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Evaluation of optimum inclusion levels of biostrong® 510 15% as a replacement for antibiotic growth promoters in broiler chicken production under field conditions in Nigeria

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Abstract

An experiment was conducted to evaluate the efficacy of Biostrong® 510 15% (BSG) in broiler chickens diet. It was included at 0 g, 75 g, 100 g and 125 g/100 Kg diet, for T1-T4 respectively while T5 had Oxytetracycline. Data was collected on growth performance indices, haematological parameters, liver function indices, kidney function, blood mineral profile, immune response, antioxidant capacity, villi morphometrics, lipid profile, ileum and ceacum microbial contents, carcass quality parameters, and tibia bone quality indices. All data collected were subjected to analysis of variance and significant differences among treatment means were compared using the Tukey test of significance. Growth performance showed that broilers fed diet containing 100g BSG had optimum performance among other levels. Haematological and liver function indices were not significantly (P>0.05) different. Birds fed BSG showed decreased cholesterol, triglycerides and high-density lipoprotein compared to the control treatment. Birds fed levels of BSG and AGP had significantly higher bone weight, bone density, bone dry matter and bone ash as compared to birds on the control group. All carcass evaluation parameters were significantly (P<0.05) different among treatments except for dressed weight, breast and kidney. Levels of BSG in the diet significantly lowered the cost of production than AGPs of finisher broilers improved liver health, bone strength, villi characteristics, increased beneficial bacteria population and also prevent the colonization of the gut by pathogenic bacteria thus improving the health of the birds. Biostrong® 510 15%, can therefore effectively replace antibiotic growth promoters in broiler chicken production.

Keywords: Biostrong® 510 15%, Antibiotic growth promoter, Performance, broiler chickens

Introduction

The use of antibiotics as growth promoter intended not only as therapeutic but also as feed additive of continuous use in animal started appropriately five decades ago (Segura and De Bloos, 2008). Antibiotics have been also used to promote growth rate, improve feed conversion ratio (FCR) and reduce mortality in broiler flocks. Usually, Antibiotic Growth Promoters (AGPs) are administered at low doses, absorbed minimally from the gut and when incorporated into the feed, they act by specifically reducing the number of pathogenic bacteria (Dafwang *et al.,* 1987). However, repeated use of antibiotics in poultry diets resulted in severe problems like resistance of pathogen to antibiotics, accumulation of antibiotics residue in their products and environment, imbalance of normal microflora and reduction in beneficial intestinal microflora (Barton, 2000). The use of feed antibiotics and some other antimicrobial compounds, used as performance enhancers became the target of increasing public criticism. By January 2006, the EU placed a total ban on the use of feed antibiotics. This total ban on the use of antibiotics as growth promoters has been integrated into a new EU regulation concerning feed additives (Broz and Paulus, 2015). The alternatives to antibiotics being currently promoted are Eubiotics. 'Eubiosis' as used in the feed industry refers to a healthy balance of the microbiota in the gastrointestinal tract. Some traditional Feed additives such as probiotics, prebiotics, organic acids, acidifiers, essential oils Zn and Cu compounds with claim to affect the composition or activity of intestinal microbiota are often referred to as eubiotics (Broz and Paulus, 2015). Biostrong® 510 15% (BSG) an essential oil; is a phytogenic eubiotic formulation from Delacon Nutrional Company in Austria. It is a blend of high-grade essential oils, bitter substances, pungent substances and saponins.

Healthy balance of micro-flora in the gastrointestinal tract is closely related with performance in broilers. This is of greatest interest among broiler producers because of its impact on economic profitability. The Increase in bacterial resistance to antibiotics in both humans and livestock has caused an increase in public and governmental interest in eliminating sub-therapeutic use of antibiotics in livestock.as it raises concerns for food safety, environmental conservation and producing safer human foods from animal sources more efficiently and at lower cost. This has given impetus to continued search for new feed additives that would positively modulate the gut micro-flora, increase rate of growth and level of production. (Dhama *et al*., 2011; Youssef *et al*., 2013). Virtually, all the Eubiotics currently been used were developed in the temperate region and their dosages were determined in a controlled environment. Therefore, there is need to ascertain the optimum level and the efficacy of Biostrong® 510 15% in the tropics and also in our environment under field conditions.

The aim of the study was to evaluate the efficacy of Biostrong® 510 15% as alternative to the conventional antibiotics used in poultry production.

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° 09Σ01.78ΣN and latitude 7°39Σ14.79ΣE, 671m above sea level. The climate is characterized by well-defined dry and wet seasons and relatively dry with annual rainfall ranging from 700-1400mm (Meteorological Unit, Institute for Agricultural Research, Ahamadu Bello University Zaria, 2017).

Experimental design and management of birds

Five hundred and ten day-old Ross broiler chicks were allocated to five dietary treatments, each replicated three times with 34 chicks per replicate each in a completely randomized design. The birds were housed in deep litter pens and managed with all necessary routine management practices and routine vaccinations

Experimental diets

Five maize-soyabeans 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 (NRC, 1994) and (Olomu, 2011). Biostrong® 510 15% was added as non-inclusive part of the diets as shown below. Five diets were formulated each for both starter and finisher phases as shown in Tables 1 and 2.

Diet 1: 0 g of Biostrong® 510 15%/100kg diet (Control); Diet 2: 75g of

Biostrong® 510 15%/100 Kg diet; Diet 3: 100g of Biostrong® 510 15%/100 Kg diet; Diet 4: 125g of Biostrong® 510 15%/100 Kg diet; Diet 5: Oxytetracycline at 60g/100Kg diet (as recommended by manufacturer). The manufacturer's recommendation for Biostrong® 510 15% 1000g/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.

Liver function test

At the end of the starter phase, 2 mLs of blood samples were taken from one chicken per replicate that is three birds per treatment into sterilised sample bottles containing no anticoagulant. samples were allowed to clott and then centrifuged, 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 Lamb (1991).

