EFFECT OF ENZYME SUPPLEMENTED DIET ON GUT MICROFLORA, DIGESTA PH AND PERFORMANCE OF BROILER CHICKENS

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ABSTRACT

This study was designed to determine the effect of enzyme supplementation and type of diet on gut microflora, gut pH and performance of broiler chickens. A day – old broiler chicken was used for the experiment. A total of 150 chicks were randomly distributed into three treatment diets having 5 replicates and 10 birds per replicate. The experiment was arranged as a completely randomized design and lasted for 49 days. The control diet contained maize –soya bean meal. The treatment diets had maize replaced with 200g/kg of wheat offal with and without enzyme supplementation. Data were collected on gut microflora population and pH in three sections (crop, ileum and caecum) of the gut. Performance related data were measured including weight gain, feed intake and FCR (feed conversion ratio). Gut microflora enumerated included *coliform*, *Escherichia coli* and *Lactobacillus*. Results obtained show that c*oliforms* and *E.coli* were consistently higher (*P<0.05*) in the control than feeds supplemented with wheat offal with or without enzyme addition in the crop, ileum and caecum. The population of *coliform* and *E.coli* were lowest in the diet containing wheat offal and RoxazymeG2 G. The results also show that *E. coli* accounted for nearly 100% of total *coliform* in the gut of the birds used for the present study. The overall pattern of the results indicated that the wheat offal in the poultry diet stimulated *Lactobacillus* growth which was further enhanced by the addition of enzyme. The stimulation of *Lactobacillus* by the diet containing wheat offal with or without enzyme coincided with the reduction in *coliform* and *E. coli* population, which suggests the efficacy of diet composition and enzyme supplementation in controlling pathogenic organisms in broiler chickens. The pH results of the digesta of the three treatments (i.e. diet compositions) where not significantly different (p >0.05), in the crop and ileum. But in the caecum, the pH was significantly different (p <0.05), being slightly more acidic, than the control diet. Performance results showed that partial replacement of maize soya beans meal with wheat offal in the presence of Roxazyme G2 G increased weight gain, but decreased feed intake and feed conversion ratio, but all these differences were not statistically significant (*p>0.05*).

Keywords: Broilers, enzyme supplementation, gut microflora, gut pH

INTRODUCTION

The poultry industry is one of the most important sources of protein all over the world today. However, commercial poultry production is challenged by microbial infections and unavailability of good quality feeds on a sustainable basis at stable prices (**Ohimain and Ofongo, 2012**). Poultry feeds compete with human nutrition especially in the use of wheat, rye, barley and maize. To further add is the current use of maize as biofuel. These grain despite being used for human nutrition, are major components of poultry diets. Conventional feed ingredients such as wheat, maize and soya beans, which are human staple, constitute about 90% of the total feed ingredients used in making poultry feeds (**Onuh et al., 2010**). Feed remains the most important cost in animal production (**Omojola and Adeshinwa, 2007**) accounting for 55-70% of the total cost in poultry production (**Adeniji and Jimoh, 2007**). The escalating cost of conventional feed stuff has stimulated research into alternative feedstuff with the aim of reducing the cost of poultry production (**Tuleun et al., 2009**).

Several alternative feedstuff have been considered as possible replacement either partially or wholly of conventional feedstuff. Some of the alternative feedstuff that have been tested include sweet orange (*Citrus sinensis*) peelings (**Agu et al., 2010**), bambara nut (*Voanddzeia subterfuges*) wastes (**Ani et al., 2012**), palm kernel cake (**Onuh et al., 2010; Lawal et al., 2010**), guinea grass, *Panicum maximum* (**Oluwasola et al., 2008**), leaf meals of *Amaranthus cruentus* (**Fasuyi and Akindanunsi, 2009**) and *Moringa oleifera* (**Ogbe and Affiku 2011**); maize offal/bran (**Ademola et al., 2012; Nnennh et al., 2006**), rice offal. (**Tuleun et al., 2009**), wheat offal and Brewers spent grain (**Ademola et al., 2012**). However, the use of these feedstuffs in poultry diet is limited because chickens do not possess the requisite enzymes to completely breakdown some of the anti –nutritional factors in these feedstuff such as non-starch polysaccharides (NSPs), phytate, oxalates, saponins, tannins, trypsin inhibitors and cyanogenic glucosides (**Ogbe**

and **Affiku, 2011**). **Zahran et al. (2012)** reported that about 70 – 90% of plant cell wall consist of NSP.

