Effect of Garlic (*Allium sativum*) oil inclusion on Nutrient Retention and Caeca Microbial Population of Broiler Chickens.

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Abstract *- This study was carried to evaluate the "Effect of Garlic (Allium sativum) oil inclusion on nutrient digestibility and caeca microbial population of broiler chickens". A total of 180 one- day-old (Arbo acre) broiler chicks of mixed sex purchased from a reputable hatchery was used in this study which lasted for eight (8) weeks. The birds were divided into four experimental groups; each group was further subdivided into three replicates of fifteen birds per each in a Complete Randomized Design (CRD). The garlic (Allum sativum) oil was added to the basal diet at (0.00, 0.1, 0.2, 0.3 %) level respectively. The treatment were T1, T2, T3, and T4 with T1 serving as control. Parameters measured covered nutrient retention and caeca microbial population. The results on nutrient digestibility showed a significant difference (P<0.05) among the treatments. Percentage digestibility of crude protein and crude fibre was significantly (P<0.05) higher in birds fed 0.3% of garlic oil. Birds on 0.2% garlic oil had highest ash digestibility (65.88%) and least value (64.60%) was recorded in birds on 0.3% garlic oil inclusion. However, ether extract percentage digestibility (66.67%) was recorded in birds on 0.3% garlic oil inclusion. At the end of 56 days of age, Lactobacilli and Escherichia coli counts were significantly (P<0.05) influenced by the inclusion of garlic oil in the diets. Garlic oil inclusion increased significantly (P<0.05) the number of lactobacilli with a decrease the Escherichia coli counts. It was concluded that garlic oil repopulates the gastro intestinal tract (GIT) with beneficial bacteria which curbs the action of pathogens and controls their population.*

Key words: *Allium sativum; nutrient retention; Escherichia coli; lactobacilli.*

1.Introduction

Garlic, (*Allium sativum),* belongs to the lily family whose relatives are onions. Once a wild herb, today's garlic is cultivated throughout the world, though some still grow wild. It consists of a bulb about the size of a child's fist. The positive effects of herbal addictive on broiler performance, carcass quality and quality traits of meat have been demonstrated (Schleicher *et al*., 1998). A variety of herbal additive including garlic have been widely used to maintain and improve the health of humans (Freeman and Kodera, 1995). Garlic supplements in broiler chickens have been recognized for their strong stimulating effect on the immune system and its very rich aromatic oils enhance digestion in birds (Gardzielewska*et al*., 2003). The key active ingredient in garlic is a powerful chemical called allicin which rapidly decomposes to several volatile organosulphur compounds with bioactivities (Chang and Cheong, 2008).

Garlic is used both as condiments and medicaments, anticoagulant, antioxidant, hypolipidaemic, antihypertensive, antiageing, antiplatelet and heavy metal detoxifier (Agarwal, 1996 and Marilynn, 2001). The antioxidative influence of garlic in meat becomes more imperatives in less developed nations, considering storage problems and increasing use of alternative feed resources without due consideration for meat quality (Onibi*et al*., 2007).

Antibiotic have been added to poultry diets to maintain health and production efficiency in the past 80 years. Various mechanism has been proposed which are include: (a) the nutrients are more efficiently absorbed and less are utilized by the gut, (b) more nutrients are available to the host because of reduced intestinal micro flora, (c) there is a reduction in harm full gut bacteria, (d) production of growth suppressing toxins or metabolites is reduced, (e) microbial de-conjugation of bile acids is decreased (Roozbeh *et al.*, 2012; Alagbe *et al*., 2019). But, continuous and misuses of antibiotics in poultry industry resulted many concerns about development of drug resistant bacteria, drug residues in the body of the birds, and imbalance of normal micro flora (Behrouz et al., 2012), this led to the ban of these products by the European Union in January 2006. (Jimoh*et al*., 2013). This decision has therefore stimulated a search for alternatives: Essential oils have been proven to control pathogens due to their antimicrobial activity (Dorman and Deans, 2000), to have ant oxidative potential (Hui, 1996) by delaying lipid oxidation in broiler meat, and to enhance digestion (Brugali, 2003) by stimulating the indigenous enzymes.

Garlic essential oils have gained prominence due to their wide range of properties not only in improving performance of broilers but, many other ways where the almost aim is to improve nutritive value of poultry meat products (Bamidele and Adejumo, 2012). Several studies have identified the separate use of this plants extracted oils in broiler nutrition as natural feed additives.

