RESEARCH IN
AGRICULTURE, LIVESTOCK AND FISHERIES
ISSN : P-2409-0603, E-2409-9325

Open Access
Research Article

Res. Agric., Livest. Fish.
Vol. 1, No.1, December 2014: 81-86

# THE VIABILITY OF DIETARY PROBIOTICS (BACTOSAC®) INFLUENCING BACTERIAL COLONIZATION OF BROILER CHICKENS

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## ARTICLE INFO

**Received**
01.11.2014

**Accepted**
23.11.2014

**Online**
27.12.2014

**Key words:**

Probiotics
Bacterial colonization
Lactobacillus
Broiler

## ABSTRACT

The efficacies of probiotic with regard to clearing bacterial infections and regulating the gut flora have been clearly demonstrated in this study. One hundred day old broiler chicks were randomly divided into four groups as group A (Vaccinated probiotics fed group), B (Non-vaccinated probiotics fed group), C (Vaccinated conventional fed group) and D (Non-vaccinated conventional fed group). Groups A and B were taken as experimental birds fed with commercially available feed with the addition of probiotics as per schedule whereas groups C and D were taken as control birds fed with commercial ration. The ratio of total viable count (TVC) and total lactobacillus count (TLC) obtained from the bacteriological examination of cecum samples of probiotics fed group (vaccinated and non-vaccinated) at the 2nd, 4th, and 6th week of age were 1:0.96 and 1:0.94; 1:0.97 and 1:0.98; 1:0.99 and 1:0.99 respectively and 1:0.39 and 1:0.41; 1:0.43 and 1:0.43; 1:0.44 and 1:0.41 for conventional fed group (vaccinated and non-vaccinated) respectively. The recovery of TLC obtained from the cecum samples of probiotics and conventional fed broilers were found always on the increase in probiotics fed broilers as compared to conventional fed broilers at the 2nd, 4th and 6th week of age. The present research suggests that vaccination of broilers has no influence on the propagation of intestinal microflora.

**To cite this article:** SML Kabir, MA Islam, MEH Kayesh, KMM Hossain, MMH Mustafa, MB Rahman and K Poonsuk, 2014. The viability of dietary probiotics (Bactosac®) influencing bacterial colonization of broiler chickens. Res. Agric., Livest. Fish. 1(1): 81-86.

## INTRODUCTION

Antibiotic feed supplements have been used in commercial poultry farming for over 50 years due to their growth-promoting and prophylactic properties (Bunyan *et al.,* 1977; Coates *et al.,* 1963; Forbes and Park, 1959). However, the extensive utility of antimicrobial agents has resulted in the occurrence of an antibiotic residue problem in poultry meat and an increase of antimicrobial resistance among pathogenic bacteria which is a great problem of public health. As a result, natural alternatives for substituting the prohibited growth promoter antibiotics with probiotics have received much attention in the recent past. Probiotics beneficially affect the host animal by improving its intestinal balance (Fuller, 1989; Kabir, 2009).

Probiotics have been shown to ensure the optimum microflora balance in order to stimulate and maintain the natural immune system of the host. They enhance immune effects and thus help to prevent illness. Numerous research efforts have dictated the use of *Lactobacillus* species, isolated from poultry (Gusils *et al.* 1999). Jin *et al.* (1996 a, b) found growth inhibiting effects of lactobacilli against *Salmonella enteritidis*, but none of the tested *Lactobacillus* species were able to reduce the attachment of *Salmonella enteritidis* to chicken intestinal epithelial cells in vitro, in contrast with *Salmonella pullorum* and *S. typhimurium*. However, when $10^8$ CFU *Lactobacillus salivarius,* CTC2197 was dosed by oral lavage together with $10^6$ CFU *Salmonella enteritidis* directly into the proventriculus in 1-day-old chicks, the Salmonella bacteria were completely removed from the birds after 21 days (Pascual *et al.* 1999). Another organism *Enterococcus faecium J96*, isolated from the intestinal tract of a free-ranging chicken, inhibited the growth of *Salmonella pullorum*, *S. gallinarum, S. typhimurium and S. enteritidis* in vitro (Audisio *et al.* 2000). Despite several reports on probiotic feeding, information with respect to their effects on gut flora is meager. The present study was, therefore, undertaken to demonstrate the viability of dietary probiotics (bactosac®) influencing bacterial colonization of broiler chickens.