Villi morphometric

Intestinal segment samples (approximately 2 cm in length) ileum was taken from birds to evaluate villi morphometric. Intestinal Tissues were harvested and\fixed in 10% formolsaline. They were histologically processed according to the method of Bancroft and Stevens, 2008. They were dehydrated through ascending grades of alcohol (70%, 90% and 100%) for 2 hours each. theTissues were cleared in xylene for 2 hours they were impregnated and embedded in paraffin wax. they were sectioned at 5micron thickness using Rotary microtome machine (Leica RT 25 made in England). Sectioned tissues were mounted on slides, dried and stained using Hematoxylin and Eosin (H and E) stain. Stained sides of the tissues were photomicrograhed using Amscope Digital Camera for microscope verision 2.0, made in Japan. Histomorphometric analysis for the villi was carried out using Digimizer image analysis software version 4.5 made in USA. (Bancroft and Stevens 2010). The morphometric indices that were evaluated include villi area, perimeter, villus height, from the tip of the villus to the crypt, crypt depth from the base of the villi to the submucosa, and the villus height to crypt depth ratio. This was carried out at the Histology Laboratory of the Department of Anatomy Ahmadu Bello University Zaria.

Intestinal bacterial profile count

At day 28, bacterial cell population in the ileum and caecum was determined for *Escherichia coli spp, Lactobacilli spp, Clostridium spp, Salmonella spp, Bacillus spp* via by using different selective media for isolation of bacteria groups and characterization based on sugars fermentation using Microbact 12E kit and conventional biochemical methods. (O.M.P[abc] 2015). This was carried out at the Microbiology Department, Ahmadu Bello University, Zaria.

Bone quality determination

The tibia bones of the birds used for the carcass analysis were removed carefully. 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 °C in a muffle furnace for 6 hours to obtain the percent ash content of the bone. The bone ash was analysed for percent calcium, and magnesium content using Atomic

Table 1: Composition of broiler Starter diet

	Levels of Biostrong® 510 15%				
Ingredients (%)	**0g**	**75g**	**100g**	**125g**	**Oxytet**
Maize	56.00	56.00	56.00	56.00	56.00
GNC	13.00	13.00	13.00	13.00	13.00
SBC	27.00	27.00	27.00	27.00	27.00
Bone Meal	3.00	3.00	3.00	3.00	3.00
Limestone	0.50	0.50	0.50	0.50	0.50
Common Salt	0.25	0.25	0.25	0.25	0.25
Lysine	0.05	0.05	0.05	0.05	0.05
Methionine	0.20	0.20	0.20	0.20	0.20
Vit/min Premix [A]	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis					
ME Kcal/kg	2,901	2,901	2,901	2,901	2,901
Crude Protein (%)	23.21	23.21	23.21	23.21	23.21
Crude Fibre (%)	3.81	3.81	3.81	3.81	3.81
Ether Extract (%)	3.33	3.33	3.33	3.33	3.33
Calcium (%)	1.32	1.32	1.32	1.32	1.32
Phosphorus (%)	0.87	0.87	0.87	0.87	0.87
Lysine (%)	1.36	1.36	1.36	1.36	1.36
Methionine (%)	0.50	0.50	0.50	0.50	0.50
Feed cost (₦ /Kg)	145.20	147.99	148.91	149.84	152.7

[A] Vitamin- mineral premix provide per kg of diet: vit. A, 13,340 i.u; vit. D_3, 2680 i.u; vit. E, 10 i.u; vit. K, 2.68 mg calcium pantothenate, 10.68mg; vit. B_{12}, 0.022mg; folic acid, 0.668mg; choline choride, 400mg; chlorotetracycline, 26.68mg; manganese, 13mg; iron, 66.68mg; zinc, 53.34mg; copper, 3.2mg; iodine, 1.86mg; cobalt, 0.268mg; selenium, 0.108mg. Oxytet = Oxytetracycline; GNC = Groundnut cake; SBC = Soya beans cake

Table 2: Composition of broiler finisher diet

	Levels of Biostrong® 510 15%				
Ingredients (%)	**0g**	**75g**	**100g**	**125g**	**Oxytet**
Maize	58.00	58.00	58.00	58.00	58.00
GNC	13.00	13.00	13.00	13.00	13.00
SBC	18.00	18.00	18.00	18.00	18.00
Maize offal	6.85	6.85	6.85	6.85	6.85
Bone Meal	3.00	3.00	3.00	3.00	3.00
Limestone	0.30	0.30	0.30	0.30	0.30
Common Salt	0.30	0.30	0.30	0.30	0.30
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.05	0.05	0.05	0.05	0.05
Vit/min.premix [A]	0.25	0.25	0.25	0.25	0.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated Analysis					
ME (Kcal/kg)	2937	2937	2937	2937	2937
Crude Protein (%)	20.28	20.28	20.28	20.28	20.28
Crude Fibre (%)	4.25	4.25	4.25	4.25	4.25
Ether Extract (%)	3.33	3.33	3.33	3.33	3.33
Calcium (%)	1.25	1.25	1.25	1.25	1.25
Phosphorus (%)	0.88	0.88	0.88	0.88	0.88
Lysine (%)	1.10	1.10	1.10	1.10	1.10
Methionine (%)	0.53	0.53	0.53	0.53	0.53
Feed cost (₦ /Kg)	143.01	145.79	146.72	147.65	150.51

[A] Vitamin- mineral premix provide per kg of diet: vit. A, 13,340 i.u; vit. D_3, 2680 i.u; vit. E, 10 i.u; vit. K, 2.68 mg calcium pantothenate, 10.68mg; vit. B_{12}, 0.022mg; folic acid, 0.668mg; choline choride, 400mg; chlorotetracycline, 26.68mg; manganese, 13mg; iron, 66.68mg; zinc, 53.34mg; copper, 3.2mg; iodine, 1.86mg; cobalt, 0.268mg; selenium, 0.108mg. Oxytet= Oxytetracycline; GNC = Groundnut cake; SBC = Soya beans cake

Absorption Spectrophotometry, while phosphorus content using flame photometry. Bone density was calculated as bone weight/bone length. (A.O.A.C 2005).

Data analysis

All data obtained from the 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 tukey Test (SAS, 2002).