Historically it is believed that Louis Pasteur first scientist who demonstrate the antimicrobial effects of garlic 'juices' in 1858, however, no reference is available. Recently it is proved that garlic is effective against many acid-fast, gram-positive and gram-negative bacteria. These include *Escherichia coli* (*E. coli*), Salmonella (Johnson *et al*., 2006), Clostridium (Witt *et al*., 1979), *Staphylococcus aureus*, Pseudomonas, Proteus (Jezowa*et al*., 2001), Klebsiella (Jezowa*et al*., 2001), Micrococcus, *Bacillus subtulis* (Sharma *et al*., 2009) and Helicobacter (Gara*et al*., 2000). So, garlic can be used to treat *E. coli*, Salmonellosis and Cholera in poultry. Garlic exerts a differential inhibition between beneficial intestinal microflora and potentially harmful Entero bacteria (Rees *et al*., 2003). For the same garlic dose inhibition zone observed in *E. coli* was more than 10 times than that seen in *Lactobacillus casei* (Skyrme, 2007). The exact mechanism of this differential inhibition is not known, but one of the possible reasons may be the change in chemical composition of membranes of different bacteria and their absorptivity to allicin (Miron*et al*., 2000). An inhibitory synergism of antimicrobial properties of garlic was observed when it was used in combination with vancomycin (Jonkers*et al*., 2000).

The objective of the study was to evaluate the effect of Garlic (*Allium sativum)* oil inclusion on caeca microbial population, nutrient retention of broiler chicken.

2. MATERIALS AND METHODS

Site of the Experiment

The experiment was undertaken at the University of Abuja Teaching and Research farm, Animal pavilion section of the Faculty of Agriculture, University of Abuja, Nigeria. The site has latitude 8.55^0 and 90^0N, longitude 7^0 00^0N and 7^005^0E.

Collection of test material and preparation

Fresh garlic cloves were purchased from a local market in Gwagwalada, Abuja, Nigeria. The transparent covering of garlic was removed manually and sun-dried for three weeks. Mortar and pestle was used to pound the dry garlic cloves into powder, then sieved and stored in a covered plastic container and was further subjected to proximate analysis according to AOAC (2000) as presented in Table 4. Garlic oil (GOL) was extracted by soxhlet extractor using n-hexane (boiling point of 40ºC - 60ºC) for six hours according to the method outlined by Gafar*et al*. (2012).

Animals and their management

One day old 180 (Arbo acre) broilers of mixed sex were obtained from a commercial hatchery in Ibadan. The birds were divided into four experimental groups; each group was further subdivided into three replicates of fifteen birds per each in a Complete Randomized Design (CRD). Anti-stress was added in the drinking water of the birds. The light (electric bulb) was continuous and the initial brooding temperature was 34ºC for the first week of age and it was gradually reduced by 2ºC per week. Vaccines were administered according to the prevailing vaccination schedule in the environment. A vitamin was added in water a day before and after each vaccination. Clean feed and water was provided unrestricted throughout the experimental period which lasted for 8 weeks.

Diets were formulated to meet the nutritional requirements of birds according to NRC (1994) and Garlic oil was included in the diet at 0 %, 0.1%, 0.2% and 0.3 % for treatment 1, 2, 3 and 4 respectively.

Data collected

Caeca microbial count

At the end of 8^{th} weeks, two birds were randomly selected per replicate; they were slaughtered. After evisceration, one gram of each sample of the caeca content was collected and transferred into the test tube and was then diluted with 9ml of 1% peptone broth and homogenized. Counts of bacteria and *lactobacillus* were determined according to farmer (2003).

Nutrient retention trial

A nutrient retention trial was carried out on the 8^{th} week of the experiment; two birds were selected from each replicate birds were housed in cages with wire bottoms. Trays were placed under each cage for faecal collection. The birds were given a known amount of feed for seven days and clean water was also given throughout the experiment. Feed consumed was measured by weighing the leftover feed daily and subtracting from amount of feed provided. Excreta was

collected for 7days, dried and mixed thoroughly before it was taken to the laboratory for further analysis.

Nutrient retention (%) = $\frac{\text{Nitrogen intake - Nitrogen in faeces}}{\text{Nitrogen intake}} \times 100$

Statistical Analysis

All data collected was subjected to one-way analysis of variance (ANOVA) using SPSS (25.0) and significant means will be separated using Duncan multiple range tests (Duncan, (1955) significant will be declared if $P \leq 0.05$.

