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Effect of ginger, garlic and negro pepper on gut microbes, gut histomorphometry and pathological assessment of selected organs of broiler chickens

[1]Shittu M. D., [2]Alagbe, O. J., [3]Alaba, O. [1]Okanlawon, E. O. [1]Adelakun, F. A., [1]Emmanuel, P. O. and [3]Adejumo, D. O.

[1]*Dept. of Animal Production and Health, Ladoke Akintola University of Technology (LAUTECH) Ogbomoso, Nigeria.*
[2]*Department of Animal Nutrition and Biochemistry Sumitra Research Institute, Gujarat, India*
[3]*Dept. of Animal Science, University of Ibadan, Ibadan.*

***Corresponding Author:** mdshittu@lautech.edu.ng or shittumdaniel@gmail.com **Phone Number:** +2348032507915*

Abstract
This study was conducted to determine the gut responses of broiler chickens fed Xylopia, ginger and garlic and their effect in controlling population of gut microbes of broiler chickens. A total of 240 Ross 308-day-old chicks were used. There were four treatments which were replicated four times. Each replicate had 15 birds. The experiment was arranged in a completely randomized design (CRD). Starter and finisher Control (T1) diets without Xylopia, ginger and garlic were formulated. T2 –T4 had 10g each of Xylopia, ginger and garlic respectively in 1000g of feed. The diets and water were offered to the birds ad libitum. At the end of the 8th week, two birds per replicate with representative weight were randomly selected, slaughtered and gastrointestinal tracts were removed. Five centimeter (5cm) cut of the jejunum of 3 randomly selected chickens per treatment were submerged inside 10% formalin and slide preparation was done. Also, the digesta from three selected sections of the gut were kept in sterile bottle, labelled and sent to reputable microbiology laboratory for microbial analysis. Data collected were analysed using SPSS version 21 and significant means were separated using Duncan's Multiple Range Test of the same statistical package. There was reduction in the population of Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter aerogens, Escherichia coli, Staphylococcus aureus in the birds fed the phtyo-additives compared to the control. The result obtained for gut morphometry of this experiment showed that garlic performed better by recording higher gut morphometry than other treatments as the gut length and gut weight of the experimental birds increased when fed garlic additives. The result of histomophometric characteristics shows significant different ($P<0.05$) across the treatments and garlic fed chickens and control had similar villi height (4401.67µ and 4171.62µ respectively) gut weight was similar across the four treatment groups except jejunum and ilium with highest value under the garlic fed chickens. The intestine histology revealed normal histo-archietecture, compact mucosa crypt and increased surface area in diets 2 and 4. Similarly, the liver histology revealed a fine histoachitecture throughout the treatment groups. This study concluded that the inclusion of Negro pepper, garlic and ginger in broilers diet improved gut ecosystem, intestine histomophometry without fear of toxicity.

Keywords: Phyto-additives, Broiler chickens, Gut morphology and histology

Introduction
Poultry production especially the broiler has potential to attain expected weight at specified number of days when optimal feed intake throughout the growing period is ensured. The optimality in feed

intake is linked with several factors such as quantity and quality of feed, type of feed, physical form of feed and proper management system (Sogunle *et al*., 2014). Likewise, the success of poultry venture depends on the birds having good health, nutrition and good management (Oluyemi and Roberts, 2000). This could be achieved by the uses of quality feed ingredients and additives. Environmental stress such as heat exposure and coccidiosis has been regarded as one of the major factors negatively affecting performance of birds in the intensive poultry industry particularly in the tropics and as a main factor in the pathogenesis of several serious diseases (Dalloul and Lillehoj, 2006). This gave rise to the supplementation of poultry feed with synthetic antioxidants (e.g., α-tocopheryl or butylated hydroxytoluene) to mitigate the oxidative stress and feed additive which is assumed to supply many micronutrients that may not be available in other ingredients involved in the feed formulation.

Feed additive are important materials that can improve the efficiency of feed utilization and animal performance. With the ban of antibiotics use in animal nutrition due to the emergence of microbe resistance, alternative growth promoters must be found. Removal of antibiotics as growth promoters has led to animal performance problems and a rise in the incidence of certain animal diseases. Therefore, the uses of plant parts as natural additives are gaining increasing interest because of the global trend of restriction in use of synthetic substances (Ahn and Fernandom, 2002). The possibility of using these natural alternative additives instead of antibiotics and hormone in animal diets has been extensively researched for the past three decades. Some plants, containing various secondary metabolites, have been used as alternative remedies by some researchers (Ceylan *et al*., 2003). Study have indicated that various plants extracts can improve feed conversion ratio, improve carcass quality, decrease the market age of broiler and reduce their rearing cost (Muhammad *et al*., 2009) which can come because of cost explored on drugs especially antibiotics.

A considerable number of studies have documented that herbs, spices and various plant extracts have digestion-stimulating and antimicrobial effects (Amad *et al*., 2011, Khan *et al*., 2012). For improvement of animal health and wellbeing, apart from an important role of spices in daily human nutrition, spices have also been efficiently used in animal nutrition. Plant-derived additives used in animal nutrition to improve performance are called “phytogenic feed additives”. This form of feed additives has recently become substance use in poultry production since the ban of in-feed antibiotics growth promoters in 2006. Herbs and spices stimulate feed intake by the secretion of endogenous enzymes, antibacterial effect and antioxidant potential (Lee *et al*., 2015), resulting in enhanced absorption of nutrients from the gut (Tehseen *et al*., 2016). Such natural feed additives have been reported to exert a wide range of beneficial effects on the production performance in broilers with respect to weight gain, feed conversion and meat quality (Aji *et al*., 2011). Example of these phyto-additives are garlic (*Allium sativum*), ginger (*Zingiber officinale*), negro pepper (*Xylopia aethiopica*) etc.

Garlic (*Allium sativum*) is widely distributed and used in all parts of the world as a spice and herbal medicine for the prevention and treatment of a variety of diseases, ranging from infections to heart diseases. Garlic is thought to have various pharmacologic properties. For example, it has been found to lower serum and liver cholesterol, inhibit platelet aggregation, inhibit bacterial growth and reduce oxidative stress. Garlic (Tollba and Hassan, 2003) is found as a natural feed additive to improved broilers growth, feed conversion ratio (FCR), and decreased mortality rate. Demir *et al.*, (2003) indicated that there was a reduction in crypt depth in the ileum of broilers given dietary natural growth promoters such as garlic.

