Food and Agricultural Immunology

ISSN: 0954-0105 (Print) 1465-3443 (Online) Journal homepage: http://www.tandfonline.com/loi/cfai20

Effect of Bacillus cereus var. Toyoi and Saccharomyces boulardii on the immune response of sheep to vaccines

T.B. Roos , V.C. Tabeleão , L.A. Dümmer , E. Schwegler , M.A. Goulart , S.V. Moura , M.N. Corrêa , F.P.L. Leite & C. Gil-Turnes

To cite this article: T.B. Roos , V.C. Tabeleão , L.A. Dümmer , E. Schwegler , M.A. Goulart , S.V. Moura , M.N. Corrêa , F.P.L. Leite & C. Gil-Turnes (2010) Effect of Bacillus cereus var. Toyoi and Saccharomyces boulardii on the immune response of sheep to vaccines, Food and Agricultural Immunology, 21:2, 113-118, DOI: 10.1080/09540100903443691

To link to this article: https://doi.org/10.1080/09540100903443691

Food and Agricultural Immunology
Vol. 21, No. 2, June 2010, 113–118

Taylor & Francis
Taylor & Francis Group

Effect of *Bacillus cereus* var. Toyoi and *Saccharomyces boulardii* on the immune response of sheep to vaccines

T.B. Roos[a], V.C. Tabeleão[a], L.A. Dümmer[b], E. Schwegler[a], M.A. Goulart[a], S.V. Moura[a], M.N. Corrêa[a], F.P.L. Leite[c]* and C. Gil-Turnes[a]

[a]*Faculdade de Veterinária, Universidade Federal de Pelotas, Pelotas, Brazil;* [b]*Centro de Biotecnologia, Universidade Federal de Pelotas, Pelotas, Brazil;* [c]*Instituto de Biologia, Universidade Federal de Pelotas, Pelotas, Brazil*

(*Received 28 January 2009; final version received 26 October 2009*)

In this study we evaluated the effects of *Bacillus cereus* var. Toyoi and *Saccharomyces boulardii* on the immune response of lambs to *Escherichia coli* K88ab and Bovine Herpes Virus type 5 (BoHV-5) vaccines. Thirty, 3-month-old lambs were randomly grouped in three lots of 10 each and vaccinated at days 0 and 30 of the experiment. They grazed on the same pasture and were fed *ad libitum* twice a day with commercial sheep feed supplemented with either *B. cereus* var. Toyoi at a concentration of 1×10^6 viable spores gr^{-1}, *S. boulardii* at a concentration of 1×10^6 CFU gr^{-1}, or non-supplemented feed. Blood samples were collected at weekly intervals over eight weeks and antibody titres were analysed by ELISA. The mean seroconversions against *E. coli* and BoHV-5 of the fed probiotics groups were higher ($p < 0.001$) than the controls. Both probiotics enhanced the humoral immune response of lambs to the vaccines.

Keywords: probiotics; ruminants; immunomodulation

Introduction

The high efficiency of farm animals during the last few decades was attained with the supplementation of the diets with subtherapeutical doses of antibiotics (Khachatourians, 1998). The indiscriminate use of these supplements, however, was questioned by the Swann Committee (1969), who first called attention to the risks the indiscriminate use of antibiotics could bring for animal and human health. The evidences obtained since then determined their ban by the European Union in 2006 (Council of the European Union, 2003).

Probiotics are promising substitutes for antibiotics as feed supplements (Gil-Turnes, Conceição, & Gil de los Santos, 2007) and although the processes involved in the enhancement of feed efficiency, disease control and immunomodulation are not completely understood, the European Union approved 19 products in 2000. Among several products available, probiotics prepared with yeasts of the genus *Saccharomyces* or bacteria of the genus *Bacillus* are especially interesting in animal production due to their resistance to the stresses animal feeds are subjected to during manipulation and storage (Coppola & Gil-Turnes, 2004; Krehbiel, Rust, Zhang, & Gilliland, 2003).

*Corresponding author. Email: fabio_leite@ufpel.tche.br

ISSN 0954-0105 print/ISSN 1465-3443 online

DOI: 10.1080/09540100903443691
http://www.informaworld.com

The restriction imposed by several markets on the use of antibacterials as food supplements also brought new attention to vaccines and the immunomodulation properties of probiotics. More potent vaccines that produce fewer undesirable effects are being developed using purified or recombinant antigens and new adjuvants. The immune modulation mediated by probiotics has been a study of several researchers (Andrellucchi et al., 2008; Galdeano, Leblanc, Vinderola, Bonet, & Perdigón 2007; Haghighi et al., 2006). Most of the information on the effects of probiotics in immunity involved lactobacilli and bifidobacteria (Perdigón, Alvarez, Rachid, Agüero, & Gobbatto, 1995), micro-organisms profusely used in humans, but that show restrictions in the practical use in large populations of animals (Gil-Turnes, Santos, Cruz, & Monteiro, 1999). Although the immunomodulation properties of several probiotics were extensively studied (Krehbiel et al., 2003) in monogastric animals such as mice, fowl and swine, there is little information in ruminants.

