Effects of neem (*Azadirachta indica*) and bitter (*Vernonia amygdalina*) leaves infusion on intake, muscle chemical composition, sensory quality of meat and hematology of broilers

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Abstract

The effects of offering neem and bitter leaf extract orally to broiler chicks as a natural feed additive were investigated in terms of the hematological serum biochemical parameters, the percentage of chick mortality and chemical composition of the meat of the broiler chicks. A completely randomized design (CRD) was used to randomly assign 192-day-old Cobb 500 broiler chicks to four treatments, each with three replications. Daily records of feed offered and refused were made, and the difference between the two on a dry matter (DM) basis was used to compute dry matter intake (DMI). The water used for the treatment contained a mixture of neem and bitter leaves at a rate of 0 ml, 2 ml, 4 ml, and 6 ml per liter of water for T1, T2, T3, and T4, respectively. Commercial broilers diet that contained 22 and 18 % CP and 3050 and 3250 kcal/kg ME for starter and finisher phases. At the end of the trial, four broilers were chosen at random from each replication and slaughtered in order to assess the hematology and meat eating quality. The addition of extract to the broiler chicks' drink water improved their blood parameters. The daily dry matter intake of broiler chicks in T4 was significantly higher ($p < 0.05$) than that of the other treatment groups, and T4's total protein (TP) of blood was significantly ($p < 0.05$) higher than that of the other treatment groups. Additionally, there was a decrease in cholesterol ($p < 0.0001$) with an increased level of neem and bitter leaf extract mixture offered with drink water. It was concluded that 6 ml of a combination of neem and bitter leaf extract in a liter of drink water can be recommended as a good natural feed additive for commercial broiler production.

Keywords: Broiler; Feed additive; Hematology; Serum biochemistry.

Introduction

The use of medicinal plants predates the arrival of antibiotics and other contemporary medications in Africa (Akinyemi *et al*., 2005). Extracts from medicinal plants were used as natural antimicrobials in food (Hsieh and Mau, 2001). Numerous researchers have tried to use herbal substances as natural feed additives in poultry feed in place of antibiotic growth promoters (AGPs), which are illegal in many countries (Jang *et al*., 2008). Due to its many therapeutic benefits, including antibacterial, antifungal, hepatoprotective, antiviral, antiprotozoal, and antiparasitic qualities, neem has gained international recognition (Kale *et al*., 2003; Jawad *et al*., 2014). Furthermore, neem and bitter leaf extracts help birds grow and feed efficiently because of their antibacterial properties (Oleforuh-Okoleh *et al*., 2015). In addition, extracts from neem and bitter leaves have an immune-stimulating impact that stimulates the cell-mediated immune response, resulting in a better defense against any future threats posed by pathogens. Therefore, providing neem and bitter leaves to immunocompromised birds can enhance cell-mediated immune responses, ultimately boosting the humoral immune effect (Oleforuh-Okoleh *et al*., 2015). *Vernonia amygdalina* and *Azadirachta indica* leaves have varying concentrations of chemical, elemental, and macronutrient components. Beneficial nutrients and bioactive compounds can be found in abundance in them. The antioxidant properties of *Vernonia amygdalina* leaves may be better than those of *Azadirachta indica* leaves. Phytochemical concentrations in both plants are low (Offor, 2014). When it comes to combating infectious diseases and fostering the growth of chickens, antibiotics have proved essential. Scientific data indicates that widespread use of antibiotics has resulted in antibiotic resistance and residues in food and the environment, especially aquatic ecosystems, which can cause issues for public health (Mehdi *et al*., 2018). Anti-nutritional factors of neem and vernonia leaf are polyphenolics such as flavenoids and their glycosides, dihydrochalcone, coumarin and tannins, and aliphatic compounds (Biswas *et al*., 2002; Atangwho *et al*., 2012).