Ginger (*Zingiber officinale*) rhizome (ginger root) is another widely used spice or condiment and medical treatment for many diseases (Arshad *et al*., 2014). Ginger may act as a pro-nutrient because of the vast active ingredients it has been reported to contain such as gingerol, gingerdione and gingerdiol that also possess strong antioxidant activity. Herbs Hands Healing Ltd (2011) reported that ginger contains a protein digesting enzyme (Zingibain- ginger protease) which is believed to improve digestion as well as kill parasites and their eggs. Piperine (1-piperoy-1-piperidine) is a major alkaloid component of ginger and is responsible for its biting taste. Among its chemical-biological activities, piperine exhibits antimicrobial, anti-inflammatory and antioxidant properties.

Negro pepper is a tree which can be 20 meters tall. The seeds and fruits are used to improve efficiency and increasing overall performance of animal. It has pharmaceutical and medicinal properties which include antibiotics, enzymes, hormones and according to (Isikwenu and Udomah, 2015), because of its phytochemical contents such as essential oils, phenols, flavonoids and carotenoids, the spice is used in diets for nursing mothers to stabilize the gastrointestinal tract (Omodamiro *et al*., 2012). Recently experience gathered on its use in human nutrition is being applied in monogastric animals due to its antioxidant and anti-microbial activities. Its positive effect on human could qualify it as an alternative growth promoter to synthetic antibiotics in broiler diets, because human being and monogastric animals have similar digestive system (Omodamiro *et al*., 2012). It could be used to improve growth performance and reduce bacterial load in the gut of broiler chickens (Isikwenu and Udomah, 2015), therefore making healthy intestine.

The small intestine is the long muscular tube of the gastrointestinal tract that is the major site for absorption and digestion of nutrients and drugs. It is made up of three structural segments: duodenum, jejunum and ileum. All the absorptive and a part of the digestive capacity of the small intestine occur around and near villi and crypts (Isikwenu *et al*., 2001). The crypt-villus is the main functional unit of absorption in the broiler small intestine. Various substances and different physiological or pathological conditions may change the proliferation rate of enterocytes in the crypt and affect their migration rate to the top of the villi (Kitt *et al*., 2001). The relationship between small intestine and nutritional factors is very complicated. Nutrients may directly cause changes or adaptations in the intestinal mucosa (Kelly, 2006). Histomorphometry of gut and liver analysis are greatly used in studies about pathophysiology. The availability of animal models and using morphometric analysis as a quantitative assessment have facilitated evaluating the morphological alterations of intestinal mucosa under various experimental conditions such as special diets and drugs (Gulbinowicz *et al*., 2004).

The gut as a powerful component of animal that help to digest feed can respond directly to different phyto-additive, therefore, this study was design to determine the gut histomorphometry and pathology of broilers chickens fed three different phyto-additives (Ginger, Garlic and Negro pepper).

Materials and Methods

Experimental Site

The study was carried out at the Poultry Unit, Ladoke Akintola University of Technology (LAUTECH) Teaching and Research Farm, Ogbomoso, it lies on $8^0 10^1$ North of the equator and longitude $4^0 10^1$ East of the Greenwich Meridian within the Derived Savannah region of Nigeria. The altitude is between 300m and 600m above sea level while the mean temperature and rainfall are 27°C and 1247mm respectively (Ayinla and Odetoye, 2015).

Procurement of Test Ingredients, Processing and Preparation of Experimental Diets

The Ginger, Garlic and Negro pepper were procured from a reputable spice market, they were peeled, cleaning, dried and ground to form meals. The dietary treatment are T1 which consist of feed with zero addition of the three additives and serve as control, T2 diet with 10g of Garlic powder per 1000g of feed, T3 diet with 10g of ginger powder per 1000g of feed and T4 diet with 10g of Negro pepper per 1000g feed. The broilers were fed on *ad libitum* bases on the same feed throughout the experiment except the additive that was added differently (Table 1)

Table 1: Nutrient composition of broiler starter and finisher diets

Ingredients	Starter Diet	Finisher diet
Maize	45.00	52.00
Soybean	28.00	22.00
Wheat offal	10.00	15.00
Corn bran	10.20	4.70
Bone meal	3.00	2.50
Fish Meal	1.50	1.00
Lime stone	1.50	1.50
Salt	0.25	0.25
Lysine	0.12	0.12
Methionine	0.18	0.18
*Premix	0.25	0.25
Total	100.00	100.00
Protein (%)	23.22	20.13
Energy (ME Kcal/Kg	2837.20	2858.38
Calcium	1.18	1.06
Phosphorous	0.70	0.58

Experimental Units and Management

A total of 240 Ross 308 strain day old broiler chicks were randomly allocated to four dietary treatments with 4 replicate which contains 15 chicks in a completely randomized design for 8 weeks. On arrival, the chicks were kept in brooding house for 21 days under a perfect brooding condition, they were given anti-stress (Livestockvit) and all necessary vaccination as instructed by the veterinary personnel on the farm. The birds were allowed to receive feed and water *ad libitum* every day for the period of 8 weeks.

Data Collection

Gut morphometry: At the end of the 8th week, three birds per replicate with representative equal weight were randomly selected for morphological analysis. The randomly selected birds were slaughtered, the gastrointestinal tracts were removed and the parameters such as oesophagus, small intestine (duodenum, jejunum and ileum), caecum and large intestine were measured using a tape rule and weighed using a sensitive scale including the proventriculus and gizzard.

Gut Histomorphometry and Gut and Liver Histopathology

According to Yusuf *et al*. (2014) *Xylopia aethiopica* phytochemical analysis showed that the sample contained tannins (4.96%), flavonoids (0.81%), saponins (2.93%) and alkaloids (1.24%), this result has prompted the pathological assessment of the liver and gut. At the end of the feeding trial, five centimetre (5cm) cut of the small intestine (also small cut from the liver) of 3 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 both intestine and liver in order to observed the slide under AmScope USB enabled microscope at x40 magnification for histopathological alteration of gut and liver while histomorphometry characteristic of gut was also measured in micrometre.

Microbial analysis methodology

Collection of digesta: At the 8th week, three birds were randomly selected from each dietary treatment and moved to the battery cage. They were slaughtered to harvest digesta aseptically from the gizzard, duodenum, jejunum and caecum into sterile bottles.

Preparation of the culture media: The Agar used were prepared according to company specifications (HIMEDIA agar) and stirred thoroughly with a stirring rod until it dissolves completely to form a solution. Each agar solution was discharged into 70 test tubes each, using a pipette. The mouth was covered tightly with cotton wool. The test tubes were placed in beakers and put in the autoclave machine to be sterilized at 121°c for 15 minutes and left to cool. Also, each agar was then dispensed into petri dishes inside the inoculating hood and left to solidify.

