Efficacy of Bacillus Subtilis (GalliPro) supplementation in Clostridium perfringens challenged necrotic enteritis of broiler chicken

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ABSTRACT

The experiment was designed for 42 days to assess dietary supplementation of Bacillus Subtilis (GalliPro-DSM 17299) on growth performance and intestinal health against C. perfringens induced necrotic enteritis in broiler birds. Birds (n=400) were divided into four groups [healthy (control), infected, healthy supplemented with B. subtilis @ 500 g/T of feed (1.2 x 10^6 cfu/g); infected supplemented with B. Subtilis@ 500 g/T ] with five replicates of 20 birds in each group. Necrotic enteritis was induced in broiler birds via inoculation of 50,000 oocysts of mixed strains of Eimeria species on 14 days of age followed by C. perfringens (10^6 cfu/mL) on 17, 18 and 19 days of age. Study revealed that necrotic enteritis challenge suppressed the body weight gain significantly, whereas infected birds supplemented with Bacillus subtilis showed significantly higher body weight gain. The feed intake and feed conversion ratio was not significantly affected in any of the groups. Significant reduction was noticed in bacterial count of C. perfringens infected birds supplemented with B. subtilis. Intestinal damage was observed in the infected control birds while it was recovered in birds supplemented with B. subtilis. The significant beneficial effect of B. Subtilis was recorded in the production performance of broiler chicken even in the absence of necrotic enteritis challenge. Thus it was concluded that supplementation of B. Subtilis helps to improve body weight gain and gut health to control necrotic enteritis.

Key words: Broiler chicken, B. subtilis, C. perfringens, Intestinal health, Necrotic enteritis.

INTRODUCTION

Use of antibiotics in the poultry feed has become an issue of concern worldwide. This has led to the formulation of regulations to restrict antibiotic use in livestock and poultry production. The use of probiotics to improve the performance and general health status of all poultry species, particularly in the broiler chicken and layer hens, has been revived by legislation to curtail the use of sub-therapeutic doses of antibiotics in poultry diets.

In spite of all the preventive advances in poultry production, necrotic enteritis an enterotoxaemic disease caused by Clostridium perfringens regularly affects chicken flocks, leading to development of necrotic lesions in gut wall causing heavy mortality leading to economic losses to the poultry farmers. The necrotic enteritis disease in its acute form may bring about increased mortality in the broiler flock for several consecutive days till the last weeks of rearing period (Kaldhushdal and Lovland, 2000). Due to development of antibiotic resistance by most of the bacterial strains in chickens including Clostridium perfringens, the current scenario demands a healthy and safe alternative to the blatant use of antibiotics in poultry industry.

Therefore, a study was conducted to evaluate efficacy of Bacillus subtilis (1.2 x10^6 CFU/g) supplementation on growth performance, colonization of Clostridium perfringens, histopathology and immunocompetence of broiler chickens under necrotic enteritis condition.

MATERIALS AND METHODS

The experiment was conducted for 42 days on day old unsexed Vencobb- 400 broiler chicks. Corn-soybean diets devoid of coccidiostat and antibiotic growth promoter (AGP) were fed to the experimental birds during entire research work. The feed was formulated as per the nutrient requirement of Vencobb- 400. A total of 400 birds were divided in to four groups with five replicates of 20 birds in each group. The various treatment groups were as follows.

Healthy Control (T1): Birds without challenge of Clostridium perfringens fed on basal diet.

Healthy supplemented with Bacillus subtilis (T2): Birds without challenge of Clostridium perfringens + Bacillus subtilis @ 500g/Ton of feed (1.2 x10^6 cfu/g feed).

Infected (T3): Birds challenged with Clostridium perfringens at 17, 18 and 19 day of age on basal diet.

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Infected supplemented with *Bacillus subtilis* (T4): Birds challenged with *Clostridium perfringens* at 17, 18 and 19 day of age + supplementation of *Bacillus subtilis* @ 500g/ Ton of feed (1.2 x 10⁶ cfu/g feed).

The standard management practices were followed with *ad libitum* water along with weighed quantity of feed during the period of experimentation. The birds were reared on deep litter system and replicate wise birds and feed were weighed weekly to generate the performance data of the experimental birds.

**Collection of Coccidial oocysts:** Mucosal scraping from the guts of field birds suspected for coccidiosis were inoculated into a two percent potassium dichromate solution followed by centrifugation at 2000 rpm for 2 min. The sediment was mixed with sheather’s sugar solution in a test tube until a convex upper meniscus was obtained, and then the tube was allowed to stand undisturbed for about 10 min. Upper layer of oocytes was transferred on to a glass slide by a cover slip and examined under a microscope. The sporulated oocysts were stored at 4ºC until further use.