Some have expressed concern that the use of antibiotics for growth promotion and therapeutic purposes may cause human and animal microorganisms to become more resistant. The potential for antibiotic resistance development has led to restricted use of antimicrobials (Patrick *et al*., 2003). Continuous antibiotic feeding causes antibiotic residues to build up in chicken meat, which can be consumed by people (Muhammed *et al*., 2009). Antibiotic-related illnesses and deaths have dramatically increased as a result of the use of antibiotics in

farm animals (Newman, 2002). Many nations have shown interest in using medicinal plant extracts for a variety of purposes, including using as feed additives to boost production (Griggs and Jacob, 2005). Since consumers are aware of the residual effects of antibiotics in poultry meat, they demand drug-free food products.

This has led to the search for alternative natural growth enhancers, such as plants and their extracts. However, no work has been conducted with regard to the effects of mixtures of neem and bitter leaf infusion on blood parameters and quality of broiler meat. Therefore, this study was designed to evaluate the effects of neem and bitter leave extracts on dry matter intake, blood parameters, chemical composition and sensory evaluation of meat of broiler chicks.

Material and methods

Study area

The experiment was carried out at the poultry farm of Haramaya University, which is located 515 km east of Addis Ababa, the capital city of Ethiopia, at an elevation of 1980 meters above sea level, and at 42° 3' East longitude and 9° 26' North latitude. The average minimum and maximum temperatures in the region are 8.5 °C and 23.4 °C, respectively, while the average annual rainfall is 780 mm (Mishra *et al*., 2004).

Collection and preparation of leaves extract and ration

Five kilograms of neem leaf was collected from Dire Dawa, Ethiopia, and 5 kg of Bitter leaf was collected from Harar, Ethiopia. The samples were dried and ground. The obtained powder, 2 kg of each, was packed in a polyethylene bag and preserved in the feed storage room until used with water for broiler chicken. Then 30 g of each leaf (*Azadirachta indica* and *Vernonia amygdalina*) powder (a total of 60 g, a mixture of neem and bitter leaf at a 1:1 ratio) was added to one liter of distilled water, shaken and placed overnight at room temperature following the procedures of Mollah *et al.* (2012). The water leaf mixture was then filtered the next morning and the filtrate (the extract) was supplied to the experimental animals via drinking water that same day. Treatment 1 (T1) was the control group which was given water only, while T2, T3, and T4 were given 2 ml, 4 ml, and 6 ml of the neem and bitter leaf extract per liter of drinking water, respectively.

The starter and finisher broiler diets were purchased from Bishoftu Alema farm, Ethiopia. The starter phase was until 3 weeks (1 - 21 days) of age and the finisher phase was from the beginning of third week up to slaughtering (22 - 42 days).

Management of experimental animals

Two weeks before the experimental animals arrived, the house was thoroughly cleaned and sanitized, the house was thoroughly cleaned and sanitized. Before the trial started, the pens were sprayed against external parasites and the feeding and drinking troughs were completely cleaned, disinfected with labeled commercial disinfectant for use in chicken farms. The chicks were kept in a deep litter experimental house which was partitioned into pens by wire-mesh. There was a 250-watt heat bulb in every pen. For the experiment, a total of 192 day-old Cobb500 breed chicks were purchased from Bishoftu Alema Farm, Ethiopia. The chicks were vaccinated against Newcastle Disease on day 7 (HB1) by ocular route, and on day 21 (Lasota) through drinking water. They were also vaccinated for Gumboro on days 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 help them recover from stress of transportation and early age acclimatization problems, according to the manufacturer's recommendation.

Experimental design and treatments

A completely randomized design was used for the experiment. The 192 broiler chicks were grouped into 4 treatments and 3 replicates, with 16 chicks per replicate. Treatment 1 was the control and was given water only, and T2, T3, and T4 were given 2 ml, 4 ml, and 6 ml of neem and bitter leaf infusions per liter of drinking water, respectively. Similar commercial broiler diets and water were offered *ad libitum* throughout the experimental period.

