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PERFORMANCE AND MOLECULAR IDENTIFICATION OF BACTERIA ISOLATED FROM THE GUT OF BROILER BIRDS AFTER ANTIBIOTIC ADMINISTRATION AND ENZYME SUPPLEMENTATION

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ABSTRACT

This study evaluated the effect of feed additives (antibiotic or enzyme) on performance and bacteria population in the ileum of one day – old broiler chicks (ANAAC 2000) randomly distributed to three treatments having eight replicates and ten birds per replicate. Completely randomized design was used and experiment lasted for 35 days.
Maize–soybean meal diet without antibiotic administration or enzyme supplementation served as control and was the same diet for all *treatments*. Antibiotic (Dicoxin plus ®) was administered to birds fed diet 2 and enzyme (Roxazyme G 2G ®) was supplemented to diet 3.Bacterial specific primers for rRNA gene sequences were used to amplify bacterial genes from samples and sequenced. Bacteria were identified from the gene sequences using Basic Local Alignment Search Tool (BLAST) against the National Centre for Biotechnology Information (NCBI).
Enzyme supplementation significantly ($P<0.01$) improved final Live body weight and weight gain compared to control or administration of antibiotic. The FCR was significantly enhanced ($P<0.01$) by enzyme and antibiotic supplementation. *Lactobacillus acidophilus (L. acidophilus)*, *Escherichia coli* (*E. coli*) and *Clostridia* were identified in digesta sampled. Partial rRNA sequences identical to *Clostridia* were the lowest (1) in control and enzyme treatment. A value of 4 was recorded in antibiotic treatment. *Lactobacillus acidophilus* was numerically high in control (8) and enzyme treatment (5) compared to antibiotic administration (1). Partial rRNA sequences identical to *Escherichia coli* sequences was however high (48) in birds administered antibiotic compared to control (8) and enzyme treatment (9). Results indicated greater improvement in weight gain, FCR and *Lactobacillus* in broilers fed enzyme supplemented diet. Feed additives may affect the biodiversity of gut bacteria in poultry birds.

Keywords: antibiotics, broilers, enzyme supplementation, molecular identification, performance