Non – starch polysaccharides are made up of three types of polymers - cellulose which is typically not soluble in water, acid or alkaline; pectic polysaccharides, which are partially soluble in water and non - cellulosic polymers which are also partially soluble in water (**Khattak et al., 2012**). The non - cellulose polymers are mostly pentosans, which comprise principally of arabinoxylans (**Alam et al., 2003**) and other polymers including β - glucans, mannans, galactans, xyloglucans and fructans (**Khattak et al., 2006**). Insoluble NSP are not inert, they have the ability to absorb large amount of water and maintain normal motility of the gut (**Stephens and Cummings, 1979**). This ability prevents increased solubilisation of NSP as reported by **Choct (1997).** The overall effect is increased passage rate of digesta giving little time for fermentative organisms (anaerobic organisms) to establish in the small intestine. The soluble NSP are known to induce viscosity in the gut of monogastric animals fed with cereal based diets (**Oswald et al., 2006**). It has been reported that soluble arabinoxylans are able to absorb water up to 10 times their own weight forming a gel-like highly viscous solution (**Alam et al., 2003**), thus reducing nutrient utilization and performance (**Zahran et al., 2012; Alam et al., 2001**).

Since poultry do not possess endogenous enzyme that can hydrolyse NSPs, the use of exogenous enzymes mostly of microbial origin has been a welcomed development in the poultry industry. Many exogenous enzymes and/or microbes have now penetrated the market including Roxazyme G2G, poultry-feed direct-feed microbial (Bionetrx international), zynerzyme (Neospark) avizyme, maxigrain, Ronozyme P, nutrase, xyla, feedzyme etc. However, in trials/experiments using alternative feed stuff for poultry feed, the researchers mostly used RoxazymeG2G (**Ademola et al., 2012; Ani et al., 2012; Agu et al.,2010; Fasyui and Akidahunsi, 2009;Tuleun et al., 2009; Lawal et al., 2010).**

Another challenge apart from feeds facing the poultry industry is infections of microbial origin. The gut of poultry contains complex populations of bacteria and other microorganisms, which can have either negative or positive effects on the host (**Torok et al., 2008**). The gut has been reported to contain one of the highest populations of microbes recorded for any ecosystem. Microbial populations in the gut have been reported to be in the order of 10^6-10^{12}cfu/g of digesta (**Torok et al., 2011; 2008; Apajalahti et al., 2004; Conway, 1997**). Some microbial populations in the gut are beneficial to the host in several ways, helping to digest feeds, protect against pathogenic microbes and conferring immunity on the host (**Ohimain and Ofongo, 2012**), while others have been associated with causing various diseases including clostridiosis, colibacillosis, and erysipelas, fowl cholera, pasteurellosis, mycobacteriossis, salmonellosis, spirochetosis (**Porter, 1998**) and coccidiosis. Antibiotic growth promoters (AGP) are both restricted or banned in Europe, USA, Japan and many other Countries, hence the poultry industry is faced with the challenge of controlling pathogenic microbes without the use of AGPs. Modulation of the gut microbes through diet and enzyme supplementation may stimulate and encourage the growth of beneficial bacterial such as lactic acid bacteria (LAB) e.g. *lactobacillus* and *Bifidobacteria*, which could in turn create undesirable conditions for pathogenic bacteria such as *E. coli, salmonella* and *Clostridia* etc. The gut microflora plays a very important role in animal health and performances influencing the hosts gut development, enzyme activities, immune response, and resistance to infections (**Torok et al., 2008**). Diet, age and environmental factors have been reported to influence gut microflora (**Torok et al., 2011**).
Ogbe et al. (2009, 2010) utilised extracts from the wild mushroom *Ganoderma lucideum* to treat chickens infected with *Eimeria*. **Willis et al. (2013, 2012, 2011, 2010a)** in several studies consistently used fungi myceliated grains containing the following mushroom shistake (*Lentinus edodes*), reishi (*Ganoderma lucidum*),

