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Effects of an *Enterococcus faecium* as a probiotic in pigs: Effects on barrier function and transport properties of pig jejunum. U. Lodemann*, K. Huebener, N. Jansen, H. Martens (Institute of Veterinary Physiology, Freie Universität Berlin, Berlin, Germany).

For young piglets, positive effects of probiotics have been described relating to performance in terms of higher daily weight gain, feed conversion and animal health, but only little is known about the underlying mechanisms. Effects of *Saccharomyces boulardii* and *Bacillus cereus* on paracellular permeability and glucose absorption have been reported. *Saccharomyces boulardii* seems to have an anti-diarrhoeal effect in the rat small intestine by stimulating chloride absorption. The aim of our study was to investigate the barrier function and transport properties of the small intestine in piglets, which were fed a diet containing the probiotic strain *Enterococcus faecium* NCIMB 10415 (*E. f.*). Sows and piglets of the experimental group received a probiotic preparation of *E. f.* On Days 14, 28, 35 and 56 after birth, 5 piglets of each experimental and control group were killed, and samples from the mid-jejunum used for conventional Using chamber measurements. Mannitol fluxes in the probiotic group were higher on the 14th day compared to the control group. The probiotic group showed a slightly higher response in I_{sc} (short circuit current) to L-glutamine compared to the control group. The addition of different glucose concentrations caused an increase of I_{sc}, which exhibited saturation. There was a tendency for a higher rise of I_{sc} of the probiotic group compared to the control group in agreement with other studies. Serosal PGE_2 addition only showed a higher response of I_{sc} on Day 28 for the probiotic group. These results indicate that the administration of the probiotic strain modified the small intestinal barrier function and transport properties as early as 14 days of age, though the observed changes depended on the age of the piglets. This work is supported by the Deutsche Forschungsgemeinschaft, Grant FOR 438/1-1.

Effects of an *Enterococcus faecium* as a probiotic in pigs: Influences on the morphology of the small intestine. K. Reiter, K.D. Weyrauch*, B. Drewes, M. Riess (Institute of Veterinary Anatomy, Freie Universität Berlin, Koserstr. 20, 14195 Berlin, Germany).

A total of 40 piglets aged 14, 28, 35 and 56 days were subjects of this study. Each age-group of 5 animals was fed a probiotic-based diet, while 5 served as control animals. Tissue samples were collected immediately following death, and were fixed in Bouin's fluid and 2.5% glutaraldehyde for light- and electron microscopic studies, respectively. All measurements were done at 10x magnification, and 50 measurements were carried out per intestinal segment of each animal. Evaluation of cell turnover rate was carried out with the help of the monoclonal antibody MIB-1. The villi of duodenum for all the probiotic groups were longer than in the control groups. In the jejunum, only the 14- and 28-day-old probiotic-fed piglets had longer villi. However, the villi were found to be longer in control animals at day 35 and 56 as compared to their probiotic supplemented counterparts. In the duodenum and jejunum, differences between crypt depths of controls and probiotic fed animals were unspectacular, though in the ileum the differences were more striking. As to cell turnover rate, it can be stated that the number of mitoticly active cells in controls differed from probiotic-fed animals to some extent and in some age groups were in agreement with the results for villus length. Scanning electron microscopic studies of the mucosal surface showed considerable variation, particularly in the duodenum and proximal jejunum within the age-groups and in the probiotic groups. However, these differences might not be ascribable to the probiotics. The DFG, Grant FOR 438/1-1.

Spray-dried animal plasma as an alternative to antibiotics for weaning piglets. R. Conde[a], J. Polo[b], D. Torrallardona[a] ([a] IRTA, Centre de Mas Bové, Apartat 415, 43280 Reus, Spain; [b] APC-Europe, 08400 Granollers, Spain).

