Intestinal histology of broiler chickens fed direct fed microbial (RE3) and antibiotics

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Abstract

This study was conducted to compare the effects of using direct fed microbial (RE3) and antibiotics on the gut morphology. Two hundred and forty unsexed, one-day old Marshal strain broiler chicks were used for the experiment. The birds were randomly allotted into three dietary treatments with each treatment replicated four times at 20 birds per replicate making a total of 80 birds per treatment. The experimental design was completely randomized design. The villous height (9.303E2), the crypt depth (1.5053E2) and the muscular thickness (2.2311E2) of the RE3 treated birds were higher ($P < 0.05$) when compared with birds fed with control diet (T1) 8.619E2, 1.3790E2 and 1.9645E2 and the control + antibiotics (T3) 7.0677E2, 1.3331E2 and 1.9027E2 respectively. The observations revealed that birds put on treatment 2 (RE3 probiotics) had better presentation and preservation of the intestinal villi, glands and intestinal wall integrity. It was concluded that supplementation of broilers diets with the direct fed microbial (RE3) will lead to maintenance of intestinal health and better utilization of nutrients to enable full expression of genetic potential.

Keywords; direct fed microbial, broilers, villous height, crypt depth, muscular thickness

Introduction

Although, the microflora capable of intense metabolic activities have both beneficial and detrimental effects on the host animal, the establishment of microbial population in the gastro intestinal tract (GIT) of all warm blooded animals soon after birth is inevitable. A bacteria population is present in the small intestine in the avian species within 24 hours following hatch (Richard, 2013). It has been hypothesized that gut microflora decrease nutrient absorption by increasing GIT thickness, the rate of digesta passage and also increase gut mucosa contents by competing with the host for a portion of the dietary energy and protein (Apajalahti *et al.*, 2004). Much of the work with antibiotic growth promoters continues to be from the standpoint of studying the effects on easily cultured bacterial population such as *Lactobacilli* and *Clostridium perfringens* and poultry health

rather than resulting changes to the GIT (George *et al.*, 1982; Engberg *et al.*, 2000, Sims *et al.*, 2004). Also, studies have demonstrated that supplementation of organic acid or probiotics to broiler diets increased growth performance, reduced disease and management problems (Jin *et al.*, 1998; Runho *et al.*, 1997).

Feed additives such as antibiotics, probiotics or organic acids can help intestinal tissue, since their supplementation in the diets decreased harmful pathogens. However, antibiotics as growth promoters have been under scrutiny for many years and have been removed from the market in many countries (Ratcliff, 2000). Direct fed microbials (Probiotics) have been utilized to improve animal performance by maintaining the normal microflora of the intestinal mucosal barrier against deleterious agents. The intestinal epithelium acts as a natural

barrier against pathogenic bacteria and toxic substances that are present in the intestinal lumen. Stressors, pathogens and chemical substances, among others, causes disturbance in the normal microflora or in the intestinal epithelium (Olivera, 1998). Consequently, there is a decrease in the villus and decrease in the digestive and absorptive activities (Vissek, 1978). Since absorption is totally dependent on the mechanisms that occur in the intestinal mucosa, the manipulation of probiotics (Live microorganisms which, when administered in adequate amounts, confer a health benefit on the host) together with prebiotics (Non – digestible ingredients/substances that are beneficial to the host because they selectively stimulate growth and/or the activity of certain bacteria in the intestine) have been used to improve performance and consequently, the energetic efficiency of the intestine (Dobrogosz *et al.*, 1991; Pelicano *et al.*, 2004). The action of probiotics can be explained by some mechanisms such as the production of antimicrobial substances and organic acids, protection of the villi and absorptive surface against toxins produced by pathogens, as well as the stimulation of the immune system (Pelicano *et al.*, 2004). However, there is a dearth of information regarding the effects of direct– fed microbials on the histomorphology of the small intestine of broiler chickens. The aim of this experiment is to investigate potency of the probiotic RE3 on villus depth, density and to compare the effects of probiotics (RE3) and antibiotics on the morphology of digestive system of broilers.

Materials and methods

Experimental Site

The experiment was conducted at the Poultry Unit of the Teaching and Research Farm, Ladoke Akintola University of

Technology, Ogbomoso, Oyo state, Nigeria. The study site is in the Derived savannah zone of Nigeria. The description of the study area had earlier been reported (Ojebiyi *et al* 2014).

Formulation of Experimental Diets

Table 1 shows the gross composition of both starter and finisher diets. Diet 1 was a control diet while diets 2 and 3 consists addition of RE3 and Fysalsp, respectively.