Dry matter intake

The amount of feed offered and refused per pen was recorded daily. The amount of feed consumed was determined as the difference between the feed offered and refused on a DM basis.

Chick mortality

Daily monitoring of the birds was a routine activity to check their health status and record any deaths. Mortality was recorded as it occurred and determined for each treatment. Calculation of mortality percentage (MP) is expressed as follows:
Mortality percentage (MP) = (Number of dead chicks/Number of total chicks) * 100

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 to 1 mm size and 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 hours. Nitrogen (N) was determined according to the Kjeldahl procedure and crude protein content of the samples was calculated as N*6.25 (AOAC, 1995). Total lipid (ether extract) content of the muscles was 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 h.

Sensory evaluation of meat

The sensory parameters determined were juiciness, tenderness, flavor, and overall acceptance. Skinless breast and thigh muscle samples were frozen until they were ready to be cooked; the thawed parts were then chopped and cut into 2.5 cm cubes at room temperature. The flesh from the breasts and thighs was cooked in a pan with vegetable oil but no salt for 15 and 17 minutes, respectively. After cooking, the pieces were cooled to room temperature. The breast and thigh meats were evaluated following the sensory profile procedure (ISO, 2003). The panel consisted of 20 trained graduating classes of undergraduate and postgraduate students from the Food Science Department of Haramaya University. Panelists were instructed to chew and taste the meat, drink water, and rinse their mouths with bottled drinking water at room temperature between each sample, pausing for 20 seconds before tasting the next sample.

Hematological parameters and serum biochemical analysis

Four broilers were picked at random from each replicate of each treatment group at the end of the trial, and blood samples were collected from the brachial vein using a syringe into a tube containing ethylene diamine tetraacetic acid (EDTA), an anticoagulant, in order to evaluate the hematology parameters (hemoglobin, packed cell volume, total white blood cells, and red blood cell counts). Before centrifuging materials, the acid hematin method was used to estimate the amount of hemoglobin (Hb) using the steps outlined by Davice and Lewis (1991). Following Campbell's guidelines, blood-filled capillary tubes were spun 1200 rpm for five minutes in a centrifuge (Hitachi, EBA 20, Germany) to calculate packed cell volume (PCV), which was then recorded on a hematocrit reader (Campbell, 1995). Using a Neubauer hemocytometer, total white blood cell (WBC) and red blood cell (RBC) counts were determined according to Campbell's (1980) protocol. Blood samples were taken for serum biochemical analysis from the same birds that were killed for carcass measurements. Anticoagulant was not used when drawing blood samples for serum biochemical examination. After centrifuged for 15 minutes at 3,000 rpm, serum was collected and kept at -20 °C until analysis. The refractometer was used to measure the total protein in the serum (George, 2001).

Statistical analysis

Collected 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:

$$Xij = \mu+Ti+eij$$

Where Xij = any observation made in the experiment, μ = Overall mean, Ti = Effect of treatments, Eij = random error

Results

Dry matter intake

The effects of mixtures of neem (*Azadirachta indica*) and bitter (*Vernonia amygdalina*) leaf extracts on dry matter intake (DMI) of broilers during the starter (1-21 days), finisher phases (22-41 days), as well as the entire growth

period (1-41 days) are shown in Table 1. The inclusion of mixtures of neem and bitter leaf extracts at different levels improved DMI as compared to the control during the starter phase. Dry matter intake during the finisher phase was higher with an increasing level of mixtures of neem and bitter leaf extracts. Whereas, intake during the entire experiment is higher in T3 and T4 as compared to T1 and T2, and T1 intake was significantly ($p < 0.05$) lower than the supplemented groups.

