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Effect of dietary supplementation of probiotic and BMD on the growth performance of broiler chickens challenged with *Clostridium perfringens* induced necrotic enteritis

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Abstract

A challenging study was carried out for the period of 35 d to investigate the bioefficacy of probiotic and bacitracin methylene disalicylate (BMD) on growth performance and intestinal health against *C. perfringens* induced necrotic enteritis (NE) in broilers. 500 day old broiler chicks (Cobb 400) were divided into five treatment groups, each with 5 replicates of 20 birds each using a completely randomized design. The treatments were an uninfected control (T1), an infected control (T2), an infected group supplemented with probiotic at 500 g/tonne of feed (T3), containing 2×10^{10} cfu/kg, an infected group supplemented with BMD at 500 g/tonne of feed (T4) and an infected group supplemented with probiotic + BMD each at 500 g/tonne of feed (T5). Necrotic enteritis was induced in the broilers by oral inoculation of 30,000 sporulated *Eimeria necatrix* oocysts on day 14 followed by *Clostridium perfringens* (MTCC No. 450, MTCC, Chandigarh) inoculation 1.0 mL (10^8 cfu/mL) on day 19 to 21 in group T3, T4 and T5. The data were recorded and analyzed by using SPSS software. The result indicated that the infected group revealed ballooning of the intestine, thickened mucosa and turkey towel appearance on postmortem examination. Probiotic supplementation significantly ($p < 0.05$) increased weight gain on par with uninfected control group. The feed intake was significantly ($p < 0.05$) reduced in challenged group. Feed conversion ratio was significantly ($p < 0.05$) superior in probiotic+BMD supplemented group and worst in an infected group. It was observed that probiotic either alone or in combination with BMD improved ileal villi length by 9.41% and 10.03% respectively when compared with infected group. *Escherichia coli* and *Clostridium perfringens* counts (log CFU/g digesta) in duodenal contents were significantly ($p < 0.01$) reduced in probiotic (T3) and probiotic+BMD (T5) supplemented groups compared with other groups. From this study, we can conclude that the supplementation of either probiotic or BMD at 500g/tonne of feed had positive effect on the growth performance of commercial broilers.

Keywords: Probiotics, BMD, necrotic enteritis, gut pathogens, intestinal health

Introduction

Necrotic enteritis (NE) is an acute disease of 2 - 4 week old broiler chickens and it is of high economic importance because in addition to 37 to 62 per cent of losses associated with clinical outbreaks. It is caused by *C. Perfringens* infection leads to decreased digestion, absorption, reduced weight gain and increased feed conversion ratio. *Clostridium perfringens* Type A and C are the causative agents of necrotic enteritis which are ubiquitous, gram positive, spore forming, toxigenic anaerobic bacteria affecting the intestinal tract of birds. It is often found in the intestinal tract of healthy broiler chickens but it can cause necrotic enteritis (NE) in many species of poultry especially in broiler flocks (Engstrom *et al.*, 2003) [1]. Normally, the number of *C. perfringens* in the intestine is low (about 10^4 cfu/g of digesta). The disease occurs when high numbers of bacteria coincide with a damaged intestinal mucosa. *Clostridium perfringens* produces many minor toxins, and the most important are β_2 and enterotoxin. Since usage of antibiotics in broilers are banned in most of the countries, it is mandatory to find the alternative solution for this problem to improve the gut health of the modern broilers. Several bacterial strains have been shown to increase broiler chickens performance (health, weight gain, feed conversion) and to prevent or reduce the incidence of diseases caused by pathogenic bacteria (Chaucheyras-Durand and Durand, 2010) [2]. One such alternative could be increase the balance of healthy bacteria to protect the gut from colonizing pathogenic bacteria. So that full genetic potential of modern broilers can be exploited.

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Hence, the present study was undertaken to find the effectiveness of probiotic over Bacitracin methylene disalicylate (BMD) in controlling necrotic enteritis.