oyster (*Peurotus ostreatus*) and cordyceps (*Codycep sinensis*) to control *Eimeria* infection in broiler chickens. **Willis et al.(2010b, 2009a, 2008)** also utilised fungi myceliated grain to stimulate the growth of *bifido*bacteria, while controlling *salmonella* population. In the control of infections, without the use of antibiotics, **Yang et al. (2009)** has proposed six kinds of alternatives to in-feed antibiotics which includes enzymes, probiotics, prebiotics, mannan - oligoscaharides, symbiotics and phytobiotics. This study was therefore designed to assess the effect of partial substitution of maize with wheat offal in a maize-Soya bean meal diet in the presence or absence of Roxazyme G2G enzyme and the effect on gut microbes (*Lactobacilus, Coliforms and E.coli*), gut pH and broiler performance.

MATERIAL AND METHODS

The experiment was carried out in the teaching and research farm of the Faculty of Agricultural Technology, Niger Delta University, Wilberforce Island, Nigeria.

Composition of experimental diets

Three experimental diets were formulated: maize – soy bean meal (M – SBM) control diet and two treatment diets in which 200g of maize was replaced with wheat offal (M – SBM/WO – enzyme) and the third diet supplemented with an enzyme (M – SBM/WO + enzyme) Roxazyme G2 G® (DSM Nutritional Products Ltd, Switzerland). Cassava starch was added to the treatment diets to meet up the energy requirement of the birds, while PKC (palm kernel cake) was added to the control diet as a source of fibre.   The gross and chemical composition of the control and treatment diets is as indicated in Table 1.

Table 1 Gross composition of experimental diets

Ingredients	M-SBM	M-SBM /WO - without enzyme	M-SBM/ WO - with enzyme
Maize, g	550	350	350
Wheat offal, g	0	200	200
SBM, g	300	300	300
Fish meal, g	40	40	40
Cassava starch, g	0	72	72
PKC, g	72	0	0
Broiler premix*, g	38	38	38
Ingredients 1000g	1000	1000	1000
ME (MJ/kgDM)	14.2	12.8	12.8
CP (g/kgDM)	213.9	218.6	218.6

M-SBM: maize-soybean meal; WO: wheat offal
*: broiler premix consisting of (2.5g); DL Methionine (1.5g); bone meal (21g); lime stone (10g) salt (3g)

Source of enzyme

The Roxazyme G2 G® is a non – starch polysaccharide (NSP) degrading enzyme. It is odourless and granular in nature and soluble in water. It contains an enzyme complex derived from *Trichoderma longibrachiatum*. It has an effective pH of 3.5 – 5.5 with an effective temperature range of 30 – 55℃. The dosage range was 200g per ton of complete feed. The specifications of the enzyme are:

- Endo – 1, 4 - glucanase activity: 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 completely randomized experimental design was used for the study. A day old broiler chicks (**ANAC, 2000**) where purchased and used for the study. A total of 150 chicks were brooded for seven days before distribution into their respective treatments and replicate group. Heating of the brooder house was controlled during the brooding period. On day 7, the birds were weighed and randomly distributed to the three dietary treatments having five replicates each and ten birds per replicate respectively. Feed and water was supplied *ad libitum*. Gumboro vaccine was administered at three weeks while feed and weight gain was determined on a weekly basis. The duration of the experiment was 49 days (seven weeks). Anti-coccidia and Antibiotics were not administered to the birds throughout the duration of the experiment.

