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Effect of dietary supplementation of prebiotic on growth performance, immune response and intestinal microbial load in broiler chickens

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ABSTRACT

The experiment was conducted to study the effect of using prebiotic (mannan oligosachharide -MOS) in place of antibiotic growth promoter (AGP) on performance, immune response and intestinal microbial load in broiler chickens. Day-old broiler chicks (192) were randomly distributed into 20 groups each of 8 chicks (4 treatments × 6 replicates). Four experimental diets T_1, T_2, T_3 and T_4 were formulated to contain no additive, bacitracin methylene di-salicylate (BMD) at 20 mg/kg diet, MOS at 0.1 and 0.2%, respectively. Body weight gain (g) was increased by the feeding of diets containing 0.2% levels of MOS, but feed intake (g), feed conversion ratio (FCR) and mortality (%) did not differ significantly. Antibody (28 d), titres were significantly higher after feeding 0.1 or 0.2% MOS and antibiotic (T_2) supplemented group. During d 35, the response to intra-dermally injected phyto-hemagglutinin, an index of the *in vivo* cell-mediated immune response, was increased in the 0.2% MOS supplemented group. Significant reduction was observed in coliforms and total plate count in cecal (28 and 42 d) and excreta (42 d) in MOS (0.1 or 0.2%) or antibiotic (T_2) supplemented groups. *Lactobacillus* count significantly increased in cecal (28 and 42 d) and excreta (42 d) in MOS @0.1 or 0.2% supplemented groups. Thus, it can be concluded that, 0.2% MOS with basal diet has a beneficial effect for growth performance, immune response and gut health status in broiler chickens, and MOS could be a good alternative to antibiotic growth promoter.

Key words: Antibiotic, Broiler, Immunity, Intestinal microflora, MOS, Performance

In last 50 years poultry production and production system in India got a spectacular explosion leading it to a high profile industry. The cost of production is governed by cost of quality ingredients and can be minimized by precise nutrient supply for augmenting nutrient utilization. Maintenance of natural gut health and gut modulation seems to be the most cost effective, sustainable, farm specific and holistic approach in commercial operation, keeping space for animal welfare. It is well accepted that there is no substitution for supplementation of quality feed ingredients for maintaining natural gut health. Antibiotics have revolutionized the intensive poultry production system as a feed additive to promote growth, production and feed conversion efficiency through improving gut health and reduction of sub-clinical infections during last 50 years (Willis and Reid 2008). Inclusion of antibiotics at low concentration maintains the gut health by reducing the pathogen load and helps the birds to prevent sub-clinical infection normally present continuously even at well-organized poultry units (Smith *et al.* 2002). Preventing the microbial adherence to the gut wall and pathogenic invasion lowers the production of toxic amines and hence stress to birds. Viscous diets are found to respond well to the inclusion of antibiotic growth promoters (AGPs).

Therefore, the poultry industry must develop alternatives to AGP to address public health concerns without compromising the efficiency of poultry production. Compounds that may have prebiotic effects are one possible way of improving intestinal health and performance in the absence of antibiotic growth promoters. A prebiotic compound was defined by Gibson and Roberfroid (2004) as a non-digestible feed ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improves gut health. Mannan oligosaccharides (MOS) are among the classes of prebiotics that beneficially affect gut health, but they do so by different modes of action (Ferket 2004).

Keeping in view the above facts, the objective of this study was to determine the effect of using mannan oligosaccharides (MOS) to substitute dietary antibiotic growth promoter on performance, immunity and gut health status of broiler chickens.

MATERIALS AND METHODS

Experimental design and diets: Day-old chicks (192) were housed and distributed randomly into 20 groups each of 8

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chicks (4 treatments × 6 replicates). The experiment had a randomized design. All the chicks were kept in battery brooder. Birds were allowed to eat and drink *ad lib*. The experiment followed the guidelines of Institutional Animal Ethics Committee (IAEC, CARI, Izatnagar). Four experimental diets T_1, T_2, T_3 and T_4 were formulated to contain no additive, bacitracin methylene di-salicylate (BMD) at 20 mg/kg diet, 0.1 or 0.2% MOS in diet, respectively. All the diets contained similar energy (2901.3 ME Kcal) and protein (22.02 and 19.5% CP) contents (Table 1).