INTRODUCTION

Supplementing poultry feed with specific enzymes improves the nutritional value of feed ingredients by increasing the efficiency of digestion and nutrient uptake. These enzymes help to increase the availability of nutrients particularly starch, protein, amino acids and minerals such as phosphorus and calcium from feed ingredients. Variability in the nutrient content of maize has been demonstrated to be as great as that observed for wheat and barley (**Leeson *et al.*, 1993; Collins *et al.*, 1998**). In practice, the average nutrient content of cereals is greater in the presence of enzymes than its absence. As a result, the addition of an enzyme allows feed formulation nutrient matrix values to be elevated. The response to enzyme addition is mediated through improvements in nutrients extraction in the small intestine by the host through accelerated digestion, reduced microbial activity as a result of substrate limitation in the ileum and active feeding of specific bacterial species. Essentially, the activity of the enzyme on viscous polymers and cell wall carbohydrates produce sugars and oligomers which are utilized preferentially by certain ileal and caecal bacterial species. The bacterial species flourish at the expense of other possible detrimental species as far as optimal growth or health of animal is concerned (**Apajalathi and Bedford, 1999**).
The importance of understanding the dynamics of intestinal microbial ecology has been recognized for a long time (**Savage, 1977**). Since the ban of in–feed antibiotic growth promoters, the concept of gut health, interaction between gut microbes and nutrient bioavailability in relation to bird performance has become important. Digestive disorders have increased in parallel to this withdrawal (**Van Immerse *et al.*, 2004**). This is often a source of underperformance due to health problems such as *necrotic enteritis* or *coccidiosis* (**Williams, 2005**). Currently, there is increasing focus on alternatives to sustain good gut flora and gut health. Potential alternatives that may be suitable include enzymes, probiotics, prebiotics, essential oils, botanicals and organic acids. Several of these products have been widely tested and the evaluation will continue in the future. These alternatives exert beneficial gut health effects on the host (**Ravindran, 2012**), but the effects of their administration on animal performance have been reported to be variable (**Partanen and Mroz, 1999; Dibner and Buttlin, 2002; Patterson and Burkholder, 2003; Ricke, 2003; Dibner and Richards, 2005; Gianneanas, 2008; Yang *et al.*, 2009**).
Maize and soybean meal are used in feeding broiler chickens worldwide. It is almost free of viscous non–starch polysaccharides (NSP) but this does not necessarily exempt enzyme use in diets containing both ingredients. Maize and soybean meal contain appreciable amounts of NSP. According to **Bach Knudsen (1997),** it contains approximately 0.9% soluble NSP and 6% insoluble NSP. According to earlier reports (**Noy and Sklan, 1995; Thrope and Beal, 2001),** corn starch digestibility rarely exceeds 85% in broilers between 4 and 21 days of age indicating opportunities for improvement. Insoluble fibre shortens retention time of digesta (**Hetland *et al.*, 2004**) and may lead to nutrient digestibility. Unlike soluble fibre, their effect on population and quantity of microflora is relatively important (**Hetland *et al.*, 2004),** although the increase of digesta passage time probably reduces settle time for fermentative microbes (anaerobic organism) especially in the small intestine.
The role of gut health in performance of poultry birds has resulted in the use of several feed additives. Gradually the use of in-feed antibiotics is no longer a favourable alternative to alleviate digestive disorders and poor performance associated with its withdrawal (**Van Immerse *et al.*, 2004).** Although these suitable alternatives (enzymes, probiotics, prebiotics, symbiotics, *etc*.) have been reported to elicit varied results in terms of growth performance, however, how they affect the biodiversity of gut bacteria may be an issue of consideration.
Due to lack of knowledge of appropriate culturing conditions, a large number of bacteria remain unidentified using basic culturing and biochemical methods. Furthermore, culturing and biochemical techniques have resulted in the misclassification of some of these bacteria **(Tellez *et al.*, 2006).** Given the profound impact of gut bacteria on performance, identification of bacteria assemblages in the gut at random will be of high biological and economic importance (**Apajalathi and Bedford, 1999; Apajalathi *et al.*, 2001, 2004; Ezenwa *et al.*, 2012).**

In the light of this, this study was designed to assess the performance of broilers fed with or without antibiotic and enzyme supplemented diet and their effects on *Lactobacillus* counts in the ileum. Considering the limitation of standard culturing methods, this study also aimed at identifying bacteria biodiversity amplified from the gut of broiler birds through molecular techniques.

MATERIAL AND METHODS

This experiment was carried out at the poultry unit of Niger Delta University Teaching and Research farm, Niger Delta University, Wilberforce Island, Nigeria.

Composition of experimental Diet

The composition of the experimental diets is presented in Table 1 below;

Table 1 Gross composition of experimental diets (g/kgDM unless otherwise stated)

Ingredients	M / SBM Diet 1(control)	M / SBM + antibiotic Diet 2	M / SBM + enzyme Diet 3
Maize	550	550	550
Soybean meal	330	330	330
Fish meal	40	40	40
Cassava starch	42	42	42
*Constant ingredients	38	38	38
Total (1000gm)	1000	1000	1000
M.E. (Kcal/kgDM)	3024	3024	3024
C.P	214.94	214.94	214.94

Legend: mineral vitamin premix (2.5g), DL Methionine (1.5g), bone meal (21g), oyster shell (10g) salt (3g). M.E.: metabolisable energy, C.P.: crude protein, M: maize, SBM: soybean meal.

Three experimental diets were formulated. All the diets were maize–soybean meal based (M/SMB), which contained 550g/kg of maize. The control diet was not supplemented with enzyme or the birds given antibiotics. An antibiotic (Dicoxin plus ®) was administered to birds fed with diet 2 at an inclusion rate of 100g/160 litres via drinking water. Diet 3 was supplemented with enzyme (Roxazyme G 2G ® - DSM Nutritional Products Ltd, Switzerland). The gross composition of the experimental diets is as indicated in Table 1.