Table 1. 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					
	T_1	T_2	T_3	T_4	SEM	*p*-value
Starter phase						
Total DMI (g)	1127.98^{c}	1260.26^{b}	1270.15^{ab}	1289.98^{a}	19.92	.0001
Daily DMI (g/bird/day)	53.73^{c}	60.00^{b}	60.47^{ab}	61.42^{a}	0.94	.0001
Finisher phase						
Total DMI (g)	3070.32^{c}	3403.69^{b}	3497.18^{b}	3624.97^{a}	64.64	.0009
Daily DMI (g/bird/day)	153.50^{c}	170.17^{b}	174.85^{b}	181.27^{a}	3.23	.0009
Entire period						
Total DMI (g)	4198.77^{c}	4663.95^{b}	4767.34^{ab}	4914.95^{a}	83.63	.0003
Daily DMI (g/bird/day)	102.40^{c}	113.74^{b}	116.27^{b}	119.87^{a}	2.03	.0003

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: 2 ml of neem and bitter leaves extract added to 1 l of water; T3: 4 ml of neem and bitter leaves extract added to 1 l of water; T4: 6 ml of neem and bitter leaves extract added to 1 l of water; DMI: dry matter intake.

Hematological serum biochemical parameters

Hematology and serum biochemical profile results in Table 2 show hemoglobin concentration (Hb), packed cell volume (PCV), and total red blood cell (RBC) count of the experimental birds were not statistically different ($p > 0.05$), however, there were 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. Total serum protein (2.91 g/dl) of the

birds in the control group (T1) was significantly lower compared with 3.71, 4.32, and 4.94 g/dl for T2, T3, and T4, respectively. In the present study, serum cholesterol concentration significantly decreased with increasing levels of neem and bitter leaf infusion. This study showed that the administration of neem and bitter leaf extract in drinking water significantly ($p < 0.05$) lowered the total white blood cell (WBC) counts.

Table 2. Effect of administering mixtures of neem and bitter leaves extract on some hematological and serum biochemistry of broiler chicks.

Parameters	Treatment					
	T_1	T_2	T_3	T_4	SEM	*p*-value
Red blood cell ($x10^6$/mm)	3.75	3.55	3.88	3.38	0.086	0.1811
White blood cell ($x10^3$/mm)	70.86^a	63.85^b	61.23^b	44.64^c	2.923	<0.0001
Hemoglobin (g/dl)	10.43	10.60	11.68	11.70	0.284	0.2394
Packed cell volume (%)	31.93	34.24	35.79	35.83	1.008	0.5388
Total protein (g/dl)	2.91^c	3.71^{ab}	4.32^{cb}	4.94^a	0.228	<0.0001
Serum cholesterol (mg/dl)	114.58^a	96.85^b	88.35^c	79.48^d	4.009	<0.0001

ab: means within a row with different superscript letters are significantly different, p <0.0; SEM: standard error of the mean, T1: 0 ml of neem and bitter leaf extract; T2: 2 ml of neem and bitter leaf extract; T3: 4 ml of neem and bitter leaf extract; T4: 6 ml of neem and bitter leaf extract

Chick mortality percentage

Mortality rate denotes the number of chickens died from a flock of a certain number of chickens. Percent mortality of broilers in the entire experimental period was 8.33, 6.25, 6.25, and 4.16 for T1, T2, T3, and T4, respectively. The difference in mortality percentage among the treatment groups was not significant ($p > 0.05$).

Table 3. 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					
	T_1	T_2	T_3	T_4	SEM	*p*-value
Mortality at starter Phase	4.16	2.08	4.16	0.00	0.92	0.36
Mortality at finisher Phase	4.16	4.16	2.08	4.16	0.92	0.86
Mortality during 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: 0 ml of neem and bitter leaf extract; T2: 2 ml of neem and bitter leaf extract; T3: 4 ml of neem and bitter leaf extract; T4: 6 ml of neem and bitter leaf extract

Sensory evaluation of meat

The panelist members did not find differences in the sensory attributes of the breast and the thigh meat (Table 4), which shows that the mixtures of neem and bitter leaf extract did not have a significant ($p < 0.05$) effect on the juiciness, tenderness, flavor, and overall acceptance of the meat from the breast and thigh of the broiler chickens.