Arrival and Management of Birds

Two hundred and forty unsexed one-day old Marshal strain broiler chicks were used for the experiment. On arrival, the birds were gently unboxed, weighed and were randomly allotted into three dietary treatments with each treatment replicated four times at 20birds per replicate to make a total of 80 birds per treatment. The experimental design was completely randomized design. After initial weight the birds were weighed on weekly basis. The birds were allowed access to feed and water *ad-libitum* throughout the 8 weeks of experiment (starter phase 1 – 4 weeks and finisher phase 5– 8 weeks).All necessary vaccinations and preventive medications were given as required for the birds. Two birds with weight closest to the mean group weight of each replicate were selected sacrificed and the gut was carefully removed for organ weight.

Histological studies

For the histological analysis, the tissues of the ileum were collected from the slaughtered birds and fixed in 10% formalin solution. Tissues were dehydrated by immersing through a series of alcohols of increasing concentrations (from 70% to absolute), infiltrated with xylene and embedded in paraffin. Casting of block was carried out in L-molds (two – L – shaped pieces) which facilitated the manipulation of size as per the requirement. The rotary type microtome was used for sectioning. The

Table 1: Gross composition of experimental diet (Starter and Finisher phase)

Ingredients	Starter			Finisher		
	Diet 1	Diet 2	Diet 3	Diet 1	Diet 2	Diet 3
Maize	55.00	55.00	55.00	57.0	57.0	57.0
Soybean	35.20	35.20	35.20	25.5	25.5	25.5
Wheat offal	1.5	1.5	1.5	10.0	10.0	10.0
Palm Kernel Cake	2.0	2.0	2.0	2.0	2.0	2.0
Fishmeal	2.5	2.5	2.5	1.5	1.5	1.5
Limestone	1.5	1.5	1.5	1.5	1.5	1.5
Bone meal	1.5	1.5	1.5	1.5	1.5	1.5
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.12	0.12	0.12	0.25	0.25	0.25
Methionine	0.18	0.18	0.18	0.25	0.25	0.25
Premix starter	0.25	0.25	0.25	—	—	—
Premix Finisher	—	—	—	0.25	0.25	0.25
RE3	—	+	—	—	+	—
Fysal Dry SP	—	—	+	—	—	+
Total	100.00	100.00	100.00	100.00	100.00	100.00
Crude protein	23.54	23.54	23.54	19.55	19.55	19.55
Energy	2982.15	2982.15	2982.15	2919.28	2919.28	2919.28
Crude fibre	3.7	3.7	3.7	3.9	3.9	3.9

*Vitamin premix per kg of diet: Vitamin A,12000IU; vitamin D3, 2500IU; vitamin E,30IU; vitamin K,2.0mg; vitamin B1, 2.25mg; vitamin B2, 6.0mg; vitamin B6, 4.5mg; vitamin B12, 0.015mg; Niacin, 40mg; Panthetonic acid, 15mg; Folic acid, 1.5mg; Biotin, 0.05mg; Chlorine chloride, 300mg; Manganese, 80mg; Zinc, 50mg; Iron, 20mg; Copper, 5.0mg; Iodine, 1.0mg; Selenium, 0.2mg; Cobalt, 0.5mg; Antioxidant, 125mg. Diet 1= Control, Diet 2=Control + RE3 Probiotics, Diet 3=Control + Fysalspp

Table 2: Villous height (µm), crypt depth (µm) and muscular thickness (µm) of broiler fed, control, direct fed microbial and antibiotics

Parameters	Treatment 1 (control)	Treatment 2 (RE3 probiotics)	Treatment 3 (antisalmonella)	Standard Error
Villous height(µm)	8.6196E2 ^b	9.3503E2 ^a	7.0677E2 ^c	5.5994E1
Crypt depth (µm)	1.3790E2 ^c	1.5053E2 ^a	1.3331E2 ^b	8.996463
Muscular thickness (µm)	1.9645E2 ^b	2.2311E2 ^a	1.9027E2 ^b	1.1121E1

^{abc}Means along the same with different superscripts are significantly different (P<0.005).

blocks were properly trimmed and the sections of 5mm thickness were cut out. Continuous ribbons (6–7 µm long) were cut and laid on the surface of slide at constant temperature of water bath. The sections were separated with a heated scalpel to spread out. The cut sections were mounted on the clean glass slides using Mayers egg albumin as the section adhesive. The mounted slides were dried in paraffin of constant temperature for one hour. The

tissue sections were stained by Harris heamatoxylin and eosin staining method. The clearing was performed in the xylene and drop of distrene plasticizer xylene (DPX) mountant was placed on a cover slip and the section on the slide pressed on it. The slides were inverted and recover slip were pressed with a rod to remove air bubbles, if any trapped according to Leslie and James (2007). The values were measured with oculimeter of a magnification of 10x under a light

Intestinal histology of broiler chickens

Histo-morphology of ileum sections of broilers fed control diet

Plate 1: Section of Ileum of Broiler Fed Control diets showing (A) Degenerated intestinal wall integrity, (B) Visible intestinal glands and (C) Villi. X100. H& E stain.