Production performance: The experimental birds were housed group wise in randomly allotted cabins or tiers of electrically heated battery brooders under uniform management. Body weight gains (BWG) were recorded during the experimental period to ascertain the weekly and overall body weight gain. A weighed quantity of respective diet was offered *ad lib.* daily to quadruplicate groups of each dietary regimen in the morning and the residue was weighed next day on daily basis to arrive at overall feed intake. Based on the data pertaining to the feed intake (FI) and BWG, the weekly and period wise FCR of birds was determined. Daily monitoring and recording on individual basis had been carried out to study the livability/mortality of the experimental birds used in the present investigation.

Immune response and lymphoid organ weight: To investigate the effect on the humoral immune response, 3 birds were selected from each of the replicated groups (that is, 12 birds/dietary treatment providing 48 birds in all) at 28 d of age and were inoculated intravenously with 1 ml of a 1% suspension of SRBC. Blood samples were obtained from the jugular vein from all SRBC injected birds at 0 and 6 d post-inoculation. All the samples were incubated at 37°C for 1 h to aid clotting and retraction then centrifuged at 15,000 g for 5 min for collection of sera. All the microtiter plates (U-bottomed) were rinsed with phosphate-buffered saline (PBS; *p*H 7.6) then dried before the haem-agglutination antibody (HA) titre was estimated by a micro haem-agglutination method (Siegel and Gross 1980) using two fold serial dilutions of sera.

The foot web index (FWI) was used as an index of the cell-mediated immune response. On 35th day, 3 birds from each replicate of the treatments were selected and 0.1 ml PHA-P mitogen (1 mg/ml PBS) was injected intradermally into the left foot web. Sterile PBS (0.1 ml) was injected into the right foot web to serve as a control. A micrometer was used to measure changes in the thickness of both foot webs. Measurements were made at 0 and 24 h after the injection (Cheng and Lamont 1988). Foot web swelling was calculated by subtracting skin thickness at 24 h post-injection from that at 0 h pre-injection.

At the end of the experiment, 2 birds from each replicate of the treatment (12 birds/dietary treatment, n=48) were selected randomly and killed to determine the relative weight of the lymphoid organs (bursa of Fabricius, spleen and thymus) and the liver. The thymus tissue was carefully dissected from each side of the neck to ensure complete removal. Organ relative weights were measured to the nearest 0.0001 g.

Microbial load: After collection of lymphoid organ 1 g of caecal contents was collected from the each bird. Samples were serially diluted and subsequently plated on duplicate in MacConkey agar media for the enumeration of coliforms. Plates were then incubated at 37°C for 24 to 72 h, aerobically. Three freshly-voided faecal samples (1 g) from each pen were diluted and plated using MacConkey and PCA for enumeration of coliforms and total anaerobes, respectively. All the media with excretal samples were incubated at 37°C in the following conditions; PCA: anaerobically for 48 h, MRS: aerobically for 48 h and MacConkey: aerobically for 24 h. A general linear model was used (week number × treatment) on logarithimic-valued counts to determine the effect of treatment and time on the levels of microbes.

Statistical analysis: The data obtained in the experiment were analysed using statistical software SPSS-20 version, following standard procedures, by one way ANOVA. The post-hoc analysis for comparing group means was done by using Duncan's (Duncan 1955) multiple range test with significance level set at $P<0.05$.

RESULTS AND DISCUSSION

Production performance: The results of BWG (Table 2) showed that birds fed MOS @0.2% supplemented diet exhibited significant ($P<0.05$) improvement in the growing phase (0–3 wk) and overall phase (0–6 wk). Feed intake did not differ significantly among treatments during the periods from 0 to 21 days of age. However, during the period from 22 to 42 days of age birds fed MOS @0.2 supplemented significantly ($P<0.05$) less feed intake than the control and antibiotic treated groups. The results of FCR showed that addition of MOS did improve FCR. Birds fed MOS supplemented diets gave almost the same values of FCR during the different intervals and the entered period.

