

## VI. Summary

### **Barbeline Justina Altherr: Studies of the influence of *Bacillus cereus* var. *toyoi* on the cellular immunity of swine**

Probiotic bacteria have been successfully used to improve the health and growth parameters in livestock. Both *in vivo* and *in vitro* studies have shown that orally administered probiotic micro organisms were able to modulate both local and systemic immune responses. However, the mechanisms for these effects have remained unclear.

The aim of this study was to characterize the effects of a probiotic bacterium on the immune system of swine under standardized conditions. For this purpose parameters of the cellular immunity of piglets after administration of *Bacillus cereus* var. *toyoi* were compared with those of an untreated group of piglets.

At the beginning of the study 10 sows were randomly divided into a probiotic-treated and an untreated control group. Sows of the probiotic group were fed *Bacillus cereus* var. *toyoi* ( $3 \times 10^8$  CFU/kg feed) from day 91 *ante partum* to day 28 *post partum*. From day 14 onwards piglets received  $1,2 \text{ CFU} \times 10^9/\text{kg}$  feed. Untreated piglets were fed with the same feed but without probiotic supplementation.

Four piglets were randomly selected from each litter and were sacrificed on days 14, 28, 35 and 56. Blood was taken from the *Vena jugularis* and tissue samples were obtained from the proximal jejunum (beginning with the *Plica duodenocolica*).

In consecutive cryostat sections taken from the proximal jejunum, absolute numbers of CD45<sup>+</sup>, CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>,  $\gamma\delta$ -T-lymphocytes, CD25<sup>+</sup>, CD21<sup>+</sup> and CD11R1<sup>+</sup> cells were determined after labeling with fluorochrom-conjugated antibodies.

The peripheral mononuclear cells (PBMCs) fraction was isolated from citrated venous blood and portions of CD45<sup>+</sup>, CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>,  $\gamma\delta$ -T-lymphocytes, CD14<sup>+</sup>, CD21<sup>+</sup> cells and their subpopulations were measured by staining with fluorochrom-conjugated antibodies and detected by flow cytometry. After stimulation with phorbol 12-myristat 13-acetate (PMA) IFN- $\gamma$  producing cells within the PBMCs fraction was analysed by flow cytometry.

Statistical evaluation was performed using an explorative data analysis and the non-parametric U-test of Mann and Whitney.

With regard to the developmental aspects of porcine intestinal immunity the obtained results were consistent with previous studies. And with few exceptions the feeding groups developed principally in the same direction.

Significant differences between with *Bacillus cereus* var. *toyoi* treated piglets and untreated control group were seen in the number of intraepithelial CD8<sup>+</sup> lymphocytes, as well as in the number of CD25<sup>+</sup> cells and  $\gamma\delta$ -T-lymphocytes in the *Lamina propria mucosae*:

After application of *Bacillus cereus* var. *toyoi*, significantly more CD8<sup>+</sup> lymphocytes, CD25<sup>+</sup> lymphocytes and  $\gamma\delta$ -T-cells were found in the probiotic group on day 35.

Analysis of the immune cell population in blood showed no significant differences between the probiotic group and untreated controls. However tendential differences in B-lymphocytes frequency was noted. The portion of CD21<sup>+</sup> lymphocytes in PBMCs was higher in the probiotic group than in the control group. The portion of IFN- $\gamma$  producing cells within the PBMC fraction in the probiotic group was approximately the same as in controls. The frequency of IFN- $\gamma$  producing cells in PBMCs increased with age in the probiotic group, while it decreased in the untreated control group.

The results of this study indicate an influence on the mucosal cellular immunity by *Bacillus cereus* var. *toyoi*. A differential analysis suggests an effect of the probiotic bacterium on the mucosal epithelium.