Inoculation of the Microbes: The inoculating hood was sterilized with ethanol, two flame sources generated from ethanol were placed on opposite ends to keep external microorganisms away. The test tubes containing each agar solution were lifted out of the autoclave machine and placed inside the inoculating hood, in between the flame sources. Nicrome loop holder was sterilized with the flame source and used to pick digesta from the sterile bottles into the test tubes for each agar, the mouth of the test tubes was sterilized with the flame source and covered with cotton wool. This process was repeated for digesta from the gizzard, duodenum, jejunum and caecum of all the treatments. The Tryptone soya broth which was inoculated was then subjected to serial dilution. The nichrome loop holder was sterilized with flame source, dipped in the serial diluted solution and smeared on the petri dish containing each agar to identify available microbes in the digesta, using a procedure called streaking. The petri dishes were placed in the incubator at 37°c for 48 hours. The cultures were observed for obvious growth characteristics that could be useful in analysing the specimen content.

Microbial identification and count

Microbes were identified using differential stain according to Chessbrough

(1985). Gram-positive microbes stain purple, Gram-negative microbes stain pink. Microbial count was taken at the 24th and 48th hour respectively. This procedure was repeated for Nutrient agar, McConkey agar, Centrimide agar and Mannitol agar, and their respective microbial count at 24 and 48 hours were taken.

Statistical Analysis: Data collected were analysed by one way analysis of variance (ANOVA) using SPSS version 21 computer software and significant means were separated using Duncan's Multiple Range Test of the same statistical package.

Result And Discussion

Results: Table 2 shows the microbial load (cfug^{-1}) in the gizzard, duodenum, jejunum and caecum of broiler chickens fed three selected phyto-additives. There were significant differences ($p<0.05$) in the population of *Enterobacter aerogenes, Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* present in the gizzard, duodenum, jejunum and caecum across all treatments.

In the gizzard, *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* had highest population in birds fed control diet (820.25cfug^{-1}, 141.75 cfug^{-1}, 1.25 cfug^{-1} respectively) and were reduced in birds fed xylopia (415.50 cfug^{-1}, 36.75 cfug^{-1}, 0.00 cfug^{-1}), ginger (1.25 cfug^{-1}, 113.50 cfug^{-1}, 0.00 cfug^{-1}) and garlic (265.50 cfug^{-1}, 5.25 cfug^{-1}, 0.00 cfug^{-1} respectively). Likewise, in the duodenum, *Enterobacter aerogens, Escherichia coli* and *Staphylococcus aureus* had the highest population in birds fed control (30.75 cfug^{-1}, 105.25 cfug^{-1}, 283.25 cfug^{-1}) and has lower quantity in birds fed xylopia (23.00 cfug^{-1}, 18.75 cfug^{-1}, 4.50 cfug^{-1}), ginger (0.00 cfug^{-1}, 49.00 cfug^{-1}, 15.25 cfug^{-1}) and garlic (1.50 cfug^{-1}, 24.00 cfug^{-1}, 23.25 cfug^{-1}). In the jejunum also, *Enterobacter aerogens, Escherichia coli* and *Staphylococcus aureus* has the highest quantity in birds fed control (3.00 cfug^{-1}, 3.50 cfug^{-1}, 521.00 cfug^{-1}) and lowest in birds fed xylopia (1.25 cfug^{-1}, 0.00 cfug^{-1}, 4.00 cfug^{-1}), they were also reduced in birds fed ginger (1.50 cfug^{-1}, 2.00 cfug^{-1}, 203.75 cfug^{-1}) and garlic (1.50 cfug^{-1}, 1.50 cfug^{-1}, 421.75 cfug^{-1}). In the caecum, *Enterobacter aerogens, Escherichia coli* and *Staphylococus aureus* has the highest quantity in birds fed control (40.00 cfug^{-1}, 146.00 cfug^{-1}, 20.25 cfug^{-1}) and lowest quantity in birds fed xylopia (0.00 cfug^{-1}, 6.00 cfug^{-1}, 4.75 cfug^{-1}).

The results obtained from this study showed that there were significant differences in the population of *Enterobacter aerogens, Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* in the GIT (gastrointestinal tract). In the GIT, bacteria participate in metabolism by nutrient absorption, hydrolyze polysaccharides to produce short-chain fatty acids, which will be absorbed and participate in important metabolic pathways in the supply of carbon and energy sources to birds. Despite the benefits of the microbiota in producing a stable intestinal environment, in unfavourable situations, they can act as pathogens, produce toxic metabolites and impair the productive performance of birds. In the gizzard, the population of *Escherichia coli* was the highest in T1 and were reduced in T2. This could be due to the active ingredients in xylopia which possess antimicrobial properties. This is in consonance with the earlier reports of Ilusanya *et al*., (2012), who reported that the reduction in microbial population of birds fed xylopia may be attributed to the presence of several compounds in the fruits (flavoniods, sterols, saponins, tannins, phlobatannins and cyanogenetic glycosides) which have biological activities such as antioxidant, antimicrobial and pharmacological effects. Flavonoids are hydroxylated phenolic substances synthesized by plants in response to microbial infection and they have been known to be antimicrobial substances against a wide range of microorganisms in vitro. The population of *Escherichia coli* was also reduced in T3 and T4. Report of Tekeli, *et al*. (2011) shows that ginger in the diets stimulate lactic acid bacteria and decreases pathogenic bacteria such as mesophilic aerobic, coliform and *Escherichia coli*.

Staphylococcus aureus had the highest population in T1, but was reduced in T2, T3 and T4. This is in tandem with the report of Tatsadjieu *et al*. (2003) who reported that xylopic acid and four other isolates from the ripe fruits of *Xylopia aethiopica*, have been examined for antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*., (Sudrashan *et al*., 2010) also obtained a significant reduction in the bacteria counts of *Staphylococcus aureus* and *Escherichia coli* when essential oil isolated from ginger was used as a decontaminating agent in chicken meat. Also, the population of *Staphylococcus aureus* was the lowest in the birds fed T4, this could imply that the active ingredient in garlic has a greater effect on *Staphylococcus aureus* in the gizzard of broiler chickens. *Pseudomonas aeruginosa* which was present in the control group was completely absent in T2, T3 and T4, which shows that xylopia, ginger and garlic has antibacterial effect and can be used to control the population of *Pseudomonas aeruginosa*.