All the birds were fed with the same type of diet except the treatment administered (i.e. antibiotic administration and enzyme supplementation). Energy and crude protein concentration was similar for all treatments and was adequate for the birds under each treatment. A hundred gram of each experimental diet was collected and set aside for proximate analysis. Proximate analysis of experimental diets was carried out according to **AOAC (1990**).The nutrient composition of the experimental diets is presented in Table 2.

Table 2 Nutrient contentof experimental diets (in g/kg DM unless otherwise stated)

Nutrient	M/SBM	M/SBM + antibiotic	M/SBM + enzyme
Dry matter (g)	739.5	733.5	651.5
Ash	154.2	148.1	170.2
Crude protein	238	229	256
Ether extract	58.1	62.7	49.1
Crude fibre	64.9	73.69	70.6

M/SBM: maize / soybean meal

Source of enzyme

The enzyme used in the current study is a non starch polysaccharide (NSP) degrading enzyme and was supplemented at an inclusion rate of 200g/t of complete feed. It is an odorless granulates which is soluble in water. It contains an enzyme complex derived from *Trichoderma longibrachiatum* with an effective pH range of 3.5 – 5.5and a temperature range of 30 – 55°C. The specifications of the enzyme are:

- Endo-1,4-glucanaseactivity: min 8,000 unit per gram (E.C.3.2.1.4.)
- Endo-1,3 (4)-glucanase activity: min 18,000 unit per gram (E.C.3.2.1.6.)
- Endo-1,4-xylanase activity: min 26,000 units per gram (E.C. 3.2.1.8.)

Animal experiment

A total of two hundred and forty (240) ANAAC 2000 one day-old broiler chicks were purchased brooded for seven days and randomly distributed to the three dietary treatments having eight replicates of ten birds per replicate. The experiment was arranged as a completely randomized design. Feed and water were supplied *ad libitum*. Since the focus of the experiment was not on the use of antibiotics, antibiotics and anticoccidiostats were not administered to the birds fed with the control diet and the enzyme supplemented diet. This was to determine the effect of exclusive antibiotic administration and enzyme supplementation on beneficial gut bacteria (*Lactobacillus*) as well as the overall performance of birds fed with a standard maize/soybean meal based diet. Feed intake and weight gain were determined on a weekly basis, while feed conversion ratio (FCR) was calculated. The antibiotic administration to birds fed with the second diet was done for 6 days from day 21 to 26. This was stopped on day 27 and discontinued till the end of the experiment. The duration of the experiment was 35 days.

Digesta collection

On day 35, two birds per replicate were slaughtered and digesta was collected to determine *Lactobacillus* counts in the ileum. The ileum was defined as 2cm posterior to merkels diverticulum and 2cm anterior to the ileal – caecal – colonic junction. After a rapid removal of this section of the gut, digesta was collected into sterile sample containers on ice. The digesta collected was taken to the laboratory for microbial analysis.

Microbial analysis

Lactobacillus was enumerated on bacteria specific agar (de man Rogossa and Sharp agar – MRS agar) after prior serial dilution of 1gm wet weight of collected digesta. The diluents were plated out in duplicate per replicate and incubated for 48 hours after which *Lactobacillus* colonies were counted. *Lactobacilli* counts were log transformed before carrying out statistical analysis.

Bacteria identification

Four birds were randomly slaughtered from each treatment and digesta was collected from the ileum into sterile sample containers and stored on ice packs. The digesta was stored at -20°C prior to molecular analysis. Metagenomic DNA was extracted and purified using ZR Fungal/Bacterial DNA MiniPrep™50 Preps. Model D6005 (Zymo Research, California, USA) according to the Manufacturer's protocol. The DNA samples were thereafter sent to Inqaba Biotechnology Pretoria South Africa for Polymerase Chain reaction (PCR) and sequencing according to **Weisburg *et al.* (1991).** The 16S rRNA partial gene sequence were targeted and amplified through PCR using primers (27-F and 1492-R) with sequences being 5'-AGA GTT TGA TYM TGG CTC AG-3' and 5'-TAC CTT GTT AYG ACT T-3' respectively **(Martin and Collen,1998**).The resulting DNA sequences were subjected to Basic Local Alignment Search Tool (BLAST) analysis on the National Centre for Biotechnology Information (NCBI) platform on the web and bacterial isolates were identified based on the resultant top hits(**Altschul *et al.*, 1990)**.

