
Enterotoxigenic Escherichia coli (ETEC) induces diarrhea and reduces absorption of water and nutrients, yet effects of ETEC on glucose transport in pigs are poorly understood. Exopolysaccharides reuteran and levan produced by Lactobacillus reuteri, but not related glycans dextran and inulin, reduce the occurrence of ETEC-induced diarrhea, and may thus also influence the expression of glucose transporters. We hypothesized that ETEC infection reduces mRNA abundance of Na+-dependent glucose transporter-1 (SGLT1) in ETEC-challenged piglets. Weaning gilts (5-wk-old; 10.2 ± 1.8 kg BW; n = 4) were prepared for small intestine perfusion with 10 jejunal segments. Five segments were infected with ETEC K88 (5 × 10⁸ cfu/mL); remaining segments were treated with saline. Five pairs of segments, 1 ETEC and 1 non-ETEC infected each, were infused with 65 mL of 10 g/L of glucans (dextran, reuteran), fructans (levan, inulin) or saline (control) for 8 h. After infusion, mRNA was extracted from mucosa, and cDNA was manufactured before quantitative reverse transcription-PCR. Gene expression in the ileum of piglets.

Key Words: enterotoxigenic Escherichia coli, glucose transporter, pig


Adhesion of enterotoxigenic Escherichia coli (ETEC) to small intestine enterocytes causes release of toxins that stimulate cytokine release. The exopolysaccharides reuteran and levan produced by Lactobacillus reuteri may reduce inflammatory responses caused by ETEC-infection. We hypothesized that these exopolysaccharides also reduce mRNA abundance of cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNFα) in ETEC-challenged piglets. Weaning gilts (5-wk-old; 10.2 ± 1.8 kg BW; n = 4) were surgically prepared for small intestine segment perfusion with 10 jejunal segments. Five segments were infected with ETEC F4 (K88) (5 × 10⁸ cfu/mL); the rest were treated with saline. Five pairs of segments, 1 ETEC infected and 1 not, were infused with 65 mL of 10 g/L glucans (dextran, reuteran), fructans (inulin, levan), or saline (control) for 8 h. After infusion, mucosa was collected, mRNA extracted, and cDNA manufactured before quantitative reverse transcription-PCR. Contrasts between ETEC- and non-ETEC-infection were analyzed as fold change using the 2-ΔΔCt method with β-actin and GAPDH as reference genes. The ETEC-infection increased IL-1β (8.2 vs. 1.0 ± 0.2; P < 0.01) and IL6 (4.1 vs. 1.0 ± 0.6; P < 0.01), but did not affect TNFα, compared with control. Without ETEC, IL-6 tended to increase for levan (6.3 vs. 1.0 ± 1.5; P = 0.09) and reuteran (7.1 vs. 1.0 ± 1.5; P = 0.07) compared with saline. With ETEC-infection, inulin decreased IL-1β (0.4 vs. 1.0 ± 0.5; P < 0.05) and IL6 (0.1 vs. 1.0 ± 1.2; P < 0.05) compared with saline. With ETEC-infection within treatments, IL-6 was increased (22.3 vs. 1.0 ± 1.3; P < 0.01) for dextran and tended to increase (5.6 vs. 1.0 ± 1.3; P = 0.07) for reuteran, but did not differ between inulin or levan, compared with non-ETEC-infection. In conclusion, ETEC-infection increased inflammatory responses based on tissue IL-1β and IL6 expression. Luminal fructan infusion may reduce these inflammatory responses whereas luminal glucan infusion did not. Thus, effects of glycans on ETEC-induced cytokine release in pigs may depend on glucan composition.

Key Words: cytokine, enterotoxigenic Escherichia coli, pig

101 Dietary inclusion of low doses of microencapsulated zinc oxide affects inflammatory cytokine and tight junction protein expression in the ileum of piglets. E. Grilli*, B. Tumolli2, F. Vitari2, and A. Piva1, 1DIMEVET, University of Bologna, Ozzano Emilia, Italy, 2Department of Health, Animal Science and Food Safety, University of Milan, Milan, Italy.