**Clostridium perfringens isolates:** The *Clostridium perfringens* isolates were obtained from Microbial Type Culture Collection (MTCC) laboratory, Chandigarh. The isolates were alpha toxin producing strains of *Clostridium perfringens* which further pooled to get a concentration of 10⁶ cfu per mL. The experimental birds were inoculated orally with *Clostridium perfringens* bacteria at the dose rate of 10⁷ cfu per ml per chick on 17, 18 and 19th day of experiment. Feed was withdrawn eight hours prior to inoculation of bacterial culture to minimize the occurrence of regurgitation.

**Histopathological studies:** At the end of 24 and 42 day age of bird, ten birds per treatment group were sacrificed in scientific manner and the entire small intestine tract was removed aseptically. About 3 cm long distal part of duodenum was preserved in 10 % formal saline in labeled plastic container for fixation till further processing.

**RESULTS AND DISCUSSION**

**Growth performance:** In the pre-starter phase of growth (0-14 days) the broiler chicks placed in healthy control group (T1) had significantly (P<0.01) lowest weight gain and poor FCR compared to all the other treatment groups (Table-1). The healthy supplemented with *Bacillus subtilis* group (T2) , infected supplemented with *Bacillus subtilis* group and infected group (T3) showed significantly higher body weight gain and better FCR before being exposed to *Clostridium perfringens* challenge on 17, 18, 19 day of age.

In the starter phase (15-28 days) the body weight gain values (Table1) among all the treatment groups were non-significantly affected. Moreover, infected (T3) and infected supplemented with *Bacillus subtilis* group (T4) revealed poor FCR value as compared to healthy control group (T1) and healthy supplemented with *Bacillus subtilis* group (T2). However; no significant differences (P>0.01) were observed in between healthy control group (T1) and its counterpart healthy supplemented with *Bacillus subtilis* (T2) group.

Considering the overall performance (0-42 days), healthy supplemented with *Bacillus subtilis* group (T2) had significantly (P<0.01) highest body weight gain as compared to any of the treatment group birds. However; the infected group (T3) recorded significantly (P<0.01) lowest body weight gain as compared to any of the treatment group. Also, the infected birds supplemented with *Bacillus subtilis* group (T4) showed significantly (P<0.01) higher live body weight gain as compared to its counterpart viz. infected group (T3).

**Table 1:** Live body weight gain (g) and FCR in response to *Clostridium perfringens* challenge and *Bacillus subtilis* supplementation in broiler chicken.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>SEM</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy control</td>
<td>Healthy control</td>
<td>Healthy control</td>
<td>T3 +B. subtilis</td>
<td>0-14 d</td>
<td></td>
</tr>
<tr>
<td>BW gain,g</td>
<td>255⁷</td>
<td>317⁷</td>
<td>309⁷</td>
<td>311⁷</td>
<td>6.49</td>
<td>0.01</td>
</tr>
<tr>
<td>FCR</td>
<td>1.38⁷</td>
<td>1.24⁷</td>
<td>1.21⁷</td>
<td>1.20⁷</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>15-28 d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW gain,g</td>
<td>510</td>
<td>554</td>
<td>504</td>
<td>501</td>
<td>11.02</td>
<td>0.295</td>
</tr>
<tr>
<td>FCR</td>
<td>1.59⁷</td>
<td>1.62⁷</td>
<td>1.79⁷</td>
<td>1.84⁷</td>
<td>0.01</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>28-42 d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW gain,g</td>
<td>961⁷</td>
<td>1049⁷</td>
<td>887⁷</td>
<td>995⁷</td>
<td>22.13</td>
<td>0.054</td>
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<tr>
<td>FCR</td>
<td>1.70⁷</td>
<td>1.68⁷</td>
<td>1.93⁷</td>
<td>1.76⁷</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>0-42 d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW gain,g</td>
<td>1726⁷</td>
<td>1920⁷</td>
<td>1700⁷</td>
<td>1807⁷</td>
<td>25.06</td>
<td>0.01</td>
</tr>
<tr>
<td>FCR</td>
<td>1.62⁷</td>
<td>1.59⁷</td>
<td>1.75⁷</td>
<td>1.68⁷</td>
<td>0.03</td>
<td>0.01</td>
</tr>
</tbody>
</table>

SEM is standard error of difference between mean values
P value is probable significance value

d age of birds in days
The overall results of the current study demonstrated that body weight gain of broiler chicken were significantly (P<0.01) improved due to supplementation of dietary *Bacillus subtilis* (500g/ton of feed) as compared to non-supplemented control birds; however, significant body weight gain was reported in the healthy birds supplemented with *Bacillus subtilis* group (T2) as compared to the infected group (T3) devoid of *B. Subtilis* supplementation under the challenge conditions.