Spray-dried animal plasma (SDAP) was evaluated as an alternative to medication with colistin for weaning piglets in three experiments. Four dietary treatments were tested in a factorial arrangement: control, SDAP, colistin and SDAP + colistin. First, the four diets were tested on 96 piglets weaned at 21d of age. SDAP improved ($P > 0.05$) average daily gain (ADG) and feed conversion ratio (FCR) at d14, and FCR at d35. Colistin improved ($P > 0.05$) FCR at d14 and d35. No interaction between SDAP and colistin was observed. Later, the four diets were tested on 64 piglets weaned at 22d and on 64 piglets weaned at 32d. SDAP improved FCR at d14 and d28 ($P = 0.08$ and $P < 0.05$, respectively). Colistin did not affect performance in this trial. No interactions between SDAP, colistin and age at weaning were observed. Finally, the diets were tested on 48 piglets weaned at 24d that received an oral dose of *Escherichia coli* K99 at weaning. SDAP improved ADG at d7 and ADG and FCR at d14 ($P < 0.05$). Colistin, on the other hand, only improved ADG and FCR at d14 ($P < 0.05$). Six piglets per treatment were slaughtered to study the morphology of the small intestine mucosa, and ileal and caecal digesta microbiology. Colistin resulted in mucosa with longer villi ($P < 0.10$). SDAP favoured the growth of Lactobacilli in the ileum ($P < 0.10$) and in the caecum ($P < 0.05$), whereas colistin reduced the number of Enterococci in the caecum and of *E. coli* both in the ileum and the caecum ($P < 0.05$). These results suggest that SDAP may be an alternative to medicated feed with antibiotics, as it provided similar performance improvements and protection against an experimental challenge with E. coli K99 compared with those treated with colistin.

Effects of high quality animal proteins on performance and gut morphology and microbiology of weaning piglets. D. Torrallardona[a], X. Córdoba[b], E. Angulo[c] ([a] IRTA, Centre de Mas Bové, Apartat 415, 43280 Reus, Spain; [b] Bioiberica, c/ Lluçà 28, 08028 Barcelona, Spain; [c] Universitat de Lleida, Rovira Roure 177, 25198 Lleida, Spain).

Different sources of high quality animal proteins for weaning piglets were tested for their effect on performance, gut morphology and microbiology. In the first trial, 180 piglets weaned at 19d were offered three iso-nutritional diets with either enzymatically hydrolysed porcine intestinal mucosa (HPM), spray-dried animal plasma (SDAP) or fishmeal (FM) for 13d. Average daily weight gain (ADG) during this period was similar for the pigs on HPM and SDAP, whereas that of pigs on FM tended to have lower weight gains. On d14, all the animals were offered a common diet until d41. During this period, the pigs that had been on the FM diet had a lower ADG than those on HPM ($P < 0.05$). The ADG of the pigs that ate SDAP was intermediate and was not significantly different from the other treatments. In the second trial, 48 piglets weaned at 21d were offered three diets with either HPM, SDAP or soybean meal 48 (SBM) for 14d. Small intestinal morphology and the microbiology of their digesta were studied. No differences in performance were observed between the three treatments, though numerically the animals on SBM had the lowest ADG and feed conversion ratio. The jejunal mucosa had shorter villi for the SBM pigs than for the SDAP and HPM pigs ($P < 0.05$). This was supported by the observation of a lighter small intestine in the SBM pigs than in the other two treatments. The piglets on HPM had lower counts of *Escherichia coli* in ileal digesta than pigs on SBM ($P < 0.1$), and lower counts in caecal digesta than SDAP and SBM pigs ($P < 0.05$). We conclude that enzymatically hydrolysed porcine intestinal mucosa is a good protein source for weaning piglets. It gave better results than fishmeal and soybean meal, and was comparable to a protein source such as spray-dried animal plasma.

Improved piglet gut health without the use of antibiotic growth promoters: addition of organic acids in the diet. N. Canibe, O. Højberg, L.L. Mikkelsen, B.B. Jensen (Danish Institute of Agricultural Sciences, Department of Animal Nutrition and Physiology, Research Centre Foulum, PO Box 50, 8830- Tjele, Denmark).