Table 3 shows the performance characteristics of broiler chicks fed levels of Biostrong® 510 15% (BSG 510 15 %) as a natural growth promoter. There were significant ($P < 0.05$) differences in final weight, weight gain, feed consumption, feed conversion ratio and feed cost/gain (N/Kg gain). Broiler chicks fed diet supplemented with 100g BSG 510 15 % showed high performance in terms of final weight, weight gained, best and least feed cost per kg gain (N 247.73) and feed conversion ratio (1.66) though lower than the AGP. BSG 510 15 % at 100g/100 Kg feed, reduced feed intake, increased body weight gain and also improved feed conversion of broiler chicks better than other levels. This level of inclusion equally significantly decreased the cost of production, thereby resulting to higher profits for the farmer. The result of this work agrees with the report of Guo *et al*. (2000) who reported that herbs and herbal products as natural feed additive have a positive effect on broiler growth performance. Nmerole (2011) also reported significant effects when comparing weight gains of birds fed diets containing *Aleo vera* feed supplement and those fed diets containing antibiotic growth promoter. In constrast however, Onimisi *et al.* (2016) did not find statistical differences in the performance parameters of birds fed diets supplemented with Biostrong® 510 an earlier product.

Table 4 shows the performance characteristics of broiler chickens fed levels of Biostrong® 510 15% (BSG 510 15 %) as a natural growth promoter. There were significant ($P < 0.05$) differences in final weight, feed consumption, feed conversion ratio and feed cost/gain (N/Kg gain) Birds on Oxyteracycline (AGP) and birds fed 100g/100kg BSG 510 15 % had significantly ($P < 0.05$) higher final weight than other levels of BSG 510 15 % and control. Feed consumption was significantly highest for the control but are at par with birds on antimicrobials. Feed conversion was significantly lowest for birds fed antimicrobials.

Table 3: Effect of Biostrong® 510 15% on the performance of broiler chicks 0-4 weeks

	Levels of BSG 15% / 100 kg feed					
Parameter	**0g**	**75g**	**100g**	**125g**	**oxytetracycline**	**SEM**
Initial weight (g/b)	47.48	47.98	47.12	47.55	47.96	0.74
Final weight (g/b)	924.50^b	931.37^b	948.23^b	943.18^b	1020.39^a	20.14
Weight gain (g/b)	877.01^b	885.39^b	901.10^b	893.63^b	969.43^a	20.46
Feed intake (g/b)	1615.10^a	1549.43^b	1499.70^b	1530.97^b	1652.00^a	29.00
Feed conversion ratio	1.84^c	1.75^b	1.66^a	1.71^{ab}	1.71^{ab}	0.04
Feed cost (₦/Kg)	145.2^a	148.0^b	148.9^c	149.8^d	152.7^e	0.00
Feed cost/gain (₦/Kg gain)	267.41^b	259.02^{ab}	247.73^a	256.90^{ab}	260.90^b	6.15
Mortality (%)	1.63	1.31	0.65	0.98	1.31	0.73

a,b,c,d e Means with different superscripts on the same row are significantly different (P<0.05).
SEM; Standard error of means

The result showed that antimicrobials had positive effect in improving production efficiency similar to the report of FAO (2014). Researchers have also stated that feed additives improve the overall efficiency of the chicken gastro intestinal tract. (Donoghue, 2003; Apajalahti *et al.,* 2004). Broiler chickens fed diet supplemented with 100g/100Kg BSG 510 15 % showed best performance in terms of final weight, weight gain, feed conversion and least cost of production than other levels and the control. The value obtained with this level of inclusion is also similar to that of the starter phase and also corresponds to the manufacturer's recommendation. The result in the present study also agrees with the report of Guo *et al*. (2000) who reported that herbs and herbal products as natural feed additive have a positive effect on broiler growth performance. In contrast however, experiments with broilers (Demir *et al*., 2003; Lee *et al*., 2003; Hernández *et al*., 2004) did not find statistical differences in the performance parameters of birds fed diets supplemented with different types, concentrations, or combinations of plant extracts. According to Lee *et al*. (2003), the absence of effect on bird performance may be related to the composition of the basal diet and/or to the environmental conditions of the experiment. The mortality recorded for this phase was generally minimal across the treatments and showed no particular trend which may not be as a result of treatment effect.

Table 4. Effect of Biostrong® 510 15% on the performance of broiler chickens 5-7 weeks

	Levels of BSG 510 15%/ 100 kg					
Parameter	**0**	**75**	**100**	**125**	**oxytetracycline**	**SEM**
Initial weight (g/b)	924.50	931.40	948.23	943.20	1020.40	20.14
Final weight (g/b)	2413.50^{c}	2431.37^{bc}	2535.73^{a}	2518.13^{ab}	2557.00^{a}	55.09
Weight gain (g/b)	1489.00	1499.97	1587.50	1574.93	1536.60	52.45
Feed intake (g/b)	2898.10^{a}	2826.07^{ab}	2839.97^{ab}	2822.80^{ab}	2887.20^{ab}	32.14
Feed conversion ratio	1.94^{b}	1.85^{ab}	1.79^{a}	1.80^{a}	1.88^{ab}	0.06
Feed cost (₦/Kg)	143.0^{a}	145.8^{b}	146.7^{c}	147.7^{d}	150.5^{e}	0.00
Feed cost/gain (₦/Kg gain)	278.77^{ab}	274.84^{ab}	265.78^{a}	262.93^{ab}	283.36^{b}	8.62
Mortality (%)	2.29^{b}	0.65^{a}	1.96^{b}	0.98^{ab}	1.96^{b}	0.55

a,b,c,d Means with different superscripts on the same row are significantly different (P<0.05); SEM; Standard error of means

Table 5 shows the liver function indices of broiler chickens fed levels of Biostrong® 510 15% there were no significant (P > 0.05) difference in all parameters measured. The results indicate that the birds could tolerate the levels of the additives without any deleterious effects on liver functions. These results also showed that the birds were in normal physiological state. However, liver enzymes AST, ALP and ALT though not significantly different were lower for birds fed Biostrong® 510 15%. The lower level of these liver enzymes in the blood of birds fed levels of Biostrong® 510 15% may indicate an improvement in the health of the liver and showed that the material does not impair the liver in its function. Such impairment or damage to the liver would have caused a leakage of these enzymes, leading to high levels in the blood. Analysis of serum enzyme is an important application in diagnosis of avian diseases (McDaniel *et al*., 1964). Table 6 shows the lipid profile of broiler chickens fed levels of Biostrong® 510 15% there were significant (P< 0.05) difference in total cholesterol, triglycerides and high-density lipoprotein. The results showed that broilers fed with diets supplemented with Biostrong® 510 15% had significantly lower cholesterol, triglycerides and high-density lipoprotein compared to the control treatment. The results obtained in the current study are in agreement with results reported by Ali *et al.* (2007) who showed that adding thyme to hen diets significantly decreased plasma HDL, total cholesterol, triglycerides and total lipids. Kirkpinar *et al.* (2011) showed that broilers fed with diets supplemented with oregano had significantly lower cholesterol and triglycerides compared to the control treatment According to Elson and Qureshi (1995), plant extracts may lower the blood cholesterol level in broilers via inhibition of the controlling enzyme for cholesterol synthesis (3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Furthermore, significant decreases in the blood cholesterol and/or LDL were observed due to supplementation of broiler chickens with licorice extract (Myandoab and HosseiniMansoub, 2012). In contrast however, Toghyani *et al.* (2010) added thyme in broiler diets in concentrations of 5 g/kg and 10 g/kg and did not find any effect on triglyceride, total cholesterol and LDL cholesterol concentration. Also El-Deek and Al-Harthi (2004) did not observe significant differences in the total lipid and cholesterol levels due to supplementation of the chicken diets with green tea powder.