Table 1: Percentage composition of experimental diet (Starter 0-4 weeks)

Ingredients	T1	T2	T3	T4
Maize	53.00	53.00	53.00	53.00
Wheat offal	1.6	1.6	1.6	1.6
GNC	10.00	10.00	10.00	10.00
Soya beancake	30.00	30.00	30.00	30.00
Bone meal	3.00	3.00.	3.00	3.00
Methionine	0.20	0.20	0.20	0.20
Premix	0.25	0.25	0.25	0.25
Limestone	1.50	1.50	1.50	1.50
Salt	0.30	0.30	0.30	0.30
Lysine	1.50	1.50	1.50	1.50
Garlic Oil (%)	**0.0**	**0.1**	**0.2**	**0.3**
Calculated Analysis				
Crude Protein (%)	22.67	22.67	22.67	22.67
Crude Fibre (%)	3.65	3.67	3.65	3.65
Phosphorus (%)	0.54	0.54	0.54	0.54
Ether Extract (%)	3.83	3.84	3.85	3.86
Energy (Kcal/kg)	2922	2922	2922	2922

Table 2: Percentage composition of experimental diet (Finisher 5-8 weeks)

Ingredients	T1	T2	T3	T4
Maize	62.00	62.00	62.00	62.00
Wheat offal	1.00	1.00	1.00	1.00
GNC	6.00	6.00	6.00	6.00
Soya beancake	26.1	26.1	26.1	26.1
Bone meal	3.00	3.00	3.00	3.00
Methionine	0.15	0.15	0.15	0.15
Premix	0.25	0.25	0.25	0.25
Limestone	1.50	1.50	1.50	1.50

Salt	0.25	0.25	0.25	0.25
Lysine	0.15	0.15	0.15	0.15
Garlic Oil (%)	**0.0**	**0.1**	**0.2**	**0.3**
Calculated Analysis				
Crude protein (%)	20.03	20.03	20.03	20.03
Crude Fibre (%)	3.32	3.32	3.32	3.32
Phosphorus (%)	0.54	0.54	0.54	0.54
Ether Extract (%)	3.79	3.79	3.79	3.79
Calcium	1.13	1.13	1.13	1.13
Energy (Kcal/kg)	2998	2998	2998	2998

Table 3: Proximate composition of Garlic (*Allium sativum*) powder

Items	Values
Constituents (%)	
Dry matter	87.3
Crude protein	6.29
Fat	0.20
Crude fiber	1.50
Ash	2.01
Mineral (mg/100g)	
Ca	290
P	120
Fe	1.2
K	129
Mg	36
Na	6.3

Table 4: Nutrient retention of birds fed with Garlic (*Allium sativum*) oil

Parameters (%)	T1	T2	T3	T4	SEM
Dry matter	84.2	80.45^{c}	85.40^{b}	85.32^{a}	4.90
2^{b}					
Protein	72.2	69.88^{b}	70.15^{b}	73.32^{a}	2.33
retention 1^{a}					
Fat retention	59.9	51.44^{b}	63.40^{a}	62.09^{a}	2.88
1^{a}					
Fibre retention	40.0	40.33	41.55	42.40	5.88
0					
Ash	65.2	65.88	65.60	64.60	3.03
1					

NFE	67.7 1	66.44	65.72	66.67	1.88

Means in the same row with different superscripts differ significantly ($P<0.05$)
NFE = nitrogen free extract, SEM = standard error of mean

Table 5: Effect of Garlic oil on the caeca microbial population of broilers

Parameters (cfu/g)	T_1	T_2	T_3	T_4	SEM
Lactobacillus	30.33^{c}	34.31^{c}	54.51^{b}	61.88^{a}	3.52
E.coli	25.60^{a}	21.80^{a}	18.44^{b}	13.50^{c}	2.11

Means in the same row with different superscripts differ significantly ($P<0.05$)
T1: Control (without Garlic oil), T2: 0.1% Garlic oil, T3:0.2% Garlic oil, T4: 0.3% Garlic oil SEM = standard error of mean