Data collection and analysis

A hundred gram of each experimental diet was collected and set aside for proximate analysis. Proximate analysis of dry matter, crude protein, ash, ether extract concentration was analyzed in the diets according to **AOAC (1990).**

Digesta collection

Four birds per replicate were slaughtered by cervical dislocation and digesta collected from different sections of the gut – crop, ileum and caecum. Digesta was collected from the ileum (2cm posterior to merkels diverticulum and 2cm anterior to the ileal-caecal-colonic junction) after a rapid removal of this section. Digesta collected was transferred into sterile container on ice after a rapid removal of each section of the gut. The digesta collected from two birds in each replicate was used to determine pH of the ileum, crop and caecum while the digesta collected from the remaining two birds slaughtered was used exclusively for enumeration of gut microflora.

Determination of gut pH

The pH of the ileum, crop and caecum was determined using a pH meter (Model PHS – 25 pH meter – B. Bran Scientific and Instrument Company England). Distilled water (30mls) was added individually to the collected digesta, crop and caeca samples respectively. The samples were homogenized prior to pH determination.

Microbiological Analysis

One gram (wet weight) of digesta sample from the different gut sections was placed in individual test tubes for serial dilution. The serially diluted samples were inoculated into growth media for *coliform, Lactobacillus* and *Escherichia coli. Coliform* bacteria were enumerated on McConkey agar (Merck kgaa b4271). *Escherichia coli* were enumerated on fluorescent VRB agar (Merck Kgaa) while *Lactobacillus* was enumerated on M. R.S. (de Man Rogosa and Sharp) agar. Data collected on microbial counts were first log transformed prior to statistical analysis.

Statistical Analysis

Descriptive statistics and one way analysis of variance (ANOVA) followed by multiple comparison (Duncan Multiple Range Test) was carried out at α = 0.05 using SPSS (SPSS/PC + Statistics. 17.0 SPSS Inc., Chicago, IL, 2008).

RESULTS AND DISCUSSION

The proximate analysis (chemical composition) of the control and experiment diets is presented in Table 2. Dry matter concentration of the control diet was 782g while M – SBM/WO diets with and without enzyme supplementation was 870.3 and 844gm respectively. Concentration of crude protein (calculated) ranged between 213.9 – 218.6g/kg DM. However results of the chemical analysis recorded the least crude protein concentration (214.17g/kg DM) in M – SBM/WO without enzyme supplemented diet. The highest value was recorded in the M – SBM/WO diet supplemented with enzyme (233.05kg/kg DM). A value of 224.81g/kg DM was recorded for the M – SBM control diet without enzyme supplementation. Ether extract concentration ranged from 14.81 – 20.97g/kgDM with the highest value reported for the control diet. This was also the case with ash content of the diets. Its value ranged from 24.13 - 32.48g/kg DM. The value obtained during this study is close to what was reported by other authors. **Lawal et al. (2010)** reported the following composition of palm kernel cake (PKC) 88.43% dry matter, crude protein 20.2%, crude fibre 3.97% either extract, 13.9% ash and 49.9% Nitrogen force extract (NFE). **Ani et al. (2012)** presented the percentage composition of bambara nut used for poultry feed as follows, 88.64 - 92.79% dry matter, 23.01-24.6% crude protein, 4.28-7.85% crude fibre, 4.2-5.56% ether extract, 6.86 - 8.03% ash and 44.54 - 51.06% NFE. Similarly, **Fasuyi and Akindahusi (2009)** reported the composition of *Amaranthus cruentus* leaf meal based broiler diet to consist of 22.76 - 23.12% crude protein, 5.02 - 6.38% crude fibre and 7.61 - 7.83 ether extract. Apparently the individual constituent of the ingredients that constitute a diet tend to influence the concentration of the components analysed. This could be the result of the rather much higher concentration of ash and ether extract in the control diet where PKC was added as a source of fibre.

