Nigerian J. Anim. Sci. 2017 (2):62 - 71

Effect of Different Inclusion Levels of CRINA® Poultry Plusas Replacement for Antibiotic Growth Promoters on the Performance of Broiler Chickens Reared under Field Conditions in Nigeria

Moses, O.[1]; Onimisi, P.A[1] and Jegede, J.O[2]

[1]Department of Animal Science Ahmadu Bello University Zaria Nigeria
[2] National Animal Production Research Institute Ahmadu Bello University Zaria Nigeria

Corresponding author:onimisiphil@gmail.com +234 8057228486

Target Audience: Poultry farmers, Poultry Researchers, Feed millers.

Abstract

A total 396 day old Ross broiler chicks were used in a feeding trial to evaluate the response of broiler chickens fed diets supplemented with different levels of CRINA® Poultry Plus, as replacement for AGPs. Chicks were allotted randomly to six treatments each replicated thrice, with 22 chicks per replicate. CRINA® Poultry Plus was included at 0g, 30g, 35g and 40g/100 Kg diet representing T1-T4 respectively while T5 and T6 had Oxytetracycline and water-grade Neocyril plus respectively. Data was collected on growth performance, haematology, liver function, ileum and ceacum microbes, carcass quality, tibia bone quality, and litter quality. All data collected were subjected to analysis of variance and significant differences among treatment means were compared using the Dunnett test of significance. In the starter phase, broilers fed diet containing Oxytetracycline had significantly ($P<0.05$) highest values for final weight, weight gain, feed consumption, and better feed conversion ratio. Significant ($P<0.05$) differences were observed for albumin and blood urea nitrogen while ALT, ALP AST were not significantly ($P > 0.05$) different. Birds fed levels of CRINA® Poultry Plus had significantly higher bone dry matter and bone ash than birds in the control group and on antibiotics. There was a significant ($P < 0.05$) increase in dry matter and a significant decrease in excreted nitrogen in the litter for treatments containing CRINA® Poultry Plus.CRINA® Poultry Plus did not improve growth in broiler chickens; it however significantly improved bone quality and litter quality which have positive implication on the health of birds.

Key words: CRINA® Poultry Plus, Antibiotic growth promoter, Performance, broiler chickens.

Description of Problem

Antibiotics have been used worldwide in animal feed for about 50 years ever since the discovery not only as an anti-microbial agent, but also as a growth-promoting agent resulting in improvement in performance. However, all uses of antimicrobial drugs, in both humans and animals, contribute to the development of antimicrobial resistance, hence antimicrobial-resistant bacteria have become a major

threat to public health. Illnesses caused by drug-resistant strains of bacteria are more likely to be potentially fatal when the medicines used to treat them are rendered less effective (1). A simple way to help overcome the health problems caused by antibiotic resistance is to stop adding antibiotics to animal feed. By January 2006, the EU placed a total ban on the use of feed antibiotics. The alternatives to antibiotics being currently promoted probiotics, prebiotics, organic acids and essential oils (2).

Essential oil compounds are a group of feed additives showing a potential for the replacement of antibiotics growth promoters (AGPs). They are active ingredients present in various plants and spices (e.g. thymol, carvacrol, eugenol). Due to their antibacterial activity they might be able to modify the composition of intestinal microbiota and to exert beneficial effects on performance of poultry (3, 2). CRINA® Poultry Plus is a feed additive from DSM Nutritional products that acts to promote eubiosis in the gastro-intestinal-tract of broilers. The product is a blend of eubiotics, a combination of benzoic acid and essential oil compounds (thymol, eugenol and piperine).

The aim of this work therefore was to evaluate the efficacy of CRINA® Poultry Plus as alternative to the conventional antibiotics used as growth promoters in poultry production. The specific objective was the Evaluation of the optimum level of CRINA® Poultry Plus in broiler diets under field conditions and the effect on growth performance, intestinal microbiota, haematology, liver function, bone quality and litter quality characteristics.

Materials and Methods

Experimental site

The experiment was conducted at the Poultry Unit of Animal Science Departmental Teaching and Research farm, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. Zaria is located in the Northern Guinea Savannah Ecological zone on longitude 11° 09' 01.78'N and latitude 7°39' 14.79'E, 671m above sea level. The climate is characterized by a well-defined dry and wet seasons and relatively dry with annual rainfall ranging from 700-1400mm (4).