Materials and methods

A total of 500 day old broiler chicks from a single hatch, was individually weighed, wing banded and randomly allocated into five treatments each with five replicates of 20 birds each. The treatments were Non challenge control group (T1), *Clostridium perfringens* challenge group (T2), *C. Perfringens* challenge + Galli ProTect (*Bacillus licheniformis*) group (T3), *C. Perfringens* challenge + BMD group (T4) and *C. Perfringens* challenge + Galli Pro Tect (*Bacillus licheniformis*) + BMD group (T5). The probiotic mixture contains *Streptococcus faecalis* (2*10)¹⁰, *Bacillus mesentericus* (2*10)⁸, *Clostridium butyricum* (2*10)⁹, Yucca extract 10%. The birds were fed with pre starter (0-7 day), starter (8-21 day) and finisher (22-42 day). Coccidial inoculation was carried out with 30,000 sporulated *Eimeria necatrix* oocysts on day 14 followed by challenged with *Clostridium perfringens* (MTCC No. 450, MTCC, Chandigarh) inoculation 1.0 mL (10⁸ cfu/mL) on day 19 to 21 in group II, III and IV. The group I was kept as control and fed with diet supplemented with coccidiostat. Standard managerial practices were followed. The birds were fed *ad libitum* with experimental diet and provided with clean, fresh potable water throughout the experimental period. The experimental diets were formulated and prepared according to BIS (2007)¹³ standard in mash form. The chemical composition of the experimental rations was analyzed as per the procedure of AOAC (1990)¹⁴. Whereas the calcium, available, lysine, methionine plus cystine and metabolisable energy content were calculated from the composition of the feed ingredients, according to BIS 2007¹³.

Data on body weight and gain, feed consumption and feed conversion ratio, livability, microbial count (*Escherichia coli* and *Clostridium perfringens*) in duodenal contents. The *Escherichia coli* (*E.coli*) count in the duodenal content was enumerated using serial 10-fold dilutions with 1% peptone solution onto the Mac Conkey Agar (Laboratorios Britania, Mendoza, Argentina). Tenfold serial dilutions up to 10⁷ of each sample were prepared in 9 ml of 0.1% sterile clostridial broth, further plated on Perfringens agar (Himedia Laboratories, India). These plates were then incubated anaerobically at 37°C for 48 h. Counts from two plates were averaged. Numbers of colony-forming units are expressed as log colony forming units per gram of digesta.

Data recorded in this biological experiment were analyzed by one way analysis of variance (ANOVA). The statistical analyses were carried out with the Statistical Package for Social Science (SPSS, 1999¹⁵). Means were compared by using Duncan multiple range comparison test with level of significance ($P < 0.05$). All the experimental procedures used in this study were approved by the Institutional Animal Ethics Committee of Tamil Nadu Veterinary and Animal Sciences University, Chennai -600 051.

Results and discussions

The effects of Probiotic and BMD supplementation either

alone or in combination to broilers challenged with *Clostridium perfringens* on body weight, weight gain, feed intake, FCR, livability, ileal histomorphometry, gut pathogens and economics are presented in table. The day-old body weight showed no significant difference and ranged from 50.32 to 50.54 g. The sixth week body weight was significantly ($P < 0.01$) higher in control (T1) and challenged group supplemented with Galli-Pro Tect (T3) followed by BMD (T4), BMD+Galli-Pro Tect supplemented group (T5) and the lowest was recorded in challenged group (1767.60g). Similar trend was observed in body weight gain also. Where supplementation of Galli-Pro Tect in challenged group had significantly ($P < 0.01$) improved 6th week body weight gain of 2096.80 g on which is compared with control. In this study, increase in body weight and body weight gain was noticed in challenge+GalliPro Tect group may be due to the beneficial action of probiotics which may alter the gut microbial profile thereby improves the productive performance in poultry. The 6th week cumulative feed consumption was significantly ($P < 0.01$) different between treatments groups. It was significantly more in non-challenged control (3705.20 g) followed by Challenged+GalliProTect (T3), Challenged+BMD (T4), Challenged+GalliProTect+BMD (T5) of 3680.20g, 3558.80g and 3493.90g respectively. The challenged group without any supplementation recorded the lowest feed consumption of 3705.20g. Overall, the best FCR was recorded in Challenged+GalliProTect+BMD (T5) of 1.79 and the worst in Challenged group without any supplementation (T2). The intermediary response was observed in Control (T1), Challenged +GalliProTect and Challenged+BMD with no significant difference among them. Similar findings were reported by Lensing *et al.*, 2010¹⁶ who reported that the lowered growth rate and feed consumption in broilers challenged with *C. Perfringens* at day 14. From day 14 onward, daily feed intake decreases until the peak of infection at day 16. After day 16, daily feed intake recovers but stays consistently lower than the healthy control group until day 19. Dietary probiotics have shown improved BW gain, reduced mortality, and enhanced feed conversion, resulting in an increase of broiler productivity (Torres-Rodriguez *et al.*, 2005; Willis *et al.*, 2007)^{17, 81}.

