IMMUNE RESPONSES IN BROILER CHICKENS SUPPLEMENTED WITH PREBIOTIC, PROBIOTIC, THEIR COMBINATION AND G-PROBIOTIC SPL.

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ABSTRACT

The immune responses in terms of relative spleen, bursa of Fabricius and thymus weights, absolute lymphocyte count (ALC) and hemagglutination inhibition titers (HIT) against New Castle disease were studied upon supplementation of lactose (2.5% in drinking water) as prebiotic, Lactobacillus acidophilus (0.1% in drinking water) as probiotic, their combination and G-Probiotic Spl® (0.5% in diet), a commercial probiotic preparation in broiler chickens on day 21 and 42 of age. The relative organ weights were numerically increased in supplemented groups indicating the improvement of immune response in broiler chickens. The ALC values (8481.1 ± 265.2 to 1088.3 ± 141.4) on both day 21 and 42 were significantly increased (P<0.05) in supplemented groups indicating better immune response that could be attributed to immunostimulatory effect of either prebiotics or probiotics in the intestines. The HIT levels (6.5 ± 0.24 to 6.9 ± 0.16) did not differ significantly (P>0.05) between the supplemented groups and control groups. It was concluded that the prebiotics, probiotics, their combination and G-Probiotic Spl® could improve the immune response in broiler chickens and maintain good health status.

Key words : Immune response, Prebiotic, Broiler chickens

INTRODUCTION

Maintenance of good immune response in broiler chickens is necessary for maintenance of good health that helps in harvesting profits from the poultry rearing. This will reduce the medication cost. Many substances are used as immunostimulants in various animals. Among them, the combined use of prebiotic and probiotic is one of the choices for maintenance of good immune system that fights against invasion of pathogenic microorganisms not only by competitive exclusion but also by maintaining good gut health in broiler chickens. Prebiotics are defined as “non digestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of beneficial microorganisms already residing in the colon, thus help to improve host health” (Gibson and Roberfroid, 1995). Dietary lactose along with used litter was useful against cecal and organ colonization of Salmonella enteritidis in Leghorn chicks and hens (Corrier et al., 1993). Lactose, as prebiotic, when used with probiotic increased the body weight in Turkey (Rodriguez et al., 2007). The probiotics are the beneficial microorganisms that can be used as feed additives to maintain balanced intestinal micro-flora (Fuller, 1992). A study was conducted to evaluate the effects of prebiotics and probiotics on immune response in broiler chickens.

MATERIAL AND METHODS

A total of 100 day-old commercial broiler chickens were randomly allocated to five groups of 20 each. They were fed with starter feed up to 21 days of age and thereafter with finisher feed up to 42 days of age. Group I was control, Group II was treated with prebiotic (lactose-2.5 % in drinking water), Group III was treated with probiotic (L. acidophilus-0.1% in drinking water), Group IV was treated with combination of prebiotic and probiotic (lactose-2.5% + L. acidophilus-0.1% in drinking water) and Group V was treated with G-Probiotic Spl® (0.05% in diet). The lactose, L. acidophilus and G-Probiotic Spl® were procured from M/s. Tetragon Chemie Pvt. Ltd., Bangalore. Randomly selected ten birds from each group were sacrificed at the end of third week and remaining ten birds at the end of sixth week. Before each sacrifice, five ml of blood was collected from right carotid artery into a sterile test tube with ethylene diamine tetraacetic
acid (EDTA) as an anticoagulant for hematological studies. The organs like bursa of Fabricious, spleen, liver and thymus, were collected in petri dishes from ten birds in each group on 21st and 42nd day. The collected organs immediately were weighed individually using an electronic digital balance. The relative weight of the organs of individual birds were calculated by dividing the organ weight by body weight and multiplied by 100. The total leucocyte count (TLC) by using Natt – Herrick diluent and the differential leukocyte count (DLC) by Wrights staining technique (Arun and Lokhande, 1994) was performed to calculate the absolute lymphocyte count (ALC) as detailed below.

\[
\text{ALC} = \frac{\text{TLC} \times \text{Lymphocyte percentage in DLC}}{100}
\]

The serum collected from the birds was subjected to hemagglutination inhibition test for New Castle Disease and measured as explained by Charles (1981). Finally, the data was analyzed by One-way ANOVA with Tukeys post test employing computerized GraphPad Prism software (GraphPad Prism, 2004) and the level of significance was recorded.