Low gastric pH values, low numbers of enterobacteria, high numbers of lactic acid bacteria, and high concentrations of organic acids characterise, in general, a healthy piglet gut. Addition of organic acids to the diet is being evaluated for obtaining a healthy piglet gut. Addition of 2.8% lactic acid to a weaner diet resulted in lower pH values in the stomach, caecum and colon of piglets, whereas addition of 0.7% and

1.4%, although decreasing the pH values along the GI-tract, did not result in significant differences compared to the control. Other studies could not detect significant differences between the pH along the GI-tract of piglets fed a control diet or diets with added 1.8% Formi (potassium diformate), or 1% Formi, 1% sorbic acid or 1% benzoic acid. Lactic acid (from 0.7% to 2.8%) tended to increase the number of lactic acid bacteria and yeasts, and to decrease the number of coliform bacteria along the GI-tract. Formic acid (1.4%) and Formi (1.8%) decreased the density of lactic acid bacteria, coliform bacteria and, as opposed to lactic acid, yeasts along the GI-tract of piglets. The antimicrobial effect of formic, propionic, butyric, lactic, benzoic, and fumaric acids was compared in a batch culture system with stomach contents at pH 4.5. Benzoic and fumaric acids showed the strongest antibacterial capacity against coliform bacteria, but these substances were also able to kill lactic acid bacteria. Feeding organic acids at the appropriate dose results in a more healthy/robust porcine GI-tract, which can help ameliorating the digestive disorders suffered by piglets around weaning.

Improved piglet gut health without the use of antibiotic growth promoters: fermented liquid feed in the diet. N. Canibe, O. Højberg, L.L. Mikkelsen, B.B. Jensen (Danish Institute of Agricultural Sciences, Department of Animal Nutrition and Physiology, Research Centre Foulum, PO Box 50, 8830- Tjele, Denmark).

Low gastric pH values, low numbers of enterobacteria, high numbers of lactic acid bacteria, and high concentration of organic acids characterise, in general, a healthy piglet gut. Therefore, feeding strategies promoting such characteristics without the use of antibiotic growth promoters are highly desirable. Feeding fermented liquid feed (FLF) is one of the feeding strategies evaluated for maintaining a healthy piglet gut. Studies carried out in our laboratory have shown that a temperature of 20 °C, a soaking time of 8 h, and a residue of 50%, results in FLF with pH values never exceeding 4.5 (considered as the maximum pH value if the growth of enterobacteria is to be inhibited), low levels of coliform bacteria, high levels of lactic acid bacteria and yeasts, and high levels of lactic acid. Feeding FLF prepared following the above-mentioned procedure decreased the gastric pH, increased the numbers of lactic acid bacteria and yeasts (especially in the proximal GI-tract), and decreased the number of coliform bacteria in the GI- tract of piglets. According to the criteria mentioned above, feeding FLF resulted in a healthier porcine GI-tract, which can help ameliorate the digestive disorders suffered by piglets around weaning.

Improved pig gut health without the use of antibiotic growth promoters: changes in feed processing and structure. L.L. Mikkelsen, O. Højberg, N. Canibe, B.B. Jensen (Danish Institute of Agricultural Sciences, Research Centre Foulum, 8830 Tjele, Denmark).