Table 4. Effect of administering mixtures of neem and bitter leaves extract on meat sensory characteristics of broilers.

Parameters	Treatments					
	T_1	T_2	T_3	T_4	SEM	*p*-value
Juiciness						
Breast	4.10	3.5	4.00	4.00	0.09	0.08
Thigh	4.00	4.00	3.95	4.58	0.12	0.19
Tenderness						
Breast	4.00	3.50	3.96	3.90	0.09	0.15
Thigh	3.80	3.95	4.61	4.21	0.13	0.12
Flavor						
Breast	3.66	3.97	3.97	3.58	0.10	0.42
Thigh	3.91	3.95	4.20	4.43	0.09	0.22
Overall acceptance						
Breast	3.92	3.65	3.98	3.82	0.06	0.21
Thigh	3.90	3.96	4.25	4.41	0.08	0.06

[ab]: means within a row with different superscript letters are significantly different, $p < 0.05$; SEM: standard error of the mean, T_1: 0 ml of neem and bitter leaf extract; T_2: 2 ml of neem and bitter leaf extract; T_3:4 ml of neem and bitter leaf extract; T_4: 6 ml of neem and bitter leaf extract

Chemical composition of meat

The laboratory results in Table 5 showed no significant ($p > 0.05$) difference in proximate composition of the breast and thigh meat in CP % between the control (T1) and experimental groups (T2, T3, and T4).

Table 5. 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	75.24	75.54	75.67	75.65		
Breast					0.11	0.6
Thigh	73.98	73.82	73.27	73.51	0.12	0.16
Crude protein						
Breast	21.84^{b}	21.33^{b}	20.92^{b}	24.34^{a}	0.45	<.005
Thigh	23.68	21.83^{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: 0 ml of neem and bitter leaf extract; T2: 2 ml of neem and bitter leaf infusion; T3: 4 ml of neem and bitter leaf extract; T4: 6 ml of neem and bitter leaf extract

Discussion

The results obtained for dry matter intake were in agreement with Singh *et al.* (2015), who reported that a group of broilers that were fed neem leaf powder at a rate of 1, 2, and 3 g per kg of feed had higher feed intake. Conversely, Oleforuh-Okoleh *et al.* (2015) reported a group of birds treated with levels of *Vernonia amygdalina* leaf extract at 25 ml, 50 ml, and 75 ml per liter of drinking water showed no significant difference in daily feed intake. The high DMI at higher levels of infusion extract of neem and bitter leaves could be related to the beneficial effect of bioactive compounds in neem and bitter leaf, which enhance gastro-intestinal enzyme secretion, thereby improving digestion, and

could cause increased utilization of nutrients (Ezeonu *et al*., 2012; Oleforuh-Okoleh *et al*., 2015).

This finding concurs with the report by Oleforuh-Okoleh *et al*. (2015) which indicates administration of bitter leaf extract for broiler shows higher total protein levels than control group. A similar result was reported by Shihab *et al.* (2017) which showed that addition of neem leaf powder at a rate of 1, 2, and 3 g/kg in broiler diets significantly ($p < 0.05$) increased total protein value.
The decrease in cholesterol content with increased level of neem and bitter leaf extract mixture offered with drinking water is in agreement with the study by Shihab *et al*. (2017) who reported inclusion of neem leaf powder at a rate of 2 g/kg to the diet decreased cholesterol content (107.54 mg/dl) compared to control diet (150.71 mg/dl). *Vernonia amygdalina* leaf extract elicited beneficial effects by lowering the levels of cholesterol in the broiler chickens, and this result is also in agreement with the observation by Owen *et al.* (2011) who reported that addition of *Vernonia amygdalina* leaf up to 15 % decreased cholesterol content of blood of broiler chicken compared to the controls. Similarly, Ojiako and Nwanjo (2006) documented that administration of bitter leaf significantly reduced the cholesterol level of streptozotocin-diabetic rats.