Table 5: Liver function indices of broiler chickens fed levels of Biostrong® 510 15%

	Levels of BSG 510 15%					
Parameter	**0**	**75g**	**100g**	**125g**	**oxytetracycline**	**SEM**
TP (g/dl)	3.63	3.57	4.23	4.00	4.03	0.35
ALB (g/dl)	1.30	1.43	1.50	1.43	1.57	0.11
Glucose (mg/dl)	43.70	50.07	53.70	51.47	45.33	0.05
GLB (g/dl)	2.33	2.13	2.73	2.53	2.47	0.32
ALT (μL)	67.30	61.00	62.33	63.33	64.33	1.16
ALP (μL)	220.33	215.33	217.52	211.65	219.30	27.36
AST (μL)	209.33	203.33	205.22	206.27	208.33	22.39

a,b ; Means with different superscripts on the same row are significantly different (P<0.05): Aspartate amino transferase (AST) Alkaline Phosphatase (ALP) : Alanine amino transferase (ALT): Albumin(ALB): Globulin (GLB); SEM; Standard Error of Means

Table 6: Lipid profile of broiler chickens fed levels of Biostrong® 510 15%

	Levels of BSG 510 15%					
Parameter	**0g**	**75g**	**100g**	**125g**	**oxytetracycline**	**SEM**
Total Cholesterol (mg/dl)	91.10^{a}	72.15^{ab}	70.87^{ab}	73.40^{ab}	83.81^{a}	3.94
Triglycerides (mg/dl)	38.65^{b}	24.89^{c}	37.43^{b}	33.10^{b}	40.79^{a}	5.62
HDL (mg/dl)	60.58^{a}	30.70^{ab}	50.17^{a}	40.53^{ab}	44.00^{ab}	8.22
LDL mg/dl	25.56	29.42	19.29	22.90	20.65	8.45

a,b,c ; Means with different superscripts on the same row are significantly different (P<0.05): HDL; High density lipoprotein. LDL;Low density lipoprotein; SEM; Standard Error of Means

The body synthesizes about 1500-2000 mg of new cholesterol each day, dietary cholesterol Intake in chicken ranges from 28 – 116 mg/dL (Collins, 2016) against the desirable cholesterol level of 200mg (<130 LDL, >40 HDL and <135mg/dl triglyceride). Cholesterol in the bile can crystalize to form gallstones that may block the bile duct, and in the development of atherosclerosis (fatty deposits that form inside the blood vessels) leading to heart attack. However, the major culprit seems to be LDL (Low Density Lipoprotein) that are in excess of the body need (Ultranet, 2006). High triglycerides levels mean there is a good chance of having abnormal cholesterol numbers. Low level of HDL signifies good cholesterol while high level of LDL signifies bad cholesterol. High triglycerides are harmful and increases risk of heart diseases (Jessie 2016). Table 7 shows the result of tibia bone characteristics of broiler chickens fed different levels of Biostrong® 510 15%. There was significance (P<0.05) difference across the treatment for all parameters measured for Tibia bone characteristics except for bone length. Birds fed levels of Biostrong® 510 15% had significantly higher bone weight, bone density, bone dry matter and bone ash than birds on control and AGPs.

Table 7: Tibia bone characteristics of broiler chickens fed levels of Biostrong® 510 15%

	Levels of BSG 510 15 %					
Parameter	**0**	**75g**	**100g**	**125g**	**oxytetracycline**	**SEM**
Bone length (cm)	9.03	9.10	9.63	9.63	9.03	1.53
Bone weight (g)	11.67^{b}	13.00^{ab}	15.00^{a}	15.00^{a}	14.00^{ab}	0.32
Bone density (g/cm)	1.30^{b}	1.56^{ab}	1.65^{a}	1.56^{ab}	1.41^{ab}	0.15
Bone dry matter %	45.42^{b}	47.31^{ab}	51.62^{a}	47.97^{ab}	47.57^{ab}	1.35
Bone ash (%)	34.45^{b}	35.79^{ab}	43.41^{a}	37.47^{ab}	40.25^{ab}	3.35

a,b, Means with different superscripts on the same row are significantly different (P<0.05)
SEM: Standard error of means.

The higher values for bone density, bone dry matter and bone ash observed for the birds fed levels of Biostrong® 510 15% show that the material increased bone strength of the birds. The results obtained agress with the findings of Elkomy and Elsaid (2015) who reported that feeding diets supplemented with herbs (sage, rosemary and thyme) exhibited positive effects on bone mineral density. Also, Muhlbauer *et al*. (2003) found that bone resorption was inhibited by the essential oils extracted from these herbs.

Burton *et al.* (1981) reported that tibia is the fastest growing bone in the chicks while, Driver *et al* (2006) stated that the tibia ash is a very sensitive tool used to evaluate calcium and phosphorus requirement based on the degree of mineralization.

Increased bone strength is a critical need in modern day broiler breeds that have been developed for very rapid growth and high weight gain and need strong bones to bear the weight. Bones must also grow fast and with improved rate of mineral deposition to forestall leg deformities and the associated poor performance and degraded chicken meat.