The objective of this study was to evaluate the influence of *B. cereus* var. Toyoi and *S. boulardii* in the humoral response of ovines to BoHV-5 and *Escherichia coli* vaccines.

Material and methods

Experimental design

Thirty, 3-month-old lambs were randomly divided into three groups of 10. The animals grazed on a natural pasture and were fed twice a day with a commercial feed devoid of antimicrobials, supplemented with the respective probiotic. Feed of group 1 was supplemented with 1×10^6 viable spores gr^{-1} of *B. cereus* var. Toyoi, group 2 with 1×10^6 CFU gr^{-1} of *S. boulardii* and group 3 remained as control with non-supplemented feed. Every animal was vaccinated with an *Escherichia coli* K88ab aluminum hydroxide ($Al(OH)_3$) adjuvanted bacterin and with an inactivated BoHV-5 oil adjuvanted vaccine (Marcol 52-Esso Standart Oil Co.) on days 0 and 30 of the experiment.

Antibody titration

Blood samples were collected by venipuncture on days 0, 7, 14, 21, 28, 35, 42 and 49 of the experiment, and the antibodies against both antigens were titrated by ELISA, using 96-well PVC plates (Greiner Labortechnik, Germany). The plates were sensitised with a suspension of either *E. coli* K88ab containing 10^9 CFU mL^{-1} or with 2 μg/mL recombinant BoHV-5 glycoprotein D (Dümmer et al., 2007) in carbonate–bicarbonate buffer pH 9.6, following standard procedures. The plates remained overnight at 4°C and were then washed three times with PBS-T. Subsequently 50 μl of serum samples diluted 1/20 in PBS-T pH 7.6 were added to the wells and incubated at 37°C for 90 min. After three washings with PBS-T, 50 μl of peroxidase conjugated rabbit anti-sheep immunoglobulins (Dakopatts, Dakopatts A/S, Denmark) were added to each well and incubated at 37°C for 90 min. After a further five washings with PBS-T, the substrate/chromogen solution was added and the absorbances read at 450 nm in a MR 700 Microplate Reader (Dynatech Laboratories, Germany). The ELISA data was expressed by seroconversion where

the absorbance of each sample was divided by that of the serum of the same animal at day 0.

Statistical analysis

Means of seroconversions of each group were $\log_{10}$ transformed and analysed by an ANOVA with means separated by the LSD test using the SAS® program (SAS®, 1997).

The experimental animals were maintained in accordance with the guidelines of the Ethics Committee in Animal Experimentation of the UFPel throughout the experimental period.

Results

Serologic response to the E. coli bacterin

Seroconversions of the animals supplemented with *Saccharomyces boulardii* were higher ($p < 0.001$) than those of the other two groups. Mean seroconversions of the *S. boulardii* group reached 1.9 while in the other groups they did not surpass 1.3. The higher seroconversions of the *S. boulardii* group were observed on days 7, 21 and 49, the *B. cereus* var. Toyoi group on day 49 and in the control group on day 7 (Figure 1).

Serologic response to the BoHV-5 vaccine

The higher seroconversion to the BoHV-5 ($p < 0.001$) was produced in the *B. cereus* var. Toyoi group, reaching 5.2 on day 49, followed by the *S. boulardii* group that reached 2.8 on days 42 and 49, significantly higher ($p < 0.001$) than the control group, that reached 1.7 on day 49 (Figure 2).

Discussion

The effects of probiotics in the prevention and treatment of human diseases are known (Erikson & Hubbard, 2000; Gomes & Malcat, 2006), and among them is immunomodulation which has called the attention of several groups (Meydani & Ha, 2000; Morais & Jacob, 2006). There is experimental evidence, using *B. cereus* var. Toyoi and *S. boulardii*, of enhanced immune response in mice (Coppola, Conceição, & Gil-Turnes, 2005), broilers (Gil de los Santos, Storch, & Gil Turnes, 2005) and other animal species (Gil Turnes et al., 2007), but information on the immunomodulatory properties of probiotics in ruminants is scarce.

In this paper we reported the effects of feed supplementation with *B. cereus* var. Toyoi and *S. boulardii*, probiotics previously tested in animal models (Coppola et al., 2005; Gil de los Santos et al., 2005), on the serologic response to an *E. coli* bacterin and a BoHV-5 inactivated vaccine.