Experimental Design and management of birds

Three hundred and ninety six (396) day-old Ross broiler chicks were allocated to six (6) dietary treatments, each replicated three times with 22 chicks per replicate each in a completely randomized design (CRD). The birds were housed in deep litter pens and managed with all necessary routine management practices and routine vaccinations.

Experimental diets

Six maize-soya beans cake based diets were formulated at both the starter and finisher phases of the feeding trial to meet standard requirements of broiler chickens as recommend by (5) and (6). CRINA® Poultry Plus was added as non-inclusive part of the diets as shown below.

Diet 1: 0 g of CRINA® Poultry Plus/100kg diet (Control)

Diet 2: 30g of CRINA® Poultry Plus /100 Kg diet

Diet 3: 35g of CRINA® Poultry Plus /100

Kg diet
Diet 4: 40g of CRINA® Poultry Plus /100 Kg diet
Diet 5: Oxytetracycline at 120g/100Kg diet (as recommended by manufacturer)
Diet 6: Control diet but birds were given water-grade Neocyril at recommended levels
The manufacturer's recommendation for CRINA® Poultry Plus is 300g/ton of feed.

Growth Study

Initial and final weights of birds were taken at the beginning and at the end of both starter and finisher phases. Feed intake was measured weekly while, weight gain feed/gain ratio and cost per Kg gain were computed for both phases. Mortality was recorded as they occur.

Haematological and Blood Serum Biochemical investigation

At the end of the starter phase trial, 2ml of blood samples was collected from each of three birds per replicate via the wing veins into sterile tubes containing an anticoagulant (ethylene diamine tetra acetic acid, EDTA) for the determination haematological parameters like Packed Cell Volume (PVC) which was determined by the microhaematocrit method, haemoglobin concentration (HB) which was determined photometrically at the wavelength of 540nm, the erthtocyte (RBC) and leucocytes (WBC) were done using the improved Neubauer haemocytometer, Differential leucocyte counts were determined by the thin slide method (7).

Liver Function Test

At the end of the starter phase, 2 mls of blood samples were taken from 1 chicken per replicate that is three birds per treatment into sterilised sample bottles containing no anticoagulant and were allowed to cloth and then centrifuged and serum was separated and stored at -20°C at the Clinical Pathology laboratory of the Ahmadu Bello University Teaching Hospital for determination of parameters related to liver function; blood glucose, blood urea nitrogen, alanine aminotransferase (ALT), albumen (ALB), aspartate aminotransferase (AST) and alkaline phosphatise (ALP), according to the methods described by (7).

Bone Quality determination

Two birds selected to represent the average weight of the each replicate group were used for the tibia bone analysis. The tibia bones of the birds were removed carefully after slaughtering procedures. They were weighed in grammes using a top loading digital scale to obtain fresh weight of bones. The length of the tibia bones was measured in cm using a graduated ruler. The fresh bones were oven dried at 100 ^{0}C until a constant weight was obtained. The dry bones were ashed at 550 ^{0}C in a muffle furnace for 6 hours to obtain the percent ash content of the bone. Bone density was calculated as bone weight/bone length.

Litter Quality Analysis

At four weeks, the litter from each pen was mixed and representative samples were taken for laboratory analysis. The dry matter, nitrogen and pH were determined. The process was repeated at week 8.

Data Analysis

All data obtained from the two feeding trials were each statistically analysed using the General Linear Model Procedure of Statistical Analysis Systems and Significant difference between treatments means were

separated using Dunnets Test (8).

Results and Discussions

Table 1 shows the growth performance of broiler chicks fed different levels of CRINA® Poultry Plus. There were significant (P<0.05) differences in all the parameters measured. Birds on Oxytetracycline had significantly highest values for final weight, weight gain, feed consumption and better feed conversion ratio. No trend was observed for growth parameters measured for birds fed different levels of CRINA® Poultry Plus.

The observed high performance by the AGP (Oxytetracycline) shows that antibiotic in animal feed increases feed efficiency and growth rate. Many authors have speculated that antibiotics had effect on growth and feed efficiency through the action of microorganism in the intestinal tract. This study agrees with the report of (9), who fed caged broilers a mixture of essential oils or 20 mg/kg Virginiamycin. Addition of Virginiamycin resulted in improved growth and feed conversion but no significant response to the mixture of essential oils was noted. Chlortetracycline, Oxytetracycline and penicillin also show an improved growth rate when supplemented in animal feed (10).

