7. Summary

Björn Seelig:

Influence of the probiotics *Enterococcus faecium* SF 68 (NCIMB 10415) and *Bacillus cereus* var. toyoi on acidic and alkaline phosphatase reactivity as well as on the endocrine cells of the intestinal mucous membrane in piglets

Probiotic dietary supplementation is increasingly applied in recent years in both, human and veterinary medicine, although many mechanisms of action of the probiotic substances still remain unclear.

In the present study the influence of the two bacteria *Enterococcus faecium* and *Bacillus cereus* var. toyoi on the enzymes and hormones of the intestinal mucous membranes in piglets was examined.

During three different feeding trials, piglets fed on a diet with probiotic supplementation were compared to a control group.

During the first feeding trial, already the pregnant sows (Duroc x Deutsche Landrasse), and, subsequently, 20 piglets from the 15th day post natum, respectively, were fed with the probiotic *E. faecium*. Intestinal samples were taken from 5 animals at a time at the age of 14, 28, 35, and 56 days p.n..

During the second identical feeding trial, the sows and piglets were fed with a *Bac. cereus* var. toyoi –supplementation, and likewise compared to 20 control animals.

During the last feeding trial, only the weaned piglets from the 29th day p.n. were fed with an *E. faecium* supplementation. In this trial, samples from the duodenum and jejunum of 4 animals aged 35 days, or 56 days, respectively, were examined and compared to equivalent control animals.

The samples were taken directly after euthanasia of the pigs. Intestinal tissue samples were taken from the duodenum, the proximal and distal area of the jejunum, from the ileum, the caecum, as well as from the ascending and descending colon.

For the evaluation of acidic phosphatase in 2% glutaraldehyde with 0.1M cacodylate buffer (pH 7.4) and for the detection of hormone-producing cells, the intestinal samples were fixed in a modified Bouin solution (acetic acid was replaced by copper acetate).

For the quantitative evaluation of alkaline phosphatase, tissue samples from the duodenum and the jejunum were deep-frozen in liquid nitrogen.

Employing the substrate p-nitrophenylphosphate, the content of the enzyme alkaline phosphatase was detected within the mucous membrane samples.

The detection of acidic phosphatase within the histological slides was carried out according to the method of BARKA (modified according to BURSTONE 1926). In total, 7 quantitation criteria regarding distribution and reactivity of acidic phosphatase-positive cells were examined by light microscopy.

Polyclonal antibodies against gastrin (RahGastrin, DAKO A.568), somatostatin (RaSomatostatin, Amersham RPN.1612) and serotonin (BioPrime SE-100) were used on 3 - 5 µm sections of paraffin-embedded tissue, and the hormone-reactive cells were detected per unit of area employing the computer-aided picture analysis-programme Lucia 32-G. The measured area was delineated by the Lamina muscularis mucosa on the basal side, and by the villous epithelium on the luminal side.

Results of the evaluation of the quantitative detection of the alkaline phosphatase showed that - regardless from supplementation strategies - the duodenum always displayed a higher reactivity compared to the jejunum.

Furthermore, a decrease of alkaline phosphatase reactivity within the first postnatal weeks was detected within the duodenum and the jejunum.

In comparison to the control animals, inconsistent results were achieved regarding both, *E. faecium* and *B. cereus* var. toyoi-supplementation within the trial groups. *E. faecium*-fed animals showed a tendency towards increased alkaline phosphatase activity in the mucous membrane of experimental animals compared to animals of the control group. *Bacillus cereus*-fed piglets, on the other hand, displayed a tendency towards decreased enzyme activity measurements.

When only the weaned piglets and not the sows (third trial) were fed *E. faecium*, the experimental animals also displayed lower enzyme activities than the control animals.

The evaluation of the different quantitation criteria of the acidic phosphatase revealed only slight deviations after supplementation of both probiotics. In general, the mucous membranes of the lower intestinal areas achieved lower enzyme activity measurements than the upper intestinal areas. The supranuclear areas of the enterocytes of the duodenum and the jejunum of

the experimental animals displayed a tendency to higher activity measurements than that of the respective control animals.

The cell count of the endocrine cells per unit of area revealed that their number decreased regularly from proximal to distal along the longitudinal axis of the intestinal tract. This applies to both, the control as well as the probiotic-supplemented experimental animals, and also to all examined hormones (gastrin, somatostatin, serotonin).

None of the three feeding trials revealed differences in the density of endocrinocytes within the different age groups. Accordingly, no influence of both probiotics employed in this study on the number of hormone-producing cells was detected in comparison to the control animals.

The results of the present study show that alterations of enzyme acitivty in the intestinal mucous membrane of piglets induced by oral application of *E. faecium* and *B. cereus* var. toyoi is possible, while an influence on the gastrin-, somatostatin- and serotonin-producing cells is not detected.