

**ORGAN WEIGHT AND INTESTINAL HISTOMORPHOLOGY OF
BROILER CHICKENS FED DIETS SUPPLEMENTED WITH
ANTIBIOTICS AND A PROBIOTIC**

Cosmas C. Ogbu*¹, Gloria Daniel-Igwe² and Ibeneme Nwabueze¹

¹Department of Veterinary Biochemistry and Animal Production and ²Department of Veterinary Pathology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

ABSTRACT

The study was designed to evaluate the effects of inclusion of antibiotics (Neomycin sulphate and Oxytetracycline) and a probiotic in diets on body weight, organ weight and intestinal histological indices of broiler chicks. A total of one hundred and twenty day-old Abor Acres broiler chicks were randomly allotted to four experimental groups: unsupplemented basal diet (group 1 or control), supplementation with, Saccharomyces cerevisiae at 0.8g/kg (group 2), neomycin sulphate at 0.5g/kg (group 3), and oxytetracycline at 0.3 g/kg (group 4). Feed and water were provided ad libitum to all experimental birds. Data were collected on live body weight, internal organ weights and intestinal histomorphology at 7 weeks of age using standard procedures. Results showed that diet supplementation with probiotic and antibiotics improved body weight, and the weight of liver and crop compared to the control group. Feeding antibiotics to broiler chicks resulted in decrease in the thickness of the colon but birds fed probiotics generally had thicker ileum, colon, and caecum. Feeding probiotic also enhanced intestinal villus characteristics which were better in some variables compared to those of birds fed antibiotics. Within treatments, intestinal sections showed differences in villus characteristics and number of goblet cells that reflected the functional characteristics of the different intestinal segments. It was concluded that probiotics could replace antibiotic growth promotants in broiler diets to enhance intestinal histomorphology and function.

Keywords: Organ weight, broilers, intestinal histomorphology, antibiotics, probiotic.

INTRODUCTION

Probiotics are biostimulants containing live or lyophilizing microbial cultures which regulate and optimize the beneficial intestinal microbiota, ensuring the maintenance of gastrointestinal balance and homeostasis [1,2]. Probiotics are among the alternatives to antibiotic growth promotants employed in livestock and poultry production [2]. They exert stimulating effects on gastrointestinal cells and tissues, enhance digestion and absorption of nutrients, and influence the

histological and microbiological characteristics of the intestinal tract. The beneficial effects of probiotics are attributed to enhanced intestinal health resulting from three principal effects: (i) enhanced colonization of the gut by beneficial micro-organisms [3] and competitive exclusion of pathogenic microbes; (ii) enhanced digestive and absorptive capacity of the gut which improves digestion, nutrient absorption, and utilization, and (iii) enhanced secretory and immunomodulatory mechanisms of the gut mucosa and glands [4] resulting in protection against assault by pathogenic microbes and their toxins and enhanced mutualism between the intestinal microbiota and the potent effector cells of the intestinal innate and adaptive immune systems [5]. Pelicano *et al.* [6] reported that probiotics have been used to improve the health, function and the energetic efficiency of the intestinal tract.

Oxytetracycline and neomycin are antibiotics commonly added to feed or fed in water by farmers for prophylactic and growth promoting effects in poultry production. The effects of antibiotics and probiotics on intestinal histomorphology have been extensively studied in chickens but with emphasis on the small intestine [7,8,9] probably on account of its primary role in digestion and absorption of nutrients. Relatively few studies have included an evaluation of the effects of antibiotic and probiotic feed additives on the large intestine leading to very scanty information on their impact on the histomorphology of the large intestine (colon, caecum, and rectum). The large intestine is a vital segment of the gastrointestinal tract being densely colonized by microorganisms and is the main site of microbial fermentation, electrolyte and water absorption in chickens and other animals [5,10]. Consequently, the large intestine should be considered in evaluating the effects of feed additives on performance, intestinal histomorphology, health and function. The present study was designed to evaluate the histomorphological and organ weight changes in broiler chickens fed diets supplemented with a probiotic and antibiotics at sub-therapeutic levels.

MATERIALS AND METHODS

Management of experimental animals

A total of 120 one day old Abor acres broilers were brooded for three weeks and then shared into four groups in a completely randomized design namely: unsupplemented basal diet (group 1 or control); supplementation with *S. cerevisiae* at 0.8 g/kg (group 2); supplementation with neomycin sulphate at 0.5 g/kg (group 3) and supplementation with oxytetracycline at 0.3 g/kg (group 4). Feed and water were provided *ad libitum* to all experimental birds. All experimental animals in all groups were housed on floor pens bedded with wood shaven. The study was carried out in the Poultry Unit of the Department of Veterinary Biochemistry and Animal Production of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

Data collection

Body and internal organ weights

At 7 weeks of age, three birds were randomly selected from each experimental group, weighed and then sacrificed humanely for gross and histomorphological studies. The internal organs including the heart, liver, spleen, gizzard, crop, proventriculus, and intestine were carefully removed and the weights of heart, liver, spleen, gizzard, crop, and proventriculus obtained with a digital weighing scale while the length of the intestine was determined using a flexible meter rule.

Histomorphological studies of the intestinal tract

Samples of the ileum, caecum, colon and rectum of the slaughtered birds were collected and fixed in 10% buffered formalin for 24 hours, dehydrated by passing through ascending concentrations of alcohol (70%, 80%, 90%, absolute 1 and absolute 2) for 2 hours, respectively, cleared in xylene, and then embedded in paraffin wax [11]. Following solidification, the embedded tissues were trimmed, mounted on wooden blocks and appropriately labeled. The embedded tissues were sectioned to 5 µm thickness using sliding microtome (KD202, Kedi, China). The sections were floated on warm water at 45°C in a floatation bath to stretch and then mounted on slides. The slides were transferred to slide warmer to dry at 70°C and thereafter de-waxed by washing in xylene. The sections were then stained by standard haematoxylin and eosin method (Sigma Chemicals, Germany) for microscopic studies. Slides were evaluated using a motic camera (Optika, Germany) and images were analyzed at x10 optical lens for measurement of crypt depth, villus height, villus width and thickness of the muscularis. The height of the villus was measured from the tip of the villus to the crypt – villus junction while three measurements taken from the epical region, middle and base of each villus were averaged to obtain the villus width. For all determinations, ten intact villi were randomly assessed for each sample and the linear values were expressed in micrometer. The villus area and villus height: crypt depth (VH:CD) were calculated. The number of goblet cells on each villus was obtained by counting at x40 magnification [12,13].

Data analysis

Data collected were subjected to Multivariate Analysis of Variance in Completely Randomized Design using the Statistical Package for Social Sciences [14]. Significantly different means were separated using the Duncan New Multiple Range Test in SPSS [14].