In contrast however (11) supplemented a wheat-barley diet for broilers with avilamycin or 150 or 300 ppm of a plant extract containing capsaicin, carvacrol, and cinnamic aldehyde. Administration of the antibiotic and the two levels of the plant extract improved body weight by 4.7, 5.4, and 8.1% and improved feed conversion by 5.8, 3.1, and 7.1%, respectively. CRINA® Poultry Plus did not improve growth performance above the control diet

Table 1. Growth Performance of Broiler chicks fed different levels of CRINA® Poultry Plus

Parameters	Level of CRINA® Poultry Plus				Oxytet[1]	Neocyr[2]	SEM
	0g	30g	35g	40g			
Initial Wt (g/bird)	41.90	42.40	41.90	41.90	43.30	42.70	1.40
Final Wt (g/bird)	1114.20^{b}	1075.90^{b}	1130.00^{b}	1055.00^{b}	1322.70^{a}	1112.30^{b}	26.72
TWG (g/bird)	1072.30^{b}	1033.50^{b}	1088.10^{b}	1013.10^{b}	1279.40^{a}	1069.60^{b}	26.46
DWG (g/bird)	38.20^{b}	36.90^{b}	38.80^{b}	36.20^{b}	45.60^{a}	38.20^{b}	0.95
TFC (g/bird)	1798.60^{bc}	1778.40^{bc}	1801.10^{bc}	1689.90^{c}	2073.50^{a}	1833.60^{b}	35.03
DFC (g/bird)	64.20^{bc}	63.50^{bc}	64.30^{bc}	60.40^{bc}	74.10^{a}	65.50^{b}	1.25
FCR	1.68	1.72	1.66	1.67	1.62	1.72	0.98
Feed cost/Kg gain (₦/Kg)	181.90^{b}	179.46^{a}	172.91^{a}	174.56^{a}	179.86^{b}	189.15^{c}	3.26
Mortality (%)	1.52^{a}	0.00^{b}	1.52^{a}	0.00^{b}	1.52^{a}	1.52^{a}	0.05

a,b,c. Means with different superscript differ significantly across the row (P<0.05) FCR= Feed conversion ratio DFC= Daily fe ed consumed TWG= Total weight gain DWG= Daily weight gain TFC= Total feed consumed Oxytet[1]: Oxytetracycline Neocyr[2]: Neocyril plus SEM: Standard error of means

Table 2 shows the growth performance of broiler finisher chickens fed levels of CRINA® PoultryPlus. There was significant ($P < 0.05$) difference in final weight, weight gain and feed consumption. Birds on AGP (Oxytetracycline) had higher values for final weight, weight gain and feed consumption as compared to other treatments. The high values observed for birds on AGP based diet showed that antibiotic increases feed efficiency and growth rate. Since there was also a significantly higher feed consumption, it may mean that Oxytetracycline as AGP simply stimulated higher feed consumption which was converted to meat.

Feed conversion ratio did not differ significantly ($P>0.05$). Although no trend was observed for birds fed CRINA® Poultry plus but 35g inclusion level in the diet proved to be the optimum and the least performance was observed for birds on 40g inclusion level of CRINA® Poultry plus. This may indicate that at that level, growth performance was less compared to the other treatments. The result of this study are in line with the findings of (12) who reported significant effects when comparing weight gains of birds fed diets with Aloe vera feed supplement and those fed diets containing antibiotic growth promoters. In contrast however,(13) did not observe significant effect on commercial essential oils on growth performance. Research results on the use of alternatives to antibiotic growth promoters in broiler chickens nutrition seem not completely consistent. Some authors stated significant positive effect on broiler performance (14, 15); others established no influence on gain, feed consumption or conversion of feed (16). The differences in results are consequences of numerous factors including type and part of plant parts used and their physical properties, preparation, method of photogenic additive and compatibility with other dietary components; health and environmental conditions of the chickens

Table 2. Growth Performance of Broiler finisher chickens fed different levels of CRINA® Poultry Plus