Table 2 Chemical composition of experimental diets (in g/kg DM unless otherwise stated)

Parameter	M – SBM diet	M – SBM/WO without enzyme	M – SBM/WO with enzyme
Dry matter (g)	782	844	870.3
Crude protein	224.81	214.17	233.05
Ether extract	20.97	14.81	16.50
Ash	32.48	26.90	24.13

The populations of microbes in the gut of the chicken following the supplementation of their diets with the enzyme Roxazyme G2G is presented in Table 3. The result shows that the chickens fed with wheat offal supplemented diet (with the Roxzyme enzyme) had significantly higher concentration of *Lactobacillus* compared to those fed control diet (*p*<0.05) in all the gut sections – crop, ileum and caecum. In addition, the chickens fed with wheat offal supplemented diet (without the Roxzyme enzyme) showed also significantly higher concentration of *Lactobacillus* compared to those fed control diet (*p*<0.05) but was detected only in crop and caecum.

Table 3 Effect of enzyme supplementation on gut microflora in broilers fed enzyme supplemented diet (log cfu/g)

Gut section	M-SBM (control)	M-SBM/WO without enzyme	M-SBM/WO with enzyme	SEM	*P value*
Crop					
Coliform	7.10[c]	6.93[a]	6.97[ab]	0.04	0.096
E. coli	7.05[c]	6.71[a]	6.83[b]	0.03	0.000
Lactobacillus	6.63[a]	6.93[b]	7.04[c]	0.03	0.000
Ileum					
Coliform	7.20[b]	7.17[b]	6.97[a]	0.04	0.007
E. coli	7.02[c]	6.70[a]	6.80[b]	0.03	0.000
Lactobacillus	6.66[a]	6.72[a]	7.03[b]	0.02	0.000
Caecum					
Coliform	7.05[b]	7.00[ab]	6.98[a]	0.02	0.041
E. coli	6.98[b]	6.64[a]	6.98[b]	0.01	0.000
Lactobacillus	6.73[a]	7.01[b]	7.03[b]	0.01	0.007

a, b, c: Means along the same row with different superscripts are significantly different (*P<0.05*)

The wheat offal diet supplemented with Roxazyme G2G had significant higher population of *Lactobacillus* compared to the diet without enzyme. The findings of this study therefore suggest that the presence of wheat offal in the diet stimulated proliferation of *Lactobacillus*, which was further enhanced by enzyme addition. Coliforms and *E.coli* were consistently higher (*P<0.05*) in the control

than feeds supplemented with wheat offal with or without enzyme addition in the crop and ileum. The results also show that *E. coli* accounted for nearly 100% of total *coliform* in the gut of the birds used for the present study. The overall pattern of the results indicated that the wheat offal in the poultry diet stimulated *Lactobacillus* growth which was further enhanced by the addition of enzyme. The stimulation of *Lactobacillus* by the diet containing wheat offal with or without enzyme coincided with the reduction in *coliform* and *E. coli* population. This suggests the efficacy of diet composition and enzyme supplementation in controlling pathogenic organisms in broiler chickens. As previously reported by Ofongo *et al.* (2012), higher and better *Lactobacillus* count in the ileum of birds fed the enzyme supplemented diet indicates better nutrient availability for digestion and absorption. Replacement of 200g of maize with wheat offal with and without enzyme supplementation further resulted in modulation of gut microflora towards proliferation of beneficial microflora (**Schutte and Langhout, 1999**) in this case *Lactobacillus*. Composition of the diet influences the species and number of bacteria in the gut as stated in previous reports by other authors (**Varel and Pond, 1985; Jensen *et al.*, 2003; Hedermann *et al.*, 2003**).

