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Influence of Dietary Mannan-oligosaccharide and α-Tocopherol on Intestinal Microbiology and Histomorphology in Broiler Chickens

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ABSTRACT
Day-old Vencobb broiler chicks (240) were randomly assigned to 4 dietary treatment groups having 3 replicates of 20 birds each to study the effect of mannan oligosaccharide (MOS) and α-tocopherol (AT) on intestinal microbiology and histomorphology. The trial lasted for 7 weeks. The chicks of the control group (T_1) were fed a basal diet. The birds in group T_2 were fed control diet as in T_1 + α-tocopherol at 400 IU per kg of feed; whereas, those in group T_3 were fed control diet as in T_1 + mannan-oligosaccharide at 0.4% and in group T_4 control diet was supplemented with α-tocopherol at 400IU per kg feed + mannan-oligosaccharide at 0.4% in feed. The results of study revealed that supplementation of MOS and AT in the diet of birds showed significant ($P<0.05$) lowering in cfu count of pathogenic bacteria, *E.coli* and *Salmonella spp.* as compared to the control group. The effect on the histomorphology of the birds also showed a significant ($P<0.05$) increase in the villi height (VH) and surface area (SA) of different parts of the intestine on supplementing the feed with MOS and AT. Thus, it was concluded that supplementation of MOS and AT improved the gut health and also had a positive influence on the microarchitecture and integrity of the intestine.

Keywords: Alpha-tocopherol, Broiler, Histomorphology, Intestinal microbiology, Mannan- oligosaccharide

INTRODUCTION

Past few years, with the ban on the use of sub-therapeutic level of antibiotics in poultry feed to prevent disease or promoting growth has resulted into an increasing interest in alternative safe feed additive having similar action (Waldroup *et al.,* 2003). These may include acidifiers, probiotics, prebiotics, enzymes, vitamins and immuno-modulators. Prebiotics, an alternative to antibiotics is defined by Gibson and Roberfroid (1995) are non-digestible food ingredients that selectively stimulate the growth and activity of one or a limited number of bacteria residing in the colon mainly that improves host health and performance. In view of this, mannan oligosaccharide (MOS), a prebiotic, derived from the cell wall of yeast, *Sacchromyces cerevisiae* is regarded as safe and cheap compound (Ferket, 2004). It has the ability of inhibiting colonization of enteric pathogens by blocking bacterial adhesion to gut lining (Spring *et al.,* 2000; Duncan *et al.,* 2005; Yang *et al.,* 2008a, 2008b, 2008c; Paul *et al.,* 2013), as well as maintain the integrity and lining of the intestinal tract of poultry healthy, thus, safeguarding the animal health status and performance (Ferket, 2002; Loddi *et al.,* 2004; Kocher., 2005; Hooge, 2006). The avian gastrointestinal tract harbours diverse and dynamic population of microorganism living in symbiotic relationship with their host which is important for host nutrition, metabolism and performance (Sohail *et al.,* 2010). But this balance is very delicate and under any stress condition changes towards more of pathogenic type. This led towards finding of such functional foods which maintain this balance and at the same time maintain the integrity of the intestinal wall (Patterson and Burkholder, 2003; Sohail *et al.,* 2011). Intestinal histomorphology is described on the basis of villi height (VH), villi width (VW), crypt depth (CD)

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and surface area (SA).The ratio of villi height (VH) and crypt depth (CD) gives the gut health index (Pluske, 1997) and if the ratio is low, the intestinal environment is more favourable to nutrient absorption and *vice-versa.* At the same time antioxidant vitamins such as vitamin E in the broilers diet enhanced histology of terminal ileum (Yoo *et al.,* 2016). The present study was conducted to see the effect of supplementing Mannan-oligosaccharide (MOS) and α-tocopherol (AT) in the diets of broiler chickens on intestinal microbiology and histomorphology.

MATERIALS AND METHODS

A total of 240 day-old Vencobb broiler chicks of either sex were randomly assigned to 4 treatment groups of 3 replicates of 20 chickens for a period of 7 weeks. The starter and finisher ration contained 23 and 20 percent crude protein and 2800 and 2900 kcal ME/kg feed respectively. The chicks were fed with standard basal diet as per BIS (1992). Ingredient composition of broiler starter and finisher diets are presented in Table 1. The control group T_1 was fed a basal diet. The birds in group T_2 were fed control diet as in T_1 + α-tocopherol @ 400IU per kg of feed ;whereas, those in group T_3 were fed control diet as in T_1 + mannan-oligosaccharide at 0.4% and in group T_4 control diet was supplemented with α-tocopherol at 400IU per kg feed + mannan-oligosaccharide at 0.4% in feed. Proper mixing of the feed supplement was ensured by initially mixing a small amount of feed by hand and then mixing into complete feed. The birds were raised in well ventilated house with proper lightening and were supplied with *ad-libtum* water during the experimental period.