Parameters	Level of CRINA® Poultry Plus				Oxytet[1]	Neocyr[2]	SEM
	0g	30g	35g	40g			
Initial Wt (g/b)	1142.90	1112.10	1161.60	1055.60	1226.20	1142.90	42.75
Final Wt (g/b)	2579.40^{ab}	2455.00^{ab}	2546.00^{ab}	2318.90^{b}	2722.20^{a}	2479.40^{ab}	80.59
TWG (g/b)	1336.5^{b}	1343.2^{b}	1385.3^{a}	1263.3^{b}	1496.0^{a}	1336.5^{b}	73.80
DWG (g/b/d)	63.50^{b}	64.00^{b}	66.00^{a}	60.20^{b}	71.20^{a}	63.60^{b}	3.58
TFC (g/b)	3069.80^{b}	3017.90^{b}	3090.40^{b}	2912.50^{a}	3264.30^{a}	3185.70^{a}	63.63
DFC (g/b/d)	146.20^{b}	143.70^{b}	147.20^{b}	138.70^{b}	155.40^{a}	151.70^{a}	3.03
FCR	2.29	2.25	2.23	2.34	2.18	2.41	0.91
Feed cost/Kg gain. (N/Kg)	234.46^{a}	234.77^{a}	233.11^{a}	244.55^{b}	240.92^{a}	265.65^{b}	12.19
Mortality (%)	0.00^{a}	1.53^{b}	0.00^{a}	6.15^{e}	3.07^{c}	4.62^{d}	1.10

a,b,c,d,e Means with different superscript differ significantly across the row ($P < 0.05$) Oxytet [1]: Oxytetracycline Neocyr [2]: Neocyril plus TWG=Total weight gain TFC=Total feed consumed DWG=daily weight gain DFC=Daily feed consumed SEM: Standard error of means

Table 3 shows the haematological indices of broiler chickens fed levels of CRINA® Poultry Plus. There were significant (P > 0.05) differences for values of PCV, HB, RBC, heterophils. Lymphocytes and monocytes. Birds fed Oxytetracycline and 30g inclusion level of CRINA® Poultry Plus had the highest value (33.0%) PCV. Although it was observed that the PCV values obtained from all the treatment groups (29.7 to 33.0%) fell within the normal range (22.00-35.00%) for healthy chickens. This result is in consonance with the result obtained by (17) who reported a value of 21-35% for apparently healthy chickens. This result showed that the birds were immunologically balanced and were not anaemic and that the diets were ideal and adequate for broiler chickens.

The haemoglobin count observed in this study varied from 9.87-11.00 g/dl which fell within the normal range of 7-15g/dl recommended for healthy chickens indicating that the birds had sufficient blood pigment for proper transportation of oxygen, thus healthy living. The aim of estimating haemoglobin content is to determine the oxygen carrying capacity of birds' circulatory system.

Birds with low oxygen capacity can easily succumb to any form of respiratory diseases while birds with high haemoglobin concentration can be regarded as having high oxygen capacity and such birds are likely to withstand some level of respiratory stress. Reduction in the concentration of haemoglobin and packed cell volume suggest the presence of toxic factor such as haemaglutinin which can have adverse effect on blood formation (18).

The values obtained across the treatment groups for the red blood cells (5.07-5.83) were higher than the normal range $2.19x10^6$ µl or 1.58-3.82 µl as reported by (18).

Treatment effect was observed for heterophils which is a component of white blood cell although the values (10.33-17.67%) did not follow a particular trend. It has been reported that the higher the value of WBC the better phagocytosis and hence the ability to

Table 3. Haematological indices of Broiler chickens fed different levels of CRINA® Poultry plus in starter diet