Table 2: Gizzard, Duodenum, Jejunum and Caecum Microbial Load (cfug^{-1}) of Broiler Chickens Fed Three Selected Phyto-additives

Treatments	Site	Control T1	Xylopia (1%) T2	Ginger (1%) T3	Garlic (1%) T4	SEM
Ea		13.25^{b}	107.25^{a}	0.50^{c}	4.00^{c}	11.41
Ec	Gizzard	820.25^{a}	415.50^{b}	1.25^{d}	265.50^{c}	77.08
Sa		141.75^{a}	36.75^{c}	113.50^{b}	5.25^{d}	14.39
Pa		1.25^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.15
Ea		30.75^{a}	23.00^{b}	0.00^{d}	1.50^{c}	3.45
Ec	Duodenum	105.25^{a}	18.75^{c}	49.00^{b}	24.00^{bc}	9.78
Sa		283.25^{a}	4.50^{b}	15.25^{b}	23.25^{b}	30.26
Pa		0.00	0.00	0.00	0.00	0.00
Ea		3.00^{a}	1.25^{b}	1.50^{b}	1.50^{b}	0.23
Ec	Jejunum	3.50^{a}	0.00^{c}	2.00^{b}	1.50^{b}	0.35
Sa		521.00^{a}	4.00^{d}	203.75^{c}	421.75^{b}	51.83
Pa		0.00	0.00	0.00	0.00	0.00
Ea		40.00^{a}	0.00^{c}	0.00^{c}	3.25^{b}	4.36
Ec	Caecum	146.00^{a}	6.00^{d}	23.75^{b}	18.25^{c}	14.63
Sa		20.25^{b}	4.75^{d}	26.50^{a}	16.50^{c}	2.07
Pa		0.00	0.00	0.00	0.00	0.00

Key: Ea = *Enterobacter aerogens*, Ec = *Escherichia coli*, Sa = *Staphylococcus aureus*, Pa=*Pseudomonas aeruginosa*

^{abcd}=Means within the same row bearing different superscript are significantly different (p<0.05).

The result shown in table 3 and 4 below contains the gut morphometry characteristics of broiler chickens fed Xylopia (1%), Ginger (1%) and Garlic (1%). From table 2, the oesophagus and large intestine showed no significant difference across the treatment ($p<0.05$) while the duodenum, jejunum, ileum and caecum showed significant differences along the treatment ($p<0.05$). Table 4 shows the result of the gut weight characteristics of the broiler chickens fed three selected phyto-additives. Oesophagus, Proventiculus, Whole Gizzard, Empty Gizzard, Duodenum, Ceacum, Large Intestine did not differ significantly ($p>0.05$) among the treatment while Jejunum and Ileum differs significantly among the treatment means ($p<0.05$) and the highest values were recorded under garlic fed broiler chickens. Increase in the jejunum and ilium length and weight of the gut may be an indication of positive effect of diet on the absorptive surface area. Semi permeability potential of the gut can be influenced when the surface area is affected positively. Transmembrane process of the gut may help to either improve or increase with increase in the length of the GIT especially the jejunum and ilium of birds since this section is believed to experience massive absorption of useful nutrients. Research had earlier speculated that different fibre content of the diet may be beneficial to human health and that better understanding of each common component, how they work will give us better understanding of why dietary fibre can result into visco-intestinal variability (James and Mark, 2010). Although research on the GIT is still scanty especially for animal but according to (Hunt *et al*., 2014) symptoms originating from the gastrointestinal tract, such as heartburn, indigestion/dyspepsia, constipation and bloating are very common in every community. In certain circumstances, they may indicate the presence of an underlying gastrointestinal disease process, such as esophagitis, peptic ulcer or cancer. The result obtained for relative gut length and weight in this experiment shows that garlic fed broilers performed better than the other treatments and this is in line with the report of Karangiya *et al*. (2016) who said that garlic supplementation was superior in comparison to ginger and mixture of garlic and ginger. This shows that garlic fed broilers has a gut morphometry-promoting substance compared to ginger and Negro pepper. Oladele *et al.* (2012) also stated that garlic can increase the digestive and absorptive capacity of the intestine of commercial broilers by increasing the cryptal depth as well as the absorptive surface area of the intestine. Garlic has a prebiotic effect which reduces the harmful microbes in the gut and enables a proper environment for growth. The author stated that garlics can improve the intestinal structure through alteration through its phytochemical properties. Ginger also had a slight increment in the relative gut length and weight in this experiment when compared to the birds fed control and this can be also due to the phytochemical properties possessed by it. Any additive with anti-inflammatory property may reduce the retention of waste in the gut and help to modify the gut weight. Pathogenic bacteria can also cause morphological changes. For example, chickens that are co-infected with *Eimeria sp*. and *Clostridium perfringens* have significantly reduced length of intestinal villi. This was also shown in chickens infected with *Salmonella Typhimurium* (Karangiya *et al*., 2011).

Table 3: Relative Gut length characteristics of broiler birds fed three selected phyto-additives

Parameters	T1Control (0%)	T2 (Xylopia) (1%)	T3 (Ginger 1%)	T4 (Garlic 1%)	SEM
Oesophagus	1.21	1.08	0.98	0.97	0.06
Duodenum	2.28^b	2.24^b	2.18^b	2.76^a	0.08
Jejunum	5.71^a	4.85^a	5.05^a	2.21^b	0.47
Ileum	5.55^a	5.32^a	4.81^a	1.92^b	0.50
Ceacum	1.30^{ab}	1.31^{ab}	1.19^b	1.47^a	0.04
Large Intestine	0.68	0.63	0.55	0.59	0.04

^{a,b}: Mean along the same row with different subscript are significantly different (p>0.05). SEM = standard error of the mean.

Table 4: Relative gut weight characteristics of broiler chicken fed three selected phto-additives

Parameters	T1 (Control 0%)	T2 (Xylopia1%)	T3 Ginger 1%)	T4 (Garlic 1%)	SEM
Oesophagus	0.63	0.67	0.65	0.82	0.42
Proventiculus	0.46	0.48	0.42	0.49	0.24
Whole Gizzard	2.79	2.64	2.83	2.84	0.74
Empty Gizzard	1.86	1.99	2.04	2.02	0.52
Duodenum	0.97	0.94	0.92	0.99	0.03
Jejunum	2.02^b	2.11^b	3.12^b	6.68^a	0.54
Ileum	1.72^b	1.45^b	2.68^b	7.16^a	0.64
Ceacum	0.60	0.55	0.66	0.64	0.05
Large Intestine	0.27	0.17	0.21	0.19	0.02

^{a,b}: Mean along the same row with different subscript are significantly different (p>0.05), SEM = Standard error of the mean.