In this study, the population density of *Lactobacillus* in the control birds was 6.63 log cfu/g i.e. 3.8-5.4 x 10^6cfu/g in the crop, 6.6log cfu/g (ileum) and 6.73 log cfu/g (caecum). This indicates that the population of *Lactobacillus* increased sequentially from the proximal end of the gut to the distal end. A significantly higher population (*P<0.05*) was observed in the wheat offal based diet without enzymes. However, the population of Lactobacillus in the gut of chickens fed with maize–soya beans diet containing wheat offal and Roxazyme G2G enzyme was relatively constant from the crop, through the ileum to the caecum.

The population of *E.coli* in the control maize-soya beans diet was 7.05 log cfu/g, 7.02 log cfu/g and 6.98 log cfu/g in the crop, ileum and caecum respectively. **Tollba and Mohammed (2009)** reported *E. coli* population of 10^6cfu/g in the ileum, caecum and feacal matter of broiler chickens. **Falaki *et al.* (2011)** reported a decrease in *coliforms* population from 7.92logcfu/g to 7.70 logcfu/g in the ileum, with an increase in *Lactobacillus* from 7.10 logcfu/g to 7.45 log cfu/g in the crop when the probiotic Primalac® was added to poultry diet.

Langhout (1999) reported the differential responses of gut microbes of chicken fed with maize soya beans diet containing the NSP called highly methylated pectin (HMP). The presence of the NSP (HMP) increased the population of microflora in the gut of 22 days old chickens. *E.coli* increased from 5.3 to 6.8 log cfu/g, *Clostridium spp* from 1.8 to 40 log cfu/g and bacteroids from 4.1 to 5.0 log cfu/g. On the order hand, beneficial microbes decreased; *Lactobacillus spp* from 7.0 to 6.0 log cfu/g and *Bifiobacteria* spp from 7.0 to 6.9 log cfu/g. This result further substantiates the result of the current study to our findings, where the addition of wheat offal presumably containing NSPs stimulated beneficial *Lactobacillus* while decreasing the population of pathogens including E.coli and *Coliform*. This could be as a result of the concentration of insoluble NSP in wheat offal which is higher than insoluble NSP that can elicit the same effect as adding highly methylated pectin. Notwithstanding, **Torok *et al.* (2011)** reported that facultative and microaerophilic bacteria such as *Lactobacillus* dominate the ileum of chickens while obligate anaerobes, mostly *Clostridium* dominate the ceacum.

The results of pH measurements of the chicken gut content revealed that pH is generally slightly acidic for all the diets except in the caecum of the control where the pH was neutral (Table 4). The pH results of the digesta of the three treatments (i.e. diet compositions) where not significantly different (p >0.05) in the crop and ileum. But in the caecum, the pH was significantly different (p <0.05) being slightly more acidic than the other 2 diets. Two reasons may be responsible for the observed differences in pH. Firstly, it has been well documented that *Lactobacillus* secretes organic compounds (mostly lactic acid) which tend to reduce the pH of the digesta. Secondly, hydrolysis of insoluble NSP present in wheat offal may further release intermediate products such as fructo – oligosaccharides and xylo – oligosaccharides. Digestion of these low molecular weight oligosaccharides will generally result in an increase in the number of *Lactobacilli* and *Bifidobacterium* with a consequent decrease in *Clostridia* and *Enterobacterium* as reported by **Nemcova *et al.*(1999)**. The Roxazyme G2G added to the feed is mostly active over acidic pH range of 3.5 - 5.5. Hence, the findings of this study support the notion that *Lactobacillus* countered pathogenic organisms by creating acidic conditions that inhibit growth and proliferation of competing pathogens. In a previous study (**Ofongo *et al.*, 2011**), enzyme supplementation of maize – soya bean meal based diet did not alter the gut pH. However the authors reported that enzyme supplementation of M-SBM diet resulted in enhanced weight gain and FCR. The result of that study was in agreement with that previously reported by **Cowieson (2005) and Jia *et al.* (2009)**. **Ofongo *et al.* (2011)** further stated that enzyme supplementation did not indicate any significant influence on gut pH which may be as a result of the type of diet fed. The authors suggested further investigation using different diet type to assess the effect on gut microflora and gut pH.