Both probiotics enhanced the serologic response against the vaccines, although the responses followed different patterns and attained different levels, suggesting that the effect of the probiotic was related to the characteristics of the antigens and the adjuvants used. Also we cannot exclude the grazing effect, since the animals were

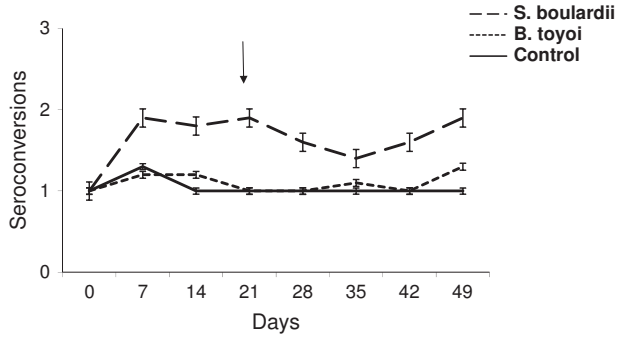

Figure 1. Mean seroconversions (±SEM) of lambs vaccinated with *E. coli* bacterin, supplemented with *B. cereus* var. Toyoi, *S. boulardii* and non-supplemented. Arrow shows day of revaccination (28).

allowed to graze freely; one might suggest that the graze may have an influence in the immune response observed.

The group that received *S. boulardii* showed higher seroconversion than the other two groups, attaining similar levels to those obtained in pigs and mice, supplemented with yeast as a probiotic, with the same vaccine (Coppola et al., 2005; Da Mota 1998). This group produced seroconversions of 1.9 one week after the first dose of vaccine, keeping this level until day 28, when they were revaccinated. The group supplemented with *B. cereus* var. Toyoi also showed seroconversions, although those obtained between two and three weeks after the first and second vaccinations, and were higher ($p < 0.001$) than those of the control group.

Seroconversions induced by the viral vaccine, in contrast to the bacterin, induced immune responses in all the groups. The control group reached a seroconversion of 1.5 two weeks after the primary vaccination, dropping to 1.2 at 21 days, and maintaining the same seroconversion level until two weeks after the booster. The revaccination effect was observed only after three weeks, when the seroconversion level reached 1.7, even though lower ($p < 0.001$) than the other two groups.

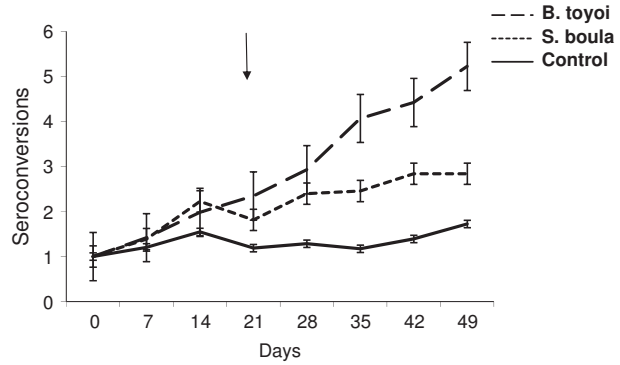

Figure 2. Mean seroconversions (±SEM) of lambs vaccinated with a Bovine Herpes Virus-5 vaccine, supplemented with *B. cereus* var. Toyoi, *S. boulardii* and non-supplemented. Arrow shows day of revaccination (28).

The group supplemented with *S. boulardii* seroconverted to higher ($p < 0.001$) levels than the control group, attaining 2.5 at the time of revaccination, and 2.8 at 42 days, showing a mild increase due to revaccination. The lambs supplemented with *B. cereus* var. Toyoi showed a steady increase in the seroconversions throughout the experiment, reaching seroconversions of 3.5 and 5.2 at revaccination and the end of the experiment, respectively. During the experiment, the seroconversions of this group were higher ($p < 0.001$) than the other groups, in accordance with the results of Coppola et al. (2005) in mice immunised with virus vaccine. We found that *B. cereus* var. Toyoi induced the expression of proinflammatory cytokines *in vitro* and *in vivo*, suggesting that these cytokines might play a role in the immunomodulation mediated by this probiotic.

Our results showed that the probiotics have immunomodulating properties enhancing the immune response to vaccines, and suggested that this effect might depend on the characteristics of the probiotic and the nature of the antigen.

Acknowledgements

The financial support of FAPERGS (Grant No. 0523299) and CNPq (Grant No. 474509/2004-4) is acknowledged. TBR had a scholarship from CAPES (Ministry of Education, Brazil) and CGT a research productivity grant from CNPq (Ministry of Science and Technology, Brazil).

