015.26 ^{a}	83.63	.0004
Daily Feed Intake (g/bird/day)	104.49 ^{c}	116.07^{b}	118.65 ^{b}	122.32 ^{a}	2.03	.0004

^{abc} *Means within a row with different superscript letters are significantly different; (P<0.05); SEM=Standard error of the mean;T1=0% of neem and bitter leaves extract; T2=2ml of neem and bitter leaves extract added to 1lt of water;T3=4ml of neem and bitter leaves extract added to 1litter of water;;T4=6ml of neem and bitter leaves extract added to 1litter of water*

3.3. Body Weight Gain

Table 4 displays the growth rate of experimental chicks during starter, finisher, and the entire growth period. The infusion of neem and bitter leaves extract significantly impacted body weight change and average daily weight gain at varying levels (P< 0.05). The study found that T4 treatment resulted in significantly higher(P< 0.05) weight gain during the starter phase, while T1 treatment resulted in significantly lower daily and final weight gain. The study found that T4 showed a significantly higher (P< 0.05) weight gain during the starter phase, while T3 did not show any significant difference in finisher phases or the entire experiment period. The report indicates a significantly lower daily and final weight gain in the supplemented group compared to the T1 group. The lower growth of broilers in T1 might be related to the feed intake. These results coincide with those of Chakravarty and Prasad (1991) who achieved high body weight gain as compared to control when offered Neem leaf extract to broilers from 1 to 6 weeks. This finding is in line with Nusrat *et al*. (2015) who reported that the higher body weight gain in broilers consuming neem Leaves infusion. The experiment done on neem leaves extract reported by Sese *et al*. (2013) showed that the final body weight gain ranges from 1733 to 2283g, also Vivian *et al* (2015) showed that the body weight of broilers fed bitter leaf ranges from 1966.43g to 2380g. Similarly, Ndelekwute *et al.,* (2017) report also shows that bitter leaf meals are a good ingredient to be included in the diets of finisher broiler chickens. Final live weight, daily gain and total feed intake were higher in groups of birds that consumed diet containing 75g bitter leaf meal per kg diet at the finisher phase. Similarly, Odoemelam *et al.,* (2013) and Ahmed *et al*. (2020) reported that the inclusion of bitter leaf in broiler diets leads to improvement in body weight. The same report from Sarker *et al.* (2014) that supplementation with 1% aqueous extract of Neem Leaves and Khatun *et al.,* (2014) combination of neem and Tulsi leaves extract at 1-3 ml/L drinking water causes significant increase in live body weight and improvement in weight gain and feed efficiency as compared to that of control group of broiler. Mohammed and Zakariyau (2012) contrarily, observed that inclusion of bitter leaf as a feed additive did not significantly improve weight gain. The probable reason for this increment of body weight could be created an environment of intestinal tract balance and which results in better utilization of feed and eventually weight gain. Hernandez *et al.,* (2004) reported the better FCR was due to the effect of medicinal plant leaf extract that increases the production of digestive enzymes and improved utilization of digestive products through enhanced liver function and might be due to anti-protozoal and immune-stimulatory properties of neem leaves that help to reduce the microbial load and improved the performance (Wankar *et al.,* 2009).

Table 4. Effect of mixture of neem and bitter leaves extracts on body weight change of broilers during the starter and finisher phases as well as the entire growth period

Parameters	Treatments				SEM	P-value
	T1	T2	T3	T4		
Starter phase						
Initial body weight (g) (g)	40.860	41.78	41.42	42.00	0.29	.290
Final body weight (g)	814.55 ^{c}	836.72 ^{b}	845.97 ^{b}	863.91 ^{a}	5.72	.0006

Body weight gain (g/bird)	773.67 [c]	794.76 [b]	804.27 [b]	821.42 [a]	5.55	.0005
Average daily gain (g/bird/day)	36.84 [c]	37.84 [b]	38.29 [b]	39.11 [a]	0.26	.0005
Finisher phase						
Final body weight (g)	1924.95[c]	2367.63[b]	2416.53[ab]	2582.67[a]	77.16	.0003
Body weight gain (g/bird) Intake (g/bird/day)	1151.28[c]	1572.87[b]	1612.26[ab]	1761.24[a]	72.27	.0006
Average daily gain (g/bird/day)	57.56[c]	78.64[b]	80.61[ab]	88.06[a]	3.61	.0006
Entire period						
Final body weight (g)	1924.95[c]	2367.63[b]	2416.53[ab]	2582.67[a]	77.16	.0003
Body weight gain (g/bird) Intake (g/bird/day)	1884.07[c]	2325.38[b]	2374.84[ab]	2540.17[a]	77.00	.0003
Average daily gain (g/bird/day)	45.95[b]	56.72[a]	57.92[a]	61.95[a]	1.88	.0003