The result of the jejunal histomorphometry indicates all parameters except villi width were significantly (P<0.05) affected by the effect of the dietary treatments. The control fed broiler chickens (4401.67µm) and garlic fed chickens (4171.62µm) had the highest significant (P<0.05) value for villi height, while negro pepper fed chickens had the lowest significant (P<0.05) value 3028.93µm**.** Garlic fed broiler chickens also had the best significant (P<0.05) value for crypt depth and muscular thickness with 1366.24µm and 1048.77µm respectively. The results obtained from the table of the histomophometry of broiler chickens fed three selected phytoadditives shows the increase in villus height and villus and crypt depth observed in the present study for chickens fed phyto additives may be related to the stimulation of epithelial cell mitosis by phyto-additives. According to Ivana *et al*. (2019) longer villi are associated with activated cell mitosis. Therefore, the gut integrity can be influence by the phyto-additives maybe through gut activity which afterward may allow birds to absorbed nutrient and prevent damaged to epithelial layer of the gut. The use of phytogenic as a replacement for synthetic antibiotics suffices as a better option in improving livestock performance as well as meat quality and safety for humans (Hedayati *et al*., 2013). The crypt can be regarded as the villus factory, and a large crypt indicates fast tissue turnover and a high demand for new tissue (Yason *et al*., 1987, Anonymous, 1999). A decrease in either villus height or crypt depth may lead to a reduction in nutrient absorption. The result of villus height to crypt depth ratio is a measure used to assess the health and functionality of small intestine's lining. It is calculated by dividing the height of the villus by the depth of the crypt (Nain *et al*., 2012). The results give insight into the absorptive capacity and turnover rate of the intestinal epithelium, the higher ratio indicates sufficient intestinal integrity and increase surface area for better absorptive capacity.

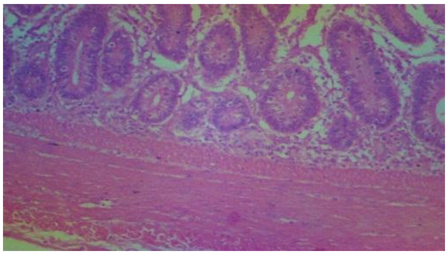
Table 5: Histomorphometry of broiler chickens fed three phyto-additives

Parameters	T1(Control)	T2(Xylopia1%)	T3(Ginger1%)	T4(Garlic1%)	SEM
Villi Height (µm)	4401.67^{a}	3028.93^{b}	3198.48^{b}	4171.62^{a}	176.59
Villi width (µm)	555.01	500.57	444.92	388.73	33.36
Crypt Depth (µm)	1113.13^{a}	1272.90^{a}	809.25^{b}	1366.24^{a}	65.44
VH:CD Ratio	4.12^{a}	2.44^{b}	4.14^{a}	3.08^{ab}	0.25
Muscular Thickness (µm)	880.03^{b}	807.06^{b}	927.91^{ab}	1048.77^{a}	27.03

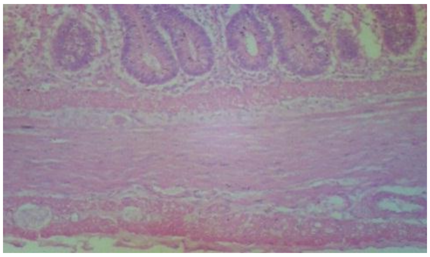
VH:CD = Villi Height Crypt Depth ratio

Histological changes/responses of the intestinal tissue of broiler chickens fed three selected phyto-additives

1a

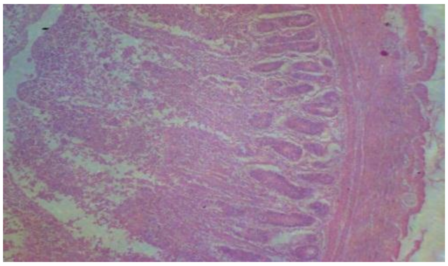


1b

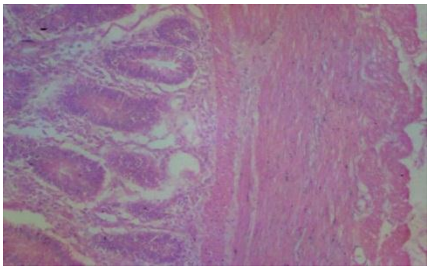


PLATES 1a and 1b: Photomicrograph of the intestinal tissue of broiler chickens fed control diet showing fine histo-architecture, well-arranged crypts with goblets within the mucosa appears normal, same with the sub-mucosa. Muscularis externa is thick, and the smooth muscle appears normal and well arranged. The muscularis mucosa and lamina propria are distinct and well preserved. The adventitia shows normal histo-architecture.

2a

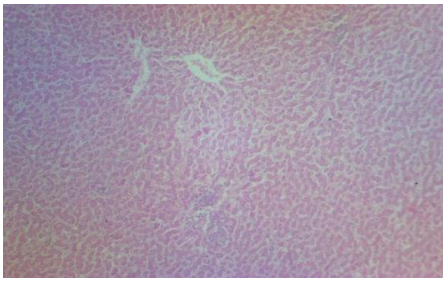


2b

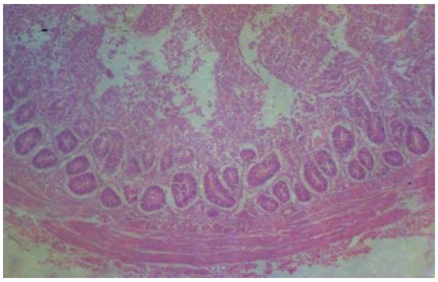


LATES 2a and 2b: Photomicrograph of the intestinal tissue of broiler chickens fed 1% Xylopia (dietary treatment T2) showing normal histo-architecture with thicker Muscularis externa, more compact mucosa crypts and more surface area for more efficient absorption.