RESULTS

Body and organ weights, and length of intestine

Final body weight did not differ significantly between probiotic and neomycin groups but these were significantly ($p < 0.05$) higher compared to the values for control and oxytetracycline groups (Table 1). Weight of heart, proventriculus and spleen, and length of intestine did not differ significantly ($p > 0.05$) between treatment groups while weights of liver and crop were significantly ($p < 0.05$) higher in birds fed neomycin compared to other treatments. Weight of liver from birds fed neomycin was 30.6, 26.0, and 24.8 % higher than those from birds in control, probiotic and oxytetracycline groups, respectively. For weight of crop, the corresponding values were 72.4, 50.4 and 30.5 %, respectively. Weight of liver did not differ significantly between control, probiotic and oxytetracycline groups. Birds fed oxytetracycline had crops that were heavier ($p < 0.05$) by 28.7 and 60.3 % compared to those from groups fed probiotic and basal diets, respectively while weight of crop from probiotic group exceeded that of control group by 44.4 %.

Table 1. Body and Organ weights (in g) and intestinal length (in cm) of the experimental birds at 7 weeks of age

Organ weight (g)	Control	<i>S. cerevisiae</i>	Neomycin	Oxytetracycline	SEM
Body weight	2113.33 ^b	2352.17 ^a	2468.18 ^a	2170.83 ^b	57.01
Heart	9.03	11.43	12.53	9.63	1.10
Liver	49.13 ^b	52.40 ^b	70.77 ^a	53.20 ^b	2.62
Spleen	2.63	1.93	3.45	2.57	0.35
Gizzard	60.87	67.70	71.93	71.63	3.91
Crop	29.50 ^d	53.03 ^c	107.00 ^a	74.40 ^b	4.58
Proventriculus	10.00	10.17	11.73	9.50	1.15
Intestine length	173.67	197.33	175.33	191.33	13.27

^{abcd}Means on the same row with different superscripts are significantly different ($p < 0.05$)

Histological variables of ileum and colon.

The values for the histological variables namely, height of villus (HV), mucosal height (MH), width of villus (WV), crypt depth (CD) and thickness of the muscularis mucosa (TM) of ileum (panel A) and colon (panel B) are shown in Fig. 1 while Table 2 contains the values for area of villus (AV), HV:CD and number of goblet cells for the intestinal segments. Plate 1, and 2 is the photomicrograph of ileum and colon, respectively of birds in the experimental groups. There were no significant differences in the measured ileal and colonic variables of the treatment groups except for thickness of colonic muscularis mucosa, colonic villus area, and ileal goblet cell number. Colonic muscularis mucosa was thicker in birds fed probiotic compared to those fed antibiotics (Fig. 1, panel B) while colonic villus area was significantly ($p < 0.05$) higher in birds fed neomycin compared to those fed probiotic and oxytetracycline. Goblet cell number was highest in ileum of birds fed oxytetracycline followed by those of birds fed probiotic but least in ileum of birds fed basal diet (Table 2).

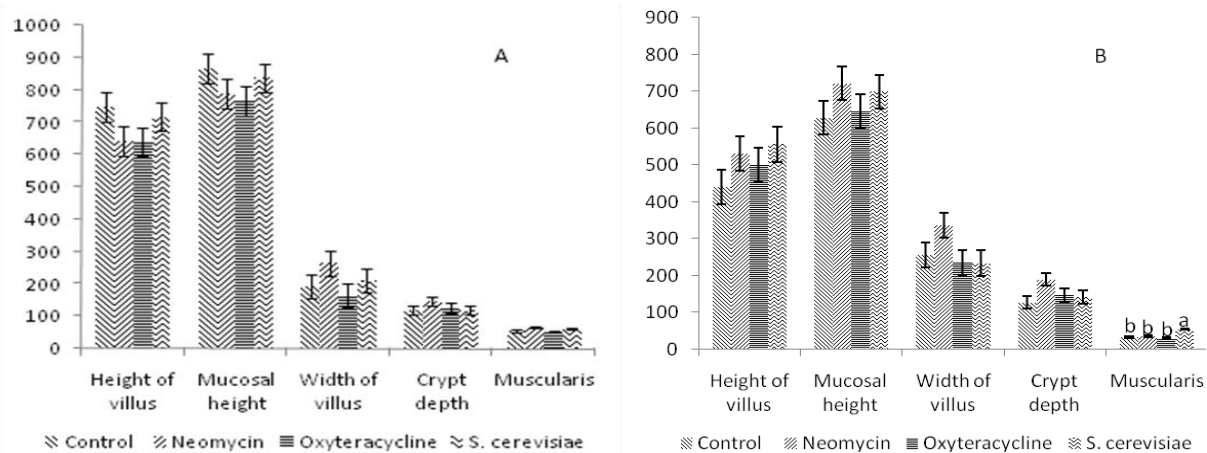
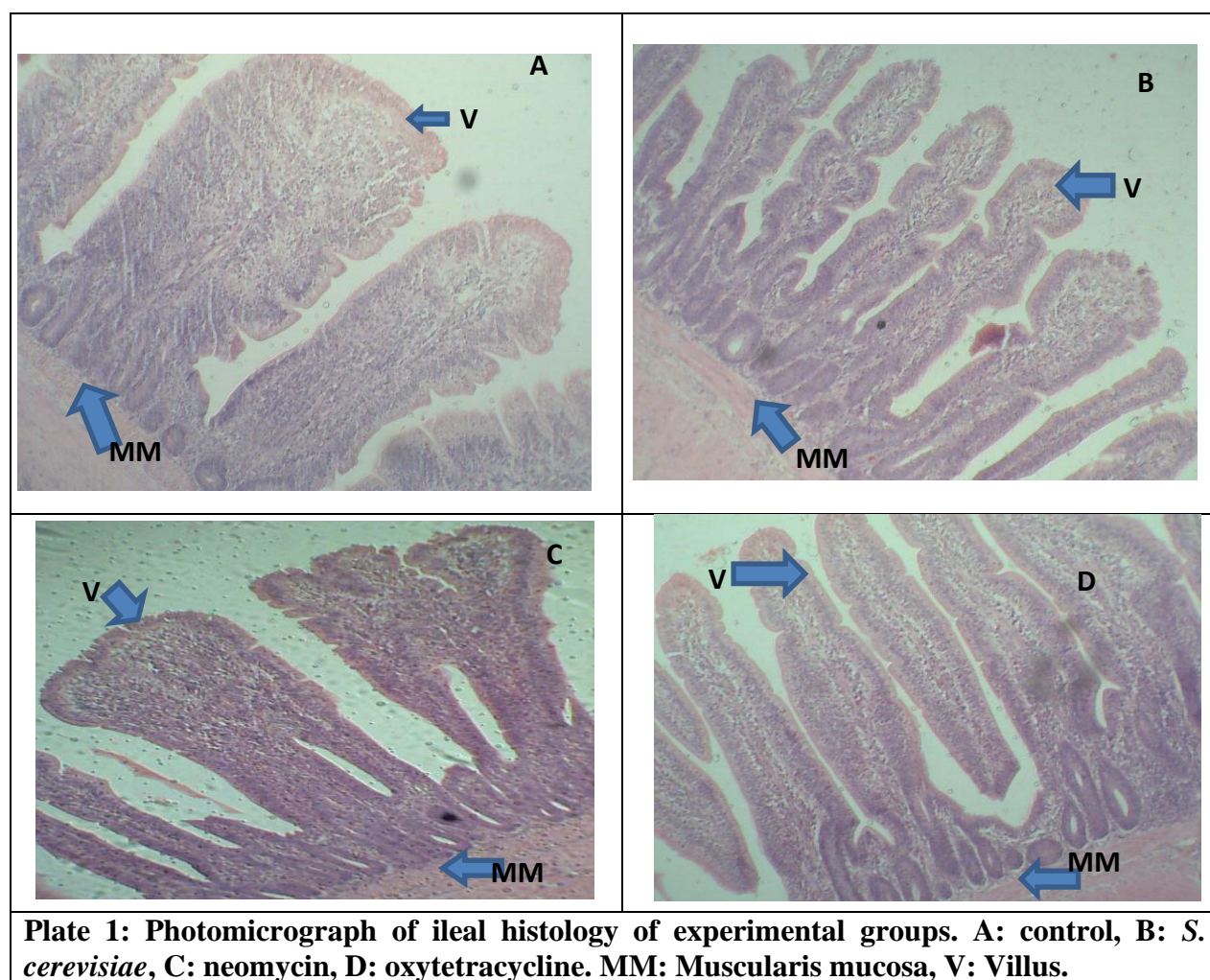