[abc] *Means within a row with different superscript letters are significantly different; (P<0.05);*
SEM=Standard error of the mean;T1=0% mixture of neem and bitter leaves extract; T2=2ml of neem and bitter leaves extract added to 1litter of water;T3=4ml of neem and bitter leaves extract added to 1litter of water;;T4=6ml of neem and bitter leaves extract added to 1litter of water.

3.4. Feed Conversion Ratio

Table 5 displays the feed conversion ratio of broilers infused with varying neem and bitter leaves extract mixtures during the starter, finisher, and growth phases. There was significant difference (P< 0.05) in feed conversion ratio among treatments during the starter phase. Starter phase chickens in control show lower in FCR than other groups (T2, T3 and T4) at (P< 0.05). But during finisher phase and the entire period, FCR of T4 was significantly lower (P< 0.05 than that of T1 and displayed lower FCR numerically than T2 and T3 in finisher phase and the entire experiment period. The improvement observed in weight gain is correlated with better FCR observed in the treated group. The lower the FCR the higher it is for the birds to convert feed consumed to meat.

The results of this study showed that inclusion of different levels of mixtures of neem and bitter leaves extracts improves feed conversion ratio of the broilers. The result is in line with the findings of Tangka (2003) and Durunna *et al*. (2011) who reported improved growth performance of animals fed bitter leaf. Ansari *et al*. (2008) observed that the FCR of broilers fed with Azadirachta indica significantly improved as compared to other treatments. Better feed conversion ratio of the broiler infused neem and bitter leaves extract may be attributed to the antibacterial properties of these supplements, which resulted in better absorption of the nutrients present in the gut and finely leading to improvement in feed conversion ratio of the rations (Ezeonu *et a*l., 2012; Vivian *et al.,* 2015).

Table 5. Effect of mixtures of neem and bitter leaves extract on FCR of broilers during the starter and finisher phases as well as the entire growth period.

Parameters	**Treatments**					
	T1	**T2**	**T3**	**T4**	**SEM**	**P-value**
FCR						
Starter	1.48 [b]	1.61 [a]	1.61 [a]	1.60 [a]	0.018	0.0004
Finisher	2.74 [a]	2.21 [b]	2.21 [b]	2.11 [b]	0.080	0.011
Entire period	2.28 [a]	2.04 [b]	2.04 [b]	1.97 [b]	0.040	0.040

[a,b,c] *Means within a row with different superscript letters are significantly different, (P<0.05);*
FCR=Feed Conversion Ratio, SEM=Standard error of the mean, T1=0 ml/lt of neem and bitter leaves extract; T2=2ml/l of neem and bitter leaves extract; T3=4ml/l of neem and bitter leaves extract; T4=6ml/l of neem and bitter leaves extract.

3.5. Carcass Parameters

The results of the carcass parameters of the birds given 0, 2, 4 and 6 ml of bitter and neem leaf mixing extract are shown in Table 6. There were significant variations (p<0.05) between the experimental treatments in slaughtered weight, carcass weight, dressing percentage, drumstick weight, thigh weight, breast weight, and giblets weights, especially, between T1 and other treatments. Odoemelam *et al*., (2013) reported that the inclusion of bitter leaf in broiler diets leads to improvement in dressing percentage and significantly promoted higher dressed weight and carcass quality. There is also affect of the inclusion of bitter reported by Odoemelam *et al.,* (2013), inclusion of bitter leaf in diet shows improvement in dressing percentage and significantly promoted higher dressed weight. The higher body weight gain in broilers consuming neem leaves infusion was due to its diversified effect on intestinal micro-flora, thereby avoiding stressful conditions (Nusrat *et al*., 2015).