Table 8 Shows the results of the effect of Biostrong® 510 15% on villi morphometrics of section of the ileum. Significant (P<0.05) differences were observed villi height, villi height/crpth depth ratio. Villi height, and villi height/crpth depth ratio were higher for birds fed higher dosage of Biostrong® 510 15% and AGP (Oxyteracycline). The observed result showed that antimicrobials had positive effect on gut morphology. (Oladele *et al.,* 2012; Erdogan *et al*., 2010) reported significantly higher villi length and crypth depth for broilers fed phytogenics feed additives. In contrast however, Abdulkarim *et al.* (2013) reported no significant effect on villi length and crypth depth on the phtogenic feed additive used in their study.

Table 8 Villi Morphometrics of broiler chickens fed levels of Biostrong® 510 15%

Parameter	**0g**	**75g**	**100g**	**125g**	**oxytetracycline**	**SEM**
Area $(\mu m)^2$	29359.00	33856.00	33096.00	33814.00	25917.00	6552.79
Perimeter (µm)	858.40	867.10	984.20	973.00	871.60	90.70
Villi height (µm)	324.33^{b}	329.15^{b}	421.62^{a}	412.86^{a}	368.42^{ab}	33.28
Villi width (µm)	133.31	160.32	150.67	144.14	133.31	17.80
Crypth depth (µm)	158.93	155.64	146.00	156.93	151.40	8.46
Villiheight/crypt (µm)	2.06^{b}	2.11^{b}	2.89^{a}	2.65^{a}	2.45^{a}	0.24

a,b , ; Means with different superscripts on the same row are significantly different (P<0.05)
SEM; Standard error of means

Effect of Biostrong® 510 15% on intestinal gut microbial contents

The effect of including Biostrong® 510 15% in the diet of broiler chickens on gut microbes are presented in figures 1 – 5 (ileal bacteria) and 6 – 10 (caecal bacteria). Figure 1 shows that supplementation of Biostrong 510® 15% reduced the concentration of Escherichia coli on birds on antimicrobials than the control. Reports show that Essential oils such as oregano and garlic oil inhibit microorganisms like *E. coli* and other Enterobacterial counts, *Clostridium* count, *Staphylococcus aureus*, *Salmonella typhimurium* and *Listeria monocytogenes* (Aligiannis *et al*., 2001; Friedman *et al*., 2002; Kirkpinar *et al*., 2011). The DSM (2013) reports that CRINA® Poultry Plus (an essential oil) reduces the levels of pathogenic bacteria and thus lowers the incidence of digestive disease and disorders in a flock. Figure 2 shows that supplementation of ***Biostrong 510® 15%*** inhibits the population of potentially pathogenic as they were not present in birds fed antimicrobials but present in the control. It therefore indicated that antimicrobials inhibited the population of potential pathogenic bacteria. The AGP Prevented exponential multiplication of common pathogenic bacteria (*E. coli*, *Salmonella* spp., *Streptococcus* spp., *Hemophilus* etc.), reduced incidences of non-specific diarrhoea or enteritis of chicken (Brennan *et al*., 2003; Huyghebaert *et al*., 2011). Figure 3 shows that supplementation of *Biostrong 510® 15%* decreased the population of *Clostridium spp*. This showed that Biostrong *510® 15%* improved the condition of the gut as it inhibited the population of potentially pathogenic clostidium. The DSM (2013) reports that CRINA® Poultry Plus (an essential oil) inhibits potentially detrimental bacteria. Both Gram positive, particularly *clostridium perfringes*, but also Gram negative such as *E.coli. Clostridium perfringens* can cause a subclinical disease associated with necrotic enteritis (NE) which is characterized by damage to the intestinal mucosa that decreases digestion, absorption and reduces weight gains which can result in great economic losses in poultry production (Wu *et al.,* 2008).

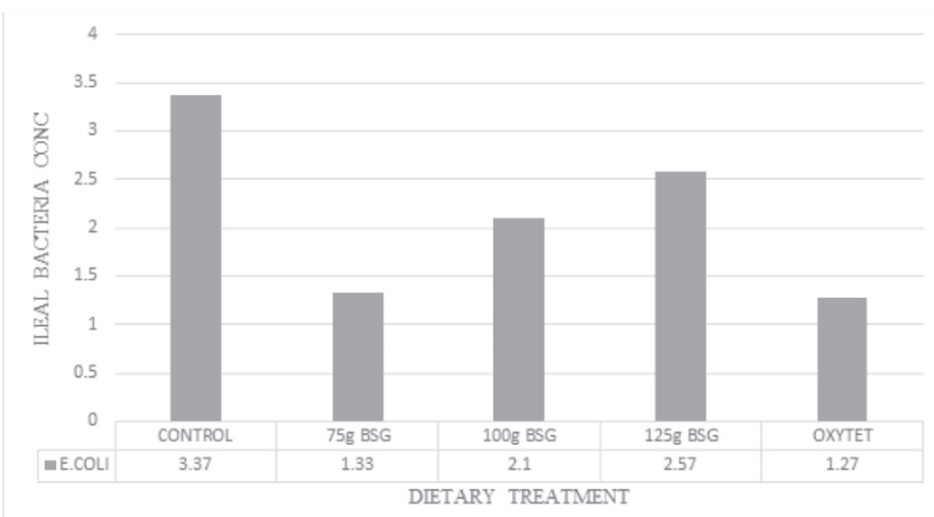

Figure 1: Effect of *Biostrong® 510 15%* on ileal *Escherichia coli specie*

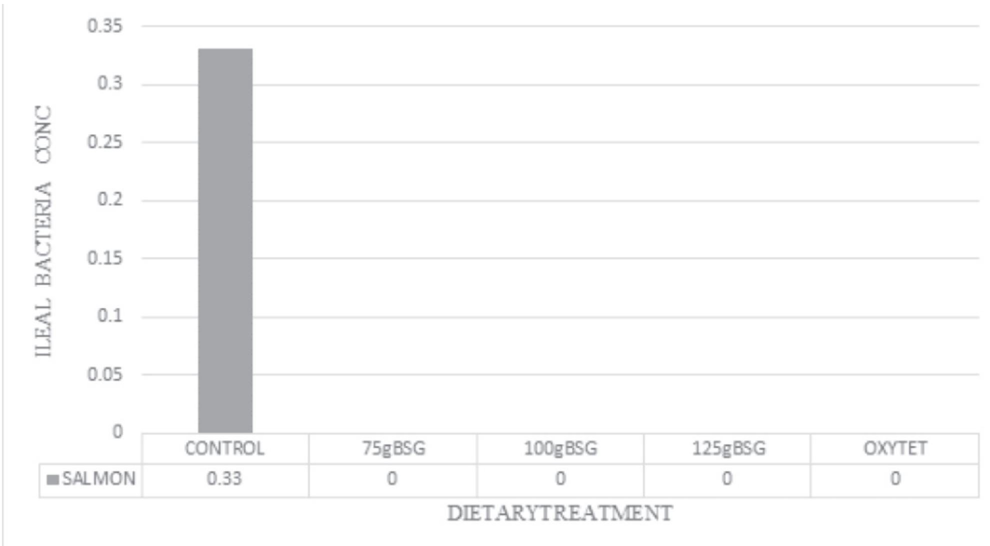

Figure 2: Effect of *Biostrong® 510 15%* on ileal *Salmonella* spp.