Fig. 1. Histological variables of ileum (panel A) and colon (panel B) of experimental birds at 7 weeks of age. Muscularis: thickness of muscularis mucosa.

Table 2. Villus area, VH:CD, and goblet cells count in ileum and colon of experimental birds

Variable	Control	<i>S. cerevisiae</i>	Neomycin	Oxytetracycline	SEM
Ileum					
Villus area (μm^2)	145107.25	150214.59	158863.18	107265.59	19413.48
VH:CD	6.59	6.13	4.83	5.32	0.77
Goblet cell (no.)	46.40 ^c	85.40 ^{ab}	56.20 ^{bc}	90.00 ^a	14.38
Colon					
Villus area (μm^2)	113108.40 ^b	125905.21 ^b	177010.33 ^a	114022.37 ^b	14520.17
VH:CD	3.62	4.07	2.94	3.77	0.57
Goblet cell (no.)	56.2	66.60	55.80	69.60	13.07

^{abc}Means on the same row with different superscripts are significantly different ($p < 0.05$)



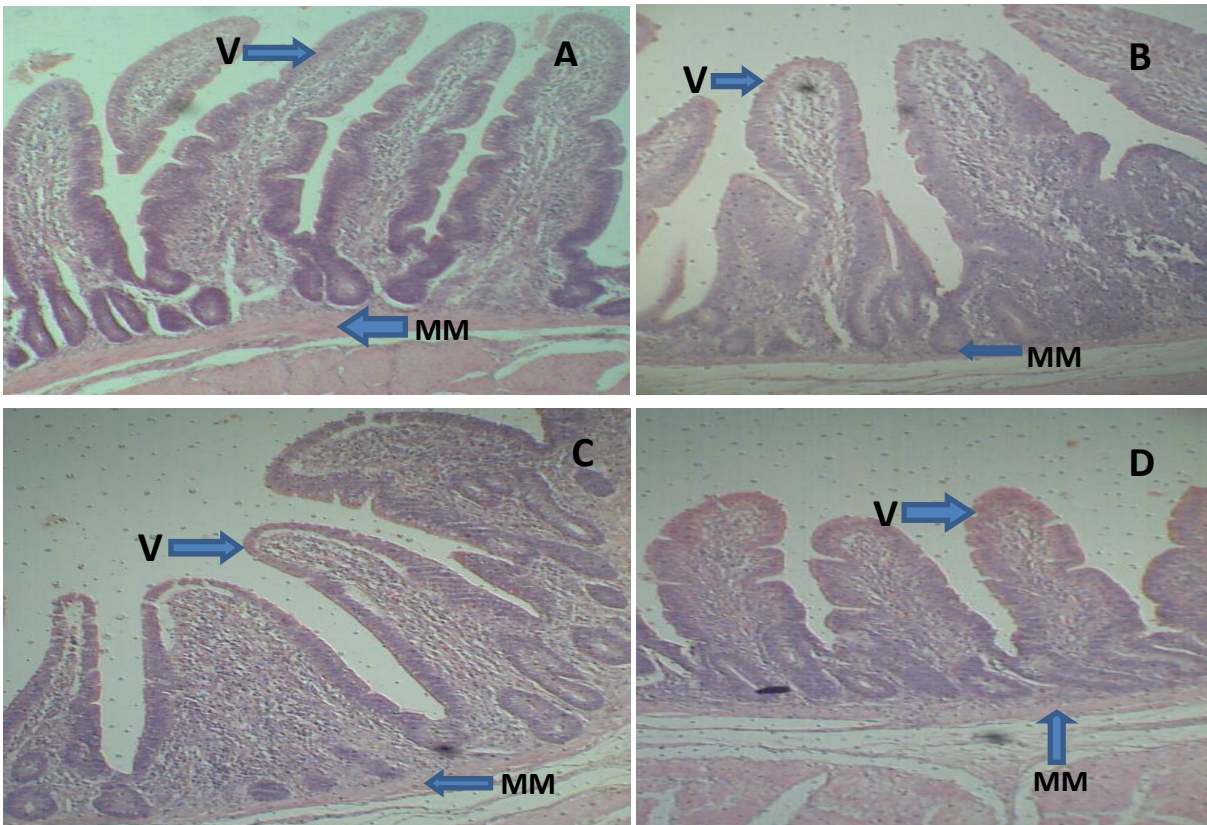


Plate 2: Photomicrograph of colonic histology of experimental groups. A: control, B: *S. cerevisiae*, C: neomycin, D: oxytetracycline. MM: Muscularis mucosa, V: Villus.