Similar to the observation of this study, Bonsu *et al.* (2012) reported that birds fed diets that contained neem leaf meal (NLM) at 2%, 1.5%, and 2.5% levels had significantly lower WBC count 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 the absence of potential disease threats before the body's system could be stimulated to produce WBC (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 a significant decrease in total WBC count in the treated groups compared to the negative control. The values obtained for all hematological parameters in this study were within the normal range (Douglas *et al*., 2010).

Administering a combination of neem and bitter leaf extract in drinking water decreased mortality. This can be related to the findings of Nodu *et al*. (2016), who implied that up to 5 g of ground neem extract per one liter of drinking water for a broiler has the potential to stimulate the best growth rate and decrease mortality percentage. Low percentage mortality was observed in treatments where neem extract was administered. There was also a similar result

from Bonsu *et al.* (2012), which suggests that the neem leaf meal inclusion in broiler ration reduced chicken mortality.

The results of the sensory evaluation of this study are comparable to the findings of Bonsu *et al.* (2012), who suggested that meat from broilers fed on up to 2.5 % neem diets were acceptable for sensory attributes.

Significant variations in the CP content of meat made it abundantly evident that blood protein content and muscle protein content are directly related. Ihsan (2017) reported similar findings, indicating that broiler diets supplemented with neem powder had a significantly ($p < 0.05$) higher CP value. The broilers' breast and thigh meat moisture, ether extract, and ash content remained unaffected by the treatments. The chemical compositions of breast and thigh muscles recorded in the present trial were within the range reported for broiler meat (Abdullah *et al*., 2010).

The limitations of the study include that the bioactive compounds content of the leaves was not analyzed and the effect of higher levels of the extracts was not studied.

Conclusion

The present study demonstrated that supplying 6 ml of a combined neem and bitter leaf extract per liter of drinking water to broiler chicks can serve as a natural feed additive and may be used in commercial broiler production. Further research should be conducted to determine the effects of higher levels of inclusion of the extracts.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper. The study was conducted independently, with funding provided by the university. No financial or personal relationships influenced the research, results, or interpretation of the data. All materials, equipment, and resources were obtained through standard academic and research channels without any additional sponsorship or funding from entities that could benefit from the study's findings.

Ethical consideration

The research was carried out as a part of project funded by Haramaya University after the approval of competent authority of the director of research and vice president of research, Haramaya University, Ethiopia and it is acceptable from ethical perspective, originality and technical competence points of view. Hence the project is allowed to be performed provided that all procedures and conditions stipulated in the proposal are respected and the project activities open for occasional supervision by the school researcher sub-thematic leader whenever this is deemed necessary.

References

Abdullah, Y.A., Al-Beitawi, N.A., Rjoup, M.M.S., Qudsieh, R.I. and Ishmais, M.A.A. 2010. Growth performance, carcass and meat quality characteristics of different commercial crosses of broiler strains of chicken. *Poult. Sci*., 47, 13-21.

Akinyemi, K.O., Oladapo, O., Okwara, C.E., Ibe C.C. and Fasure, K.A., 2005. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. *J. Complement. Altern. Med*., 5, 5-11.

AOAC, 1995. Association of Official Analytical Chemists. Official Methods of Analysis of AOAC International (16th Edition). Virginia. USA.

Atangwho, I.J., Edet, E.E., Uti, D.E., Obi, A.U., Asmawi, M.Z. and Ahmad, M., 2012. Biochemical and histological impact of *Vernonia amygdalina* supplemented diet in obese rats. *Saudi J. Biol. Sci.,* 19, 385–392.