Data collection and analysis

Data collected include weight gain, feed intake and calculated feed conversion ratio (FCR) in addition to data collected on log CFU *Lactobacillus* counts were subjected to general linear model analysis using SPSS package (SPSS Inc, Chicago, IL. 2008) version 17 and significant means separated using Duncans

Multiple Range test (**Steele and Torrie, 1995**).

RESULTS AND DISCUSSION

Analyzed crude protein and ash concentration was higher in enzyme supplemented diet as shown in Table 2. This could be attributed to hydrolysis of NSP and release of minerals and proteins attached to high molecular weight carbohydrates. The crude protein concentration was the least (229g/kgDM) in diet 2 to which antibiotic were administered to the birds fed this diet. A value of 256g/kgDM was recorded in the enzyme supplemented diet which was the highest value recorded across the treatments. The value recorded for the control diet was 238g/kgDM. A similar trend was observed for the ash concentration across the treatments respectively. The best crude protein concentration recorded in the enzyme supplemented diet compared to the control and antibiotic administered diet is in line with previous work reported by **Ohimain and Ofongo (2013**) an increased crude protein concentration in an enzyme supplemented maize/soybean meal based diet containing 200g of wheat offal. Although the maize does not contain viscous NSP, enzyme supplementation can improve birds' performance by increasing digestibility of nutrients found in maize kernel (**Meng and Slominski, 2005)**. A probable mode of action of Roxazyme G2G with regards to the enzyme supplemented diet could be via the hydrolysis of certain types of carbohydrate-protein complexes (glycoproteins, proteoglycans) in which the protein component is resistant to proteolysis because of its substitution with bulky carbohydrate groups **(Shibuya and Iwasaki, 1985; Meng and Slominski, 2005**).In addition, the release of such proteins from high molecular weight carbohydrate-protein complexes could enhance protein availability for digestion and absorption.

Table 3 Performance and *Lactobacillus* counts (*log CFU*) in broilers fed with feed additive supplemented diets

Performance indices g/bird except FCR	M/SBM	M/SBM+ antibiotic	M/SBM+ enzyme	SEM	*P value*
Initial live weight	138.33	148.75	143.75	-	-
Final live weight	1909.70^{a}	2249.50^{b}	2464.30^{c}	64.76	0.000***
Weight gain	1771.30^{a}	2103.29^{b}	2320.80^{c}	65.44	0.000***
Feed intake	3811.84	3700.96	4072.23	130.23	0.143^{ns}
FCR	2.19^{b}	1.77^{a}	1.78^{a}	0.072	0.001***
Gut section (ileum)					
Lactobacillus	7.58^{bc}	7.33^{a}	7.76^{c}	0.06	0.034**

abc: means along the same row with different superscripts are significantly different (*P<0.05*)
M/SBM: maize-soybean meal; FCR: feed conversion ratio; SEM: standard error of mean

Results on performance variables (Table 3) showed significantly higher final live body weight and weight gain (*P<0.01*) in broilers fed with enzyme–supplemented diet compared to antibiotic administration or no supplementation at all. From the results, antibiotic administration and enzyme supplementation significantly (*P<0.01*) improved weight gain and FCR than feeding the maize/soy bean meal diet without either treatment.