A coarsely ground meal diet favours the growth of lactic acid bacteria in the stomach of pigs leading to increased production of lactic acid and a low pH. Pathogenic E. coli and zoonotic Salmonella are killed at high levels of lactic acid at low pH. Such properties in the stomach may prevent these bacteria entering and/or proliferating in the lower parts of the gastrointestinal tract. Four groups of 6 Danish Landrace x Yorkshire pigs (25–60 kg) were fed either a coarse non-pelleted (C-NP), coarse pelleted (C-P), fine non-pelleted (F-NP) or fine pelleted (F-P) standard pig diet. After 4 weeks, the pigs were killed and the gastrointestinal tract divided into eight segments. Digesta contents from each segment were analysed. Pigs fed the C-NP diet showed reduced pH and significantly increased concentrations of organic acids (lactic, acetic, propionic and butyric acids) in the stomach. The number of total anaerobic bacteria was significantly higher in pigs fed the C-NP diet, and T-RFLP (Terminal Restriction Fragment Length Polymorphism) indicated that these pigs had a higher microbial diversity in the stomach as compared to pigs fed the other diets. In vitro studies showed a significantly higher death rate of *Salmonella enterica* DT12 in the stomach contents from pigs fed the C-NP diet. In the gastrointestinal tract, reduced numbers of coliform bacteria were also observed in the caecum and colon of pigs fed the C-NP diet as compared to pigs fed the other diets. The present results support that feeding pigs a coarsely ground meal feed improves health status of the animal.

Effects of an *Enterococcus faecium* probiotic in pigs: Modification of intestinal bacterial metabolic activities. W. Vahjen*, M. Macha, D. Taras, O. Simon (Institute of Animal Nutrition, Free University Berlin, Berlin, Germany).

An interdisciplinary research project called "Integrative analysis of modes of action of probiotics in pigs" studied identical sample material from piglets receiving a probiotic microorganism and respective control piglets to investigate the probiotic influence on morphology, physiology, microbiology, immunology and animal performance. Sows and piglets of the experimental group received a probiotic preparation of *Enterococcus faecium* NCIMB 10415. Lumen samples from stomach, proximal jejunum, terminal jejunum, ileum and ascending colon, were taken from suckling- and weaned piglets of each group at the age of 14, 28, 35 and 56 days. Ammonia in sample dilutions was directly measured with an ion-selective electrode, lactate and peroxide concentrations were determined by enzymatic means. All data were subject to high individual variation. The only significant differences in total lactate concentrations were found in stomach and colon samples of 14-day-old animals, which displayed a fivefold increase in probiotic fed piglets. Ammonia concentrations were similar or higher in probiotic fed piglets at the age of 14 days. Beginning from day 28, a trend for higher ammonia concentrations in the control piglets was noted at all intestinal sampling sites. This trend became significant for jejunum and ileum samples one week after weaning. All stomach samples of probiotic-fed piglets, exhibited lower ammonia concentrations than the control group throughout the trial period.

Effects of an *Enterococcus faecium* probiotic in pigs: Influence on enteropathogenic bacteria. P. Schierack, M. Nordhoff, M. Pollmann, P. Schwerk, D. Taras, A. Lübke-Becker, L.H. Wieler, K. Tedin* (Institut für Mikrobiologie und Tierseuchen, Freie Universität Berlin, Berlin, Germany).

The influence of a probiotic strain of *Enterococcus faecium* on total bacterial counts in faeces and intestinal contents was determined in two groups of sows and their offspring, one of which received *E. faecium* NCIMB 10415 as a feed additive, the other remaining as an untreated control group, respectively. Sows were followed over a period of three months prior to the birth of piglets, which were also examined before and after weaning. No significant differences were observed for the two groups both in terms of total anaerobe and coliform bacterial counts. Despite the lack of clinical symptoms, up to 40% of piglets in the control group samples were found to harbour "classical" swine-associated enteropathogenic *Escherichia coli* O138, O139, O141, and O149 serogroups, as determined by agglutination tests of *E. coli* enriched cultures and purified isolates. In contrast, there was up to 50% reduction in the occurrence of the O141 serogroup in piglets belonging to the probiotic group. Similar results were observed for total β-haemolytic E. coli for which no specific serogroup could be assigned. Of the purified isolates, PCR-based assays verified the presence of genes encoding virulence factors such as Shiga toxin Stx2e, indicating the presence of these serogroups. Since no significant differences were observed in the total coliform bacterial counts in either group, the probiotic *E. faecium* NCIMB 10415 strain does not appear to confer protection against enteropathogenic *E. coli* through a general exclusion effect directed against *E. coli* strains, a conclusion supported by in vitro assays using a porcine intestinal epithelial cell line. This work was supported by the Deutsche Forschungsgemeinschaft, Grant FOR 438/1-1.