**RESULTS AND DISCUSSION**

The results of mean relative spleen, bursa of fabricius and thymus weights are presented in Table 1. These organs weights were not statistically different \(P > 0.05\) between the groups on both day 21 and 42. But, the mean relative weight of spleen, bursa of fabricius and thymus were numerically increased. These increased values in the present study were in agreement with the observations of the earlier workers such as Arun (1992), Devegowda et al. (1994) and Upendra (1999), and the numerical increase in relative organ weights could be possibly due to boosting of immune status that has improved health status and performance in prebiotic and probiotic fed broilers (Flickinger and Fahey (2002). Huang et al. (2007) assessed the weights of spleen, bursa and thymus for immune status in broiler chickens. The immunostimulation was responsible for increased relative spleen weight (Cotter, 1997), whereas immunosuppression resulted in decreased relative spleen weight (Rao, 1992; Dwivedi and Burns, 1994).

It was observed that supplementation of prebiotic and probiotic resulted in a significant \(P < 0.05\) increase in mean absolute lymphocyte count (ALC) in broiler chicks as compared to control group (Table 2). Increased mean values of absolute lymphocyte count were supported by the findings of Upendra (1999). These findings could be due to improved immune response in broiler chicks (Weir and Blackwell, 1983; Berg, 1985; Klupsch, 1985; De Simone, 1986; De Simone et al., 1987). The observations of earlier workers such as, the increased cellularity of Payer’s patches in Lactobacillus supplemented layers (Perdigon et al., 1995), modulation of mucosal intestinal immunity with the interaction with microflora of gut (Rundles, 2004).

**Table 1 :** The mean ±SE values of relative weight of spleen, bursa of Fabricius and thymus weights.

<table>
<thead>
<tr>
<th>Organ</th>
<th>Day</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>21</td>
<td>0.10 ± 0.006</td>
<td>0.13 ± 0.006</td>
<td>0.11 ± 0.010</td>
<td>0.11 ± 0.007</td>
<td>0.11 ± 0.008</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>0.18 ± 0.004</td>
<td>0.19 ± 0.006</td>
<td>0.20 ± 0.012</td>
<td>0.19 ± 0.004</td>
<td>0.21 ± 0.010</td>
</tr>
<tr>
<td>Bursa of Fabricius</td>
<td>21</td>
<td>0.31 ± 0.011</td>
<td>0.32 ± 0.011</td>
<td>0.32 ± 0.008</td>
<td>0.31 ± 0.006</td>
<td>0.32 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>0.26 ± 0.003</td>
<td>0.27 ± 0.003</td>
<td>0.28 ± 0.005</td>
<td>0.27 ± 0.005</td>
<td>0.28 ± 0.006</td>
</tr>
<tr>
<td>Thymus</td>
<td>21</td>
<td>0.73 ± 0.009</td>
<td>0.74 ± 0.009</td>
<td>0.75 ± 0.014</td>
<td>0.76 ± 0.011</td>
<td>0.73 ± 0.006</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>0.26 ± 0.003</td>
<td>0.27 ± 0.003</td>
<td>0.28 ± 0.005</td>
<td>0.27 ± 0.005</td>
<td>0.28 ± 0.006</td>
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</tbody>
</table>

**Table 2 :** The mean values of Absolute Lymphocyte Count (cells/μl) and Haemagglutination inhibition titer (log₂).

<table>
<thead>
<tr>
<th>Day</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute</td>
<td>21</td>
<td>7402.5</td>
<td>8549.8</td>
<td>8481.1</td>
<td>8899.8</td>
</tr>
<tr>
<td>Lymphocyte Count (cells/μl)</td>
<td>42</td>
<td>8684.1</td>
<td>10883.3</td>
<td>10857.3</td>
<td>10804.8</td>
</tr>
<tr>
<td>H I titer (log₂)</td>
<td>21</td>
<td>6.8 ± 0.16</td>
<td>6.7 ± 0.16</td>
<td>6.9 ± 0.16</td>
<td>6.6 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>6.4 ± 0.27</td>
<td>6.7 ± 0.30</td>
<td>6.5 ± 0.24</td>
<td>6.8 ± 0.16</td>
</tr>
</tbody>
</table>
and improvement in immune system by feeding inulin as a prebiotic in poultry and using oligochitosan as prebiotic that improved the immune status in broilers (Huang et al. 2007) might be the additional causes for significant increase in ALC in the present study.

The results of mean HI titer levels did not differ significantly (p > 0.05) between the groups on both day 21 and 42 (Table 2) which was in accordance with the findings of Balevi et al. 2001. However, Upendra (1999) reported that the HI titer levels in probiotic group significantly differed with the control group of broiler chickens.

REFERENCES

GraphPad Prism, Version 4.01 for Windows. (2004) GraphPad Software Inc. San Diego, California. USA.