Histological variables of caecum and rectum

The values for the histological variables of the caecum and rectum are presented in Fig. 2 and Table 3 while Plate 3, and 4 is the photomicrograph of caecum and rectum, respectively of the experimental birds. Caecal mucosal height, CD and TM (Fig. 2, panel A) as well as AV, and HV: CD (Table 3) differed significantly ($p < 0.05$) between treatments. Mucosal height was significantly higher ($p < 0.05$) at 553.46 μm in birds fed neomycin compared to those of other treatments which had similar values for this parameter (446.71, 430.86, and 387.79 μm for control, oxytetracycline and probiotic groups, respectively). Crypt depth did not differ significantly between the control (188.54 \pm 15.69 μm), neomycin (231.15 \pm 15.69 μm) and oxytetracycline (197.53 \pm 15.69 μm) groups but these values were significantly ($p < 0.05$) higher compared to the group fed probiotic (129.97 \pm 15.69 μm). Height of villus to crypt depth ratio was also not statistically different between the control (1.38 \pm 0.18), neomycin (1.42 \pm 0.18) and oxytetracycline (1.23 \pm 0.18) groups but these values were significantly lower ($p < 0.05$) compared to the probiotic group (2.04 \pm 0.18). Values observed for TM were significantly higher in the probiotic group (62.60 \pm 4.80 μm) compared to the control (38.45 \pm 4.80 μm), neomycin (25.38 \pm 4.80 μm), and oxytetracycline (47.06 \pm 4.80 μm) groups. No significant differences were observed between the control and neomycin groups (38.45 \pm 4.80 vs 25.38 \pm 4.80 μm) as

well as between the control and oxytetracycline groups ($38.45 \pm 4.80 \mu\text{m}$ vs $47.06 \pm 4.80 \mu\text{m}$), but the value for the group fed oxytetracycline exceeded ($p < 0.05$) that of birds fed neomycin (47.06 ± 4.80 vs $25.38 \pm 4.80 \mu\text{m}$). Area of villus was significantly higher in the neomycin group at $48,156.98 \mu\text{m}^2$ compared to other treatments which did not differ significantly in this parameter. For the rectum, significant ($p < 0.05$) differences were observed in mucosal height, WV, CD, and TM (Fig. 2, panel B). Values for mucosal height in broilers fed probiotic was significantly ($p < 0.05$) higher compared to the control group ($695.94 \pm 45.12 \mu\text{m}$ vs $484.75 \pm 45.12 \mu\text{m}$) but not statistically different from those of neomycin and oxyteracycline groups ($695.94 \pm 45.12 \mu\text{m}$ vs 580.21 ± 45.12 and $568.59 \pm 45.12 \mu\text{m}$, respectively). Width of villus was significantly higher ($p < 0.05$) in the control group compared to the values in other treatment groups which were not statistically different for this parameter. Thickness of the muscularis mucosa (TM) was similar in birds fed antibiotics (oxytetracycline: $62.10 \pm 3.72\mu\text{m}$ and neomycin: $51.34 \pm 3.72 \mu\text{m}$) but higher in the oxytetracycline group compared to control group ($62.10 \pm 3.72\mu\text{m}$ vs $48.42 \pm 3.72 \mu\text{m}$). Birds fed probiotic had the least value for TM ($30.46 \pm 3.72 \mu\text{m}$). Crypt depth (CD) was higher in birds fed probiotic compared to other treatments which did not differ significantly in this variable.

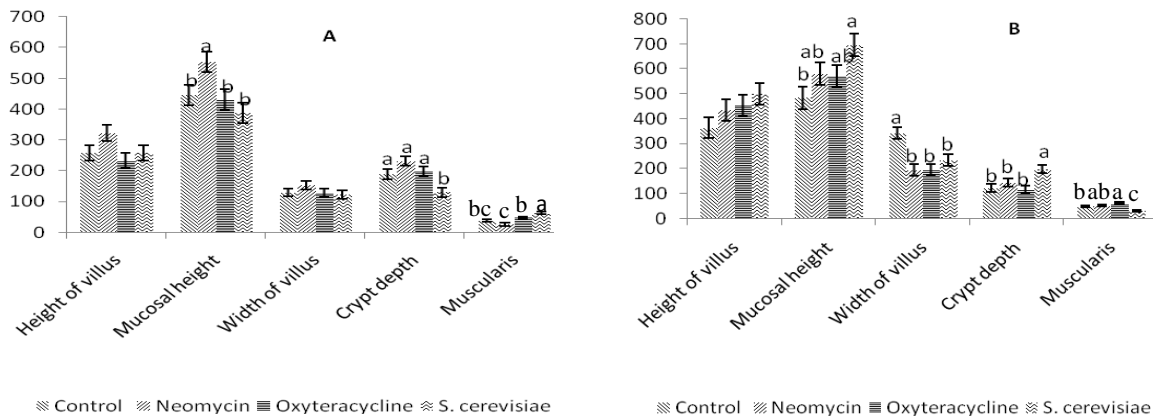


Fig. 2. Histological variables of caecum (panel A) and rectum (panel B) of experimental birds at 7 weeks of age. Muscularis: thickness of muscularis mucosa.