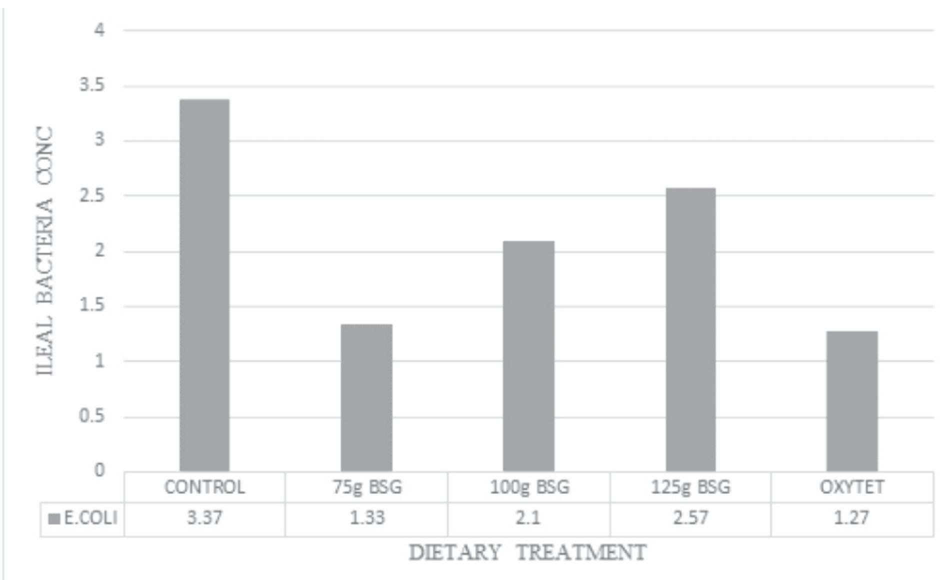

Figure 3: Effect of *Biostrong® 510 15%* on ileal *clostridium* spp

Figure 4 showed that supplementation of ***Biostrong 510® 15%*** increased the concentration of *Bacillius spp* in the ileum, it was however absent in the control. *Bacillius spp* are one of the beneficial harmless microbes in the microbiota. Liu *et al. (2012) reported that* oregano oil could improve the intestinal ecosystem of animals via promoting the growth of beneficial bacteria species and suppressing the growth of potential pathogenic bacterial species. Various *Bacillus spp* are being use as direct fed microbials enhanced chick performance as well as enriched the birds with a more diverse and complex bacterial composition in ileum, which expectedly provides a more robust microbiota less susceptible to diseases and infections (Lan *et al*., 2003). Figure 5 showed that supplementation of *Biostrong 510*® *15%* increased the concentration of *lactobacillius spp* in the ileum as the dosage of *Biostrong* 510® 15% increased. *Lactobacillus spp* are commensal bacteria that have long been known for their ability to activate the intestinal immue system and increase the resistance to disease (Muir et al., 2000). These bacteria have been reported to produce a wide variety of short chain fatty acids which are bacteriostatic for a subset of *bacteria spp*. Many essential oils stimulate growth of beneficial microbes and limit number of pathogenic bacteria in poultry (Wenk, 2000)