3. Results and Discussion

Table 3 reveals the proximate composition of garlic powder. The proximate components contained dry matter, crude protein, ether extract, crude fibre and ash at 87.30 %, 6.29 %, 0.30%, 1.50 % and 2.01 % respectively. The mineral analysis of the garlic powder indicated its richness in sodium, Phosphorus, Iron, Zinc, Copper, Manganese, Phosphorus and magnesium. According to Murray *et al* (2000); Oluwafemi et al. (2020) minerals are structural components of the body tissues are involved in the maintenance of acid base balance, regulation of body fluids, transport of gases and in muscle contractions. Calcium plays a vital role in providing rigidity and support to animals (Ibrahim *et al*., 2001); Alagbe and Motunrade (2019) Magnesium, zinc, iron, manganese are important co-factors found in the structure of certain enzymes and are indispensable in numerous biochemical pathways (Soetan *et al*., 2010). Sodium is responsible for acid-base balance and regulation of plasma volume (Brown and Pentland, 2007; Alagbe *et al*., 2020)

The nutrient retention of birds fed with Garlic (*Allium* sativum) oil of the experimental birds is presented in Table 4. Significant difference *(P<0.05)*were observed in the digestibility of Dry matter, Crude Protein, crude fibre, ash, ether extract and nitrogen free extract in birds on the experimental diets. Percentage digestibility of crude protein and crude fibre was significantly (P<0.05) higher in birds on 0.3% of garlic oil. Birds on 0.2% garlic oil had highest ash digestibility (65.88%) and least value (64.60%) was recorded in birds on 0.3% garlic oil inclusion. However, least ether extract percentage digestibility (66.67%) was recorded in birds on 0.3% garlic oil inclusion. This result is in agreement with the observation of Mohammed *et al*. (2016) when garlic extract was fed to broiler chicken.

Table 5 showed the effect of Garlic (*Allium sativum*) oil on caeca microbial population of broilers. The *E.coli* count ranged from 30.30 – 61.88 (cfu/g) while those of lactobacillus ranged from 13.50 – 25.60 (cfu/g). There was a significant difference *(P<0.05)* in the *E.coli*count among the treatments, the values decreased from treatment 1 to 4 while those of *lactobacilli* increased from treatment 1 to 4. This was comparable to the findings of Musa *et al*. (2020) Alagbe *et al.* (2019); Alagbe (2019); Olafadehan *et al*. (2020); Shittu et al. (2020) who observed a significant *(P<0.05)* difference in the lactobacillus and *E. coli* count among the treatment but contrary to the reports of Hanan (2014) ; Akintayo and Alagbe (2020) thus acting as a probiotic. According to Tiwari and Jyoti (2008), Lactic acid bacteria produce several bactericidal/antibiotic like substances, which have been found effective against enteric pathogens. These bacteriocins can kill pathogenic bacteria, prevent colonization actions and also performs competitive exclusion which refers to the physical blocking of opportunistic pathogens.

4. CONCLUSION

The garlic oil inclusion reduced the mortality rate of the birds, increases productivity and food safety. Garlic is king of medicinal plants and it has wondrous effects in poultry. The garlic oil shown better performance of birds,

ultimately enhancing the production potential. Additionally, garlic oil reduces the number of pathogenic bacteria like Campylobacter, *Escherichia coli* and salmonella, clostridium etc. it has beneficial effect on consumer's immunity. So, it can effectively use to replace the antibiotics growth promoter in poultry feed.

5. RECOMMENDATIONS

Farms may include 0.3% Garlic oil inclusion in both starter and finisher diets since it supports optimal performance and compared favorably with conventional antibiotics.

