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Research Paper

Effect of Administration of Lab Isolated L.Plantarum from GI Tract of Guinea Fowl on Intestinal Histomorphometry in Broilers

Veterinary Science

KEYWORDS : Bacitracin methylene disalicylate, Lactobacillus plantarum, Lactobacillus acidophilus.

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ABSTRACT

The study was aimed to investigate the effect of feed supplements viz: Lactobacillus plantarum (lab isolate from gastrointestinal tract of Guinea fowl), Lactobacillus acidophilus (NCDC, Karnal) and in- feed antibiotic bacitracin methylene disalicylate (BMD) on intestinal histomorphometry in broilers. During the entire experimental period of 35 days in broilers, four treatment groups were provided with different dietary treatments (T1-basal diet (Control-1), T2-Antibiotic growth promoter BMD @ 20g /100kg feed (Control-2), T3 - 1x108cfu of L.acidophilus/gm fermented feed +MOS @ 1g /kg feed T4-1x108 cfu of lab isolated L.plantarum (L.I.L.p) /gm fermented feed+ MOS @ 1g /kg feed. There were 20 birds per each treatment group in Guinea fowl and 25 chicks in broilers. Histomorphological examination of duodenum, ileum of broilers fed different dietary treatments was conducted at 35th day of experiment. Six birds from each group were sacrificed and sections of intestine were cut and processed for histomorphological study and slides were prepared and examined using an optical microscope. In terms of histomorphology of duodenum was concerned, duodenal villous height (VH) and crypt depth (CD) was significantly higher for T4 and T3 and lowest values obtained for antibiotic fed T2 group. Whereas, ileal villous height didn't show any significant difference among treatment groups. Ileal crypt depth was lowest for antibiotic supplemented T2 group over the other three groups. Duodenal villous height:crypt depth ratio was highest for T4 and T3 over T2 and control birds T1. The increment in the height of villi and villous height crypt depth ratio in Lactobacillus plantarum fed T4 group in broilers suggests that guinea fowl specific lab isolated L.plantarum may be used in commercial broiler production for improving growth by augmenting digestion and absorption of nutrients by modifying intestinal histomorphology.

INTRODUCTION

In recent year's use of probiotics, prebiotics and synbiotics that enrich certain bacterial population in the digestive system are considered as alternatives to antibiotic growth promoters in poultry nutrition (Patterson and Burkholder, 2003). Probiotics, microbial cell preparations that are mono or mixed cultures of live protective microorganisms beneficially alter the intestinal microflora balance, inhibit the growth of harmful bacteria, promote good digestion, boost immune function and increase resistance to infection (Helland et al., 2004). Other physiological benefits of probiotics include removal of carcinogens, lowering of cholesterol, immunostimulating and allergy lowering effect, synthesis and enhancing the bioavailability of nutrients (Parvez et al., 2006).

The intestinal epithelium acts as a natural barrier against pathogenic bacteria and toxic substances that are present in the intestinal lumen. Since absorption is totally dependent on the mechanisms that occur in the intestinal mucosa, the use of probiotics together with prebiotics (non-digestible ingredients 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 (Hofacre et al., 2003, Pelicano et al., 2004).The present study evaluated the effects of probiotics and their association on the histological and morphological indexes of the intestinal mucosa of broilers aged 35 days.

MATERIALS AND METHODS

L.plantarum strain isolated from guinea fowl GIT was used as probiotic strain in broiler birds (CARIBRO-Dhanraja maintained at experimental broiler farm, CARI, Izatnagar) in comparison with L.acidophilus (culture obtained from NCDC, Karnal) and BMD in feed antibiotic in broiler ration through feeding trial.

A total of 100 Chicks were obtained from hatchery CARI, Izatnagar.They were vaccinated for Ranikhet disease and Marek's disease at the hatchery. The experiment was conducted for 0-35 days. The birds were kept under deep litter system of rearing and provided with standard a standard corn-soybean meal based diet starter and finisher ration and water ad libitum. No medication, deworming was given throughout the experiment.Two phase feeding program was adopted with a starter diet from 1 to 21 days and a finisher diet from 22 to 35 days. All diets were formulated to meet requirements for macro and micronutrients

Table1. Ingredient and nutrient Composition of broiler ration

Composition/100 kg feed	Broiler starter	Broiler finisher
Maize	55.5	62.425
DORB	2.14	1.55
Soyabean	30.6	20.5
Guar korm	4	4
RSM	4	4
Fish meal	0	4
Marble chips	0	0.6
Limestone	0.8	0.5
DCP	2	1.6