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Plate 3: Section of Ileum of Broiler Fed Control diets showing (G) Thin intestinal wall, (H) Visible intestinal glands and (I) Short damaged Villi. X100. H& E stain

Histo-morphology of ileum sections of broilers fed control+direct fed microbial

Plate 9: Section of Ileum of Broiler Fed Control diets + RE3 probiotics showing (A) Preserved intestinal wall integrity, (B) Visible intestinal glands and (C) Elongated Villi. X150. H& E stain.

Irregular intestinal glands arrangement and (L) Damaged Villi. X100. H& E stain

Plate 5: Section of Ileum of Broiler Fed Control diets showing (M) Preserved intestinal wall, (N) Visible intestinal glands and (O) Damaged Villi. X100. H& E stain

Plate 6: Section of Ileum of Broiler Fed Control diets showing (P) Ruptured intestinal wall, (Q) Visible intestinal glands and (R) Damaged inflamed Villi. X100. H& E stain.

Plate 7: Section of Ileum of Broiler Fed Control diets showing (S) Wearing intestinal wall, (T) Visible intestinal glands and (U) Damaged Villi. X100. H& E stain.

Plate 10: Section of Ileum of Broiler Fed Control diets + RE3 probiotics showing (D) Preserved intestinal wall integrity, (B) Visible intestinal glands and (C) Elongated Villi. X150. H& E stain

Plate 11: Section of Ileum of Broiler Fed Control diets + RE3 probiotics showing (G) Preserved intestinal wall integrity, (H) Visible intestinal glands and (I) Elongated Villi.X150. H& E stain

Plate 12: Section of Ileum of Broiler Fed Control diets + RE3 probiotics showing (J) Normal intestinal wall integrity, (K) Visible intestinal glands and (L) Elongated Villi.X150. H& E stain.

Plate 19: Section of Ileum of Broiler Fed Control diets + Fysalspp showing (G) Wearing intestinal wall, (H) Visible deformed intestinal glands and (I) Short severely clustered villi with closing lumen.X100. H& E stain.

Plate 20: Section of Ileum of Broiler Fed Control diets + Fysalspp showing (J) Mildly Preserved intestinal wall, (K) Visible intestinal glands and (L) Broken Villi with closing lumen.X100. H& E stain.

Histo-morphology of ileum sections of broilers fed control + antibiotics

Plate 17: Section of Ileum of Broiler Fed Control diets + Fysalspp showing (A) Mildly degenerated intestinal wall, (B) Disrupted intestinal glands and (C) Damaged Villi with closing lumen.X100. H& E stain.

Plate 18: Section of Ileum of Broiler Fed Control diets + Fysalspp showing (D) Damaged intestinal wall, (E) Visible intestinal glands and (F) Short clustered villi with closing lumen.X100. H& E stain.

Plate 21: Section of Ileum of Broiler Fed Control diets + Fysalspp showing (M) Mildly Preserved intestinal wall, (N) Visible intestinal glands and (O) Abnormal Villi Presentation with closing lumen.X100. H& E stain.

Plate 22: Section of Ileum of Broiler Fed Control diets + Fysalspp showing (P) Preserved intestinal wall integrity, (Q) Visible intestinal glands and (R) Elongated Villi.X100. H& E stain.

microscope fitted with the stage micrometer.

Feed Analysis

Proximate compositions of the experimental diets were undertaken, according to the procedures of AOAC (2005).

Data Analysis

Data were subjected to analysis of variance using the General Linear Model of SAS (2000). Means were separated using Duncan's Multiple Range Test of the same package.