Biswas, K., Chattopadhyay, K.I., Banerjee, R.K. and Bandyopadhyay, U., 2002. Biological activities and medicinal properties of neem (*Azadirachta indica*). *J. Curr. Sci.,* 82(11), 1336-1345.

Bonsu, F.R.K., Kagya-Agyemang, J.K., Kwenin, W.K.J. and Zanu, H.K., 2012. Medicinal response of broiler chickens to diets containing neem *(Azadirachta indica*) leaf meal, hematology and meat sensory analysis. *World Appl. Sci. J*., 19, 800-805.

Campbell, M.J., Wagner, M.F., Scott, M.P. and Brown, D.G., 1980. Sequential immunological studies on an asbestos exposed population. II. Affecting lymphocyte function. *Clin. Exp. Immunol*., 39, 176-182.

Campbell, T.W., 1995. Avian haematology and cytology. In: Veterinary Clinical Pathology. 3rd ed., Iowa State University Press, Ames, Iowa, pp 104.

Davice, J.U. and Lewis, S.M., 1991. Practical Hematology (8th edition). Longman Ltd London, pp. 22-48.

Douglas, J., Weiss, K. and Wardrop, J., 2010. Veternary Hemathology. 6th Edn., Blackwell Publishing Ltd.

Ezeonu, I.M., Akobueze, E.U. and Chah, K.F., 2012. Prebiotic effects of *Vernonia amygdalina* and *Ocimum gratissimum* aqueous leaf extracts in rabbit (*Oryctolagus cuniculus). Afr. J. Biotechnol*., 11, 2537-2547.

George, J.W., 2001. The usefulness and limitations of hand-held refractometers in veterinary laboratory medicine: an historical and technical review. *Vet. Clin. Pathol*., 30, 201-210.

Gotep, J.G., Tanko, J.T., Forcados, G.E., Muraina, I.A., Ozele, N., Dogonyaro, B.B., *et al*., 2016. Therapeutic and safety evaluation of combined aqueous extracts of *Azadirachta indica* and *Khaya senegalensis* in chickens experimentally infected with *Eimeria* oocysts. *J. Parasitol. Res*., 1, 4692424. https://doi.org/10.1155/2016/4692424.

Griggs, J.P. and Jacob, J.P., 2005. Alternatives to antibiotics for organic poultry production. *J. Appl. Poult. Res*., 14, 750-756.

Hsieh, P.C. and Mau, J.L., 2001. Antimicrobial effect of various combinations of plant extracts. *Food Microbiol*., 18, 35-43.

Ihsan, M.S., 2017. Impact of supplementation Neem powder (Azadirachta indica) to diet broiler in immunological, physiological and productive traits. *Adv. Environ. Biol*., 11(3), 44-51.

ISO (International Standards Organization), 2003 ISO 13299. General Guidance for Establishing a Sensory Profile. ISO, Geneva, Switzerland.

Jang, A., Liu, X.D., Shin, M.H., Lee, B.D., Lee S.K. and Lee, J.H., 2008. Anti-oxidative potential of raw breast meat from broiler chicks fed a dietary medicinal herb extract mixture. *J. Poult. Sci*., 87, 2382-2389.

Jawad, Z.M., Younus, M.U., Rehman, R., Munir, A., Maqbool, W., Shahzad S.M., *et al*., 2014. Effect of *Azadirachta indica* on the hepato-renal functions in broilers chickens. *J. Anim. Plant Sci*., 24, 1012-1018.

Kale, B.P., Kothekar, M.A., Tayade, H.P., Jaju, J.B. and Mateenuddin, M., 2003. Effect of aqueous extract of *Azadirachta indica* leaves on hepatotoxicity induced by antitubercular drugs in rats. *Indian J. Pharmacol*., 35, 177-180.

Mehdi, Y., Létourneau-Montminy, M.P., Gaucher, M.L., Chorfi, Y., Suresh, G., Rouissi, T., *et al*., 2018. Use of antibiotics in broiler production: global impacts and alternatives. *Anim. Nutr.*, 4(2), 170-178.