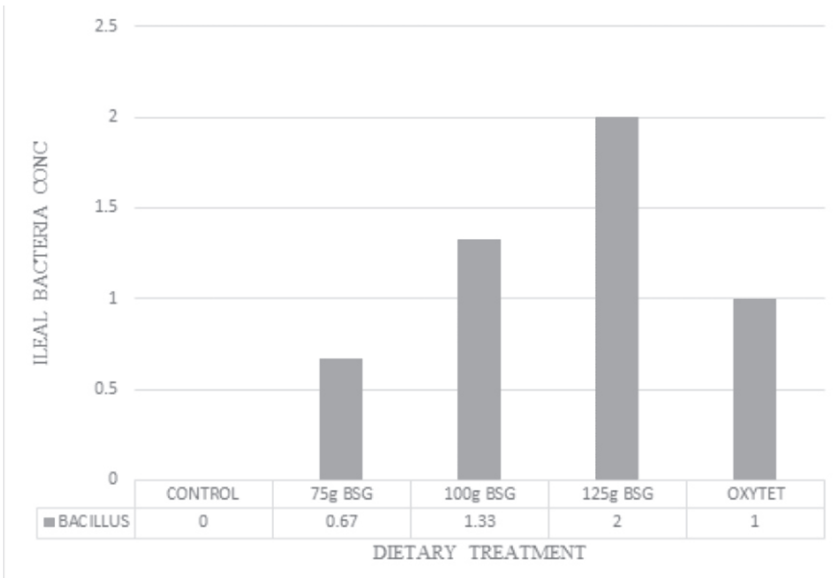

Figure 4: Effect of *Biostrong*® *510 15%* on ileal *Bacillus* spp

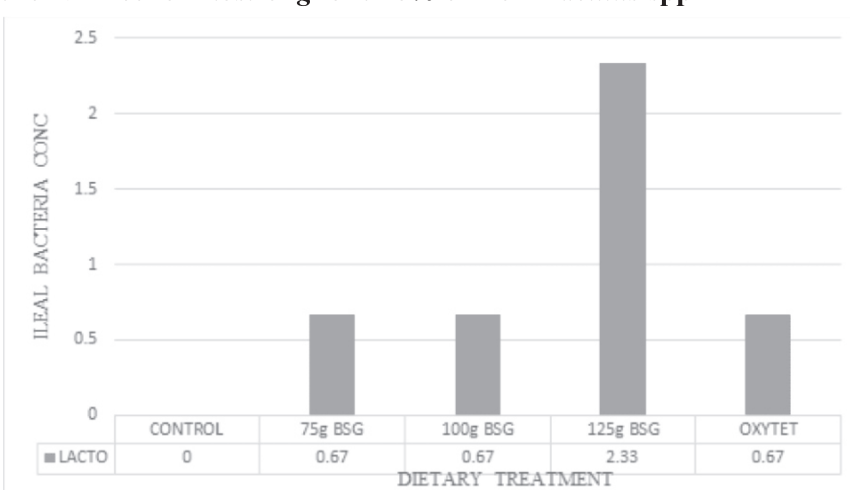

Figure 5 Effect of *Biostrong*® *510 15%* on ileal *lactobacillus* spp

Figure 6 showed that supplementation of *Biostrong®* 510 15% decreased the population of potentially pathogenic *Escherichia coli* decreased in the Ceacum. It was highest for the control, lower for birds fed 75g BSG and 125g BSG and absent for birds fed 100g BSG and AGP. This may indicate that colonization by the beneficial bacteria have suppressed the activity and habitation of the pathogenic specie. Escherichia coli may cause gastroenteritis and diarrhoea in animals (Nagy and Fekete 2005). Recent studies have also shown that Escherichia coli infection could impair intestine and induce the inflammatory response in animals (Bhandari *et al*., 2008). Figure 7 showed that pathogenic *Salmonella spp* were only identified in the control and not identified in birds fed *Biostrong® 510 15%* and AGP. This showed that antimicrobials had effects in controlling potential pathogenic bacteria. Cerisuelo *et al.* (2014) showed a clear effectiveness of low doses of EOs and sodium butyrate in *Salmonella* control in broilers. Recent studies have shown essential oils in their ability in reducing *Salmonella* colonization in chicken cecum by enhancing innate immune defense via increased synthesis of host defense peptides (Sunkara *et al.,* 2011) Both essential oils and organic acids have shown promising results in reducing *Salmonella in vitro* (Van Immerseel *et al.,* 2003). Figure 8 showed that pathogenic *Clostridium spp* were only identified in the control and not identified in birds fed *Biostrong® 510 15% and AGP.* This showed that antimicrobial had effect in controlling potential pathogenic bacteria. Essential oils offer a potential natural method of reducing pathogens in the gastrointestinal tract of poultry (Brenes and Roura, 2010).