Table 6. Effect of administering mixtures of neem and bitter leaves extract on carcass yield characteristics of broilers

Parameters	Treatments					
	T1	T2	T3	T4	SEM	P-value
Starter phase						
Slaughter weight (g)	1924.95^{c}	2367.63^{b}	2416.5^{3ab}	2582.67^{a}	77.16	0.0002
Dressing weight(g)	1663.84^{c}	1983.60^{b}	2092.90^{b}	2267.60^{a}	69.61	0.0002
Dressing percentage (%)	86.45^{a}	83.81^{b}	86.60^{a}	87.78^{a}	0.52	0.022
Eviscerating weight (g)	1364.53^{c}	1679.10^{b}	1732.32^{b}	1968.81^{a}	69.60	0.0007
Eviscerating percentage (%)	70.89^{b}	70.91^{b}	71.69^{b}	76.13^{a}	0.75	0.006
Carcass weight (g)	1331.96^{c}	1622.01^{b}	1674.27^{b}	1899.70^{a}	65.58	0.0008
Carcass percentage (%)	68.50^{b}	69.22^{b}	69.28b	73.45^{a}	0.69	0.015
Breast weight (g)	464.47^{c}	591.13^{b}	633.92^{b}	697.02^{a}	26.65	0.0001
Breast (%)	24.11^{b}	24.98^{b}	26.22^{b}	26.98^{a}	0.36	0.0009
Thigh weight (g)	176.48^{d}	218.60^{c}	251.05^{b}	273.25^{a}	11.34	0.0001
Thigh (%)	9.18^{b}	9.23^{b}	10.39^{a}	10.57^{a}	0.20	0.0002
Drumstick weight (g)	154.05^{c}	195.38^{b}	213.58^{b}	246.53^{a}	10.61	0.0002
Drumstick (%)	8.01^{c}	8.26^{bc}	8.83^{b}	9.53^{a}	0.19	0.0040
Abdominal fat weight (g)	41.00	41.00	40.67	40.67	0.22	0.53
Abdominal fat (%)	2.13^{a}	1.73^{b}	1.68^{c}	1.61^{c}	0.06	0.0001

^{a,b,c} Means within a row with different superscript letters are significantly different, (P<0.05);
SEM=Standard error of the mean, T1=0 ml/lt of neem and bitter leaves extract; T2=2ml/l of neem and bitter leaves extract; T3=4ml/l of neem and bitter leaves extract; T4=6ml/l of neem and bitter leaves extract

Average weight of giblet of broiler chickens administered the mixture of Azadirachta indica and Vernonia amygdalina leaves extract shown on Table 7, the average heart weight of control treatment was significantly (P< 0.05) lower than the supplemented animal. Average liver weight was (33.36 g) in control, followed by an average liver weight of 36.43 g, 39.53 g, and 38.70 g recorded in T2, T3 and T4 respectively. The average crop weight was (3.57 g) in control, following 4.65 g, 4.83g and 5.01g, for T2 , T3 and T4 respectively. The present study was in line with Sobayo *et al*. (2016) who report that the inclusion of Neem Leaf Meal in birds influence gizzard, liver and heart were significantly (P< 0.05) affected. In this finding, there was no significant (P> 0.05) difference observed in proventriculus, large intestine, small intestine and ceaca among treatment group.

Table 7. Effect of administering mixtures of neem and bitter leaves extract on the organ parts weight and length of broilers

Parameters	**Treatments**					
	T1	**T2**	**T3**	**T4**	**SEM**	**P-value**
Starter phase						
Heart weight (g)	9.05^{b}	10..33^{ab}	11.41^{a}	11.55^{a}	0.37	0.03
Heart (%)	0.45	0.44	0.47	0.46	0.01	0.85
Liver weight (g)	33.65^{b}	36.43^{ab}	39.53^{a}	38.70^{a}	0.81	0.01
Liver (%)	1.68^{a}	1.56^{ab}	1.63^{b}	1.53^{b}	0.02	0.07
Gizzard weight (g)	30.76^{c}	33.56^{b}	35.08^{ab}	36.83^{a}	0.74	0.003
Esophagus weight (g)	5.28^{b}	5.45^{b}	6.00^{ab}	6.7^{9a}	0.22	0.02
Esophagus length (cm)	15.16^{c}	15.50^{bc}	16.56^{ab}	17.57^{a}	0.33	0.01
Crop weight (g)	3.57^{b}	4.65^{a}	4.83^{a}	5.01^{a}	0.193	0.005
Crop length (cm)	2.83^{b}	3.50^{ab}	3.57^{ab}	4.15^{a}	0.176	0.03
Proventriculus weight (g)	8.18	9.23	9.55	9.00	0.2762	0.38
Proventriculus length (cm)	4.83	4.75	4.58	4.58	0.102	0.82
Small intestine weight (g)	58.15	72.63	64.65	55.48	3.399	0.31
Small intestine length (cm)	159.83	171.00	164.33	180.33	3.60089	0.21
Large intestine weight	3.40	4.00	5.57	4.48	0.55436	0.63