These results indicated that mannan oligosaccharides (MOS) were an effective replacer to the antibiotic growth promoter (AGP) in growth performance. The beneficial effects of prebiotic on broiler performance in the present study was in concomitance with Ghahri *et al.* (2013) and Kim *et al.* (2010) who also reported that the prebiotic could be considered as an effective growth promoter and it showed a significant improvement in body weight gain as compared to chickens fed control and antibiotic treated diet. Whereas, these results were in disagreement with Kamran *et al.* (2013) who concluded that the incorporation of prebiotic had no significant effect on growth performance of broiler chickens. The probable hypothesis of improved body weight gain on inclusion of prebiotic is that it improved the structural intestinal health resulting in increased absorption surface and improved utilization of the nutrients (feed) by the chickens. It also reduced the pathogenic bacteria and maintained the beneficial bacteria in the intestine (Biggs *et al.* 2007). Therefore, the improved performance in poultry fed MOS supplemented diet in this study could be related

to the above mentioned facts. Regarding feed intake and feed conversion ratio (FCR), the present study was in disagreement with Kim *et al.* (2010), Ghahri *et al.* (2013) and Chichlowski *et al.* (2007) who reported that a significant improvement in feed intake and FCR was observed in prebiotics supplemented group but the present study came in accordance with Kamran *et al.* (2013) who concluded that the supplementation of prebiotic had no significant effect on feed intake and feed conversion ratio. Willis and Reid (2008) also reported that supplementation of prebiotics reduced the presence of *Clostridium jejuni* but had no significant effect on feed intake and FCR when prebiotics were used in basal diet. Whereas, Mohan *et al.* (1996) reported that prebiotics incorporation with basal diet improved feed intake and FCR and remained consistent in both growing (0–21 d) as well as finishing period (21–42 d). It is generally accepted that many of the factors influenced the results of this experiment; moreover most of the trials with prebiotics reported thus far have been experimentally different with various numbers, strain of microorganisms. The production performance during this experiment supported the fact that the efficacy of prebiotic application depends on many factors such as environmental stress factors, diet administration (method, level, and frequency), farm sanitation, undefined microorganism and age of chicken (Patterson and Burkholder 2003).

Table 1. Composition of the basal diet

Ingredient	Starter (g/kg)	Finisher (g/kg)
Maize	504	580.00
Soybean	420	342.40
RSM	30	30.00
Oil	13.5	17.50
Lime stone	9	8.00
DCP	17	15.00
Salt	3	3.00
DL-Methionine	1.1	1.00
TM-Premix1	1.0	1.00
Vit-Premix2	1.5	1.50
B complex-Premix3	0.15	0.15
Ch. Chloride	0.05	0.05
Toxin binder	0.05	0.05
Calculated values		
Crude protein (g/kg)	22.02	19.53
Metabolizable energy (MJ/kg)	12.15	12.56
Calcium (g/kg)	10.13	9.05
Available phosphorus (g/kg)	4.49	4.01
Lysine (mg/kg)	125	106
Methionine (mg/kg)	49.62	45.13
Threonine (mg/kg)	98.45	86.51

Premix 1: Each 1 g of mineral mixture contained: 200 mg of $FeSO_4.7H_2O$, 20 mg of $CuSO_4$. $5H_2O$, 200mg of $MnSO_4$. H_2O, 150mg of $ZnSO_4.7H_2O$, 1mg of KI. *Premix 2*: Each 1 g of vitamin A, B_2, D_3, K provided: vitamin A (retinol) 540 mg, vitamin B_2 (riboflavin) 50 mg, vitamin D_3 (cholecalciferol) 400 mg, vitamin K (menadione) 10 mg. *Premix 3:* Each g of B-complex provided: vitamin B_1 (thiamine) 2 mg, folic acid 10 mg, pyridoxine HCl 4 mg, cyanocobalamin 10 µg, nicotinamide 12 mg.

Table 2. Effect of dietary supplementation of mannan oligosaccharides (MOS) on production performance in broiler birds (N=48)

Attribute	Groups†				Pooled SEM	P-value
	T1	T2	T3	T4		
Body weight gain (g)						
0–3 wk	360.83^{a}	377.31^{a}	379.18^{ab}	383.05^{b}	3.63	<0.05
4–6 wk	1110.37	1236.68	1165.93	1181.05	9.56	NS
0–6 wk	1520.20^{a}	1525.28^{a}	1560.23^{ab}	1608.98^{b}	10.79	<0.05
Feed intake (g)						
0–3 wk	606.19	607.47	633.23	636.40	4.75	NS
0–6 wk	2949.19^{b}	3098.86^{b}	2924.04^{b}	2898.41^{a}	25.20	<0.05
Feed conversion ratio (FCR)						
0–3 wk	1.68	1.61	1.67	1.66	–	–
0–6 wk	1.94	2.03	1.86	1.82	–	–

†Dietary groups consisted of a control with no additive (T1) or basal diet supplemented with bacitracin methylene di-salicylate (BMD) at 20 mg/kg (T2), and mannan oligosaccharides at 0.1% (T3) or mannan oligosaccharides at 0.2% (T4). ^{ab} Mean values bearing different superscript in a row differ significantly.

