7. Summary

Effects of the probiotic *Enterococcus faecium* SF 68 (NCIMB 10415) on the morphology of the intestinal mucosa of pigs

The benefit of probiotics on the host's intestinal mucosa is well established, however not much is known about their influence on the morphology of the intestinal epithelium.
The aim of this study was to examine the influence of a probiotic diet on the morphology of the intestinal mucosa in the pig.
A total of 40 piglets age 14, 28 (age of weaning), 35 and 56 days were examined.
Each age-group consisted of five animals derived from sows (Duroc x Deutsche Landrasse) which were provided with an *Enterococcus faecium* (NCIMB 10415, Cyclactin®, Roche) supplemented diet. Likewise, five control piglets of each age group were derived from sows fed a normal, unsupplemented diet. Sows of the probiotic group were fed the *E. faecium* supplemented diet beginning at day 25 of gestation.
Tissue samples were collected immediately following sacrifice of animals by euthanasia with sodium barbital at 8.30h a.m. Samples for light- and electronmicroscopic, histochemical, immunhistochemical and morphometric determinations were taken from duodenum, proximal and distal jejunum, ileum, caecum, colon ascendens and colon descendens.
The collected samples were fixed in Bouin's fluid and 2% Paraformeldehyde + 2.5% Glutaraldehyde in 0.1 M Cacodylate buffer (pH 7.5) for light- and electron microscopic studies, respectively.
Morphometric analysis was carried out with a computerized image analysis program "Lucia 32-G Corona 4.11" (Nokia) for villus length, crypt depth, ratio of villus length to crypt depth and calculation of enlargement factor for the villus and crypt surface.
Mucosal substances present in Goblet cells or surface coat were stained with the periodate acid Schiff (PAS)/ Alcian Blue method.
Evaluation of cell turnover rate was carried out with the help of the monoclonal antibody MIB-1 against the nucleus associated protein Ki67. Activities of programmed cell death were detected using the TUNEL-, Apostain- and Caspase3 –methods.
Scanning electron microscopic studies of the mucosal surface showed considerable variations particularly in duodenum and proximal jejunum within the control and probiotic group. In addition to the most common tongue-and fingershaped villi, crestlike villi were also observed particularly in the duodenum and ileum. These differences however cannot be ascribed to feeding or age.

With regard to villus lengths, for both control and probiotic- fed piglets it was found that at age of 35 days (seven days after weaning) the villi were much shorter than in the older or younger animals.

This age- dependent phenomenon was also observed in the decreasing villus: crypt ratios and the enlargement factor of the surface of the intestinal mucosa, or the decrease of the number of goblet cells in the villi of duodenum and ileum and the crypts of duodenum and cecum.

The Crypts of Lieberkühn were generally deeper in the colon than in the small intestines, depths increased in all intestinal segments with increasing age.

The ratio of villus length and crypt depth showed the highest values in the jejunum. The enlargement factor for the intestinal surface due to crypt formation in the jejunum and ileum differed significantly compared to the colon segments ($p < 0,05$). An age-dependent increase of the enlargement factor here was not as apparent as in the crypt depth. In both the proximal and distal jejunum as well as in the colon ascendens the enlargement factor increased up to day 35, but decreasing again by day 56 to a lower level.

In the area of the intestinal villi of the duodenum the number of goblet cells per mm in 14 day- old, *E. faecium* fed piglets were significantly ($p< 0.05$) higher than in controls.

The qualitative evaluation of the goblet cells indicated that these cells showed positive reactions with Alcian-blue throughout the intestinal tract. In contrast, the intestinal mucous coat showed positive reactions with Alcian-blue in the small intestines and PAS-positive mainly in the colon ascendens.

Regarding cell turnover rates, the number of mitotically active cells was highest in the crypts of the jejunum. These data clearly differed from those of the duodenum and the colon segments in all age groups.

The methods for detection of programmed cell death (apoptosis) did not produce reliable results.

The results generally showed that apart from the differences found during the quantitative evaluation of the goblet cells between controls and probiotic fed piglets, no clear group-specific effect was observed for the majority of the various parameters in the intestinal tract. Supplementation of sows and piglets with the probiotic *E. faecium* strain used in this study did not appear to influence the morphology of either the small or large intestine.