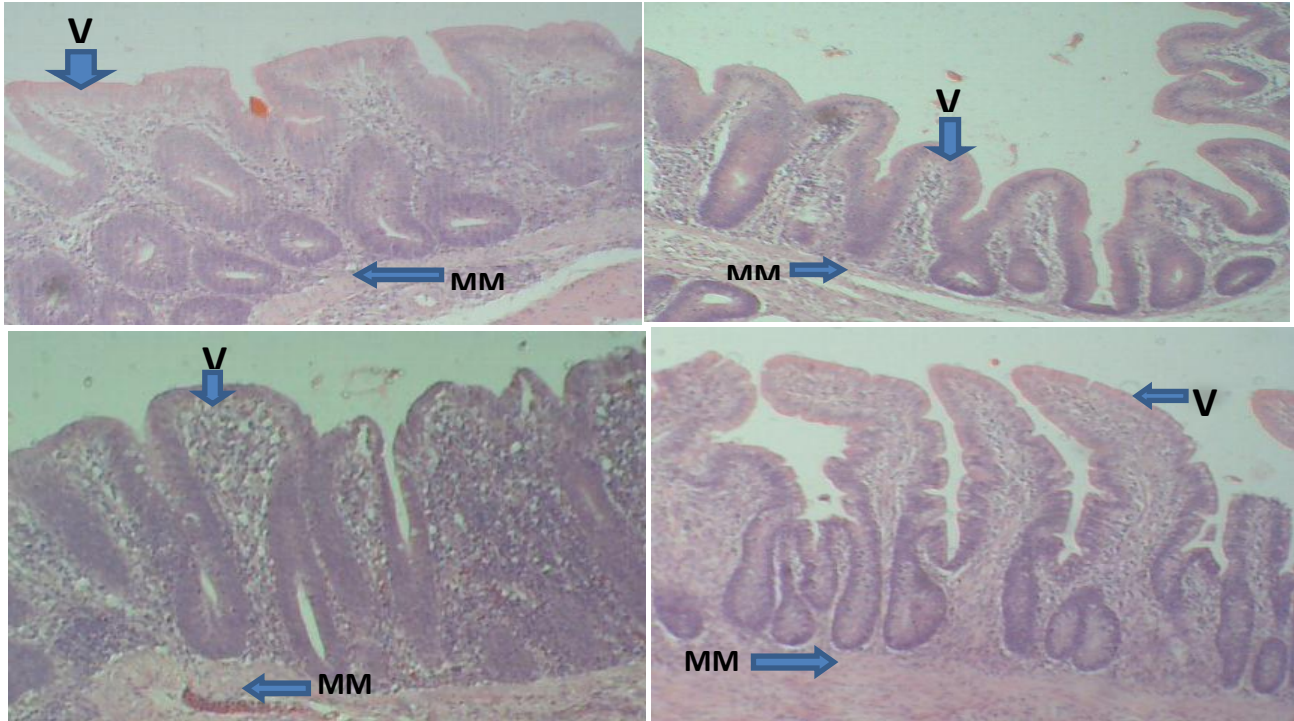
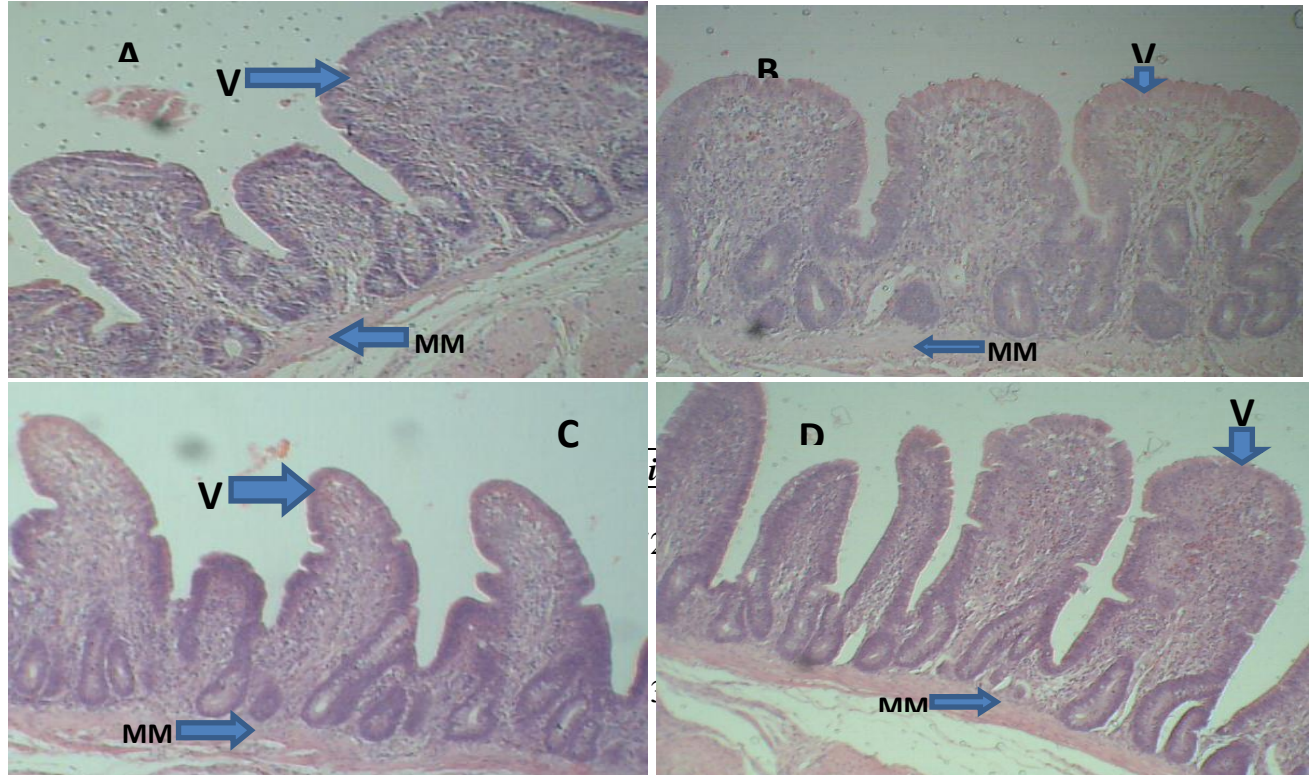


Plate 3: Photomicrograph of caecal histology of experimental groups. A: control, B: *S. cerevisiae*. C: neomycine sulphate. D: oxytetracycline. MM: muscularis mucosa. V: villus.



^{ab}Means on the same row with different superscripts are significantly different ($p < 0.05$).
 Plate 4: Photomicrograph of rectal histology of experimental groups: A: control, B: *S. cerevisiae*, C: neomycin sulphate, D: oxytetracycline. MM: muscularis mucosa, V: villus.

Within treatment histomorphological characteristics of intestinal segments

The within treatment histological values for the different segments of the intestine are presented in Fig. 3 and Table 4. Across treatments, height of villus (HV) and mucosal height (MH) were highest in the ileum followed by the colon while the caecum had the least values for these variables. Width of villus (WV) was highest in the rectum followed by the colon and least in the caecum of birds belonging to the control group whereas in birds fed probiotic no significant differences were observed between the ileum, colon and rectum but these were higher compared to the value observed in the caecum. For birds fed antibiotics (neomycin and oxytetracycline, respectively), WV was similar in the colon and ileum and these were significantly higher compared to the values observed in the caecum and rectum. Crypt depth (CD) was highest in the caecum compared to other sections of the intestine in birds fed basal diet, neomycin and oxytetracycline whereas in birds fed probiotic, CD was highest in the rectum. Other sections of the intestine did not differ in this variable in the probiotic fed group. Thickness of the muscularis mucosa (TM) differed significantly among intestinal sections with the ileum and rectum having the highest values in birds fed basal diet and antibiotics (neomycin and oxytetracycline, respectively) whereas in birds fed probiotic, it was least in the rectum but statistically similar in the ileum, colon and caecum. Table 4 shows that area of villus (AV) was significantly higher in the ileum, colon and rectum compared to the caecum in birds fed basal diet, probiotic and oxytetracycline whereas in birds fed neomycin, similar values were observed in the ileum and colon and these were higher than the values observed in the caecum and rectum. Height of villus to crypt depth ratio (HV:CD) was highest in the ileum followed by the colon, and rectum but least in the caecum across treatment groups. Goblet cell count was generally higher in the rectum followed by the ileum and colon but least in the caecum across treatments.

Table 4. Values of intestinal variables for different intestinal sections at 7 weeks of age

Treatment/variable	Intestinal sections				SEM
	Ileum	Colon	Caecum	Rectum	
Control					
Villus area (μm^2)	145,107.50 ^a	113,108.40 ^a	39258.55 ^b	124,209.87 ^a	15963.97
HV:CD	6.59 ^a	3.62 ^b	1.38 ^c	3.21 ^b	0.56
Goblet cell (no.)	46.40 ^{bc}	56.2 ^b	24.20 ^c	132.40 ^a	11.67
<i>S. cerevisiae</i>					
Villus area (μm^2)	150,214.59 ^a	125,905.21 ^a	31,677.72 ^b	120,327.32 ^a	18051.17
HV:CD	6.13 ^a	4.07 ^b	2.04 ^c	2.61 ^c	0.40
Goblet cell (no.)	85.40 ^b	66.60 ^b	20.40 ^c	127.00 ^a	11.07
Neomycin					
Villus area (μm^2)	158,863.18 ^a	177,010.33 ^a	48,156.98 ^b	84,786.93 ^b	17245.02
HV:CD	4.83 ^a	2.94 ^{ab}	1.42 ^b	3.19 ^{ab}	0.65
Goblet cell (no.)	56.20 ^b	55.80 ^b	18.00 ^b	148.60 ^a	16.14
Oxytetracycline					
Villus area (μm^2)	107,265.59 ^a	114,022.37 ^a	29,717.09 ^b	87,992.17 ^a	9948.49
HV:CD	5.32 ^a	3.77 ^a	1.23 ^b	4.07 ^a	0.55
Goblet cell (no.)	90.00 ^{ab}	69.60 ^b	20.40 ^c	109.40 ^a	11.99