Feed samples were analyzed for proximate principles (AOAC 1990). Nitrogen free extract was calculated by difference. Ca and P content of feed was also determined.

At the end of 7th week, two chicks from each pen replicate were sacrificed for determining the gut microbial population and histomorphology The intestine was taken out from each and the contents were collected from each part (duodenal, jeju-ileal, cecal) aseptically and labeled as per the group. Samples were weighed (1 g) and transferred to sterile tubes and homogenized with sterile 0.9% normal saline solution (1:1). The solutions were mixed on vortex and serial dilutions were made up to sixth dilution. Then 0.1 ml of each dilution was poured and spread uniformly on Mac Conkey agar and Eosin Methylene Blue Agar (EMB). Plates were incubated at 37°C for 48 hours. Bacterial

Table1. Ingredients and chemical composition (%) of broilers starter and finisher's diet

Feed ingredient	Starter phase				Finisher phase			
Groups	**T_1**	**T_2**	**T_3**	**T_4**	**T_1**	**T_2**	**T_3**	**T_4**
Yellow Maize	52.75	52.75	52.75	52.75	60.00	60.00	60.00	60.00
Soyabean meal	35.00	35.00	35.00	35.00	27.5	27.5	27.5	27.5
Fish meal	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Mineral mixture	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
α-tocopherol(AT)	-	400 IU	-	400 IU	-	400 IU	-	400 IU
MOS	-	-	0.4%	0.4%	-	-	0.4%	0.4%
Chemical composition %								
CP%	23.61	23.61	23.61	23.61	21.14	21.14	21.14	21.14
ME, kcal/kg	2806.85	2806.85	2806.85	2806.25	2882.25	2882.25	2882.25	2882.25
Ca	1.222	1.222	1.222	1.222	1.108	1.108	1.108	1.108
P	0.556	0.556	0.556	0.556	0.548	0.548	0.548	0.548

T_1= Basal diet; T_2= Basal diet + AT (400 IU); T_3= Basal diet + MOS (0.4%),T_4= Basal diet + MOS (0.4%) + AT (400 IU).

colonies were counted by pour plate method (Quinn *et al.,* 1992). The average number of colonies were multiplied by reciprocal of the dilution factor and expressed as cfu/g of contents. Colony forming units (CFU) were defined as being distinct colonies measuring at least 1mm in diameter. The colonies were detected for E.coli and Salmonella bacteria. The Mac conkey agar medium having lactose as one ingredient and E. coli being rapid lactose fermenting bacteria gives dark pink donut shaped surrounded with dark pink area of precipitated bile salts. Salmonella species do not ferment lactose or produce acid, their colonies appears grey on EMB agar.

For determining the villus height (VH), width (VW), crypt depth (CD), parts of the small intestine were sectioned from birds of each group and were fixed in formaldehyde 10% (v/v), buffered (pH 7.0) (Ricca Chemical Company, Arlington, TX) and then dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Five-micrometer thick sections were cut with a microtome and mounted on slides. Four discontinuous paraffin-embedded sections per broiler intestinal sample were processed for evaluation. These sections were de-paraffinized in xylene, dehydrated in ethanol, and stained with haematoxylin and eosin. The slides were scanned by light microscope and examined for determining the morphometric indices as villus height (VH) from the tip of the villus to crypt, width (VW) from one wall to next, and crypt depth (CD) from the base of the villi to the submucosa (Awad *et al.* 2008). For this morphometric investigation was made on 20 intact villi and 30 crypts chosen from the intestinal segments of broiler chickens. Villus height and Villus width data were used to calculate villus surface area (SA) [$2\pi \times (W/2) \times L$], where W= villus width and L= villus length (Sakamoto *et al.* 2000).

All the data were analyzed statistically using statistical packages for social science (SPSS), 17th Version as per Snedecor and Cochran (1980) and comparison of means was done using Duncan's multiple comparison tests.

RESULTS AND DISCUSSION

Dietary treatments had significant effect on reduction of pathogenic bacteria in all the three segments of intestine (Table 2). The average cfu count of *E.coli* and *Salmonella spp*. in the intestine were significantly ($P<0.05$) low in T4 group as compared to others which corroborates well to earlier reports (Finucane *et al.,* 1999b; Spring *et al.,* 2000; Iji *et al.,* 2001; Loddi *et al.,* 2004; Yang *et al.,* 2008a, 2008b, 2008c; Hrangkhwal *et al.,* 2013). The two supplements had a positive effect on modulating the gut ecosystem by lowering pathogenic colonization. Using prebiotics helps in to reduce pathogenic bacteria by interference with attachment via the type-1 fimbriae found on many gram negative bacteria as E.coli and Salmonella (Ferket, 2004).