Immune response and lymphoid organ weight: Our results are in agreement with the findings of Huang *et al.* (2004) who also reported that anti-vaccine titre of prebiotic treated birds were significantly higher than that of control birds. Elayreh *et al.* (2012) and Cheng and Chen (2004) also reported that prebiotics had a positive effect on immune response but reported that significant improvements were recorded after dietary incorporation of prebiotics in broiler chicken. Better immune response was measured by elevated levels of serum antibodies titre, but these results were not in line with Shahir *et al.* (2014) who reported that the prebiotic had no significant effect on haem-agglutination titre against the influenza and New Castle diseases. Prebiotics may improve bird immunity through different ways i.e. functioning as an agent and attach to bacteria to start immune response, direct promoting effect on immune system by active groups and competition with pathogen for nutrients, colonization of specific pathogen can be inhibited by prebiotics. But these pathogens are presented to immune cell as attenuated antigens. The appearance of increased diffused lympho-histiocytic infiltration and solitary lymphoid follicles in the mucosa and a stronger response indicated increased immunological response in chicken fed with prebiotic supplemented diets. Houshmand *et al.* (2012) reported that incorporation of the prebiotic in animal diet can stimulate the immune system by migrating through the intestinal wall as viable cells and multiply to a limited extent, causing production of immunogenic compounds, and mediating down-regulation of specific signalling pathways. Consequently, stimulated immunity may manifest as enhanced macrophage activity and a systemic antibody response through enhanced production of immune-globulins (IgG, IgM), interferons, IgA levels at mucosal surfaces, and expression of various pro- and anti-inflammatory cytokines.

There was no significant (P>0.05) effect on the relative

weight of the spleen and liver among the dietary treated groups. The relative weight of thymus and bursa of Fabricius increased significantly (P <0.05) with the MOS-supplemented groups (T_3 and T_4) than the control and antibiotic supplemented diet (Table 3). The relative weights of the liver and spleen were not significantly influenced by dietary MOS, consistent with the observations for growing chickens of Sabiha (2005). Increasing dietary MOS increased thymus and bursa of Fabricius relative weight, in contrast to reports for the chicken where there was no change in the thymus after supplementing diets with MOS (Yang *et al.* 2009). The exact explanation for the present findings is not known. One explanation for the considerable increase in the relative weights of the bursa of Fabricius and thymus is that MOS helps protect proliferating immature bursal B cells and thymic T-lymphocytes from oxidative stress (Sabiha 2005). As a consequence, there would be larger populations of the mature cells available for export to the peripheral tissues. Increased populations of immature B and T cells in the secondary lymphoid tissues should have a positive effect on immune responses.

Microbial load: Our results (Table 4) are in agreement with findings of Adil *et al.* (2011) who reported that supplementation has positive effect on microbial load in gut region. The results regarding coliform in ceacal digesta and faecal excreta (28 days, 42 days) did not agree with the findings of Gajewska *et al.* (2012), who reported that prebiotics had a positive effect to reduce the coliform count. Yang *et al.* (2009) also reported that supplementation of prebiotic (MOS) did not show a clear positive effect on intestinal microbial load but total plate count in faeces reduced after prebiotic supplementation. Our results regarding *Lactobacillus* count caecal digesta and faeces (28 and 42 d) are not in agreement with the findings of Kim *et al.* (2010) who reported that the antibiotic-supplemented group showed a significant (P<0.05) reduction in the total aerobic count than prebiotic-supplemented group and

Table 3. Effects of supplementing the diet with mannan oligosaccharides (MOS) on immune response and lymphoid organ weight (% of LW**) of broiler chickens

Attribute	Group†				Pooled SEM	P-value
	T1	T2	T3	T4		
HA Titre (log2)	1.83^{a}	2.80^{b}	2.80^{b}	2.86^{b}	0.12	<0.05
Foot web index (mm^2)	0.51^{a}	0.54^{a}	0.62^{ab}	0.74^{b}	0.04	<0.05
Thymus	4.21^{b}	3.98^{b}	3.06^{a}	2.98^{a}	0.13	<0.05
Bursa of Fabricius	3.28	3.21	3.01	3.00	0.05	NS
Spleen	2.52	2.11	2.09	2.35	0.03	NS
Liver	2.34	2.39	2.38	2.24	0.02	NS

†Dietary groups consisted of a control with no additive (T1) or basal diet supplemented with bacitracin methylene di-salicylate (BMD) at 20 mg/kg (T2), and mannan oligosaccharides at 0.1% (T3) or mannan oligosaccharides at 0.2% (T4). ^{ab} Mean values bearing different superscript in a row differ significantly.