## MATERIALS AND METHODS

### Experimental birds

A total number of one hundred day-old-broiler chicks (Cobb 500 strain) were obtained from the local sale centre of Kazi Farms Limited, Mymensingh, Bangladesh. At the beginning of the experimental study, the broiler chicks were equally divided primarily into two main groups- group I and group II. Group I comprised 50 broiler chicks which belonged to probiotics fed group (PFG) and the remaining in group II comprised 50 broiler chicks which belonged to conventional fed group (CFG). Initial body weight of each bird from each group was recorded just prior to keeping them in two well separated blocks. A total of 25 birds out of 50 from both groups I and II were targeted and selected for vaccination on $6^{th}$ day of age and the remaining 25 birds from both groups were tagged as non-vaccinated group. In term of the schedule of the experiment as mentioned all birds belonging to group I and II were again divided into four subgroups as group A, B, C and D on $6^{th}$ day of age.

### Feeding and management

Commercially available poultry feed (Narish Feeds Ltd., Dhaka) was used throughout the experimental study. The broiler chicks were fed with standard broiler starter for 14 days and broiler grower for 15-28 days and broiler finisher ration for 29-42 days of age, as formulated by Narish Feeds Ltd., Dhaka. Probiotics as per instruction was added to drinking water at a level of 1cc/5-litres water every day from 0 day to $2^{nd}$ week of age and 1cc/5-liter water 3 days in a week in $3^{rd}$ week of age and 1cc/10-liter water from $4^{th}$ week to rest given to birds belonging to group A and B. The remaining two groups such as group C and group D were kept as control without adding probiotics in drinking water.

**Bactosac®**

Bactosac® marketed by PVF Agro Limited (Bangladesh) and manufactured by K.M.P Biotech Co. Limited, Thailand was used in this study and containing six strains of various organisms, those are *Lactobacillus acidophilus, Lactobacillus plantarum, Bacillus subtilis, Bacillus licheniformis, Pediococcus pentosaceus, and Saccharomyces cerevisiae.*

**Immunization**

Birds were vaccinated against baby chick Ranikhet disease by BCRDV (LRI, Dhaka) following the recommendation of the vaccine manufacturer at the age of 6$^{th}$ day followed by a booster dose at 24$^{th}$ day intraoccularly. The birds were also vaccinated against Gumboro disease by Gumbo-L vaccine (ACI Limited, Bangladesh) following the instruction of the manufacturer. One ampoule Gumbo-L was diluted with 30 ml of diluents and the birds were vaccinated at the age of 11$^{th}$ day followed by a booster dose at 21$^{st}$ day intraoccularly.

**Enumeration of total viable count (TVC) and total lactobacillus count (TLC)**

Probiotic fed birds were sacrificed, dressed and their caeca were obtained aseptically. To enumerate the TVC and TLC of caecal contents, a total of 5 birds each of 2$^{nd}$, 4$^{th}$ and 6$^{th}$ weeks of age from each group were selected for this purpose. To perform this study, portions of caeca with their contents were obtained aseptically using a sterile scalpel and forceps. These portions were homogenized uniformly in a blender. The total viable bacterial count of homogenized samples was determined as per recommendation of International Organization for Standardization (1995). The results of the total bacterial count were expressed as the number of organism of colony forming units per gram (CFU/gm) of caecum samples. For the determination of total lactobacillus count, the procedures of sampling, dilution and streaking were similar to those followed in total viable bacterial count. Only in case of lactobacillus count, MRS (de Man, Rogosa and Sharpe) agar was used. The calculation for TLC was similar to that of total viable count.

**Cultural and biochemical examination of bacterial isolates obtained from samples**

The quantitation of bacteria in cecum samples was done according to the standard method (ICMSF, 1985). The examination followed detail study of cultural characteristic including colony formation, staining reactions and biochemical properties. In order to find out different types of microorganisms in cecum different kinds of bacterial colonies were isolated in pure culture and identified as per instruction of Cowan (1985).

**Statistical analysis**

The data on total viable count (TVC) and total lactobacillus count (TLC) obtained from the bacteriolgical examination of cecum samples of broilers were analyzed in Completely Randomized Design (CRD) using computer package MSTAT-C (Freed, 1992).