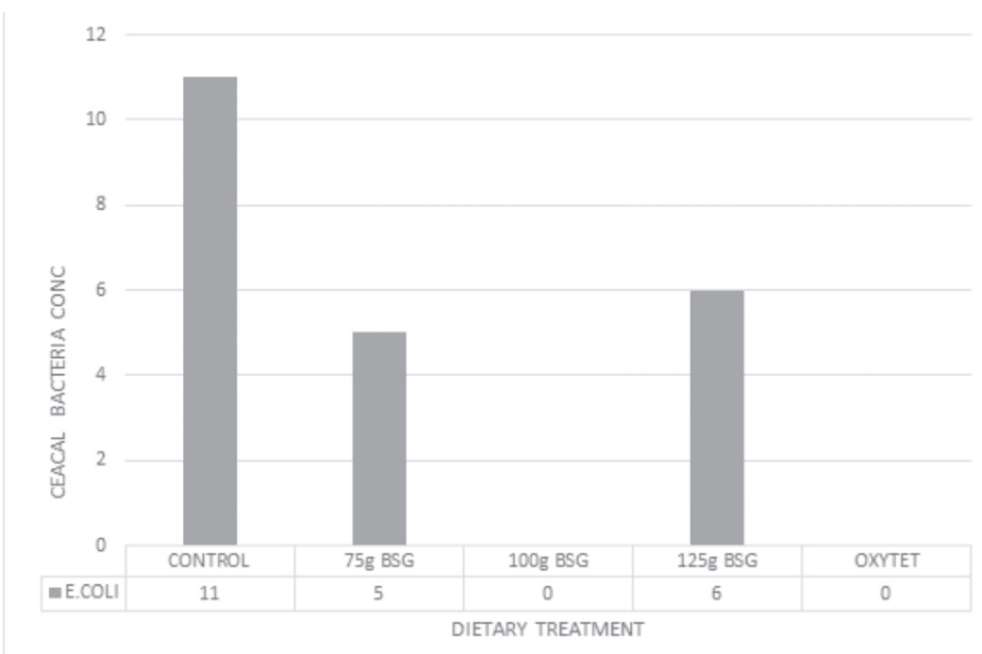

Figure 6: Effect of *Biostrong® 510 15%* on Ceacal *E.coli.* spp

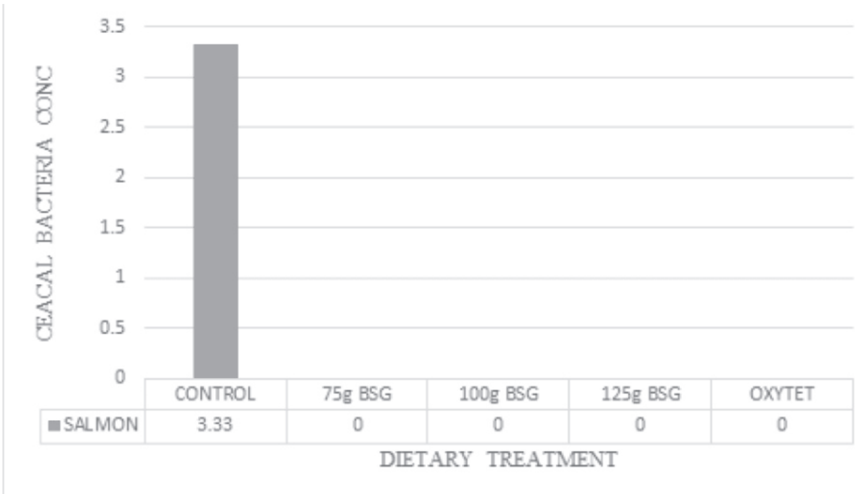

Figure 7: Effect of *Biostrong*® *510 15%* on Ceacal *Salmonella.* Spp

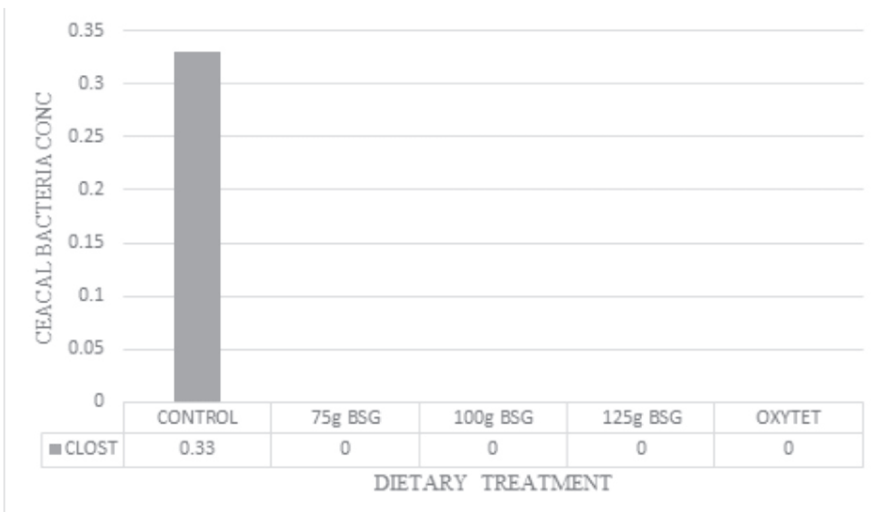

Figure 8: Effect of *Biostrong*® *510 15%* on Ceacal *Clostridium.* Spp

Figure 9 showed that supplementation of ***Biostrong*® *510 15% increased*** the population of *Bacillus spp.* The increase in population may have led to the colonization of the gut by the beneficial *Bacillus spp.* The increased population of the *Bacillus spp.* may also have provided health benefits to the host broiler. Anderson *et al.,* (1999) reported that dietary supplementation with oregano oil improved Lactobacillus or Bacillus populations and decreased Escherichia coli population of jejunal or cecal digesta in piglets. Figure 10 showed that supplementation of ***Biostrong*® *510 15% increased*** the population of *lactobacillus spp.* It was high for birds fed 75g BSG 15%, and low for the control and AGP. This indicates that essential oils suppress the growth of harmful bacteria and colonizes the gut with the beneficial bacteria to stabilize the gut thereby improving performance. Reports indicates that essential oils may be an alternative in fighting pathogenic bacteria that developed resistance to many antibiotics (Solorzano-Santos and Miranda-Novales, 2012; de Rapper *et al.,* 2013). Essential oils and organic acids help in colonization of *Lactobacillus* thus improve their activities (Elwinger *et al.,* 1998; Al-Ghazzewi and Tester, 2012; Khan *et al*., 2012). Nava *et al.* (2009) reported that essential oils increases intestinal colonization of *Lactobacillus* spp. in chicks

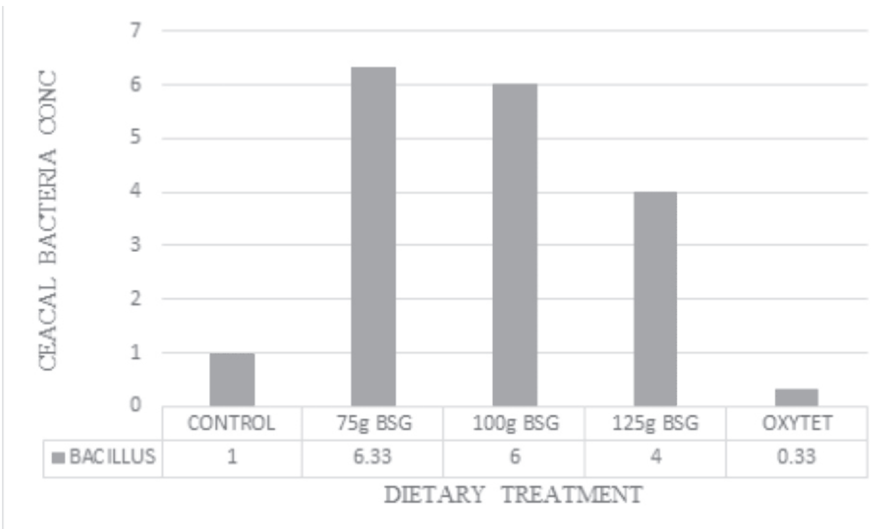

Figure 9: Effect of *Biostrong® 510 15%* on Ceacal *Bacillus.* Spp

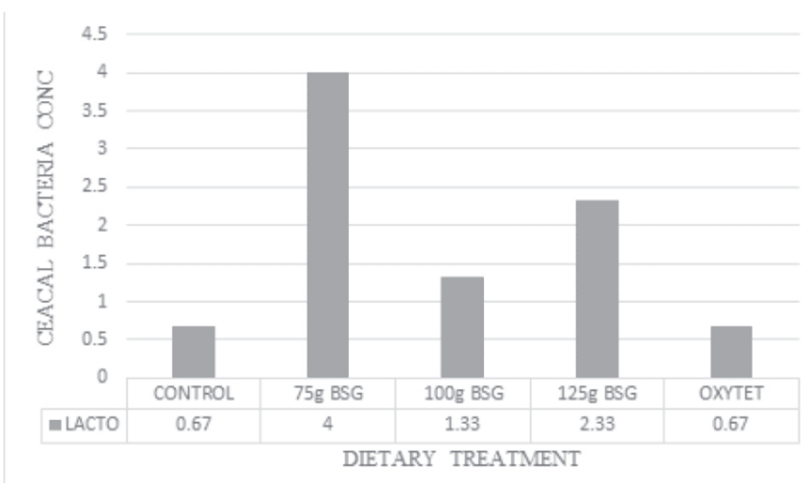

Figure 10: Effect of *Biostrong® 510 15%* on Ceacal *Lactobacillus.* Spp

Conclusions

The study showed that dietary inclusion of Biostrong® 510 15%, a natural growth promoter did not significantly improve growth of broiler chickens, but however improved feed conversion ability of broiler finishers above the control and the AGPs used and significantly lowered the cost of production than AGPs of finisher broiler. It also improved liver health, bone strength, villi characteristics; decreased cholesterol, triglycerides and high density lipoprotein thus improving the health of the birds. It also improved the activities of beneficial bacteria in the ileum and caecum of broiler chickens thereby making more nutrients particularly energy available from the feed consumed can effectively replace antibiotic growth promoters-AGP

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