The results of gut histomorphology of all the three parts of the intestine had been presented in table 3. The results showed significant ($P<0.05$) increase in VH and SA of jejunum followed with ileum and duodenum in all the three treatment groups as compared to control one. The dietary supplements affected the structure of the small intestine in broilers. Intestinal morphology is the main indicator of gut health which is characterized in particular to enhanced villus height and crypt depth. The

Table 2. The effect of supplementation of mannan-oligosaccharide and α-tocopherol individually and in combination on study of gut microbiology in broiler chicken

Attribute	E. coli, Cfu/ml				Salmonella, Cfu/ml			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
Duodenal	$1.77^{ab}\pm0.01$	$1.79^{b}\pm0.01$	$1.78^{b}\pm0.01$	$1.74^{a}\pm0.01$	$1.96^{b}\pm0.01$	$1.97^{b}\pm0.01$	$1.97^{b}\pm0.01$	$1.63^{a}\pm0.02$
Jeju-ileal	$1.64^{b}\pm0.01$	$1.67^{b}\pm0.01$	$1.56^{a}\pm0.01$	$1.55^{a}\pm0.01$	$1.97^{c}\pm0.01$	$1.97^{c}\pm0.01$	$1.94^{b}\pm0.01$	$1.58^{a}\pm0.01$
Cecal	$1.67^{c}\pm0.01$	$1.65^{c}\pm0.01$	$1.37^{b}\pm0.01$	$1.27^{a}\pm0.01$	$1.99^{d}\pm0.01$	$1.95^{c}\pm0.01$	$1.76^{b}\pm0.01$	$1.61^{a}\pm0.01$

[*]Values with similar superscript (row wise- a, b, c, d) did not differ significantly ($P<0.05$)

Table 3. The effect of supplementation of mannan-oligosaccharide and α-tocopherol individually and in combination on study of gut histology in broiler chicken

	Jejunum				Ileum				Duodenum			
	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
VH (µm)	248.6^{a}	255.56^{ab}	293.33^{b}	282.2^{ab}	180.0^{a}	180.00^{a}	271.1^{b}	255.5^{b}	168.9^{a}	173.3^{a}	215.55^{b}	206.6^{b}
	±5.93	±4.44	±20.37	±9.69	±10.18	±13.33	±4.44	±8.01	±2.22	±3.85	±9.69	±3.85
VW (µm)	6.67^{a}	12.22^{b}	13.33^{b}	12.22^{b}	11.11^{a}	32.22^{b}	62.22^{c}	28.89^{b}	57.78^{a}	46.67^{a}	53.33^{a}	49.11^{a}
	±0.01	±0.78	±0.01	±1.11	±1.11	±1.11	±2.22	±2.22	±2.22	±3.85	±3.85	±8.17
CD(µm)	86.67^{a}	86.67^{a}	133.33^{b}	68.89^{a}	115.55^{b}	48.89^{a}	133.33^{b}	55.55^{a}	37.76^{a}	66.67^{b}	35.55^{a}	95.56^{c}
	±6.67	±6.67	±24.04	±2.22	±9.69	±5.88	±0.01	2.22	±5.86	±3.85	±2.22	±8.01
SA (µmm^2)	781.51^{a}	1468.8^{b}	1842.1^{c}	1615.36	952.47^{a}	2717.85^{b}	7940.71^{d}	3460.98^{c}	4591.38^{a}	3816.84^{a}	5449.64	4786.76^{a}
	±1.84	±1.24	±1.27	±1.04	±1.52	±1.01	±2.42	±1.55	±1.18	^{a}±3.7	±6.36	±8.58

[*]VH= villus height; VW= villus width; CD= crypt depth; SA= villus surface area; [**]Values with similar superscript (row wise- a, b, c, d) did not differ significantly ($P<0.05$).

lengthening of villi may be the source of increased total luminal villus absorptive area resulting in increased digestive enzyme action and higher nutrient transport. Thus, from the table it was evident that there was positive effect of the two supplements on improving the structural integrity of the gastrointestinal tract which was in agreement to previous reports (Iji *et al.,* 2001, Zikic *et al.,* 2008, Hrangkhawl *et al.,* 2013, Yoo *et al.,* 2016). The two in combination showed positive interaction in maintaining the intestinal integrity and uniformity.

CONCLUSIONS

On the basis of these observations, it was concluded that the dietary supplementation of MOS and AT in the broilers diet had a positive influence on reducing the pathogenic bacteria in the intestinal tract. Supplementation also enhanced the intestinal microarchitecture resulting in more efficient absorption and assimilation of nutrients. This may be beneficial augmenting performance, health and welfare of chickens.

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