Aim of this study was to investigate the expression of inflammation markers and tight junctions protein (TJ) in the ileum of piglets fed with low doses of microencapsulated zinc oxide (ZnO; Zincoret, Vetagro SpA, Italy) in comparison with either a pharmaceutical dose of free ZnO (positive control) or a negative control. Twenty-four pigs weaned at 28 d and divided in 4 groups, received either a basal diet (NC) or the basal diet added with zinc oxide at 2850 mg/kg (PC), or lipid encapsulated ZnO at 187 or 437 mg/kg (Zn200 and Zn400). After 15 d, 6 pigs per group were euthanized and ileal samples were collected for cytokines (IL-6, IL-10, TNF-a, and IFN-g), zonula occludens-1 (ZO-1), occludin (OCC) and claudin-1 at both mRNA and protein level. Data were analyzed with 1-way ANOVA. Both groups receiving microencapsulated ZnO tended to have a reduced expression of IL-6 (~25%, P = 0.1), compared with both NC and PC. IFN-g expression was the lowest in Zn400 group (P = 0.02), and the protein tended to be lower in Zn400 than in PC (P = 0.07). Microencapsulated ZnO tended also to downregulate TNF-a expression compared with NC and PC (P = 0.1) and TNF content in ileal samples (P = 0.07). OCC gene expression was the lowest in Zn400 (P = 0.04), though the protein amount was 2–4 fold higher in Zn400 group compared with both NC and PC, respectively (P < 0.001). ZO-1 expression was not affected by the treatments but ZO-1 amount in Zn400 group was 1.3–1.5 fold higher than in PC and NC, respectively (P < 0.001). Claudin-1 gene expression was 1.7–2.4 higher in Zn400 compared with NC and PC, respectively (P = 0.01). Overall, Zn200 group tended to have intermediate values between PC and Zn400. The results suggest that ZnO released from a lipid matrix is available in the ileum of piglets where it modulates the local immune response which, in turn, affects the intestinal permeability via the TJ proteins. In this respect, lipid encapsulated ZnO was effective at relatively low concentrations whereas free ZnO fed at 6–15 higher doses failed to be, probably because of a rapid metabolism in the upper gut.

Key Words: microencapsulation, piglet, zinc oxide

An experiment was conducted to test for beneficial effects of dietary clays on young chicks challenged with pathogenic Salmonella and to explore potential mechanisms through which clays may produce benefits, with emphasis on barrier function. Two hundred forty 1-d-old male commercial broiler chicks (initial BW: 41.6 ± 0.4 g; Ross × Ross) were allotted in a randomized complete block design with level on the battery as the block and pen as the experimental unit. Six replicates of 5 chicks/pen were assigned to each treatment. Pens were randomly assigned to 1 of 2 infection treatments (with or without Salmonella challenge at 2 wk of age) and 4 dietary treatments: basal, 0.3% smectite A (SMA), 0.3% smectite B (SMB) and 0.3% zeolite (ZEO). The Salmonella challenge reduced (P < 0.05) the growth rate of chicks fed the basal diet by 11% during d 3–7 post-inoculation (PI), but the clays prevented this reduction. The interaction between challenge and diet occurred (P < 0.05) for ADFI d7–10 PI and ADFI and ADG during the overall period (d0–14 PI), and the pattern was similar but not significant for other measures. Goblet cell number and size were increased (P < 0.05) by the Salmonella challenge in chicks fed the basal diet, and were reduced (P < 0.05) in Salmonella-challenged chicks by feeding SMA. Villus height was reduced by the Salmonella challenge in the chicks fed dietary clays (P < 0.01) but not in chicks fed basal diet (interaction P < 0.05). The mRNA expression of IFN-γ, a canonical inflammatory marker, in cecal tissues remained low in all treatments. Clays did not alter the concentration of α-1 acid glycoprotein in the sham-challenged group but increased it in the Salmonella-challenged group. In conclusion, clays restored performance of Salmonella-challenged chicks, SMA had effects consistent with strengthening of the mucosal barrier, and the pattern of response suggests that different clays produce benefits through different mechanisms.

