

Influence of *Bacillus subtilis* on immune-response of broiler chickens

A. Kőrösi Molnár¹, B. Podmaniczky^{1*}, P.Kürti², M. Mørk Jensen², Zs. Farkas¹, Zs. Szabó¹,

¹Institute for Small Animal Research, Gödöllő, Hungary

²Chr.Hansen S/A, Hørsholm, Denmark, E-mail: amolnar@katki.hu

Abstract

The number of the solitary lymphoid follicles occurring in the connective tissue layer of the mucous membrane increased in chickens fed with *B. subtilis* supplementation. According to the microbiological examination the dietary inclusion of *Bacillus subtilis* was significantly reflected in the chicken caecal samples. The birds from the groups fed *Bacillus subtilis* consequently showed stronger response to vaccine of Newcastle disease.

Introduction

Probiotics have been administered to poultry to enhance production performance and immune responses. In addition that the probiotic supplementation has been shown to improve production parameters - body weight, FCR, mortality – (Jin et al., 1996; Mohan et al., 1996; Huang et al., 2004) and can protect the fowls against pathogens by colonization in the gastrointestinal tract (Jin et al., 1996; La Ragione et al., 2001) the probiotics have positive effect on humoral and cellular immune responses (Yunis et al., 2000; Koenen et al., 2004, Huang et al., 2004) too. In group of probiotic microorganisms, members of the genus *Bacillus* occupy a unique position since they are delivered as spores. Because *B. subtilis* is not a member of the normal intestinal microflora and is a strict aerobe, it is not able to grow or metabolise vigorously in the gut. As a result of continuous feeding to poultry, however, larger numbers of this microorganism can exist and become active in the gut and thus influence the microbial milieu of the intestine.

Bacillus subtilis spores have been shown to enhance the response of human peripheral blood lymphocytes to mitogens like phytohaemagglutinin and concanavalin A (Spreafico et al., 1980). Over a wide range of concentrations, *B. subtilis* spores are capable of inducing an increased expression of antigens MLP3, MLR4 and HLA-DR in cultures of peripheral mononuclear cells. At high concentration of *B. subtilis* spores the effect is both quantitatively and qualitatively similar to that induced by the mitogens phytohaemagglutinin and concanavalin A (Fais et al., 1987).

The gut-associated lymphoid tissue (GALT) has evolved with specialized cytological features that reflect its role as the first line of defence at mucosal surfaces. The GALT in chickens includes the bursa of Fabricius, the cecal tonsils, Peyer's patches, and lymphocyte aggregates within the epithelium and in the lamina propria of the wall of the gastrointestinal tract (Lillehoj, 1993).

Bacillus subtilis spores in broiler chicken feeds may exert their beneficial effects among others by activation of intestinal function (Samanaya and Yamauchi, 2002), to create a more favourable environment for beneficial anaerobic species by enhanced immune responses (Fiorini et al., 1985),

Material and Methods

Animals and housing: Five hundred and sixty day-old Ross 308 broiler chicks were randomly assigned to two treatment groups. There were 35 chicks (17 chicks/m²) per replicate and eight replicates per treatment. Birds were housed in floor pens.

Diet: For the experiment, antibiotic- and coccidiostat-free one-phase basal diet (12.63 MJ/kg BE, 20.0 % crude protein, 4.0 % crude fiber, 1.15 % Lysine, 0.46 % Met, 0.44 % Cystine) was used from day old to 42 days of age. Control groups were fed with basal diet containing only the carrier whey powder in a dose of 500g/t feed. The treated groups received basal diet

containing *B. subtilis* (provided by Chr.Hansen A/S) at a dose of 500 g premix (3.6E+05 spores/ g feed). The average spore count of *Bacillus subtilis* was <1E +05 in the control and 3.65E +05 CFU/g in the experimental feed. Feed samples were extracted and analysed for their viable count of *B. subtilis* according to Chr.Hansen Procedure DK-BIRA-QAM-124.

Histological examination: At three weeks old of age tissue samples were taken from the ileum and the bursa of Fabricius was removed from five birds of average body weight of each group.

Serological examination: New Castle disease (ND) vaccination was carried out at day old (by spray in the hatchery) and at three weeks of age (via the drinking water) with a neurotropic vaccine virus strain. Blood samples were taken (10 samples / treatment) at 21 and 42 days of age for serological analyses. The method of analysis was the haemagglutination inhibition (HI) test for ND as described in the European Pharmacopoeia.

Microbiological examination: At six weeks old of age, ten birds of average body weight from each group were randomly selected and killed by decapitation under anaesthesia with diethyl ether. Both caeca of the examined chickens were ligated and immediately frozen at -18 °C. Germinating spores in the caecal content were enumerated according to AM200019.

Carcass qualification: Slaughter tests for carcass qualification were performed on 60 chickens (15 females + 15 males) with average body weight per treatment.

Statistics: All records were analyzed by ANOVA as a randomised block design using the software program Statgraphics (Manugistics Inc., MD, USA).

Results and Discussion

Performance of birds: Broilers fed *Bacillus subtilis* supplemented diet had significantly higher body weight and better feed conversion ratio than those of control.