Results and Discussion

Data on villus height, crypt depth and muscular thickness (μm) of broilers are presented in Table 2. The villus height shows significant difference ($P < 0.05$) among the treatments. The RE3 treatment (T2) has the highest mean value of $9.3503 \times 10^2 \mu\text{m}$ while the treatment T3 (control + anti-salmonella) had the least value of $5.5994 \mu\text{m}$. The crypt depth shows significant difference ($P < 0.05$) among the treatments. The RE3 probiotics treatment (T2) had higher value of $1.5053 \times 10^2 \mu\text{m}$. The control (T1) and anti-salmonella (T3) has similar values of $1.3790 \times 10^2 \mu\text{m}$ and $1.3331 \times 10^2 \mu\text{m}$, respectively. There were significant differences ($P < 0.05$) in the muscular thickness with T2 (control + RE3) having higher ($P < 0.05$) value of $2.2311 \times 10^2 \mu\text{m}$ than the Control (T1) ($1.9645 \times 10^2 \mu\text{m}$) and T3 (control + Anti-salmonella) ($1.9027 \times 10^2 \mu\text{m}$) that are similar ($P > 0.05$).

Villus Height

The higher villi heights recorded in RE3 containing diet as shown in plates 9, 10, 11 and 12 could be due to the contribution of microbes (usually *Lactobacilli*, *Bifidobacterium thermophilum* and *Enterococcus faecium*) in RE3 to the growth of the villi. Similar observation was

earlier reported by Chichlowski *et al.*, (2007). The higher villi height could also be traced to the effect brought about by the microbes in probiotics by maintaining a low pH and bile acids concentration in the digestive system which could cause shortening of the villi (Amit-Romach *et al.*, 2004).

This observation contradicted the findings of Watkins and Kratver (1983; 1984) that oral administration of *Lactobacilli* strains to birds has no significant effect on the villi. Treatment 3 (control + antibiotics) treated birds had shorter and damaged villi presentation as seen in plates 17, 18, 19, 21 and 22. This might be due to the fact that the residues which are toxic caused the shortening of the villi. The implication of this is that the feed conversion efficiency of the birds would be very low. This is because the villi would be unable to access enough nutrients from the lumen, leading to feed wastage (WHO, 2003).

Also, the beneficial effect of longer villi as seen on the slides of birds fed control + RE3 probiotics in that it contributed to the absorptive surface area in birds/ animals. This will result in improve feed intake and digestion, low feed retention time according to Nahanshon *et al.* (1992; 1993), the more such bird feeds, the more improvement in growth performance is expected according to Kabir *et al.* (2004) and Samli *et al.* (2007).

Crypt Depth (μm)

RE3 treated birds having the highest value is an indication that there were an appreciable nutrients and digestive glands needed by the birds for the digestion and even for the growth of villi (plates 9, 10, 11 and 12). The crypt depth of RE3 treated birds were influenced by the microbial secretions that added more to the digestive enzymes and intestinal juices. In contrast, the effect of antibiotics toxin residues seen

caused the reduced crypt depth and increase in the crypt epithelial tissue turn-over (as seen in plates 19 and 21). This will affect the integrity of villi along the length of the tract, because epithelial cells differentiate into a variety of cells with special functions that include the secretion of various fluids, electrolytes, enzymes, and in the gizzard (Verma and Agarwal, 2005; Richard, 2013).

Muscular Thickness (μm)

The RE3 treatment bird's show the highest intestinal muscular thickness and this is also reflected on the plates 9, 10, 11, 12, 13 and 14 where the intestinal walls were preserved. The thick and preserved intestinal muscular thickness revealed that the probiotics functioned to protect the wall from antagonizing organisms, thus supporting the immune system of the birds or animals according to WGO (2008). The thin intestinal muscular wall of the T3 (control + antibiotics) as seen on plates 17, 18 and 19 could be due to the chemical residues which reacted with the mucin layer of the intestines negatively causing the mucosa, submucosa and the serosa layers to be eroded. The implication of this is that the immune system of the birds is compromised since there is no barrier limiting deleterious component of diet (Korver 2006).

Also, the thin-intestinal muscular tissues of the T3 (control + antisalmonella) birds (plates 17, 18 and 19) could result from the antibiotics acting on the beneficial organisms and interference with the microbial community of the intestinal wall (Apajalahti *et al.*, 2004). The thin intestinal muscular tissues seen on plates 1, 2, 3, 4, 5, 6 and 7 of the birds fed normal diets could be that the pathogenic microbes were able to invade the intestinal walls without any barrier.

Conclusion and recommendation

From this study dietary inclusion of RE3

can exert beneficial effects in the gastrointestinal tract of broilers sequel to possible increase in digestion and absorption of nutrients thus resulting in improved performance. Its inclusion in broiler diet is therefore recommended.

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