Pancreatic juice protects gut from pathogenic bacteria. D. Kruszewska[a], Å. Ljungh[a], S.G. Pierzynowski[b] ([a] Dept Med Microbiology, Dermatology and Infection, Lund University, Sölvegaten 23, SE-223 62 Lund, Sweden; [b] Dept Cell and Organism Biology, Animal Physiology, Lund University, Helgonavägen 3b, SE-223 62 Lund, Sweden and Marine Fisheries Institute, Kollataja 1, 82-332 Gdynia, Poland).

The role of exocrine pancreatic fluid in regulating gut microflora colonization is puzzling. In the last two decades it has been postulated that pancreatic juice possesses antibacterial activity. Our objective was to highlight the effect of pancreatic juice on the growth of pathogenic bacteria and fungi. Pancreatic juice samples were obtained from nine eight-week-old weaned pigs

with a pancreatic duct cannula. The antibacterial activity of pure and active pig pancreatic juice collected from healthy, conscious and from anaesthetized pigs was investigated with multi-drug resistant microbial isolates and laboratory strains. Studies were performed on 22 bacterial, two Candida albicans isolates, four lactic acid bacterial species (LAB), and three reference strains. Pancreatic juice was effective ($P < 0.01$) against multi-drug resistant bacterial pathogens, while laboratory strains were only moderately sensitive ($P < 0.05$) to its antibacterial action and LAB was insensitive to its activity. The antibacterial action remained stable when measured before and after small intestine enterokinase activation of trypsinogen to trypsin in vitro. The antibacterial activity of pancreatic juice lasted for several hours and was correlated with the amount of trypsin in pancreatic juice. Pancreatic juice lacking trypsin due to impaired or undeveloped synthesis did not have any effect on bacteria growth. The data point out that the antibacterial properties of pancreatic juice are independent and resistant to trypsin. It can be postulated that trypsin plays at least part of the antibacterial role of pancreatic juice in the developing pancreas. Pancreatic juice antibacterial activity can be an important factor limiting in vivo colonization of pathogenic bacteria into the gut.

Influence on immunological parameters in sows and piglets. L. Scharek, J. Guth, M.F.G. Schmidt (FU Berlin, Institut für Immunologie und Molekularbiologie, Philippstrasse 13, D-10115 Berlin, Germany).

To examine the influence of a probiotic strain of *Enterococcus faecium* on the local immune system, we isolated lymphocytes from the intestinal epithelium (upper jejunum) as well as from Peyers Patches (continuous ileal Peyers Patch (PP)) and determined the levels of several different cell types by fluorescence cytometry. The tissues were taken from 20 piglets treated with *E. faecium* and 20 untreated control animals of the same age (14, 28, 35 and 56 days). The greatest changes appeared in the lymphocyte populations isolated from the pigs' jejunal epithelium. We observed a decrease in CD8+ intraepithelial lymphocytes in piglets belonging to the probiotic group. Cell populations isolated from PP were unstable. CD8+ cells in PP of treated piglets tended to be elevated. CD8+ populations in the first round of animal studies were not further differentiated into cytotoxic and natural killer cells. Total amount of faecal IgA was not significantly affected by the treatment. Concerning the systemic immune system, neither total IgG nor IgA levels in the sera of sows and piglets were affected, nor were the amounts of total IgG or IgA in the milk of the sows influenced by the probiotic treatment. Within the first few weeks after birth, the immune system of piglets is not fully developed, and therefore unable to respond with appropriate production of antibodies against noxious antigens. In these first few weeks, they depend on their native and local immune response. The observations suggest the presence of the probiotic *E. faecium* strain appears to result in changes in the intraepithelial lymphocyte populations.