Table 4. Effects of dietary supplementation of mannan oligosaccharides (MOS) on intestinal microflora in broiler chickens (N=48)

Attribute	Day	Group†				Pooled SEM	P-value
		T1	T2	T3	T4		
Coliform count (cfu/g)							
Ceacal	28	5.75^{b}	4.94^{ab}	4.75^{ab}	3.67^{a}	0.12	<0.05
contents	42	3.30^{b}	3.09^{ab}	3.08^{ab}	2.45^{a}	0.04	<0.05
Faeces	28	6.53^{b}	5.43^{ab}	5.44^{ab}	4.62^{a}	0.17	<0.05
	42	4.21^{b}	3.88^{ab}	3.19^{ab}	3.07^{a}	0.13	<0.05
Lactobacillus *count* (cfu/g)							
Ceacal	28	4.18^{a}	3.80^{a}	4.25^{ab}	4.94^{b}	0.05	<0.05
contents	42	2.22^{a}	2.11^{a}	2.29^{a}	2.80^{b}	0.03	<0.05
Faeces	28	5.04^{a}	4.92^{a}	5.12^{a}	6.40^{b}	0.07	<0.05
	42	2.45^{a}	2.05^{a}	2.95^{ab}	3.40^{b}	0.06	<0.05
Total plate count (cfu/g)							
Ceacal	28	6.18^{b}	5.70^{a}	5.45^{a}	5.35^{a}	0.11	<0.05
contents	42	5.08^{b}	4.88^{a}	4.45^{a}	3.60^{a}	0.08	<0.05
Faeces	28	7.35^{b}	6.34^{a}	6.61^{a}	6.23^{a}	0.13	<0.05
	42	3.11^{b}	2.65^{a}	2.36^{a}	2.54^{a}	0.09	<0.05

†Dietary groups consisted of a control with no additive (T1) or basal diet supplemented with bacitracin methylene di-salicylate (BMD) at 20 mg/kg (T2), and mannan oligosaccharides at 0.1% (T3) or mannan oligosaccharides at 0.2% (T4). ^{ab} Mean values bearing different superscript in a row differ significantly.

control. Banerjee *et al.* (2013) also recorded that the dietary supplementation of BMD caused a significant reduction in the *Lactobacillus* count in broiler chicks results suggested that *Lactobacillus* count significantly decreased in 0.2% MOS supplemented group compared to BMD or control groups. These results explained the bad effect of antibiotic on the intestinal tissue healthiness and morphology and this seem consistent with Baurhoo *et al.* (2007) who reported that the antibiotic was less effective in maintaining the intestinal tissue healthiness and morphology than prebiotic due to its bad effect on the beneficial intestinal bacteria. By increasing the growth of beneficial microbes or by reduction and removal of potential pathogens, the alternatives to AGP possibly can improve the health and performance of birds (Yang *et al.* 2009). Regarding the coliform count in this experiment, significant (P<0.05) reduction in the coliform count were showed in 0.2% MOS and antibiotic-supplemented groups. These results are not in consistency with Ferket (2004) who mentioned that prebiotic (MOS) possessed inhibitory effect on intestinal pathogens which could be related to their effects on pathogenic or potential pathogenic bacteria which possess type-1fimbriae, resulting in better performance. Baurhoo *et al.* (2007) revealed that the prebiotic-fed groups based on MOS showed a significant reduction in the excreta *Lactobacillus* load than control and virginamycin-fed group. Furthermore, Kim *et al.* (2010) concluded that the addition of prebiotic (MOS) in the broiler diet caused a significant reduction in the total coliform count than the control and antibiotic received groups. In this context, our results are not in agreement with the above mentioned observations.

It could be concluded that MOS was more efficient than antibiotic growth promoter (BMD) on improving broiler performance, immunity and decreasing intestinal enteropathogen load. If prebiotic i.e. MOS is used correctly along with nutritional, managerial and biosecurity measures, they can be a powerful tool in maintaining the gut health of poultry, thus improving their performances, and can be successfully used as growth promoters.

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