Parameters	Level of CRINA® Poultry Plus				Oxytet[1]	Neocyr[2]	SEM
	0g	30g	35g	40g			
PCV (%)	31.3^{ab}	33.0^{a}	30.7^{ab}	33.0^{a}	33.0^{a}	29.7^{b}	0.71
Hb (g/dl)	10.40^{ab}	10.97^{a}	10.20^{ab}	11.00^{a}	10.97^{a}	9.87^{b}	0.24
TP (g/dl)	2.20	2.0 7	2.33	2.53	2.33	2.67	0.22
RBC ($x10^{12}$ /L)	5.13^{bc}	5.63^{ab}	5.10^{bc}	5.83^{a}	5.47^{abc}	5.07^{c}	0.16
WBC ($x10^{9}$ /L)	14.400	13.97	14.03	14.93	13.87	15.03	1.93
Heterophils (%)	10.33^{ab}	7.33^{b}	13.667^{ab}	13.67^{ab}	17.67^{a}	13.33^{ab}	2.66
Lymphocytes (%)	86.33^{a}	87.00^{a}	84.67^{a}	79.67^{b}	80.00^{b}	85.67^{a}	2.48
Monocytes (%)	82.00^{b}	83.00^{a}	84.67^{a}	79.67^{b}	80.00^{b}	85.67^{a}	1.56
Eosinophils (%)	1.33	2.67	0.33	3.00	1.00	0.00	1.53
Basophils (%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00

a,b,c, Means with different superscript differ significantly across the row (P<0.05) Oxytet [1]: Oxytetracycline Neocyr[2]: Neocyril plus SEM: Standard error of means

fight diseases. The non-significant (P > 0.05) differences in the values of lymphocytes, monocytes, eosinophils and basophils indicate no active infections. It therefore implies that the birds were well protected against microbial infection.

Table 4 shows the results of the liver function tests of starter broiler chickens fed different levels of CRINA® Poultry Plus. Significant (P<0.05) differences were observed for albumin and blood urea nitrogen. The albumin value range (41.67-54.3g/l), fell within the normal range reported by (17). Serum albumin is a strong predictor of health; a low albumin concentration is a sign of poor health and predictor of bad outcome (19). The higher the value of albumin the higher the clotting ability of blood, hence prevention of haemorrhage.

The mean value for blood urea nitrogen showed significant differences across the treatment groups with values ranging between 4.60-7.67mg/dl which is within the normal values (1.50-8.30mg/dl) as reported by (18). ALT is more specific to the liver and it's a better parameter for detecting liver injury, mean values for ALT showed no significant difference and also caused no injury to the liver since the values were within the normal range of 9.50 -37.20 as reported by (20).

It was observed that dietary treatment had no significant effect on the plasma glucose, which indicated that carbohydrate metabolism was not affected by the diet as chickens used carbohydrates as energy source rather than free fatty acid.

Table 5 showed the result of tibia bone analysis of broiler chickens fed levels of CRINA® Poultry Plus. Significant (P<0.05) differences were observed for bone dry matter and ash content. Birds fed levels of CRINA® Poultry Plus had significantly higher bone dry matter and bone ash as compared to birds on the control group and on antibiotics. CRINA® Poultry Plus showed a good potential to increase bone quality of bird. Burton *et al.* (21) reported that tibia is the fastest growing bone in the chicks while (22) stated that the tibia ash is a very sensitive tool used to evaluate calcium and phosphorus requirement based on the degree of mineralization. Improved bone quality, particularly faster mineral deposition is a critical need in modern

Table 4. Liver function indices of broiler chickens fed different levels of CRINA ® Poultry Plus

Parameters	Level of CRINA® Poultry Plus				Oxytet	Neocyr	SEM
	0g	30g	35g	40g			
Glucose (mmol/L)	262.0	252.7	252.0	236.7	249.0	255.7	11.05
Albumin (g/L)	44.3[a]	41.67[a]	43.67[a]	38.67[b]	42.33[a]	44.00[a]	2.56
(mmol/L)	7.67[a]	4.97[b]	6.73[ab]	5.33[b]	4.60[b]	5.33[b]	0.65
AST (µL)	23.33	28.0 0	29.67	24.33	20.33	26.33	3.94
ALT (µL)	12.67	15.6 7	16.67	13.33	16.00	18.67	2.09
AP (µL)	72.00	96.0 0	125.33	119.67	83.33	91.33	22.57

a,b, Means with different superscript differ significantly across the row (P < 0.05) Oxytet: Oxytetracycline Neocyr: Neocyril plus ALT= Alanine -amino tranferase AP= Alkaline phosphatise ASP= Aspartate -amino tranferase BUN= Blood Urea Nitrogen SEM: Standard error of means

day broiler breeds that have been bred for very rapid growth and high weight gain and need bones to develop fast enough to bear the weight which prevent leg deformities and the resultant poor performance and degraded chicken meat.