Key Words: goblet cell, chick, clay

103 Bacillus licheniformis and sodium butyrate protective effects on oxidative stress-induced inflammation in IPEC-J2 porcine intestinal epithelial cells. A. Ortíz*, P. Gálfi2, E. Paszti-Gere3, A. Jerzsele2, M. Puyalto1, J. J. Mallo1, Novel S.A., Madrid, Spain, Szent István University, Budapest, Hungary.

To determine whether sodium butyrate (SB) and Bacillus licheniformis (BL) spent culture supernatant (SCS) could exert protective effects under oxidative stimuli, IPEC-J2 porcine intestinal epithelial cells were treated simultaneously with hydrogen peroxide and the above mentioned active ingredients (AIs). Transepithelial electrical resistance (TER) measurement of monolayers was performed using epithelial tissue volt/ohmmeter (EVOM) to elucidate the effect of 0.5 mM peroxide and AIs on cell membrane integrity. Occurrence of cell death was monitored using 2 staining methods, Trypan blue exclusion assay (TB) and Neutral Red uptake test (NRU) by counting 300 cells per flask in 3 parallelism. Administration of sodium butyrate at 2 mM significantly (P < 0.05) increased TER values compared with positive controls (84.09% vs. 67.55%), this barrier strengthening property remained elevated after an 24-h recovery period (174.41% vs. 143.16%), SCS of Bacillus licheniformis in 10% solution significantly (P < 0.05) elevated TER values compared with control (90.10% vs. 69.32%). Sodium butyrate at 2 mM significantly (P < 0.05) elevated the number of viable cells with NRU staining compared with H2O2 treated samples (68.71% vs. 58.37%). SCS of Bacillus licheniformis in 10% solution also increased cell viability significantly (79.60% vs. 58.37%; P < 0.05) as a reduced number of dead cells was found. Sodium butyrate at 2 mM significantly (P < 0.05) elevated the number of viable cells compared with H2O2 treated samples (82.20% vs. 55.48%) with the TB assay. It is concluded that both SB at 2 mM and BL SCS in 10% solution have a protective effect on IPEC-J2 porcine intestinal epithelial cells.

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<th>Table 1.</th>
<th>Mean TER (OHM-cm2)</th>
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<td>Before treatment</td>
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<td>Trial 1</td>
<td>0.5 mM H2O2</td>
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<td>0.5 mM H2O2 + 2mM SB</td>
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<td>0.5 mM H2O2 + 10% BL</td>
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Key Words: stress, butyrate, licheniformis

104 Effect of acute water and feed deprivation event on mucin, cytokine, and tight junction gene expression in weaned pigs. N. Horn*, G. Miller1, K. M. Ajuwon1, F. Ruch2, and O. Adeola1, Purdue University, West Lafayette, IN; Enzyvia LLC., Sheridan, IN; Biomatrix, Princeton, MN.

The effect of acute water and feed deprivation events on mucin, cytokine, and tight junction gene expression in weaned nursery pigs were evaluated. Pigs (6.21 ± 0.29 kg) were allotted in a randomized complete block design to 4 post-weaning treatments on the basis of body weight at the time of weaning which consisted of a control, 24-h feed deprivation event, 24-h water deprivation event, and a 24-h feed and water deprivation event. There were 8 pigs per pen and 12 replicate mixed-sex pens per treatment. Following the deprivation events pigs were returned to normal management procedures. On d 2 and 7 post weaning one pig per pen was euthanized and ileal and jejunal mucosal samples were taken for gene expression analyses by RT-PCR for mucin (MUC2), cytokines interleukin 1 (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor (TNF-α), and tight junction proteins claudin 4, occludin, and zonula occludens 1 (ZO-1). In the jejunum there was a decrease in TNF-α expression the day following the water stress event (P = 0.011) and a significant feed x water interaction (P = 0.017). Furthermore, there was a decrease in occludin gene expression the day following the water stress event (P = 0.019). There were no differences in MUC2, tight junction, or cytokine gene expression in the jejunum 7 d post weaning. In the ileum there was a decrease in claudin 4 gene expression 7 d post weaning in water stressed pigs (P = 0.008). There was also a tendency for a decrease in ZO-1 gene expression with the water stress event (P = 0.081) and a tendency for a feed x water interaction (P = 0.096) was observed 7 d post weaning. There were no differences in MUC2, cytokine, or tight junction gene expression in the ileum 2 d post-weaning. The results from the current trial show a decrease in tight junction gene expression in the gastrointestinal tract of nursery pigs on d 24 h post-feeding a 24-h water deprivation event at weaning although expression of cytokines and MUC2 were largely unaffected by the stress events.