3a

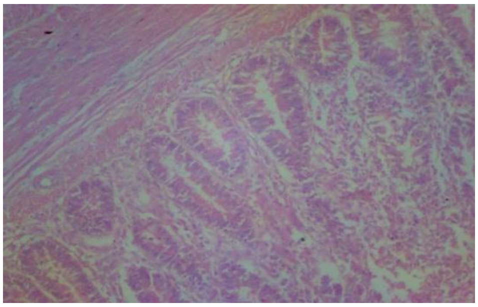


3b

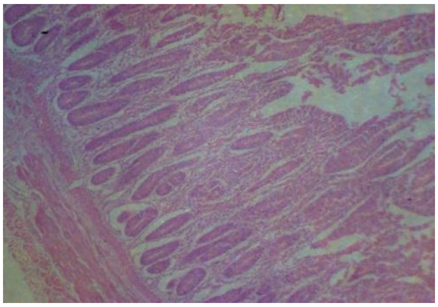


PLATES 3a and b: Photomicrograph of the intestinal tissue of broiler chickens fed 1% Ginger dietary (T_3) shows normal histoarchitecture with thicker muscularis externa, more compact mucosa crypts and surface that increases the surface area for more efficient absorption.

4a

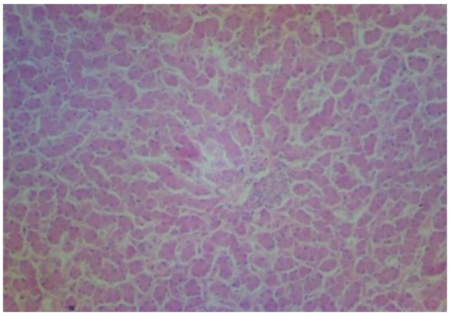


4b

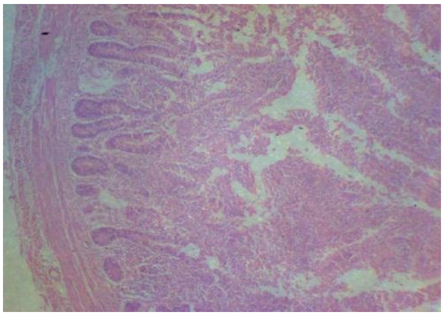


PLATES 4a and b: Photomicrograph of the intestinal tissue of broiler chickens fed 1% garlic dietary treatment (T4) showing thinner Muscularis externa, necrotizing or atrophic crypts. The thickness of the mucosa layer appears very low relative to the previous treatments

5a



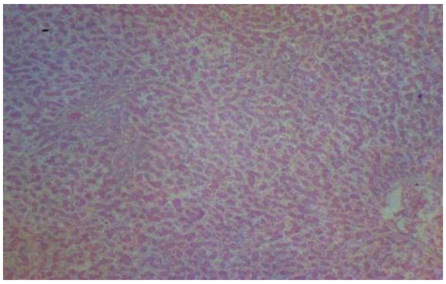
5b



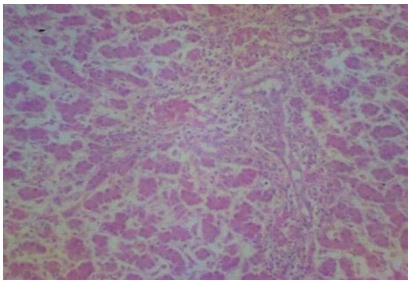
PLATES 5a and b: Photomicrograph of the liver tissue of broiler chickens fed control (dietary treatment T1) reveals normal histoarchitecture. The sinusoids are clear and normal, the cords of hepatocytes are well arranged. The portal triads and central veins are distinct, normal and well identifiable. Clear and normal sinusoids, and well-arranged cords of hepatocytes. It also shows distinct and well identifiable portal triads and central veins.

Histological changes/responses of the liver of broiler chickens fed three selected phyto-additives

6a

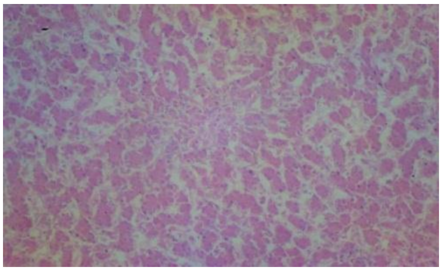


6b

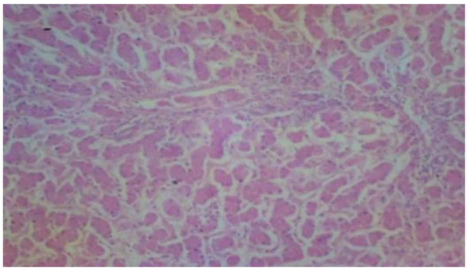


PLATES 6 and b: Photomicrograph of the liver tissue of broiler chickens fed 1% Xylopia dietary treatment (T2) showed that the sinusoids are clear and normal, the cords of hepatocytes are well arranged. The portal triads and central veins are distinct, normal and well identifiable. Clear and normal sinusoids, and well-arranged cords of hepatocytes. It also shows distinct and well identifiable portal triads and central veins.

7a

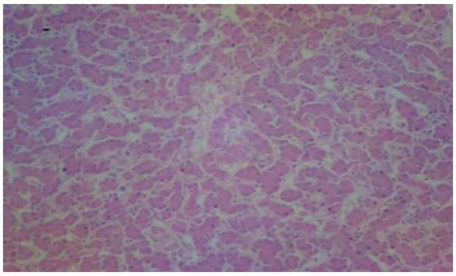


7b

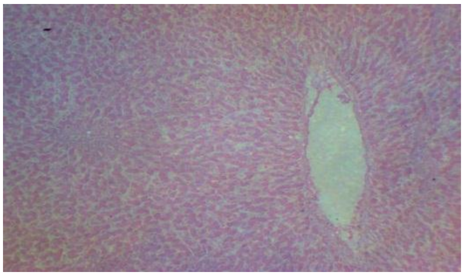


PLATES 7a and 7b: Photomicrograph of the liver tissues of broiler chickens fed 1% Ginger dietary treatment (T_3) reveals a finer histoarchitecture than observed in T1 and T2. Also, more distinct component features and circulatory organs were observed

8a



8b



PLATES 8a and 8b: Photomicrograph of the liver tissues of broiler chickens fed 1% Garlic dietary treatment (T_4) also showed finer histoarchitecture than observed in T1 and T2, more distinct circulatory organs and component features.