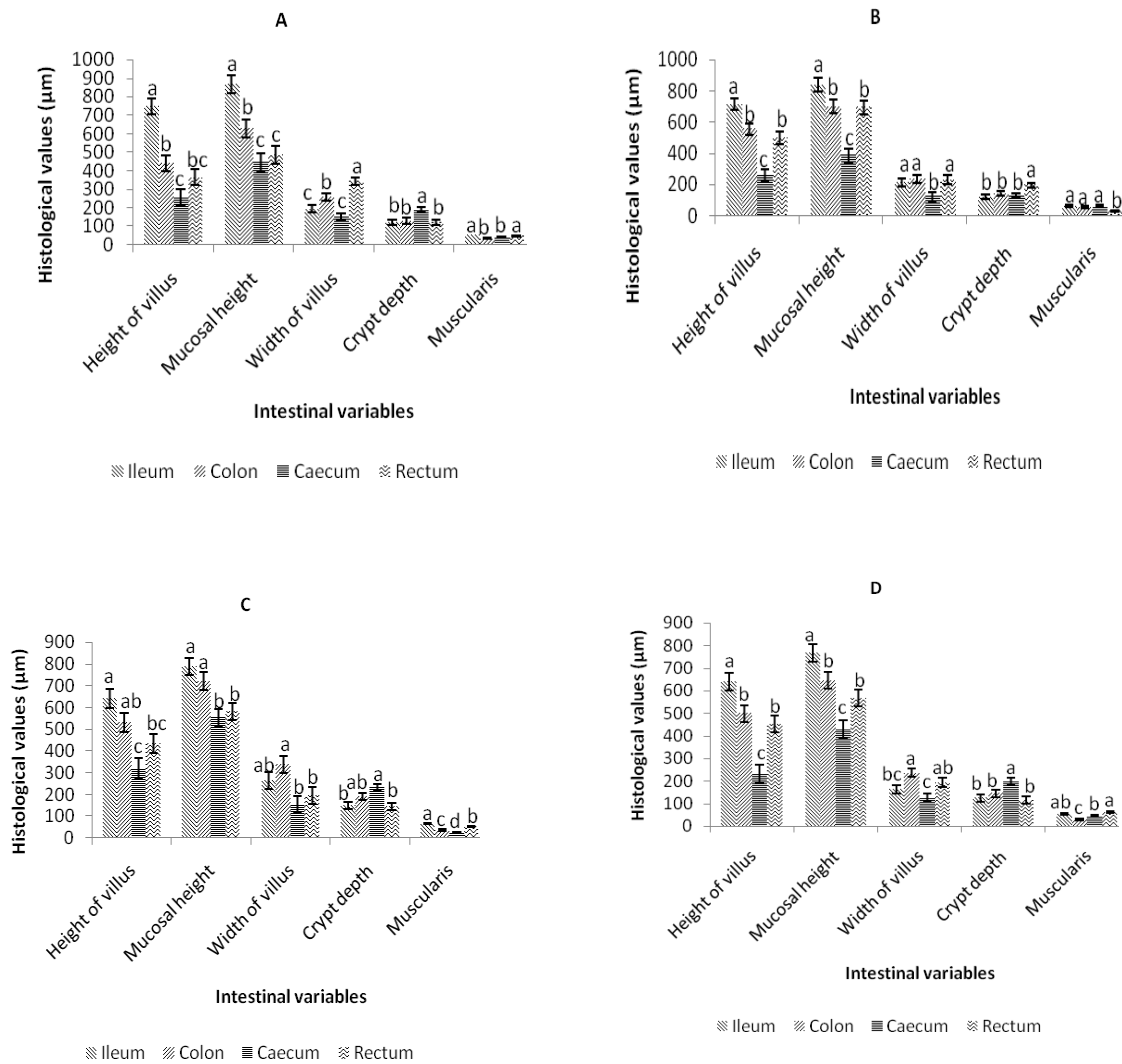


Fig. 3. Within treatment comparison between intestinal sections for intestinal variables: control group (panel A), *S. cerevisiae* (panel B), neomycin (panel C), and oxytetracycline (panel D). Muscularis: thickness of muscularis mucosa.

DISCUSSION

Suboptimal production environment (harsh climatic and weather conditions, suboptimal nutrition, challenge of endemic diseases and parasites, and suboptimal management practices) has made feed additives a critical component of poultry production to meet production targets. Antibiotic feed additives are being withdrawn from poultry production to reduce the incidence and spread of antibiotic resistance by pathogenic microorganisms of medical and veterinary importance. Probiotic as an alternative to antibiotic feed additives was evaluated in the present study for its effect on growth and histomorphology of broiler chicks. The observed higher liver weights in the antibiotic supplemented groups indicate stimulatory effect of antibiotics on this organ probably as a consequence of antibiotic metabolism or higher metabolic activities in individuals of these groups while the higher weight of crop could have resulted from the stimulatory effect of the feed additives as well as from increased capacity to hold larger

quantities of feed to support the improved growth rate of birds in these groups (Table 1). It has been shown that clinical doses of bactericidal antibiotics cause mitochondrial dysfunction and reactive oxygen species overproduction leading to oxidative damage to DNA, proteins and membrane lipids [15]. Key metabolic organs like the liver are mostly affected [15] and the accompanying inflammatory response could lead to increased organ weight. A tendency for higher weights of gizzard and longer intestines was also observed in the antibiotic and probiotic groups compared to the control. Reports on effects of feed additives on intestinal organ parameters are to some extent inconsistent. The lack of significant effect of treatments on weight of heart, proventriculus and spleen and length of intestine in the present study agrees with Corduk *et al.* [16] who fed diets supplemented with antibiotic (avilamycin), organic acid, prebiotic, plant extract and probiotic to broiler chickens and observed non-significant effect of the treatments on carcass yield, weight of pancreas and gizzard, and length of small and large intestines. Other studies [7,8,9,17] also reported that supplementation of broiler diet with antibiotics, probiotics and prebiotics had no effect on the weight of intestinal organs of broiler chickens. Ashayerizadeh *et al.* [18] had reported non-significant effect of probiotic (primalac^(R)), prebiotic (biolex-MB^(R)), and antibiotic (flavomycin) on percentage of liver, spleen, gizzard, and pancreas of broiler chickens. The same study however, reported significantly higher ($p < 0.05$) percentage heart and bursal weights in broiler chickens fed probiotic compared to the control. The increased weight of heart was attributed to enhanced metabolic rate and need for higher oxygen due to the higher growth rate of the birds. In the present study, a tendency for higher organ weights (except for weight of spleen) was observed in the probiotic group compared to the control group indicating enhanced organ development and functions to support greater weight gain in the groups [13].