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REFERENCES

1. Agarwal, K. C. (1996). Therapeutic action of garlic constituents. Med. Res. Rev. 16; 111-124.
2. Akintayo Balogun Omolere. M and Alagbe, J.O (2020). Probiotics and medicinal plants in poultry nutrition: A review. *United International Journal for Research and Technology*, 2(1): 7-13.
3. Alagbe, J.O (2020). **Caecal Microbial Population of Growing Grass Cutters (*Thyronoyms***
4. ***Swinderianus*) Fed *Phyllantus Amarus* and *Pilogstigma Thonngii* Leaf Meal Mixture as Partial Replacement for Soya Bean Meal. *Concept of Dairy and Veterinary Sciences*. 3(5): 350 – 355.**
5. Alagbe, J.O and Adegbite Motunrade Betty (2019). Haematological and serum biochemical indices of starter broiler chicks fed aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* bark mixture. *International Journal of Biological, Physical and Chemical Studies*. 1(1): 8-15.
6. Alagbe, J.O., Shittu, M.D and Eunice Abidemi Ojo (2020). Prospect of leaf extracts on the performance and blood profile of monogastric – A review. *International Journal of Integrated Education*. 3(7): 122-127.
7. Alagbe, J.O. (2019). Haematology, serum biochemistry, relative organ weight and bacteria count of broiler chicken given different levels of Luffa aegyptiaca. International Journal of Advanced
8. Biological and Biomedical Research, 7(4): 382-392.
9. Alagbe, J.O., Olanrewaju, A., Adewemimo, A, Tanimomo, B.K. (2019). Carcass, ceaca microbial population and immune parameters of broilers given different levels of mixed lemon grass and garlic extract. Academic Journal of Life Sciences, 5(11): 107-111.
10. Alagbe, J.O., Sadiq, M.R., Anaso, E.U and Grace, F.R. (2019). Efficacy of Albizia lebbeck seed oil on the growth performance and carcass characteristics of weaner rabbits. Sumerianz Journal of Agriculture and Veterinary, 2(12): 116-112.
11. AOAC, (2000): Association of Official Analytical Chemist Official Method of Analysis 15th Edition Washington, D.C. pages 70 -88
12. Bamidele and adejumo, (2012).Effect of garlic (Allium sativum L) and ginger (Zingiber officinale Roscoe) mixture on performance characteristics and 11(3):217-220.
13. Behrouz, R.D, Sajjad, H, and Afshin,Z.(2012). Effect of dietary supplementations of prebiotics, symbiotic and acidifiers on growth performance and organs weights of broiler chickens: European Journal of Experimental Biology,2(6)2125-2129.
14. Bird A, Croom W and McBride B. 2002. Dietary management of the intestinal microbiota using probiotics and prebiotics in human and animals. Proceedings of Australian Poultry Science Symposium 14: 35-43.
15. Brown, L and Pentland, S. (2007). Male fertility – improving sperm quality. Health infertility Organization Vancouver, Canada, pg 87-101.
16. Brugali, I. (2003). Alimentacao alternative: an utilization de fitoterapicosou nutraceuticoscomomoduladores da imumidade e desempenho animal. Anais do simposiosobremanejo e Nutr Cad de Aves e Suinos; Campinas, Sao Paulo., Brasil.Campainas: (BNA) pp. 167-182.
17. Chang, K. J., Cheong, S. H. (2008). Volatile organosulfur and nutrient compounds from garlic by cultivating areas and processing methods. *Federation of Ammerican Society of Experimental Biology Journal*.22: 1108-1112.
18. Duncan, D.B. (1955) Multiple Range and Multiple F-Test. Biometrics, 11, 1-5.