The plates 1a-4b shows the gut morphology of broiler chickens as affected by three selected phyto-additives. The GIT development and health is the key to productivity in all farm animals and poultry. The digestive functions could be considered the most limiting factors in performances (Jeukendrup, 2017) and enhancing it may be an added advantage to the poultry industry. The gastrointestinal tract needs a booster to enhance the capacity to secrete vital substances for effective utilization of nutrients. Young chicks lack the full capacity to utilize feed effectively because their gastrointestinal tract is not well developed and endogenous secretions such as gastric juice components (e.g. hydrochloric acid) and mucus are inadequate. Except the broilers on treatment 4 (garlic additive) that had thinner Muscularis external, necrotizing or atrophic crypts others had fine histo-architecture, well-arranged crypts with distinct goblets cells arrangement. Thinner Muscularis external can be because of inadequate intake of essential nutrients which leads to muscle wasting of the gut. Inflammation of the gut can also result into thinner Muscularis external. According to Christie and Mattew (2022) starvation and inflammation affect muscle mass and adipose tissue as well as the body's ability to utilize nutrition and hydration. The compact mucosa crypts observed in this study is contrary to the report of Demir *et al.* (2003) that used female chickens. Several researchers have studied intestinal morphology in poultry during the last decade but predominantly from the standpoint of normal development and not about effects of antibiotics and other growth promoters. The test ingredients used seem not to cause any stress to the gut except the garlic. Stress is defined as an acute threat to homeostasis, evokes an adaptive or allostatic response and can have both a short- and long-term influence on the function of gastrointestinal tract (Vikram and Bakesh, 2005). It establishes that the ginger and Xylopia additives will encourage the functionality of the enteric nervous system which according to Vikram and Bakesh (2005) was connected to bidirectionally to the brain by parasympathetic and sympathetic pathways for the brain-gut axis.

The plates 5a-8b shows histological changes/responses of the liver of broiler chickens fed varying levels of phyto-additive. Histological study on the liver showed a normal structure and no alteration in the livers of the treated broiler chickens fed the three selected phyto-additives. The histo-architectural states of the livers observed agrees with the other previously used phytoadditives as report by Uhegbu *et al.* (2015) 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 Negro pepper, ginger and garlic may contain chemical constituents that may possess hepato-protective effect. Normal hepatocytes observed help to stress the usefulness of the three selected phytoadditives 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. According to Herbert *et al.*, (2022) bidirectional crosstalk along the gut-liver axis controls gastrointestinal health and disease and exploits environmental and host mediators. Nutrients, microbial antigens, metabolites, and bile acids regulate metabolism and immune responses in the gut and liver, which reciprocally shape microbial community structure and function. Therefore, understanding of the liver-gut relationship will help to improve the gastrointestinal functionality and gut ecology.

Conclusion and Recommendation

The improvement in the growth performance and reduction in the pathogenic microbial population in the intestine of broilers chickens in this study indicate that *Xylopia aethiopica* has growth promoting potentials and can serve as a natural alternative to antibiotics in broiler production with no adverse health implication. Also, the use of xylopia, ginger and garlic can help to reduce the population of the microbes in the gut of broiler chickens. The study showed that garlic works better in improving the gut morphometry of the broiler birds than ginger and *Xylopia aethiopica* (negro pepper). It can be recommended that 1% xylopia can be given to broiler chicken to improve their growth potential and in replacement of antibiotics because of their antibacterial and antioxidants activities.

References

Ahn, I. U. and Fernandom, A. U. (2002). Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. *Journals of Food Sci*ences, 67: 1364-1369.

Aji. S. B., Ignatituius, K., Ado, A. Y., Nuhu, J. B. and Abdulkarin, A. (2011). Effect of feeding onion (*Allium cepa*) and garlic (*Allium sativum*) on some performance characteristics of broiler chickens, *Resources Journals Poultry Science 4:22-27.*

Amad, A. A., Manner, K., Wendler, K. R., Neumann, K. and Zentek, J. (2011). Effects of a phytogenic feed additive on growth performance and ileal nutrient digestibility in broiler chickens. *Poultry Science*, 90(12):2811- 2816.

Anonymous. 1999. How do mannanoligosaccharides work? Feeding Times (1):7–9.

Arshad, H R., Fahad, M. A. and Salah, M. A. (2014). Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. *International Journal of Physiol. Pathophysiology Pharmacology*: 6(2): 125–136.

Ayinla, A. K. and Odetoye, A. S. (2015). Climatic pattern and design for indoor comfort in Ogbomoso, *Nigeria. Journal of Environment and Earth Science*. 5(17): 30-37.

Ceylan, A., Bayram, E., Sahbaz, N., Otan, H. and Karaman, S. (2003). Yield performance and essential oil composition of individual plants and improved clones of *Origanum onites* L. grown in the Aegean region of Turkey. *Israel Journals Plant Science*. 51 (4):285-290.

Chessbrough, M. (1985). Medical laboratory manual for tropical countries. *Microbiology*. 2: 400 - 480

Christie, L. M. and Mathew, J. S. (2022). More than Just Weight Loss: Understanding the Toll of Malnutrition on the Body. *Nutrition Issues In Gastroenterology*, Series #221. Pages 28-43.

Dalloul, R. A. and Lillehoj, H. S. (2006). Poultry coccidiosis: Recent advancements in control measures and vaccine development. *Expert Review Vaccines* 5:143–163.

Demir, E., Sarica, S., Ozcan, M. A. and Suicmez, M. (2003). The use of natural feed additives as alternatives for an antibiotic growth promoter in broiler diet. *British Poultry Science*, 4 (1) 44-45.

Golder, H. M., Geier, M. S., Forder, R. E. A., Hynd, P. I. and Hughes R. J. (2011). Effects of necrotic enteritis challenge on intestinal micro-architecture and mucin profile. *British Poultry Science* 52:500–506.

Gulbinowicz, M., Berdel, B. and Wójcik, S. (2004). Morphometric analysis of the small intestine in wild type mice C57BL/6L, a developmental study. *Folia Morphol*ogy 63(4): 423–430.

Hedayati, M., Manafi, M., Yari, Mand Vafaei, P. (2013). Effects of supplementing diets with an acidifier on performance parameters and visceral organ weights of broilers. *European Journal of Zoological Research*. 2: 49–55.

Herbert, T., Timon, E. A. and Michael, T. (2022). Gut-liver axis: Pathophysiological concepts and clinical implications. *Cell Metabolism.* 34: 1700 – 1718.

Herbs Hands Healing Ltd (2011).Traditional Western Herbal Product, Ginger Extracts from ‘in a nutshell ginger’ by Rosemary Davies. wwwherbshandshealingcouk.

Hunt, R., Quigley, E., Abbas, Z., Eliakim, A., Emmanuel, A., Goh, K. L., Guarner, F., Katelaris, P., Smout, A., Umar, M., Whorwell, P., Johanson, J., Saenz, R., Besançon, L., Ndjeuda, E., Horn, J., Hungin, P., Jones, R., Krabshuis, J. and LeMair, A. (2014). World Gastroenterology Organisation. Coping with common gastrointestinal symptoms in the community: a global perspective on heartburn, constipation, bloating, and abdominal pain/discomfort. Journal Clinical Gastroenterology. 48(7): 567-578.