(g)						
Large intestine Length(cm)	10.16 [a]	11.75[a]	11.83[a]	11.25[a]	0.37563	0.42
Ceaca weight (g)	11.35	14.88	13.66	12.78	0.74199	0.44
Ceaca Length(cm)	17.16	16.66	17.33	16.41	0.60573	0.96

[ab] *Means within a row with different superscript letters are significantly different, (P<0.05); SEM= Standard error of the mean, T1=0ml of neem and bitter leaf extract; T2=2ml of neem and bitter leaf extract; T3=4ml of neem and bitter leaf extract; and T4=6ml of neem and bitter leaf extract.*

3.6. Hematological Serum Biochemical Parameters

Hematology and serum biochemical profiles results in Table 8 shows hemoglobin concentration (Hb), packed cell volume and total red blood cell (RBC) count of the experimental birds were not statistically different (P> 0.05), whereas, there are significant differences (P< 0.05) in total white blood cell (WBC) count, total protein (TP) and serum cholesterol concentration between the control and experimental groups. The total protein is a composite of the albumin and globulin content in the blood and is a reflection of the nutritional status of the birds. Total protein of the birds in the control group was significantly lower than 2.91g/dl in T1 compared with the other treatments T2, T3 and T4 as it was 3.71, 4.32 and 4.94 g/dl respectively during the experimental period. This finding concurs with a report by Vivian *et al.* (2015) that indicate administration of bitter leaf extract for broiler shows higher total protein than control group. Similar result has been reported by Ihsan *et al*. (2017) which depicted that addition of neem powder in broiler diets significantly (P< 0.05) increased total protein value.

In the present study the serum cholesterol concentration was shown to be significantly lower (P< 0.05) (79.48, 95.50, 96.85, and 114.58) mg/100ml, for T4, T3, T2 and T1 respectively.

The decline in cholesterol level with increased level of neem and bitter leaves extract mixture offered with drink water is in agreement with the report of Ogbuewu *et al*. (2008) who worked on dietary neem leaf meal for rabbits. Upadhyay (1990) also reported a decline in blood cholesterol levels of broilers and rats fed neem leaf meals. Vernonia amygdalina leaves extract elicited beneficial effects by lowering the levels of cholesterol of the broiler chickens and this result is also in agreement with the observation of Owen *et al.* (2011). Similarly, Ojiako and Nwanjo (2006) documented that administration of bitter leaf significantly attenuated the cholesterol level of streptozotocin-diabetic rats.

This study showed that administration of neem and bitter leaves extract in drinking water significantly (P< 0.05) lowered the total white blood cell (WBC) counts. This is similar with the finding of Bonsu *et al*. (2012) who reported that birds fed diets that contained neem leaf meal (NLM) at 2%, 1.5% and 2.5% level were significantly (P< 0.05) lowered the number of WBC when compared with the control birds. The relatively lower WBC of the birds administered with the mixture of neem and bitter leaf extract could be attributed to no fight against potential disease threats before the body's system could be stimulated to produce WBC as was similarly reported by Zanu *et al*. (2011). This is also in agreement with the findings of Gotep *et al* (2016) who administered, combined aqueous extracts of Azadirachta indica and Khaya senegalensis and found out that there was a significant decrease in total WBC count in the treated groups compared to negative control. The values obtained for all hematological parameters were within the normal range (Douglas *et al.,* 2010).

Table 8. Effect of administering mixtures of neem and bitter leaves extract on some hematological and serum biochemistry of broiler chicks

Parameters	**Treatments**					
	T1	T2	T3	T4	SEM	P-value
Red blood cell (x106/mm)	3.75	3.55	3.88	3.38	0.086	<.1811
White blood cell (x103/mm)	70.86[a]	63.85[b]	61.23[b]	44.64[c]	2.923	<.0001
Hemoglobin (g/dl)	10.43	10.60	11.68	11.70	0.284	<. 2394
Packed cell volume (%)	31.93	34.24	35.79	35.83	1.008	<. 5388
Total protein (g/dl)	2.91[a]	3.71[ab]	4.32[cb]	4.94[a]	0.228	<.0001
Serum cholesterol (mg/dl)	114.58[a]	96.85[b]	88.35[c]	79.48[d]	4.009	<.0001