## RESULTS AND DISCUSSION

The bacteriological examination of cecum samples for different bacterial counts (TVC and TVC) are presented in Tables 1a and 1b. While the values of TVC obtained from cecum samples of probiotics fed group (vaccinated and non-vaccinated) at the 2$^{nd}$, 4$^{th}$ and 6$^{th}$ week of age were log 7.57 and log 7.86; log8.02 and log 7.79; log 7.23 and log 8.04 respectively and log 8.07 and log 8.13; log 8.17 and log 8.25; log 8.31 and log 8.24 for conventional fed group (vaccinated and non-vaccinated) respectively. The beneficial effect of probiotics was evidenced by Singh et al. (1999) who found that the microbial counts tended to decrease with introduction of probiotics in feed. The study of Shoeib and Madian (2002) concluded that the addition of biogen at a rate of 2g/kg diet as a probiotic was superior to pronifer in reducing the total bacterial count and *E. coli* count. The TLC obtained from cecum samples of probiotics fed group (vaccinated and non-vaccinated ) were log 7.24 and log 7.43; log 7.78 and log 7.67; log 7.17 and log 7.98 respectively and log 3.14 and log

3.33; log 3.48 and log 3.57; log 3.67 and log 3.38 for conventional fed group (vaccinated and non-vaccinated) respectively. Fuller (1973) reported the suppression of *E. coli* by lactobacilli on gnotobiotic birds. It was observed that lactobacilli when present in sufficient numbers were directly involved in preventing the unrestricted growth of *E. coli.*

On the other hand, the ratio of TVC and TLC obtained from the bacteriological examination of cecum samples of probiotics fed group (vaccinated and non-vaccinated) at the 2nd, 4th, and 6th week of age were 1:0.96 and 1:0.94; 1:0.97 and 1:0.98; 1:0.99 and 1:0.99 respectively and 1:0.39and 1:0.41; 1:0.43 and 1:0.43; 1:0.44 and 1:0.41 for conventional fed group (vaccinated and non-vaccinated) respectively. It is interesting to note that the values of TLC obtained from the bacteriological examination of cecum samples of probiotics and conventional fed broilers were found always on the increase in probiotics fed broilers as compared to conventional fed broilers at the 2nd, 4th, and 6th week of age. The present research suggests that vaccination of broilers has no influence on the propagation of intestinal microflora. The values of TVC and TLC were almost similar both in vaccinated and non-vaccinated birds. The present findings more or less support the views of Shoeib and Madian (2002), Edens FW (2003) and Kabir et al. (2005).

**Table 1a.** Occurrence of total viable bacteria and lactobacillus counts in cecum of probiotic fed broilers at the 2nd, 4th and 6th week of age

| Parameters | Probiotic fed group (PFG) | | | | | |
|---|---|---|---|---|---|---|
| | 2nd week | | 4th week | | 6th week | |
| | A | B | A | B | A | B |
| TVC | 7.57 | 7.86 | 8.02 | 7.79 | 7.23 | 8.04 |
| TLC | 7.24 | 7.43 | 7.78 | 7.67 | 7.17 | 7.98 |
| Ratio of TVC and TLC | 1:0.96 | 1:0.94 | 1:0.97 | 1:0.98 | 1:0.99 | 1:0.99 |

**Table 1b.** Occurrence of total viable bacteria and lactobacillus counts in cecum of conventional fed broilers at the 2nd, 4th and 6th week of age

| Parameters | Conventional fed group (CFG) | | | | | |
|---|---|---|---|---|---|---|
| | 2nd week | | 4th week | | 6th week | |
| | C | D | C | D | C | D |
| TVC | 8.07 | 8.13 | 8.17 | 8.25 | 8.31 | 8.24 |
| TLC | 3.14 | 3.33 | 3.48 | 3.57 | 3.67 | 3.38 |
| Ratio of TVC and TLC | 1:0.39 | 1:0.41 | 1:0.43 | 1:0.43 | 1:0.44 | 1:0.41 |

All counts are expressed in logarithms. **TVC:** Total viable count**; TLC:** Total lactobacillus count;
**A and C:** Vaccinated bird**; B and D:** Non-vaccinated bird