Enzyme supplementation significantly (*P<0.01*) improved the performance of broilers while increasing the *Lactobacillus* counts in relation to antibiotic administration. Antibiotic administration also significantly (*P<0.01)* enhanced weight gain compared to the control diet but values recorded was significantly (*P<0.01)* lower than enzyme supplementation. The least value of weight gain (1771.30g) was recorded in birds fed the control diet while values of 2103.29g and 2320.80g was recorded for birds given antibiotic and birds fed enzyme supplemented diet respectively. According to **Ofongo *et al.* (2011)** and **Ikoro(2010)** improved weight gain was reported in broilers fed enzyme supplemented maize – soybean meal based diet. The current findings further substantiate earlier report by **Cowieson (2005)** that demonstrated an improved FCR from 0.78% to 10.5% and body weight gain from 0.5% to 10.9% in enzyme supplemented maize based diet over the control. Apparently, low viscosity diets which are considered to be energy dense can have their nutrient availability improved. In another report (**Chesson, 2001**), maize kernel was stated to contain 111g/kg of total NSP of which 230g/kg is arabinose and 300g/kg is xylose. That report further warrants the use of exogenous enzymes to increase the digestibility of nutrients found in maize kernel (**Meng and Slominski, 2005**). The enzyme used in the current study had enzyme activities (over 26,000 units per gram of xylanase activity) which may have been adequate in hydrolyzing the NSP present in maize kernel. Although feed intake was not significantly (*P>0.05*) different across the treatments, however, antibiotic use and enzyme supplementation significantly (*P<0.01*) enhanced FCR compared to the control diet. Feed intake value was least in birds administered antibiotic, with a value of 3811.84g recorded in the control and 4072.23g in birds fed enzyme supplemented diets. According to **Tahir *et al.* (2005),** cellulase and hemicellulase and their combination increased body weight gain without having any effect on feed intake in broilers fed corn/soy bean meal based diet. Although maize-soy bean based diets do not induce high intestinal viscosity as other cereals, it has been shown that these diets could benefit from carbohydrase- supplementation when fed to broilers (**Cowieson, 2010**). Results from various studies however, have been to some extent inconsistent (**Zanella *et al*., 1999; Centeno *et al.*, 2006; Singh *et al.*, 2012**). The results of this study are similar to that reported by **Tahir *et al.* (2005)** but this was not the case with that observed by **Cowieson and Ravindran (2008).** The authors observed increase in both body weight gain and feed intake in response to enzyme supplementation with xylanase, amylase and protease. In this regard the enzyme cocktail may be a probable factor since amylase and protease were not part of the enzyme component in the enzyme used in this study. **Tahir *et al*. (2008)** reported a 9% gain in body weight of broilers fed diets with enzyme combination of cellulose, hemicelluloses and pectinase. The report of **Olukosi *et al.* (2007)** observed no effect on body weight gain in broilers fed enzyme supplemented corn/soy bean based diet.

The adequacy of nutrients or nutritional values of all diets used in this study were adequate since all birds in the 3 treatments were given a similar diet. Expectedly any response observed may be attributed to treatment effect and not variability in diet composition. It was suggested by **Cowieson (2010**) that there are many interacting factors involved in dictating the measured response to an exogenous enzyme of which the most influential is the nutritional value of the diet to which the enzyme is added. Furthermore, broilers fed diets that are essentially adequate in all nutrients often still respond to exogenous enzyme supplementation (**Bao *et al*., 2013**). The authors suggested that enzyme benefits may be due to changes in less tangible metrics such as appetite control, digestive physiology, immunology or microbiology i.e. net effects. Maize is a highly digestible feed ingredient due to its low NSP concentration thereby bringing to the fore the inherent digestibility of nutrients in the diet prior to enzyme addition. This digestibility has been demonstrated to be a good indicator of the magnitude of the enzyme response (**Cowieson and Bedford, 2009; Cowieson, 2010**). This was obvious in birds fed diets 2 and 3. In the case of diet 2, antibiotic administration minimized competition for nutrients between host and gut microorganisms. Enzyme supplementation on the other hand must have improved nutrient digestibility via reduction in cell wall integrity, generation of fermentable disaccharides, low-molecular weight polysaccharides and oligosaccharides, improving protein solubility, decreasing endogenous losses and overcoming anti-nutritional factors (**Cowieson and Ravindran, 2008**).