3.7. Chick Mortality Percentage

Mortality rate denotes to the number of chickens died from a flock of certain number of chickens. The results showed that the mortality rate of the broilers (Table 9) was statistically not significant (P>0.05) Percent mortality of broilers in entire experimental period was 8.33, 6.25, 6.25 and 4.16 for T1, T2, T3, and T4, respectively. The mortality percentage in all treatments was not significantly different, possibly due to a high growth rate or rapid metabolism. Postmortem examinations revealed watery fluid in all dead birds. Nodu *et al*. (2016) found that combining neem and bitter leaves extract in drinking water can decrease mortality rates in broilers, potentially boosting growth rates. Low percentage mortality was observed in treatments in which neem extract was administered. There was also similar result from Bonsu *et al.* (2012) and Ahmed *et al*. (2020) who suggest that the neem extract acted positively as an antibiotic at all levels.

Table 9. Effect of administering mixtures of neem and bitter leaves extract on mortality percentage of broilers during the starter and finisher Phases as well as the entire growth period.

Parameters	Treatments					
	T1	**T2**	**T3**	**T4**	**SEM**	**P-value**
Mortality starter Phase	4.16	2.08	4.16	0.00	0.92	0.36
Mortality finisher Phase	4.16	4.16	2.08	4.16	0.92	0.86
Mortality Entire period	8.33	6.25	6.25	4.16	0.76	0.33

[ab] Means within a row with different superscript letters are significantly different, (P<0.05);, SEM= Standard error of the mean, T1=0ml of neem and bitter leaf extract; T2=2ml of neem and bitter leaf infusion; T3=4ml of neem and bitter leaf extract; and T4=6ml of neem and bitter leaf extract.

3.8. Chemical Composition of Meat

The laboratory results in Table 10 shows not significantly (P>0.05) affect in proximate composition of the breast and thigh meat on CP% of the breast and thigh meat. Crude protein of the breast and thigh meat between control and experimental groups was recorded (21.68, 23.52) in T1 compared with the other treatments T2 (21.17, 21.67), T3 (20.92, 24.07) and T4 (24.34, 24.79) respectively in the experiment period. This result clearly shows that blood protein content is directly related to muscle protein content. Similar results have been reported by Ihsan *et al*. (2017) and these authors indicated the addition of neem powder in broiler diets significantly ((P<0.05) higher crude protein value of the meat. This result clearly showed that blood protein content is directly related to muscle protein content.

Table 10. Effect of administering mixtures of neem and bitter leaves extract on meat chemical composition of broilers

Parameters (%)	Treatments					
	T1	**T2**	**T3**	**T4**	**SEM**	**P-value**
Moisture						
Breast	75.16	75.38	75.51	75.49	0.11	0.6
Thigh	73.82	73.66	73.11	73.35	0.12	0.16
Crude protein						
Breast	21.68^{b}	21.17^{b}	20.76^{b}	24.18^{a}	0.45	<.005
Thigh	23.52	21.67^{c}	24.07^{b}	24.79^{a}	0.36	<.002
Ether Extract						
Breast	5.89	5.61	5.8	6	0.17	0.91
Thigh	7.06	7.57	6.74	6.35	0.18	0.09
Ash						
Breast	5.23	5.75	5.26 5	.45	0.19	0.8
Thigh	5.21	5.24	4.56	4.4	0.16	0.14

[ab] Means within a row with different superscript letters are significantly different, (P<0.05);, SEM= Standard error of the mean, T1=0ml of neem and bitter leaf extract; T2=2ml of neem and bitter leaf infusion; T3=4ml of neem and bitter leaf extract; and T4=6ml of neem and bitter leaf extract

4. CONCLUSION

Oral administration of neem and bitter leaves extract increased broiler chick feed intake and weight gain, primarily due to improved health and digesting system, compared to control groups. The study found significant differences in giblet body organ weight among broiler chickens treated with a mixture of Azadirachta indica and Vernonia amygdalina leaves extract. Based on the finding, it can be concluded that neem and bitter leaves extract, when administered in a ratio

of 6 ml per liter of water, significantly improved feed intake, growth rate, body gain, and carcass weight of 6 ml mixtures of neem and bitter Leaves extract per one liter of water for broiler chicks has good performance on the feed intake, growth rate, average daily body gain, feed conversion ratio, and eviscerated carcass weight. The study recommends neem and bitter leaves extract mixed with drink water for broiler chick performance, but further research is needed to understand its effects on performance, hematological parameters, and meat sensory.

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