**Histopathological studies:** The birds exposed to necrotic enteritis challenge (T3) showed severe distortion of the jejunal villi of intestine; infiltrations of inflammatory cells in the mucosal layer along with sloughing of epithelial cells from the villi as well as presence of coccidial gametocytes in epithelial lining cells of villi (Fig.1). Although infected birds supplemented with *Bacillus subtilis* group (T4) showed lesions of infiltration with inflammatory cells (Fig.2) the severity of the infiltration with inflammatory cells was very less as compared to the infected group (T3). The higher villi length was noticed in necrotic enteritis challenged birds supplemented with *B. subtilis* (T4) as compared to necrotic enteritis challenged birds not supplemented with *B. subtilis* (T3). Similarly the non-challenged birds supplemented with probiotic (T2) showed better intestinal health with highest villi length along with a healthy mucosal layer than non-supplemented control group birds (T1).

**Colony forming unit (CFU) count:** The birds were sacrificed on 24th and 42nd day; jejunal contents were collected from the sacrificed birds and analyzed for *Clostridium perfringens* count. The statistical analysis revealed that mean *Clostridium perfringens* count (log10 cfu/g of jejunal content) was significantly (P<0.01) high in the birds exposed for necrotic enteritis challenge devoid of supplementation of *B. subtilis* (T3) as compared to any of the other treatment groups (Table 2) or healthy control birds (T1). While comparing in between the treatment groups the significantly (P<0.01) lowest concentration of *Clostridium perfringens* count was recorded in healthy supplemented with *B. subtilis* group (T2).

The present observations i.e. presence of higher villi length in necrotic enteritis challenged birds supplemented with *B. subtilis* and in non-infected group supplemented with *B. subtilis* are in consistency with Samanaya and Yamuchi (2002) as they have also reported improved histologies in probiotic supplemented birds in terms of villus length. It was also reported by Jayaraman *et al* (2013) that *B. subtilis* supplemented birds showed better villi health in comparison to any other groups which indicated that it could be possible that *Bacillus subtilis* acts independent of the infection in improving the gut health.

![Fig 1: Duodenal section of *C. perfringens* challenged birds (T3; 24 d age) showing damaged villi and severe infiltration of leucocytes (HandE x 10X)](image1)

![Fig 2: Duodenal section of *C. perfringens* challenged birds supplemented with *B. subtilis* (T4; 24 d age) showing mild infiltration of leucocytes (HandE x 10X)](image2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>uninfl Control</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>SEM</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>24 d</td>
<td>6.67bc</td>
<td>6.36c</td>
<td>7.65a</td>
<td>7.00c</td>
<td>0.13</td>
<td>0.012</td>
</tr>
<tr>
<td>42 d</td>
<td>6.90bc</td>
<td>6.65c</td>
<td>7.30a</td>
<td>7.12c</td>
<td>0.08</td>
<td>0.013</td>
</tr>
</tbody>
</table>

SEM is standard error of difference between mean values
P is probable significance value
The presence of higher mean *Clostridium perfringens* count in the birds exposed for necrotic enteritis challenge devoid of supplementation of *B. subtilis* (T3) as compared to any of the other treatment groups or healthy control birds and decrease in mean concentration of *Clostridium perfringens* count in healthy supplemented with *B. subtilis* group is comparable with results of Jayaraman *et al* (2013) where they reported significantly lower count of *Clostridium perfringens*. Maruta *et al* (1996) also observed protection of birds against *Clostridium perfringens* using *B. subtilis* C-3102 in the feed. The intestinal microbial analysis for *Clostridium perfringens* count concluded that supplementation of *Bacillus subtilis* reduces the damage caused in necrotic enteritis infection in broiler birds as well as under disease free condition supplementation of *B. subtilis* helps in improving gut health and overall growth performance of birds.

The present study concludes that broiler body weight gain was significantly benefited due to supplementation of *B. Subtilis*, whereas; feed conversion was improved without affecting livability percentage. Supplementation of *Bacillus subtilis* (1x10^6 cfu/g feed) not only controlled *C. perfringens*-induced necrotic enteritis in broiler birds, but also improved the gut health and gut morphology. The supplementation of *B. subtilis* could serve as an alternative approach in controlling necrotic enteritis infection in broilers. Further study is needed in this context in order to curtail the use of antibiotics in feed as growth promoters.

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**REFERENCES**