Histological findings: The thickness and morphological appearance of the different layers (mucous membrane, muscle layer and serous membrane) were similar in sections of small intestine samples taken from the control and treated groups. The length of the villi found on the mucous membrane, the depth of the crypts of Lieberkühn, the ratio of the villi and the glands, as well as the morphology and composition of the cells constituting them did not show any significant deviation either among the groups or between individual animals. This is at variance with the findings of Samanaya and Yamauchi (2002), who observed greater intestinal villus height in chickens that received *B. subtilis* var. *natto* supplementation. The length of microvilli on the surface of enterocytes (at the cuticular margin) was also not different in the groups studied. The number of the solitary lymphoid follicles occurring in the connective tissue layer of the mucous membrane increased in chickens fed *B. subtilis* supplementation. The appearance of solitary lymphoid follicles, indicating gradual lymphoblast cell production, is suggestive of the higher immunological activity of the mucous membrane caused by any stimulating effect.

In the *bursa of Fabricius* samples, the number and dimension of the lymphoid follicles, the cell composition of the cortex and medulla, and the rate of lymphocyte colonization were not different either between the different groups or between individual animals.

Serological results: The birds from the groups fed *Bacillus subtilis* consequently showed a numerically stronger response to NDV, especially at 21 days of age.

Microbiological findings: The dietary inclusion of *Bacillus subtilis* was significantly reflected in the chicken cecal samples. The viable spore count of the examined strain of *Bacillus subtilis* in the caecal samples was significantly higher ($P \leq 0.05$) in treated group than in the control group. This result shows that the probiotic, *Bacillus subtilis*, actually reached the lower part of the intestinal tract and it could be recovered from the intestinal content.

Table 1. Effect of *Bacillus subtilis* on serological, histological and microbiological responses in broiler chickens

Groups, diet		Control	with <i>B. subtilis</i>
Number of chicks		280	280
Repetition		8	8
Live weight at 42 days, g		2271.88±332.35	2406.92±351.8***
FCR kg/kg	0-42 days	1.94	1.86
Mortality %	0-42 days	3.2	2.5
Samples with solitary lymph follicles / total samples		0 / 5	2 / 5
NDV titre	at 21 days	171.2	524.8
	at 42 days	662.8	723.2
Viable spore count of <i>Bacillus subtilis</i> in caeca, CFU/g faeces		3.08 x 10 ³	1.36 x 10 ⁴ *

* P≤ 0.05, *** P≤ 0.001

References

- CHR. HANSEN *Procedure DK-BIRA-QAM-124 European Pharmacopoeia*, 5th Edition, 2004.
- FIORINI, G., CIMMINIELLO, C., CHIANESE, R., VISCONTI, G. P., COVA, D., UMBERTI, D. (1985): *Bacillus subtilis* selectively stimulates the synthesis of membrane bound and secreted IgA. *Chemioterapia* 4: 310-312.
- HUANG, M. K., CHOI, Y. J., HOUDE, R., LEE, J.-W., LEE, B., ZHAO, X. (2004): Effects of Lactobacilli and an Acidophilic Fungus on the Production Performance and Immune Responses in Broiler Chickens. *Poultry Science* 83: 788-795.
- JIN, L. Z., HO, Y. W., ABDULLAH, N., JALALUDIN, S. (1996): Influence of dried *Bacillus subtilis* and Lactobacilli cultures on intestinal microflora and performance in broilers. *Asian-Australas Journal Animal Science* 9 (4) 397-403.
- KOENEN, M. E., KRAMER, J., VAN DER HULST, R., HERES, L., JEURISSEN, S. H. M., BOERSMA, W. J. A. (2004): Immunomodulation by probiotic lactobacilli in layer- and meat-type chickens. *British Poultry Science* 45:355-366.
- LA RAGIONE, R. M., CASULA, G., CUTTING, S. M., WOODWARD, M. J. (2001): *Bacillus subtilis* spores competitively exclude *Escheria coli* O78:K80 in poultry. *Veterinary Microbiology* 79:133-142.
- LILLEHOJ, H. S. (1993): Avian Gut-Associated Immune System: Implication in coccidial vaccine Development. *Poultry Science* 72:1306-1311.
- MOHAN, B., KADIRVEL, R., NATARAJAN, A., BHASKARAN, M. (1996): **Effect of probiotic supplementation on growth, nitrogen utilisation and serum cholesterol in broilers.** *British Poultry Science* 37:395-401.
- SAMANYA, M., YAMAUCHI, K. (2002): Histological alterations of intestinal villi in chickens fed dried *Bacillus subtilis* var. natto. *Comparative Biochemistry and Physiology Part A* 133: 95-104.
- SPREAFICO, F., POLENTARUTTI, N., VECCHI, A., FILIPPESCHI, S., TAGLIABUE A., SIRONI, M., MORAS, M. L. (1980): L'effetto immunostimolatore delle spore di *B. subtilis*: aspetti sperimentali. *Chemioterapia Antimicrobiologia* 4 :259, 1980.
- YUNIS, R., BEN-DAVID, A., HELLER, E. D., CAHANER, A. (2000): **Immunocompetence and viability under commercial conditions of broiler groups deferring in growth rate and in antibody response to *Escherichia coli* vaccine.** *Poultry Science* 79:810-816.