Mishra, B.B., Kidan, H.G., Kibret, K., Hassen, M. and Eshetu, B., 2004. Soil and land resource inventory at Haramaya University research farm with reference to land evaluation for sustainable agricultural management and production. Synthesis of working papers, Soil Science. Bulletin No. 1, 123p. Alemaya University, Dire Dawa, Ethiopia.

Mollah, M.R., Rahman, M.M., Akter F. and Mostofa, M., 2012. Effects of nishyinda, black pepper and cinamon extract as growth promoter in broiler. *Bangladesh Vet*., 29, 69-77.

Muhammed, F., Aktar, M., Rahman, Z.U., Javedand I. and Anwar, M.I., 2009. Role of veterinarians in providing Residue-free veterinary food. *Pak. Vet. J*., 29, 42-46.

Newman, M.G., 2002. Antibiotic resistance is a reality: novel techniques for overcoming antibiotic resistance when using new growth promoters. Nutrition Biotechnology in the Feed and Food Industries. Proceeding of Alltech's 18th Annual Symposium. Nottingham University Press 98-106.

Nodu, M.B., Okpeku, M., Akpoveta, Z.A. and Iroegbu, D.O., 2016**.** Evaluation of *Azadirachta indica* leaves extract on hematology and biochemical profiles, organs weight and growth parameters of broiler chickens. *Int. J. Agric. Environ. Biotechnol*., 32, 1879-1884.

Offor, C.E., 2014. Comparative chemical analyses of *Vernonia amygdalina* and *Azadirachta indica* leaves. *Int. J. Pharm. Biol. Sci*., 9, 73-77.

Ojiako, A. and Nwanjo, U., 2006. Is *Vernonia amygdalina* hepatotoxic or hepatoprotective? Response from biochemical and toxicity studies in rats. *Afr. J. Biotechnol*., 5, 1648-1651.

Oleforuh-Okoleh, V.U., Olorunleke, S.O. and Nte, I.J., 2015. Comparative response of bitter leaf (*Vernonia amygdalina*) infusion administration on performance, haematology and serum biochemistry of broiler chicks. *Asian J. Anim. Sci*., 9(5), 217-224.

Owen, O.J., Amakiri, A.O. and Karibi-Botoye, T.A., 2011. Lipid-lowering effects of bitter leaf (*Vernonia amygdalina*) in broiler chickens fed finishers' mash. *AAAS Annu. Meet. Sci. Innov. Expo*., 2, 1038-1041.

Patrick, B., Devriese, L.A. and Haesebrouck, F., 2003. Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on gram-positive bacteria. *J. Clin. Microbiol*., 16, 175-188.

SAS, 2002. SAS User's Guide. Statistics. SAS Institute, Inc., Cary, NC. USA.

Shihab, I.M., AL-Zuhariy, M.T., Abdullah, S.M. and Mutar, S.S., 2017. Impact of supplementation Neem powder (*Azadirachta indica*) to diet broiler in immunological, physiological and productive traits. *Adv. Environ. Biol*., 11(3), 44-51.

Singh, M.K., Singh, S.K., Sharma, R.K., Singh, B., Kumar, S., Joshi, S.K., *et al*., 2015. Performance and carcass characteristics of guinea fowl fed on dietary Neem (*Azadirachta indica*) leaf powder as a growth promoter. *Iran. J. Vet. Res.,* 16(1)*, 78-82.*

Zanu, H., Kagya-Agyemang, J.K., Kwenin, K.J., Bonsu, F., Antwi, E. and Ateni, S., 2011. Physiological response of broiler chickens to neem (*Azadirachta indica*) and akakapenpen (*Rauvolfia vomitoria*) decoctions: performance and carcass characteristics. *Int. J. Poult. Sci*., 10(9), 730-733.