Table 5 Tibia bone characteristics of broiler chickens fed different levels of CRINA ® Poultry Plus

Parameters	Level of CRINA® Poultry Plus				Oxytet[1]	Neocyr[2]	SEM
	0g	30g	35g	40g			
Bone length (cm)	10.50	10.80	10.77	10.30	10.47	10.27	0.21
Bone weight (g)	22.33	22.33	23.33	20.67	21.67	20.00	1.02
Bone density (g/cm)	2.13	2.0 7	2.12	2.17	2.07	1.95	0.09
Bone dry matter %	70.64^{b}	73.21 ^{ab}	72.12^{ab}	74.71^{a}	71.28^{b}	71.19^{b}	0.46
Bone ash (%)	30.62^{b}	34.63^{a}	34.72^{a}	35.97^{a}	31.32^{ab}	31.27^{b}	1.18

a,b, Means with different superscript differ significantly across the row (P<0.05) Oxytet[1]: Oxytetracycline Neocyr[2]: Neocyril plus
SEM: Standard error of means

Table 6 shows the litter quality characteristics of broiler chickens fed different levels of CRINA® Poultry Plus. There was a significant (P<0.05) increase in dry matter and a significant (P<0.05) decrease in excreted Nitrogen for treatments containing CRINA® Poultry Plus. The pH values obtained were similar for all treatments and within a range of 5 - 6.5 reported by (23). In the course of the feeding trial, it was observed that the litters for birds fed CRINA® Poultry Plus was generally of better visual qualities as samples appear drier, lighter in colour and more powdery and without caking; compared to the darker, caked litter observed in birds on antibiotics and to a lesser extent those in the control group. A similar observation was made in the feeding trial in which Biostrong® 510 was used.

The significant (P<0.05) increase in dry matter may mean that birds excreted less water possibly as a result of improved gut efficiency by the action of CRINA® Poultry Plus. The reduced nitrogen levels in litters of birds fed CRINA® Poultry Plus is an indication that birds utilized feed nitrogen more efficiently resulting in less concentration of nitrogen in the wastes excreted. The combined effect of increased dry matter and reduced nitrogen contents of litter may have helped in minimizing ammonia build up in poultry pens as wet litters often generate ammonia. Ammonia toxicity has negative consequences on the health and performance of birds. Therefore, CRINA® Poultry Plus improves the quality of litter and consequently the health of birds.

Table 6 Litter quality characteristics of Broiler chickens fed different levels of CRINA ® Poultry Plus

Parameters	Level of CRINA® Poultry Plus				Oxytet	Neocyr	SEM
	0g	30g	35g	40g			
Dry matter (%)	86.21^{b}	89.69^{a}	93.41^{a}	94.44^{a}	78.55^{b}	80.77^{b}	3.24
pH	2.66	2.54	2.56	2.52	2.53	2.45	0.09
Nitrogen concentration (%)	6.30^{a}	6.17^{b}	6.13^{b}	6.10^{b}	6.20^{a}	6.27^{a}	0.05

a,b, Means with different superscript differ significantly across the row (P < 0.05) Oxytet: Oxytetracycline Neocyr: Neocyril plus
SEM: Standard error of means

Conclusion and Applications

It can be concluded that:

1. CRINA® Poultry Plus used as natural growth promoter, did not significantly improve growth of broiler chickens above those fed the control, diet
2. Cost of production was lowered (35g/100 Kg) feed than the control and AGPs in finisher broilers.
3. It improved liver health, bone strength and litter quality.

Acknowledgement

The authors wish to acknowledge the contributions of DSM Nutritional Products Company through their representative in Nigeria in supplying the product, CRINA® Poultry Plus used for this experiment.