Percentage distribution of selected bacteria present in cecum samples of probiotics fed broilers are presented in Table 2 which revealed *Lactobacilli*, 82.19%; *Staphylococci*, 5.48%; *Streptococci*, 5.48%; *Bacilli*, 4.11%; *Escherichia coli*, 2.74%. It is noteworthy that *Micrococci, Salmonella, Proteus* and others unidentified were not found in cecum samples of probiotics fed broilers. In this study *Lactobacilli* occurred the highest percentage of occurrence. Next to *Lactobacilli, Staphylococc*i ranked the second position, Streptococci and *Bacilli* secured the third position and *Escherichia coli* obtained the fourth position. Soerjadi et al. (1981) evidenced in their study that the

out numbering of antagonistically reduced the number of *Salmonellae* adhering to the cecum mucosa by 1 to 2 logarithms. Tarakanov et al. (1999) observed that number of *Escherichia coli* was reduced by 37% and *Salmonella* by 2 times in the experimental broilers, whereas intestinal count of amylolytic and *lactobacillus* increased. The count of *coliforms* and *enterococci* decreased during feeding of the acidophilus milk was reported by Patidar and Prajapati (1999). They also found that on average, feeding of *L. acidophilus* $I_4$ and $C_2$ caused to lower *coliform* count, while $I_4$ and $V_3$ reduced *enterococci* count. Kabir et al. (2005) evidenced that the probiotic organisms inhibited some nonbeneficial pathogens by occupying intestinal wall space.

**Table 2.** Frequency distribution of bacterial flora isolated from cecum samples.

| Sl. No. | Name of isolates | Number of isolates | | Percentages (%) | |
|---|---|---|---|---|---|
| | | PFG (A+B) | CFG (C+D) | PFG (A+B) | CFG (C+D) |
| 1. | *Lactobacillus* spp. | 60 | 19 | 82.19 | 17.92 |
| 2. | *Staphylococcus* spp. | 4 | 20 | 5.48 | 18.87 |
| 3. | *Streptococcus* spp. | 4 | 17 | 5.48 | 16.04 |
| 4. | *Bacillus* spp. | 3 | 15 | 4.11 | 14.15 |
| 5. | *Escherichia coli* | 2 | 23 | 2.74 | 21.70 |
| 6. | *Micrococcus* spp. | 0 | 5 | 0 | 4.72 |
| 7. | *Salmonella* spp. | 0 | 4 | 0 | 3.77 |
| 8. | *Proteus* spp. | 0 | 2 | 0 | 1.87 |
| 9. | Others (Unidentefied) | 0 | 1 | 0 | 0.94 |
| | **Total** | **73** | **106** | **100** | **100** |

**PFG:** Probiotic fed group; **A:** Vaccinated birds; **B:** Non-vaccinated birds;
**CFG:** Conventional fed group; **C:** Vaccinated birds; **D:** Non-vaccinated birds

On the other hand, percentage distribution of selected bacteria found in cecum samples of conventional fed broilers is presented in Table 2. These microbes were *Escherichia coli,* 21.70%; *Lactobacilli*, 17.92%; *Staphylococci*, 18.87%; *Streptococci*, 16.04%; *Bacilli,* 14.15%; *Micrococci*, 4.72%; *Salmonella*, 3.77%; *Proteus*, 1.87%; and others 0.94%. The presence of high percentage of pathogenic *Escherichia coli, Staphylococci* and *Bacilli* in addition to the presence of *Micrococci, Salmonella* and *Proteus* are alarming for poultry industry. Many researchers are also of the same view that the presence of the above organisms could be potential hazard not only to human health but also to establishment of poultry industry.

## CONFLICT OF INTEREST

Authors have declared that no conflict of interests exist.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial contributions from K.M.P Biotech Co. Limited, Thailand under the guidance of PVF Agro Limited, Dhaka, Bangladesh for successful completion of the research work and also express their grateful thanks to Dr. Kh. Md. Mahmud Hossain, Director-Head of Sales & Marketing, PVF Agro Limited, Dhaka, Bangladesh for his assistance, encouragement, constructive and informative suggestions.

We are grateful to Professor Dr. M. Mansurul Amin, Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh, Bangladesh for critically reading the manuscript.

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