The overall mortality was higher in challenged group with value of 34 per cent due to necrotic enteritis which is higher than the standards prescribed for commercial broilers. Necropsy of dead birds revealed pathological lesions that were attributable to necrotic enteritis. There was no mortality in challenged groups supplemented with Galli-ProTect @ 500g/MT dose in the feed. The livability was significantly ($P < 0.01$) improved in the challenged groups fed with diet supplemented with either GalliProTect @ 500g/MT or BMD and their combination also improved the survivability of the challenged groups. Livability was seems to be low in the challenged group without GalliProTect @ 500g/MT and BMD. This finding was in agreement with Hofacre *et al.*, 2003¹⁹ who reported that Lactobacilli were successful in decreasing mortality due to necrotic enteritis from 60% to 30% in an experimental challenge trial, when they were given orally at day 1 of life.

Table 1: Effect of different dietary treatments on the on the growth performance of broilers (Mean \pm S.E)

Parameter Studied	Non challenged Control	Challenged	Challenged + Galli-ProTect	Challenged +BMD	Challenged +Galli-ProTect+BMD
Day old weight (g) ^{NS}	50.38 \pm 0.05	50.54 \pm 0.12	50.35 \pm 0.06	50.37 \pm 0.06	50.32 \pm 0.06
Body weight (g)**	2099.90 ^a \pm 11.02	1767.60 ^c \pm 11.40	2096.80 ^a \pm 12.25	1988.60 ^b \pm 13.90	2000.00 ^b \pm 12.74
Weight gain (g)	2049.10 ^a \pm 11.02	1717.10 ^c \pm 11.40	2046.50 ^a \pm 12.25	1938.20 ^b \pm 13.91	1949.80 ^b \pm 12.75
Feed consumption(g)**	3705.20 ^a \pm 38.19	3297.10 ^e \pm 30.16	3680.20 ^b \pm 76.23	3558.80 ^c \pm 58.66	3493.90 ^d \pm 21.83
Feed conversion ratio*	1.81 ^b \pm 0.01	1.92 ^a \pm 0.00	1.80 ^b \pm 0.01	1.84 ^b \pm 0.01	1.79 ^c \pm 0.01
Livability (%)*	97.00 ^a \pm 1.22	66.00 ^b \pm 3.67	97.00 ^a \pm 2.00	96.00 ^a \pm 1.87	94.00 ^a \pm 2.92
<i>Escherichia coli</i> count (log 10 CFU/g)*	6.34 ^a \pm 0.21	6.21 ^a \pm 0.20	3.11 ^c \pm 0.12	4.12 ^b \pm 0.21	3.67 ^c \pm 0.13
<i>Clostridium perfringens</i> (log 10 CFU/g)*	3.74 ^a \pm 0.10	3.87 ^a \pm 0.20	2.12 ^b \pm 0.10	2.67 ^b \pm 0.11	2.56 ^b \pm 0.10
Net profit/ kg live weight (Rs)	2.94	0.74	6.34	4.43	4.21

Means with at least one common superscript in the same raw do not differ significantly

*($P < 0.05$), **($P < 0.01$)

Escherichia coli, *Clostridium perfringens* and *Salmonella sps* counts (log CFU/g digesta) in duodenal contents were significantly ($p < 0.01$) reduced in probiotic (T3) and probiotic+BMD (T5) supplemented groups compared with other groups. Intestinal content of *C. perfringens* in control group was found to be 108 cfu/g. whereas in case of challenge group the increasing level of intestinal content of *C. perfringens* was observed (10¹¹ cfu/g). In the challenge group supplemented with BMD, Gallipro Tect and BMD +Gallipro Tect, slight increase in the intestinal count of *C. Perfringens* when compared to the control group (10⁹ cfu/g). This was supported by the findings of Teo and Tan, 2005^[10] who found that supplementation of *B. subtilis* PB6 in necrotic challenged broilers significantly ($P < 0.05$) reduced the intestinal *C. perfringens* counts and controlled NE. This can be attributed to the production of anticlostridial factors by *B. subtilis* PB6.

Conclusion

In conclusion, supplementation of probiotics not only controlled *C. perfringens*-induced necrotic enteritis in broilers, but also improved the weight gain and FCR. The supplementation of probiotics also reduced gut pathogens compared with the infected group. These findings indicate that probiotics can be used as a replacement for antimicrobial growth promoters to improve gut health in broilers.

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