References

1. FDA, (2015). Phasing Out Certain Antibiotic Use in Farm Animals. U.S. Food and Drug Administration. *_Toc464041291http://www.fda.gov/ForConsumers/ConsumerUpdates/ucm378100.htm_Toc449680403_Toc449682154_Toc464041291.*
2. Broz, J and Paulus, C. (2015). Eubiotics: Definitions and concepts. DSM Nutritional products. *http://www.dsm.com/animal-nutrition-health.*
3. Mitsch, P. Zitterl-Eglseer, K. Kohler, B. Gabler, C., Losa, R. and Zimpernik, I. (2004). The effect of two different blends of essential oil components on the proliferation of *Clostridium perfringens* in the intestines of broiler chickens. *Poultry. Science.* 83:669-675.
4. Ovimaps, (2015). Ovi location map; Ovi earth imagery. July 30th, 2015.
5. NRC, (1994). Nutrient Requirements of Poultry. (9th revised edition.). National Research Council. National Academy Press. Washington, D.C., USA.
6. Olomu, J.M. (2011). Monogastric animal nutrition. Principles and practice. Jachem publishers Nigeria. Pp 68-69.
7. Lamb, G.N. (1991). *Manual of Veterinary laboratory technique*. CIBA-GEIGY, Kenya, PP: 96-107.
8. S.A.S. (2002). Statistical Analysis System Institute, User's Guide. Version 9 for Windows. North Carolina, U.S.A.
9. Vogt, H. (1990). The effect of a mixture of essential oils in broiler diets. *Landbauforshung Volkenrode*, 40:157-159.
10. Anonymous, (2015). Effects of Antibiotics on Animal Feed. *http://www.udel.edu/chem/C465/senior/fall97/feed/present.html.*
11. Jamroz, D.J. Orda, C. Kamel, A. Wiliczkiewicz, T. Wertelecki and J. Skorupinska. (2003). the influence of phytogenic extracts on performance, nutrient digestibility, carcass characteristics, and gut microbial status in broiler chickens. *Journal of Animal Feed Science*. 12: 583-596.
12. Nmerole, F.U.C. (2011). Evaluation of dietry inclusion of Aleovera as an alternative to antibiotic growth promoter in broiler production.

*Pakistan Journal of nutrition*10 (1);1-5.

13. Jang I.S, ko Y.H, kang S.Y. Lee, C.Y. (2007). Effect of Commercial essential oils on growth performance, digestive enzyme activity and intestinal microflora population in broiler chickens. *Animal Feed Science Technology* 134:304-315.
14. Eratas, O.N. Guler, T, Cifti, M, Dalkilic B. and Simek, U.G (2005). The effect of an essential oil mix derived from oregano, clove and anise on broiler performance. *International Journal of Poultry Science* .4.Pp.879-884.
15. Cross D. E, McDevitt R. M, Hillman K, and Acamovic, T. (2007). The effect of herbs and their associated essential oils on performance, dietary digestibility and gut microflora in chickens from 7-28 days of age. *British Journal Poultry Science,* 48: 496-506.
16. Ocak, N, Erener, G. Burck, A.K.F. Sungu, M. Altop, A. and Ozmen, A (2008). Performance of broilers fed diets supplemented with dry peppermint (*Mentha Piperita L.)* or thyme (*Thymus Vulgaris L)* leaves as growth promoter source *Czech Journal of Animal Science* 53(4):169-175.
17. Oladele, S.B and Ayo, J.O (1999). Comparative studies on haematocrit, haemoglobin and total protein values of apparently healthy and clinically sick indigenous chickens in Zaria, Nigeria. *Bulletin of Animal Health and Production in Africa*, 47(4): 163 – 165
18. Oyawoye, E.O and Ogunkunle, M. (1998); Physiological and Biochemical effects of raw jack beans on broiler. *Proceedings of 23rd Conference of Association of Nigerian Society for Animal Production* held at Gateway Hotel, Abeokuta. Nigeria. September, 1998.Pp. 141-142.
19. Mitruka, B.M. and Rawnsley, H.M. (1997). Clinical Biochemical and Heamatological references values in normal experimental animal. Masson Publishing U.S.A. Inc .New York.
20. Kastow.com (2009). Globulins. Total protein Available. *http/://www.drkastow.com/htm/protein. Albumin.globulin.html*.
21. Burton, R. W., Sheridan A. K., and Howlett C. R. (1981). The incidence and importance of tibialdyschondroplasia to commercial broiler industry in Australia. *British Journal Poultry Science,* 222:153–160.
22. Driver, J. P., G. M. Pesti, R. I. Bakalli, and H. M. Edwards, Jr. (2006). The effect of feeding calcium- and phosphorus-deficient diets to broiler chickens during the starting and growing-finishing phases on carcass quality. *Poultry Science,* 85:1939-1946.
23. Nahm, K.H (2003). Evaluation of the nitrogen content in poultry manure. *World's Poultry Science.*