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Composition/100 kg feed	Broiler starter	Broiler finisher
Salt	0.3	0.3
DL-Methionine	0.1	0.07
Lysine	0.135	0.07
CP%	22.1586	19.711
ME	2816.66	2878.785
Cacium	1.06984	1.05871
Available P	0.4906	0.4138
Lysine	1.2408	1.0038
Methionine	0.4907	0.4294

(TM premix-0.1%, vit.premix- 0.15%, Bcomplex-0.015%, Ch.chloride 0.05%,Toxine bind-0.05%, Coccidiostat-0.05%, Soda.bicarb-0.5%, Composition of trace minerals includes FeSO4 80 Mg/kg of diet, ZnSO4and CusSO4 8 mg/kg diet, MnSO4 65 mg/kg diet and KI 1.2 mg/kg diet. Composition of Vitamin Premix includes Choline chloride 500 mg/kg of diet, Niacin12 mg/kg of diet, Pyridoxine hydrochloride1.6 mg/kg of diet, Vitamin A 8250 IU/ kg diet, Vitamin B10.8 mg/kg of diet, Vitamin B2 8 mg/kg of diet, Vitamin D31200 IU kg diet, Vitamin E 10 mg/kg of diet and Vitamin K 1 mg/kg of diet.)

25 experimental chicks were randomly allotted to one of the four treatments. Various treatment groups were T1- Basal standard feed only (Control), T2- Basal ration + BMD @20g/100kg, T3-Basal ration + (108cfu of L.acidophilus/ gram fermented feed+ Prebiotic (MOS) @ 1g/kg feed, T4-Basal ration+ (108cfu of lab isolated Lactobacillus plantarum (LI.L.p) / gram fermented feed) + Prebiotic (MOS) @1g/kg feed. All the supplements were given in feed throughout the experimental period.

Titration of LI.L.p and L.acidophilus for dose standardization was done by serial dilution, plating and counting on MRS agar plates. Thereafter, the aliquots were adjusted to108CFU/ml using sterile PBS.Basal feed fermentation with titrated dose of LI.L.p was done in such a way that 20% of daily ration for broiler chicken was autoclaved and daily inoculated with 15% of lactobacillus isolate broth culture having viable count of 108cfu/ml and fermented at 37°C for 24 hours before adding to daily ration afresh and was mixed well.

The effect of feeding supplements on the morphology of the vital organ ie., intestine of broilers was examined. At the end of experiment, birds were sacrificed and sections of intestine were cut and slides were prepared and examined using an optical microscope (Chiou et al., 1999).The villus height, crypt depth, and villus height crypt depth ratio measured. Sample collected from duodenum, ileum of broiler birds were processed for histomorphological study.

Histological technique involved processes like Fixation of tissue, Dehydration, Clearing, Embedding, Cutting and Staining. Fixation in 10 % formalin with approximately 10-20 times the volume of the specimen was done. Tissues were dehydrated by using increasing strength of alcohol like 50%, 70%, 90% and 100%. Clearing was done by replacing alcohol by xylene for 0.5 – 1 hour. Impregnation of tissue with Wax was done at melting point temperature of paraffin wax and the volume of wax was about 25-30 times the volume of tissues for a total duration of 4 hours. Impregnated tissues were placed in a mould with their labels and then fresh melted wax was poured in it and allowed to settle and solidify. Then section cutting was done followed by hematoxylin–eosine staining for histomorphological examination of duodenum and ileum. Histomorphological examination was done in terms of measurement of parameters like villous height, cryptal depth and villous crypt ratio of 6 birds each of different dietary treatments using image capturing microscope using image analysis softwares for histopathology (Image Pro).

Statistical analysis

The data collected were analyzed using SPSS 20.0.Differences among groups were determined using Duncan's multiple-range test. Statements of statistical significance were based on $P< 0.05$.

RESULTS AND DISCUSSION

In terms of histomorphology of intestine was concerned, duodenal villous height (VH) and crypt depth (CD) was significantly higher for T4 (VH-1060.56±20.44 µm, CD-235.57±4.23 µm) and T3 (VH-1047.63±18.44µm, CD-231.65±4.77 µm) and lowest values obtained for antibiotic fed T2 (VH-925.55±18.22 µm, CD-212.51±4.14 µm) group. Whereas, ileal villous height didn't show any significant difference among treatment groups, ileal crypt depth was lowest for antibiotic supplemented T2 (159.35±5.84 µm) group over the other three groups. Duodenal villous height:crypt depth ratio was highest for T4 (4.50±.09) and T3 (4.54±.15) over T2 (4.36±.09) and control birds T1 (4.45±.14).Thus the probiotic the dietary supplementations resulted in an increase in the villus height and crypt depth of intestinal mucosa of broilers. The increase in the villus height and villus height:crypt depth ratio was associated with improvement of growth performance for both symbiotic groups. This indicates that the synbiotics can be used as a growth promoter in broiler diets and can improve the gut health. Histomorphology results of small intestine observed in the present study are in agreement with the findings of Rahimi and Karimi (2005), Pelicano et al. (2005) that the supplementation of probiotic in broiler diets improved VH and CD in the small intestines of treated birds compared with the control birds. The increment in the size and number of intestinal glands and villi could result in greater enzyme production resulting in better digestion and absorption of nutrients (Mohan et al., 1996). Parameters like duodenal, ileal villous height, cryptal depth and villous height cryptal depth ratio were measured and presented in Table 2.

