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 oligosaccharide -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 T1, T2, T3 and T4 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 (T2) 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 (T2) 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 T1, T2, T3 and T4 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; pH 7.6) then dried before the haemagglutination antibody (HA) titre was estimated by a micro haemagglutination 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 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, MR: aerobically for 48 h and MacConkey: aerobically for 24 h. A general linear model was used (week number × treatment) on logarithmic-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 Gahari 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

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 FeSO₄·7H₂O, 20 mg of CuSO₄, 5H₂O, 200mg of MnSO₄·H₂O, 150mg of ZnSO₄·7H₂O, 1mg of KI. Premix 2: Each 1 g of vitamin A, B₁, D₃, K provided: vitamin A (retinol) 540 mg, vitamin B₁ (riboflavin) 50 mg, vitamin D₃ (cholecalciferol) 400 mg, vitamin K (menadione) 10 mg. Premix 3: Each g of B-complex provided: vitamin B₂ (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 value |
|------------------|---------|--------|-----------|
|                  | T1      | T2     | T3        | T4        |
| Body weight gain (g) |         |        |           |           |
| 0–3 wk           | 360.83* | 377.31*| 379.18*   | 383.05*   |
|                  | 383.05* | 379.18*| 377.31*   | 360.83*   |
|                  | 3.63    | <0.05  | 3.63      | <0.05     |
| 4–6 wk           | 1110.37 | 1236.68| 1165.93   | 1181.05   |
|                  | 1181.05 | 1165.93| 1236.68   | 1110.37   |
|                  | 9.56    | NS     | 9.56      | NS        |
| 0–6 wk           | 1520.20*| 1525.28*| 1560.23*  | 1608.98*  |
|                  | 1608.98*| 1560.23*| 1525.28*  | 1520.20*  |
|                  | 10.79   | <0.05  | 10.79     | <0.05     |
| Feed intake (g)  |         |        |           |           |
| 0–3 wk           | 606.19  | 607.47 | 633.23    | 636.40    |
|                  | 636.40  | 633.23 | 607.47    | 606.19    |
|                  | 4.75    | NS     | 4.75      | NS        |
| 0–6 wk           | 2949.19*| 3098.86*| 3294.04*  | 2898.41*  |
|                  | 2898.41*| 3294.04*| 3098.86*  | 2949.19*  |
|                  | 25.20   | <0.05  | 25.20     | <0.05     |
| Feed conversion ratio (FCR) |         |        |           |           |
| 0–3 wk           | 1.68    | 1.61   | 1.67      | 1.66      |
|                  | 1.66    | 1.67   | 1.61      | 1.68      |
| 0–6 wk           | 1.94    | 2.03   | 1.86      | 1.82      |
|                  | 1.82    | 1.86   | 2.03      | 1.94      |

†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.
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 (T3 and T4) 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 caecal 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 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-1 fimbriae, 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.

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 |
|----------------|--------|------------|---------|
| HA Titre (log2) | T1     | 1.83a      | 0.12 <0.05 |
|                | T2     | 2.80b      |         |
|                | T3     | 2.80b      |         |
|                | T4     | 2.86b      |         |
| Foot web index (mm²) | T1     | 0.51a      |         |
|                | T2     | 0.54a      |         |
|                | T3     | 0.62ab     |         |
|                | T4     | 0.74b      |         |
| Thymus         | T1     | 4.21b      |         |
|                | T2     | 3.98b      |         |
|                | T3     | 3.06a      |         |
|                | T4     | 2.98a      | 0.13 <0.05 |
| Bursa of Fabricius | T1   | 3.28       |         |
|                | T2     | 3.21       |         |
|                | T3     | 3.01       |         |
|                | T4     | 3.00       | 0.05     |
| Spleen         | T1     | 2.52       |         |
|                | T2     | 2.11       |         |
|                | T3     | 2.09       |         |
|                | T4     | 2.35       | 0.03     |
| Liver          | T1     | 2.34       |         |
|                | T2     | 2.39       |         |
|                | T3     | 2.38       |         |
|                | T4     | 2.24       | 0.02     |

†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 | P-SEM | value |
|----------------|-----|--------|--------|-------|-------|
| Coliform count (cfu/g) |     |        |        |       |       |
| Ceacal contents       | 28  | 5.75b  | 4.94ab | 4.75ab | 3.67a | 0.12 <0.05 |
| Faeces contents       | 42  | 3.30b  | 3.09ab | 3.08ab | 2.45a | 0.04 <0.05 |
| Lactobacillus count (cfu/g) |     |        |        |       |       |
| Ceacal contents       | 28  | 4.18a  | 3.80a  | 4.25ab | 4.94b | 0.05 <0.05 |
| Faeces contents       | 42  | 2.22a  | 2.11a  | 2.29a  | 2.80b | 0.03 <0.05 |
| Total plate count (cfu/g) |     |        |        |       |       |
| Ceacal contents       | 28  | 6.18b  | 5.70a  | 5.45a  | 5.35a | 0.11 <0.05 |
| Faeces contents       | 42  | 5.08b  | 4.88a  | 4.45a  | 4.36a | 0.08 <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.
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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