In the present study non-significant treatment effects were observed in most ileal and colonic intestinal variables, caecal villus height (VH), VW, and goblet cell count, and rectal villus area (VA), VH:CD, and goblet cell count indicating lack of significant beneficial effects of the antibiotic additives over other treatments. Non-significant effects of antibiotic growth promotants on intestinal histological variables have been reported by other studies [7,9,19]. Miles *et al.* [19] reported non-significant effect of treatments on duodenal histomorphology (relative intestinal weight and length, height, width and area of villus, thickness of muscularis interna and mucosa, and depth of crypts of Lieberkuhn) of broiler chicks fed control diet and diets supplemented with bacitracin methylene disalicylate and virginiamycin. Olnood *et al.* [7] reported non-significant treatment effect on villus height, crypt depth, and muscle depth in broiler chickens fed novel probiotics and antibiotics while Wang *et al.* [9] reported non-significant effects of prebiotic, probiotic, pre- + pro-biotics and antibiotics on length and width of villus, crypt depth, muscle thickness, and goblet cell size of broiler chickens from day 14 to day 40 of age. On the other hand, Miles *et al.* [19] reported thinner ileal muscularis mucosa, and muscularis interna in groups fed antibiotics compared to counterparts fed control diet contrary to the observed non-significant effect of treatments on ileal muscularis mucosal thickness in the present study. The authors attributed the reduced thickness of the ileum to the thinning effect of antibiotics on intestinal epithelial lining. Results of numerous studies however, indicate that the effect of feed additives on thickness of intestinal wall vary from one section of the intestinal tract to another. For instance, Pani Padihari *et al.* [20] reported decreased thickness of tunica muscularis in the duodenum, jejunum, and ileum but increased thickness in the colorectum following mannan Oligosaccharides and *S. cerevisiae* supplementation in diets of broiler

chickens. These reports support the thicker colonic muscularis mucosa in birds fed probiotic in the present study and indicate enhanced colonic epithelial integrity. Pani Padihari *et al.* [20] also reported significant treatment effects on ileal villus area and height, as well as ileal crypt depth contrary to our findings in the ileum but in support of the observed significantly enhanced colonic villus area in the neomycin group. The higher ileal goblet cell number in the supplemented groups could be the result of enhanced immune status in response to the stimulatory effects of the supplements on the intestinal epithelium.

The higher caecal mucosal height in birds fed neomycin corresponds with the higher VH observed in this group which also had significantly higher VW and VA. These results indicate higher absorptive surfaces in this group compared to other groups. Increased villus height and area has been associated with improved digestive and absorptive efficiency in broiler chickens and other animals [21,22]. The observed higher crypt depth (deeper crypt) in control, neomycin and oxytetracycline groups indicate increased epithelial turnover rate probably to compensate for losses in caecal villi from sloughing and atrophy [23,24]. Thus the lower CD and higher VH:CD observed in birds fed probiotic suggest better protected caecal epithelial surfaces and villi [6] and hence lower caecal epithelial maintenance requirements in this group. The probiotic group also had thicker caecal muscularis mucosa which further implies better caecal epithelial integrity. The observed higher mucosal height in the probiotic group compared to the control indicates the favourable effects of the feed additive on intestinal villi. This group also had numerically higher rectal villus height compared to the control group. The higher VW observed in the control group was probably to compensate for the shorter villi observed in this group. The thicker rectal muscularis mucosa in the antibiotic groups means that inclusion of antibiotics enhanced rectal epithelial integrity of the birds which may explain the significantly lower values for crypt depth observed in these groups compared to the probiotic group [23,24].

The observed higher values for VH, VW, VA and VH:CD in the ileum, colon and rectum compared to the caecum across treatments is in agreement with the greater digestive and absorptive roles of the ileum, colon, and rectum than the caecum, and the greater epithelial turnover characteristic of the ileum and other sections of the small intestine involved in active digestive and absorptive processes [5,19,25]. Larger villi are generally the result of activated cell mitosis which occurs in the crypts and which permits renewal of the villi [13]. De Verdal *et al.* [13] reported decreasing villus height to crypt depth ratio from the duodenum to the ileum in broiler chickens and this agrees with the trend observed in most of the treatments in the present study. Differences in thickness of the muscularis mucosa (TM) as was observed in the present study could reflect differences in volume and density of digester in the different sections of the intestinal tract and hence the adaptations to cope with the pressure exerted on the intestinal musculature as the digester passes to the cloaca. Thus the thicker muscularis mucosa observed in the ileum and rectum could be an adaptive reinforcement to enhance motility in these sections of the intestine [13,26]. The higher number of goblet cells in the rectum, compared to other sections of the intestine and in the ileum, and colon compared to the caecum across treatments indicate greater needs for mucus secretion in these sections as the digester becomes denser and viscous moving from the ileum to the rectum. Goblet cells are the central cell type for the production of mucus that covers the gastrointestinal tract to form protective mucous gel layer [5]. De Verdal *et al.* [13] observed a proximodistal increase in number of goblet cells per villus area moving from the duodenum to the ileum in broiler chickens. Goblet cells also secrete antimicrobial proteins

such as α -defensins, usually induced by bacterial colonization [5]. Thus, the higher frequency of goblet cells in the rectum could also be attributed to the need to protect the rectum against assault by the dense microbial population resident in this section. It has been reported that the small intestine is covered by a single loose layer of mucus to enable absorption of nutrients while the colon is covered by a stratified two-layer mucus anchored to the intestinal epithelial cell glycocalyx to protect against the dense microbial population of this section [5,27]. Faderl *et al.* [5] also argued that the evolution of different structural characteristics of the mucus layer in the small and large intestines may be attributed to changing rates of microbial colonization along the gut and that this is reflected by the distinct spatial variation and frequencies of mucin secreting goblet cells with high frequencies at sites of dense microbial colonization such as the distal colon.

CONCLUSION

Supplementation of diets of broiler chickens with a probiotic (*S. cerevisiae*) and antibiotics (neomycin sulphate and oxytetracycline) improved the weight of liver and crop over that of birds fed only the basal diet. Feeding antibiotics to broiler chicks caused decreases in the thickness of the colon while birds fed probiotic generally had thicker ileum, colon, and caecum indicating enhanced intestinal epithelial integrity. Feeding probiotic also enhanced intestinal villus characteristics (HV, AV, and HV:CD) which were largely similar and better in some variables to those of birds fed antibiotics. The results therefore suggest that probiotics could replace antibiotic growth promotants in broiler diets to enhance intestinal histomorphology and function.