19. Farmer, J.J., Enterobacteriaceae: Introduction and Identification, in Manual of Clinical Microbiology, 8th ed., Murray, P.R. *et al.*, Eds., ASM Press, Washington, DC, 2003, chap. 41.
20. Freeman, F. & Kodera, Y. (1995). Garlic chemistry: stability of S-(2-propenyl)-2 propene-1- sulfinothioate (allicin) in blood, solvents, and simulated physiological fluids. Journal of Agricultural and Food Chemistry 43, 2332–8.
21. Garfa, M.K., Itodo, A.A and Abdullahi, L. (2012). Extraction and physicochemical determination of garlic. International journal of food and nutrition science, 1(2): 4-7.
22. Gardzielewska, J., Pudyszak K., Majewska, T., Jakubowska, M and Pomianowski, J., (2003). Effect of plant-supplemented feeding on fresh and frozen storage quality of broiler chicken meat. Animal Husbandry Series of ElectronicJ.PolishAgric.Univ.6(2).
23. Hanan E. Al-Mashhadani (2014). Effect of different levels of turmeric supplementation on broiler performance, carcass characteristics and bacteria count of broiler chicken. Egyptian Poultry Science Journal.Vol 35 (1): 25-39.
24. Hui, Y.H. (1996):Oleoresins and essential oils. In: Hui,YH, editor. Bailey's industrial oil and fat products. New York, Wiley-interscience Publication, Cap.6.pp: 145-153.
25. Ibrahim, N.D.G., Abdulrahman, E.M and Ibrahim.G (2001). Elemental analysis of the leaves of Vernoniaamydalina and its biological evaluation in rats. Nigerian Journal of Natural Products and Medicine. 5: 13-17.
26. Jimoh,A.A.; Ibitoye, E.B.; Dabai, Y.U. and Graba,S.(2013). In vivo Antimicrobial Potentials of garlic against Clostridium perfringens and its promotant effect on performance of broiler chickens. Pakistan Journal of Biological Science. 16(25)1978-1984.
27. Jonkers D, Sluimer J, Stobberingh E. (2000). Effect of garlic on vancomycin resistance enterococci. Antimicrobe Agents chemother; 3043-33045
28. Marilynn L, (2001). Effect of garlic on blood lipids in particles with coronary heart disease. Am J ClinNutr 34: 2001-2103.
29. Miron T, Rabinkov A, Mirelman D, Wilchek H, Weiner L., (2000). The mode of action of allicin: its ready permeability through phospholipid membranes may contribute to its biological activity. BiochemBiophysActa: 1463:20-30
30. Mohammed, E., Mosad, A.S., Mohammed, M.S and Adel, H. (2016). Growth performance, Immune response, blood serum parameters, nutrient digestibility and carcass traits of broiler chickens as affected by dietary supplementation of garlic extract. Alexandria Journal of Veterinary Sciences, 49(2): 50-54.
31. Murray, R.K., Granner, D.K, Mayes, P.A and Rodwell, V.W (2000). Harpers Biochemistry, 25th edition, McGraw – Hill, Health Profession Division, USA.
32. **Musa, Bashir.,** Alagbe, J.O., Adegbite Motunrade Betty, Omokore, E.A. (2020). Growth performance, caeca microbial population and immune response of broiler chicks fed aqueous extract of Balanites aegyptiaca and Alchornea cordifolia stem bark mixture. United Journal for Research and Technology, 2(2):13-21.
33. NRC, National Research Council (1994) Nutrient Requirement for Poultry (9th red) National Academy Press. Washington D. C, USA.
34. **Olafadehan, O.A., Oluwafemi, R.A and Alagbe, J.O (2020). Carcass quality, nutrient retention and caeca microbial population of broiler chicks administered Rolfe (*Daniellia oliveri*) leaf extract as an antibiotic alternative. *Journal of Drug Discovery*. 14(33):146-154.**
35. Oluwafemi, R.A., Isiaka Olawale and Alagbe, J.O. (2020). Recent trends in the utilization of medicinal plants as growth promoters in poultry nutrition- A review. *Research in: Agricultural and Veterinary Sciences*. 4(1): 5-11.
36. Onibi, G.E.; Oluwatoyin, E.; Adebisi, A.; Fajemisin, N.;Ayode, V. and Adetun, J.I. (2009). Response of broiler chickens in terms of performance and meat quality to garlic (*Allium sativum L*) supplementation. African Journal of Agricultural Research. 4(5):511-517.
37. Rees, L. P., Minney, S. F., Plummer, N. T., Slater, J. H. and Skyrme, D. A., (2003) A quantitative assessment of the antimicrobial activity of garlic *(Allium Sativum).World journal of microbiology and Biotechnology* 9,303-307.
38. Roozbeh ,S.;Mehran,N.;Faramjn,J.;Ali,A.; and Hamed,K.(2012).The effect of probiotics on growth

performance of broilers. (Scholars Research Library, Annals of biological Research) 3(12):5450-5452.

39. Schleicher A, Fritz Z, Kinal S (1998). Zastosowaniewybranychziół w mieszankachtre_ciwychdlakurcz_trze_nych [The use of some herbs in concentrates for broiler chickens].Rocz.Nauk.Zootech. 25(3): 213-244
40. Sharma VD, Sethi MS, Kumar A, Rarotra JR. (2009) antibacterial property of Allium sativum Linn. In vivo and in vitro studies. India J WxpBiol; 15: 466-468
41. Skyrme DA. (1997). The antimicrobial activity of *Allum sativum.* PhD Thesis, Cardiff University, UK.
42. Soetan, K.O., Olaiya, C.O and Oyewole, O.E (2010). The importance of mineral elements for humans, domestic animals and plants: A review. African Journal of Food Science. Vol. 4(5) pp. 200-222.
43. Tiwari DP and Jyoti P. 2008. Antibiotic Probiotic and Prebiotic as feed additives: Health benefits and safety. Food Quality Assurance: a concentrated approach p 111 -130
44 Shittu, M.D., Adejumo, D.O., Ewuola, E.O., Alaba, O., Alagbe, J.O and Ojebiyi, O.O. (2020). Gut morphometric characteristic and ecological response of broiler starter fed varied levels of protein. *Asian Journal of Animal Science*, 14(1):33-39.