References

Andrellucchi, A.O., Sánchez-Villegas, A., Rodríguez-Gallego, C., Lemes, A., Molero, T., Soria, A., Peña-Quintana, L., Santana, M., Ramírez, O., García, J., Cabrera, F., Cobo, J., & Serra-Majem, L. (2008). Immunomodulatory effects of the intake of fermented milk with *Lactobacillus casei* DN114001 in lactating mothers and their children. *British Journal of Nutrition,* 834–845.

Council of the European Union. (2003). *Council regulation on the authorization of the additive avilamycin in feedingstuffs.* Retrieved September 14, 2006, from http://www.wetgiw.gov.pl/inspekcja_weterynaryjna/FUS/Srodki_z_zwierzat/dodatki_paszowe/COUNCIL%20REGULATION%20(EC)%20No%20355%202003.pdf

Coppola, M.M., & Gil-Turnes, C. (2004). Probióticos e resposta imune. *Ciência Rural*, *34*, 1297–1303.

Coppola, M.M., Conceição, R.F., & Gil-Turnes, C. (2005). Effect of *Saccharomyces boulardii* and *Bacillus cereus* var. Toyoi on the humoral and cellular response of mice to vaccines. *Food and Agricultural Immunology*, *16*, 213–219.

Da Mota, R.S.S. (1998). Avaliação do efeito de um imunomodulador de *Mycobacterium* sp. em vacinas contra a Colibacilose suína. Master in Science, Universidade Federal de Pelotas, Brasil.

Dümmer, L.A., Nizoli, L.Q., Rocha, A.S.R., Moraes, C.M., Conceicao, F.R., Gil-Turnes, C., et al. (2007). *Production and characterization of recombinant Bovine Herpes virus type 5 Glycoprotein D expressed in Pichia pastoris.* Paper presented at the meeting of the eighth International Veterinary Immunology Symposium, Ouro Preto, Minas Gerais.

Erikson, L.K., & Hubbard, E.N. (2000). Probiotic immunomodulation in health and disease. *Journal of Nutrition*, *130*, 403–409.

Galdeano, C.M., Leblanc, A.M., Vinderola, G., Bonet, M.E.B., & Perdigón, G. (2007). Minireview: Proposed model: Mechanisms of immunomodulation induced by probiotic bactéria. *Clinical and Vaccine Immunology*, *14*, 485–492.

Gil de los Santos, J.R., Storch, O.B., & Gil Turnes, C. (2005). *Bacillus cereus* var. Toyoii and *Saccharomyces boulardii* increased feed efficiency in broilers infected with *Salmonella enteritidis. British Poultry Science*, *46*, 494–497.

Gil-Turnes, C., Santos, A.F., Cruz, F.W., & Monteiro, A.V. (1999). Properties of the *Bacillus cereus* strain used in probiotic CenBiot. *Brazilian Journal of Microbiology*, *30*, 11–14.

Gil-Turnes, C., Conceição, F.R., & Gil de los Santos, J.R. (2007). *Bacillus cereus* var. Toyoi improves feed efficiency and health in animals. *International Journal of Probiotics and Prebiotics*, *2*, 21–28.

Gomes, A.M.P., & Malcat, F.X. (1999). *Agentes probióticos em alimentos: aspectos fisiológicos e terapeuticos e aplicações tecnológicas. Boletim de Biotechnologia*, *164*, 12–22.

Haghighi, H.R., Gong, J., Gyles, C.L., Hayes, M.A., Zhou, H., Sanei, B. Chambers, J.R., & Sharif, S. (2006). Probiotic stimulate production of natural antibodies in chickens. *Clinical and Vaccine Immunology*, *13*, 975–980.

Khachatourians, G.G. (1998). Agricultural use of antibiotics and the evolution and transfer of antibiotic-resistant bacteria. *Canadian Medical Association Journal*, *159*, 1129–1136.

Krehbiel, C.R., Rust, S.R., Zhang, G., & Gilliland, S.E. (2003). Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. *Journal of Animal Science*, *81*, 120–132.

Meydani, S.N., & Ha, W.K. (2000). Immunologic effects of yogurt. *The American Journal of Clinical Nutrition*, *71*, 861–872.

Morais, M.B., & Jacob, C.M.A. (2006). The role of probiotics and prebiotics in pediatric practice. *Jornal de Pediatria*, *82*, 189–197.

Perdigón, G., Alvarez, S., Rachid, M., Agüero, G., & Gobbatto, N. (1995). Immune system stimulation by probiotics. *Journal of Dairy Science*, *78*, 1597–1606.

SAS®. (1997). *SAS/STAT User's Guide*. Release 6.03. Cary, NC: SAS Institute.

Swann Committee (1969). BVA and RCVS evidence to the Swann Committee. *The Veterinary Record*, *25*, 91–92.