REFERENCES

1. Bosscher, D., Breynaeri, A., Pieters, L., Hermans, N. (2009). Food-based strategies to modulate the composition of the intestinal microbiota and their associated health effects. *Journal of Physiology and Pharmacology*, 60 (6): 5 - 11.
2. Kuo, S. M., Merhige, P. M. and Hagey, L. R., (2013). The effect of dietary prebiotics and probiotics on body weight, large intestinal indices and faecal bile acid profile in wild type and IL10-1-mice. *PLoS One*, 8 (3): e 60270.
3. Hrister, H., Bochukov, A. and Pencher, G. (2004). Comparative study on the effect of lactina^(R) probiotic on some microbiological and histological characteristics of the digestive tract of Muscovy ducklings. *Journal of Central European Agriculture*, 5 (4): 347 - 352.
4. Pelicano, E. R. L., Souza, P. A., and Souza, H. B. A. (2002). Probioticos e probioticos na nutricao de aves. *Ciencias Ararias e de Saude*, 2 (1): 59-64.
5. Faderl, M., Noti, M., Corazza, N., and Mueller, C. (2015). Keeping the bugs in check: The mucus layer as a critical component in maintaining intestinal homeostasis. *International Union of Biochemistry and Molecular Biology*, 67 (4): 275 - 285.
6. Pelicano, E. R. L., Souza, P. A., Souza, H. B. A., Oba, A., Leonel, F. R., Zeola, N. M. B. L. and Boiago, M. M. (2004). Utilizacao de probioticos e/ou prebioticos como promotores de crescimento em racoes iniciais de frangos de corte. *Revista Brasileira de Ciencia Avicola*, (suppl. 6): 17.
7. Olnood, C. G., Besk, S. S. M., Choct, M. and Iji, P. A. (2015). Novel probiotic: Their effects on growth performance, gut development, microbial community and activity of broiler chickens. *Animal Nutrition*, 1: 184 - 191.

8. Odefemi, T. R. (2016). Performance response and carcass characteristics of broilers fed dietary antibiotics, probiotics and prebiotics. *European Journal of Agriculture and Forestry Research*, 4 (1): 27 - 36.
9. Wang, X., Farnell, Y. Z., Peebles, E. D., Kiess, A. S., Wamsley, K. G. S. and Zhai, W. (2016). Effects of prebiotics, probiotics, and their combination on growth performance, small intestine morphology, and resident lactobacillus of male broilers. *Poultry Science*, 95: 1332 - 1340.
10. Nasrin, M., Siddiqi, M. N. H., Masum, M. A. and Wares, M. A. (2012). Gross and histological studies of digestive tract of broilers during postnatal growth and development. *Journal of the Bangladesh Agricultural University*, 10 (1): 69 - 77.
11. O'Dowd, G., Bell, S. and Wright Sylva (2020). *Wheater's Pathology: A Text, Atlas and Review of Histopathology*. 2nd Ed., Elsevier, Philadelphia. Pg 2 – 3.
12. Incharoen, T. and Yamauchi, K. (2009). Performance and histological changes of the intestinal villi in chickens fed dietary natural zeolite including plant extract. *Asian Journal of Poultry Science*, 3: 42 - 50.
13. De Verdal, H., Mignon-Grasteau, S., Jeulin, C., Le Bihan-Duval, E., Leconte, M., Mallet, S., Martin, C. and Newcy, A. (2010). Digestive tract measurements and histological adaptation in broiler lines divergently selected for digestive efficiency. *Poultry Science*, 89: 1955 - 1961.
14. SPSS (2001). Statistical package for Social Sciences version 9.0.
15. Foti, J. J., Devadoss, B., Winkler, J. A., Collins J. J. and Walker, G. C. (2012). Oxidation of the guanine nucleotide pool underlies cell death by bactericidal antibiotics. *Science* 336: 315.
16. Corduk, M., Ceylan, N., Dede, N. and Tel, D. Y. (2008). Effects of novel feed additives on performance, carcass traits and *E. coli*, aerobic bacteria and yeast counts in broilers. *Archiv für Geflügelkunde*, 72 (2): 61 - 67.
17. El-Hammady, H. Y., El-Sagheer, M., Hassanien, H. H. M. and Hassan, H. A. (2014). Performance and carcass traits of broilers supplemented with probiotic and neomycin antibiotic. *Egyptian Journal of Animal Production*, 51 (2): 107 - 114.
18. Ashayerizadeh, O., Dastar, B., Shargh, M. S., Ashayerizadeh, A. and Mamooee, M. (2009). Influence of antibiotic, prebiotic and probiotic supplementation to diets on carcass characteristics, hematological indices and internal organ size of young broiler chickens. *Journal of Animal and Veterinary Advances*, 8 (9): 1772 - 1776.
19. Miles, R. D., Butcher, G. D., Henry, P. R. and Littell, R. C. (2006). Effect of antibiotic growth promoters on broiler performance, intestinal growth parameters, and quantitative morphology. *Poultry Science*, 85: 476 - 485.
20. Pani Padihari, V., Tiwari, S. P., Sahu, T., Endley, M. K. and Naik, S. K. (2014). Effects of mannan oligosaccharide and *Saccharomyces cerevisiae* on gut morphology of broiler chickens. *Journal of World's Poultry Research*, 4 (3): 56 - 59.
21. Khambualai, O., Ruttannavat, J., Kitabatake, M., Goto, H., Erikawa, T. and Yamauchi, K. (2009). Effects of dietary natural zeolite including plant extract on growth performance and intestinal histology in Aigamo ducks. *British Poultry Science*, 50: 123 - 130.
22. Al-Baadani, H.H., Abudabos, A. M., Al-Mufarrej, S. I. and Alzawqari, M. (2016). Effects of dietary inclusion of probiotic and synbiotics on intestinal histological changes in challenged broiler chickens. *South African Journal of Animal Science*, 46 (2): 157 - 165.

23. Gao, J., Zhang, H. J., Yu, S. H., Wu, S. G., Yoon, I., Quigley, J. Gao, Y. P. and Qi, G. H. (2008). Effect of yeast culture in broiler diets on performance and immunomodulatory functions. *Poultry Science*, 87: 1377-1384.
24. Laudadio, V., Passantino, L., Perillo, A., Lopresti, G., Passantino, A., Khan, R. U. and Tufarelli, V. (2012). Productive performance and histological features of intestinal mucosa of broiler chickens fed different dietary protein levels. *Poultry Science*, 9 (1): 265 - 270.
25. Awad, W. A., Ghareeb, K., Abdel-Raheem, S. and Bohm, J. (2009). Effects of dietary inclusion of probiotic and symbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. *Poultry Science*, 88: 49 - 55.
26. Rougiere, N. and Carre, B. (2010). Gastric retention is key determinant for differences between chickens from D+ and D- genetic lines selected for diverent digestion efficiency. *Animal*, 4: 1227 - 1239.
27. Berg RD (1996). The indigenous gastrointestinal microflora. *Trends in Microbiology*, 4: 430 - 435.