The mechanism of antibiotic use is apparently to control intestinal microflora favoring beneficial bacteria while suppressing detrimental or pathogenic bacteria that provoke inflammation of the gut mucosa. As a result, antibiotics are used routinely in poultry to prevent and treat diseases associated with gut microflora. With the ban on their use in most developed countries, the poultry industry is faced with the challenge of controlling pathogenic microbes. Modulation of the gut microflora can be done either through diet or enzyme supplementation (**Ohimain and Ofongo, 2013**), which could stimulate and encourage the growth of beneficial bacteria such as *Lactobacillus* and *Bifidobacteria*. Due to the fact that microbial colonization of the gut takes place post hatch, a large number of microorganisms can become established in the GIT shortly after hatching. Intestinal microflora is mainly responsible for degrading the plentiful amounts of mucus produced by the goblet cells in the intestine (**Falk *et al.*, 1998**). A less or not inflamed gut with little or no bacteria competing for nutrients and less digestive disorders mean better nutrient digestion and absorption with a resultant enhanced weight gain. This might have been the probable reason for observed weight gain in birds administered antibiotics.

Lactobacillus counts (Table 3) was significantly influenced (*P<0.05*) by antibiotic administration and enzyme supplementation. It was significantly (*P<0.05*) higher in birds fed with enzyme supplemented diet than antibiotic administration even though the antibiotic was not targeted at *Lactobacillus.* This report is congruent with previous findings (**Ohimain and Ofongo, 2013**). *Lactobacillus* counts were numerically higher in enzyme supplemented diet than the control diet but this apparent difference was not significant (*P>0.05*) with the control. Gut microflora has significant effect on host nutrition, health and growth performance (**Barrow, 1992**) by interacting with nutrient utilization and the development of gut ecosystem of the host. This interaction is very complex and depending on the composition and activity of gut microflora, it can have either positive or negative effect on the health and growth of birds. For example, when pathogens attach to the intestinal mucosa, gut integrity and function are severely affected (**Droleskey*et al.*, 1994**) and immune system is threatened (**Neish, 2002**). According to **Klasing *et al*. (1987**), chicks grown in pathogen free environment grow 15% faster than those under conventional conditions where they are exposed to bacteria and viruses. The high number of *Lactobacillus* in the ileum indicated an acidic environment which could prevent pathogenic bacteria from colonizing the GIT and enhance performance with less competition for nutrients

with the bird. It is generally agreed that gut microflora is a nutritional "burden" in fast growing broiler chickens (**Dibner and Richard 2004**; **Lan*et al.*, 2005**). The focus of alternative strategies is to prevent proliferation of pathogenic bacteria and modulation of indigenous bacteria so that the health, immune status and performance of broilers can be improved **(Ravindran, 2006)** as demonstrated in the present study.

Previous study by **Tierlynck *et al*. (2009)** showed evidence and markers of gut damage, apoptosis, increased mounting of immune defense and microbial invasion of intestinal tissues in broilers fed wheat–ryediet compared to corn. This does not rule out the microbial response in the gut of broilers fed maize based diet in the presence and absence of NSP enzyme or antibiotics. Carbohydrase supplementation has been shown to reverse the negative effects of NSP mediated by gut microorganisms **(Hogberg and Lindberg, 2004; Kiarie *et al.*, 2007).** This response is mediated by increasing the proportion of lactic and organic acids, reducing ammonia production (**Kiarie *et al.*, 2007**) and increasing volatile fatty acid (VFA) concentration as reported by **Huberner *et al.* (2002**). According to the author, increased VFA concentration is an indicative of hydrolysis fragmentation of NSP and this supports growth of beneficial bacteria. Increased proportion of lactic acid promotes gut health by suppressing growth of presumptive pathogens (**Pluske *et al.*, 2001; Ohimain and Ofongo, 2013**). **Hillman *et al.* (1995**) observed that certain strains of *Lactobacillus* inhibit the growth of *coliforms* such as *E. coli*. The obviously improved *Lactobacillus* count in birds fed enzyme supplemented diet above antibiotic administration is indicative of the added benefit of enzyme addition to a maize-soybean meal diet. Also, in the absence of enzyme, *Lactobacillus*count was significantly better than antibiotic administration. **He *et al.* (2010)** reported that xylose (possible product of exogenous and endogenous carbohydrase activity) is important in preferentially enhancing the growth of beneficial *Bifidobacteria.* According to **Torok *et al.* (2007)** and **Courtin *et al.* (2008),** exogenous enzymes mediated changes that influenced gut microbial populations. The results of this study sheds light on the impact of antibiotic use and enzyme supplementation on *Lactobacillus* population in the gut and overall growth response of broilers to their application. **Bedford and Cowieson (2012)** suggested that intestinal microbial population size and composition clearly plays a very large role in determining the extent of digestion accomplished by the host and by extension, growth rate and efficiency.