Table 4 Effect of diet type and enzyme supplementation on Gut pH in broilers

Gut section	M-SBM Control diet	WO/M-SBM without enzymes	WO/M-SBM with enzymes	SEM	*P value*
Crop	6.40	6.32	6.40	0.14	0.976
Ileum	6.53	6.71	6.61	0.11	0.623
Caecum	7.13 [a]	6.95[b]	6.88 [c]	0.03	0.002

a, b, c: means along the same row with different superscripts are significantly different (*P<0.05*)

The performance characteristics of the birds feed with the maize soya bean meal supplemented with wheat offal with and without Roxazyme G2G is presented in Table 5. The results showed that partial replacement of maize beans meal with wheat offal in the presence of Roxazyme G2 G increased weight gain, but decreased feed intake and feed conversion ratio (note that the lower the feed intake and FCR the more efficient uses of feed), but all these differences were not statistically significant (*p>0.05*). However, literature reports on the role of enzyme on performance are quite contradictory. **Ademola et al. (2012)** reported that Roxazyme G and maxigrain slightly improved feed conversion but failed to improve the performance and profits of chicken fed with wheat offal diet. **Cowieson et al. (2006)** reported a FCR of 2-9% over the control of broilers quails, turkeys and layers fed with grains (wheat, rye, maize, and barley) supplemented with xylanase and/or 3-glucanase. However, **Jin et al. (2000)** studied the performance of broilers feed with diets supplemented with *Lactobacillus* cultures and found that *Lactobacillus* cultures caused significant increase in both weight gain and FCR (*p<0.05*) over the control. The inconsistency of the effects of enzyme supplementation on chicken performance is due to several factors such as environmental differences, growing conditions, cereal type, enzyme dose, age of the animal, method of processing of the feeds, nutrient density, and the nature of the microbial community in the gut (**Ademola et al., 2012; Ponte et al., 2004; Cowieson et al., 2006**). Although, enzyme supplementation of poultry diets essentially results in enhanced growth, feed conversion and increased accuracy in least – cost feed formulation. However, the performance results recorded in the current study only showed numerically better values in terms of weight gain and feed conversion.

Table 5 Performance characteristics of broilers fed diets with and without enzyme supplementation (g/bird for all parameters except FCR which is a ratio)

Parameters	M-SBM Control diet	WO/M-SBM without enzymes	WO/M-SBM with enzymes	SEM	*P value*
Initial live weight	46.80	49.8	50.00	-	-
Final live weight	1198.83	1133.99	1226.25	68.66	0.524
Weight gain	1151.99	1084.19	1176.25	68.67	0.807
Feed intake	4508.91	4261.43	4258.39	149.66	0.267
FCR	4.01	3.99	3.63	0.27	0.336

CONCLUSION

The study was designed to determine the effect of enzyme supplementation and type of diet on gut microflora, pH and performance of broiler chicken. Results obtained show that *coliforms* and *E.coli* were consistently higher (*P<0.05*) in the control than feeds supplemented with wheat offal with or without enzyme addition in the crop, ileum and caecum. The overall pattern of the results indicated that the wheat offal in the poultry diet stimulated *Lactobacillus* growth which was further enhanced by the addition of enzyme. The stimulation of *Lactobacillus* by the diet containing wheat offal with or without enzyme coincided with the reduction in *coliform* and *E. coli* population, which suggests the efficacy of diet composition and enzyme supplementation in controlling pathogenic organisms in broiler chickens. In conclusion, performance results showed that partial replacement of maize soya beans meal with wheat offal in the presence of Roxazyme G2 G increased weight gain, but decreased feed intake and feed conversion ratio, but all these differences were not statistically significant (*p>0.05*).

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