Table 2. Villous height,crypt depth (µm) and villus height/crypt depth ratio in duodenum and ileum of broilers on different dietary treatments

Treatments	Duodenum			Ileum		
	Villi height (µm)	Crypt depth (µm)	Villi height/ crypt ratio	Villi height (µm)	Crypt depth (µm)	Villi height/ crypt ratio
Broiler chicken (Mean±SE)						
Control (T1)	995.54±19.26^{b}	223.68±5.54^{b}	4.45±.14	952.15±25.14	172.40±3.84^{ab}	5.54±.13

Treatments	Duodenum			Ileum		
	Villi height (µm)	Crypt depth (µm)	Villi height/ crypt ratio	Villi height (µm)	Crypt depth (µm)	Villi height/ crypt ratio
BMD (T2)	925.55±18.22^{c}	212.51±4.14^{c}	4.36±.09	918.83±31.85	159.35±5.84^{b}	5.53±.20
L.acidophilus+MOS (T3)	1047.63±18.44^{ab}	231.65±4.77^{a}	4.54±.15	967.63±19.62	174.53±4.41^{a}	5.59±.21
LI.L.p+MOS (T4)	1060.56±20.44^{a}	235.57±4.23^{a}	4.50±.09	962.88±26.84	170.82±4.16^{ab}	5.64±.21

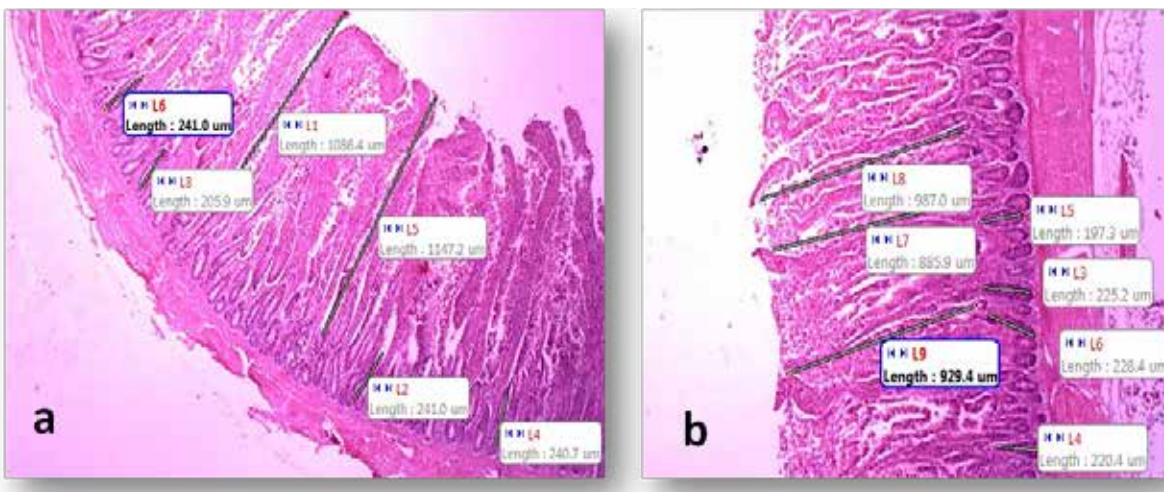

Figure 1: Representative photograph showing Villi height and crypt depth of the duodenum and ileum of broiler fed with diet containing L.plantarum .

In addition to histomorphological changes observed in the study that probiotic supplementation enhanced the growth and improved the feed conversion. The nutritional effects of the treatments have been confirmed in well-controlled laboratory studies.

CONCLUSION

In the present work, the histological changes that were brought in this experiment by feeding Lactobacillus plantarum and Lactobacillus acidophilus provided new information regarding the potential for using probiotics in broiler feed as booster of digestion and absorption of nutrients. The production of several bioactive compounds by these bacterial agents might have enhanced the villi height and crypt depth. Further, an attempt to use the Lactobacillus based probiotics in chicken feed as an alternative to antibiotics has explored avenue with scientific evidence.

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