Table 4 Relative number specific Bacteria identified in ileum content of broiler gut as affected by feed additives

Treatments	Bacteria genera		
	Lactobacillus acidophilus	*Escherichia coli*	*Clostridia*
Control	8	8	1
Antibiotic	1	48	4
Enzyme	5	9	1

The number of bacteria of economic importance according to their specific species identified using molecular techniques is presented in Table 4. Results obtained for molecular identification of gut bacteria (Table 4) showed that the relative number of *E. coli* identified was numerically high in the antibiotic treatment compared to the control and enzyme treatment. This was also the case with *Clostridia* which was least in both the control and enzyme treatment. The relative number of *Lactobacillus acidophilus* was the least in the antibiotic treatment but was numerically higher in treatment 1 and 3 respectively. Results previously reported using bacteria specific culturing methods showed that feed additives significantly affected *lactobacillus* population in the ileum (**Abule *et al.*, 2014)**. Although the antibiotic used in this study had anti-coccidia properties, molecular techniques of identification revealed the presence of *Clostridia* population in the gut of birds administered an antibiotic which was numerically higher than the control and enzyme treatment. Although *E. coli* is part of the normal flora in the lower section of the intestine of warm blooded animals, the relative number identified via molecular means in the current study was also numerically higher than that obtained in the control and enzyme treatment. Observed disparity in the relative number of *Lactobacillus acidophilus* recorded further buttresses the report of **Abule *et al.* (2014)**, which stated that antibiotics enhance performance of broilers but may not necessarily increase *Lactobacillus* counts in the gut.

According to **Apajalathi *et al.* (2004)**, recent molecular studies targeting bacterial DNA in poultry gut have yielded more detailed insight into the composition of the diverse microbial community. Furthermore, this composition may be further diversified depending on usage or non-usage of feed additives. Results of the current study also shed light on the principle of competitive exclusion in gut microflora population based on nutrient availability in the gut. Modulation of the gut microflora either through diet and enzyme supplementation (**Ohimain and Ofongo, 2013**) may stimulate or encourage the growth of beneficial bacteria such as *Lactobacillus* and *Bifidobacteria.* In spite of this, the bacteria biodiversity in the gut may also be influenced by feed additives depending on nutrient availability and population of specific bacteria present in the gut.

The interaction between gut microflora, nutrient utilization and development of the gut ecosystem of the bird is complex and can affect performance depending the composition and activity of the gut microflora. This interaction can have either positive or negative effect on health and growth of birds. For example, when pathogens attach to the intestinal mucosa, gut integrity and function can be severely affected (**Droleskey *et al.*, 1994**) and the immune system threatened (**Neish, 2002**). As previously stated **Klasing *et al.* (1987**), chicks grown in pathogen free environment grow 15% faster than those under conventional conditions where they are exposed to bacteria and viruses. The high number of *Lactobacillus* in the ileum as earlier stated indicated an acidic environment which will not favour pathogenic bacteria and better performance with less competition for nutrients with the bird. The focus of alternative strategies to prevent proliferation of pathogenic bacteria and modulation of indigenous bacteria so that the health, immune status and performance are improved (**Ravindran, 2006**) as indicated in the present study is a very welcomed idea.

CONCLUSION

It can be concluded from the findings of the current study that antibiotics enhance performance of broilers but may not necessarily increase *Lactobacillus* counts in the gut. Enzyme supplemented maize–soybean meal based diet improves weight gain as well as *Lactobacillus* counts in the gut. In-feed antibiotics may not favour the proliferation of beneficial bacteria when administered to prevent digestive disorder in broiler birds. It is of benefit to the farmer to supplement maize – soybean meal based diet with enzyme rather than antibiotic where such alternative is available. Molecular identification of gut bacteria under different additive supplementation may shed more light on the role of gut bacteria on performance, physiology of the gut and overall health of broiler birds.

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