Ilusanya, O.A.F., Odunbaku, O.A., Adesetan, T.O and Amosun, O. T. (2012). Antimicrobial activity of fruit extracts of *Xylopia aethiopica* and its combination with antibiotics against clinical bacterial pathogens. *Journal of Biology, Agriculture and Health care*. 2(9): 1-9.

Isikwenu, J. O. and Udomah, J. E. (2015). Effect of Xylopia aethiopia Dried Fruits (Grains of selim) as Additive on Performance, carcass Characteristics and Economic Prospects in Finisher Broilers. *Journal of Agriculture and Veterinary Science*, 8: 7-10.

Isikwenu, J.O., Ifeanychukuru, U., Bernard, I. O. and Theresa, O. K. (2001). Performance, Antimicrobial Effect and Carcass Parameters of Finisher Broilers Given Xylopia aethiopica Dried Fruits (Grains of Selim) as Additive. *Agricultural tropical et subtropical* 47(4): 124-130.

Ivana, P., Maja, M., Mirela, P., Ksenija, M., Valerija, B., Ivan, M. and Matija, D. (2019). Intestinal Morphology in Broiler Chickens Supplemented with Propolis and Bee Pollen. *Animals. 9*(6): 301.

James, M. L. and Mark, D. H. (2010). Effect of dietary fibre and its component on metabolic health. *Nutrient*. 2(12): 1266-1289.

Jeukendrup, A. E. (2017). Training the gut for athletes. *Sport Med*. 47(suppl. 1): 101 -110

Karangiya V.K., Savsani H. H., Shrikant Soma Patil, D. D. Garg, K. S. Murthy, N. K. Ribadiya and S. J. Vekariya (2016). Effect of dietary supplementation of garlic, ginger and their combination on feed intake, growth performance and economics in commercial broilers. *Vet World*. 9(3): 245-50.

Kelly, A. T. (2006). Mechanisms of Enteral Nutrient-Enhanced Intestinal Adaptation. *Gastroenterology*. 130 (2): S93-S99.

Khan, R. U., Naz, S., Nikousefat, Z., Tufarelli, V. and Javdani, M. (2012). Potential applications of ginger (*Zingiber officinale*) in poultry diets. *World's Poult Science Journals* 68: 245-252.

Kitt, S. J., Miller, P. S. and Lewis, A. J. (2001). Factors affecting small intestine development in weanling pigs. *Nebraska Swine Report.* 33–35.

Lee, K. W., Kim, J., Oh. S., Kang, C. and An, B. (2015). Effects of dietary sanguinarine on growth performance, relative organ weight, cecal microflora, serum cholesterol level and meat quality in broiler chickens. *Journal of Poultry Science*, 52:15–22.

Muhammad, I. R., Abdu, M. I., Iyeghe-Erakpotobor, G. T., Sulaiman, K. A., (2009). Ensiling quality of gamba fortified with tropical legumes and its preference by rabbits. *Res. J. Appl. Sci*., 4 (1) : 20-25.

Nain, S.; Renema, R.; Zuidhof, M.; Korver, D. (2012). Effect of metabolic efficiency and intestinal morphology on variability in n-3 polyunsaturated fatty acid enrichment of eggs. *Poultry Sciences*. 91: 888–898.

Oladele, O. A.; Emikpe, B. O. and Bakare, H. (2012) Effects of dietary garlic (Allium sativum Linn.) supplementation on body weight and gut morphometry of commercial broilers. Int. Journal Morphology. 30(1):238-240,

Oluyemi, J.A. and F.A. Roberts, (2000). Poultry Production in Warm Wet Climate Spectrum Books Ltd., Ibadan, Nigeria, pp: 65.

Omodamiro, O. D., Ohaeri, O. C. and Nweke, I. N. (2012). Oxytocic Effect of Aqueous, Ethanolic, n-Hexane and Chloroform Extracts of *Xylopia aethiopica* on Guinea Pig Uterus. *Asian Journal of Plant Science and Research*, 2:73-74.

Sogunle, O. M., Anthonio, R. A., Egbeyale, L. T., Ekunseitan, D. A. and O.A. Olaniy, (2014). Response of Broiler Chickens to the Interaction between Phase of Growth and Feed Forms. In: eds., Adbullah, A.R., G.O. Tayo and A.O. Okubanjo, Lynson Prints, Abeokuta, Nigeria, 571-574.

Sudrashan S., Fairoze N., Wildfred S., Shekar R. (2010). Effect of aqueous extract and essential oils of ginger and garlic as immunostimulant in chicken meat. Research. *Journal of Poultry Science* 3: 58-61.

Tatsadjieu, L.N., Ngang, J.J., Ngassoum, M.B., Etoa, F.X. (2003). Antibacterial and antifungal activity of *Xylopia aethiopica*, *Monodora myristica*, *Zanthoxylum xanthoxyloides* and *Zanthoxylum leprieurii* from Cameroon. *Fitoterapia*. *Journal of animal science.* 74:469–472.

Tehseen M, Tahir M, Khan RU, Jabbar A, Ahmad B, Ahsan T, Khan S, Abudabos AM. (2016). *The Philippine Agricultural Scientists*. 99:408–413.

Tekeli, A., Kutlu, H.R., Celik, L. (2011): Effect of Z. officinale propalis extracts on the performance, carcass and some blood parameters of broiler chicks. *Current Research in Poultry Science*. 1: 12-23.

Tollba, A. A. and Hassan, M. S. (2003). Using some natural additives to improve physiological and productive performance of broiler chicks under high temperature conditions. Black cumin (Nigella Sativa) or garlic (*Allium* Sativum). *Poultry Science*, 23:32-340.

Uhegbu, F.O., Imo, C., Ugbogu, A.E. (2015). Effect of aqueous extract of *Piper guinesse* seeds on some liver enzymes, antioxidant enzymes and some haematological parameters in albino rats. *International Journal of Plant Science Ecology* 1(4):167-17.

Vikram, B. and Bakesh, K. T. (2005). Stress and gastrointestinal tract. *Journal of Gastroenterology and Hepatology*, 20, 3232 – 339.

Yason, C. V., Summers, B. A. and Schat, K. A. (1987). Pathogenesis of rotavirus infection in various age groups of chickens and turkeys: *Pathology*. *American Journal of Veterinary Research*. 6:927–938.