Key Words: tight junction gene expression, nursery pig, stressor
Effect of dietary fructo-oligosaccharide with different polymerization degree on the cellular immune response in weaned pigs. V. Halas*1, I. Nochta2, T. Tuboly3, Cs. Szabó1, and L. Babinszky4,
1Kaposvár University, Kaposvár, Hungary, 2Provimi, Zichyújfalu, Hungary, 3Szent István University, Budapest, Hungary, 4University of Debrecen, Debrecen, Hungary.

Fructo-oligosaccharide (FOS) supports the beneficial microbiota that affects the immune status, particularly at weaning. However, it is not clear if degree of FOS polymerization can alter the animal response. Therefore the aim of the trial was to study the effect of different FOS supplementation on cellular immune response and growth performance of nursery pigs. A total of 464 piglets (7.9 kg BW) were assigned into 4 dietary treatments (6 flat deck pens/treatment) at weaning (28 d). The negative control (NC) diet was a commercial feed without growth promoter (basal diet), the basal diets was supplemented with 40 ppm avilamycin (treatment PC), 0.5 g/kg of FOS (3–5 degree of polymerization; Profeed, Allied Nutrition Ltd., South Africa; treatment FOS) or 0.5 g/kg of inulin (10 degree of polymerization; Fibrulin, Warcoing, S.A. Belgium; treatment IN). Pigs were immunized by inactivated Aujeszky’s disease virus vaccine at d1 and 14 of the trial (28- and 43 d-age). Blood samples were taken at d1, 8, 15, 22 and 32 from 2 pigs/pen for lymphocyte stimulation tests (LST) with nonspecific mitogens (concanavalin A, pokeweed mitogen, phytohemagglutinin) and with specific mitogen (Aujeszky’s virus). All piglets were individually weighed on d1, 15 and 32. Data were analyzed by ANOVA. Feed additives enhanced the nonspecific cellular immune response at d32 (P < 0.05), but no treatment effect was observed on specific LSTs at any time. At the first 2 weeks of the trial ADG was lower in FOS and IN groups (207 and 201 g/d, resp.) compared with the NC and PC groups (234 and 228 g/d, resp; P < 0.05). However, there were no differences in growth rate among treatments (P > 0.05) either in the last 18 d (NC, PC, FOS, IN: 358, 361, 339, 346 g/d, resp) or in the whole nursery period (NC, PC, FOS, IN: 300, 299, 277, 278 g/d, resp). The ADFI and FCR values were similar in each period. In conclusion, fructo-oligosaccharide supplementation results in better non-specific cellular immune response regardless of degree of polymerization ranging from 3 to 10, even if no growth promoting effect is present.

Key Words: FOS polymerization, immune response, piglet

Astragalus polysaccharide reduces inflammatory response by decreasing permeability of LPS-stimulated Caco2 cells. X. Wang, Y. Li, X. Yang, and J. Yao*, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, China.

As the major constituent of Radix Astragali, astragalus polysaccharide (APS) is known for its anti-inflammation and immunomodulatory functions. The objective of this study was to investigate the effect of APS on inflammatory response and structural changes in lipopolysaccharide (LPS)-stimulated Caco2 cells. Caco2 cells were cocultured with APS and LPS, with APS added after the addition of LPS (post-addition), before the addition of LPS (pre-addition), or simultaneously with the addition of LPS (simultaneous addition). The mRNA expression of inflammatory indicators (TNF-α, IL-1β and IL-8) and tight junctions (zonula occludens-1 (ZO-1) and occludin) was measured by RT-qPCR. Short circuit current (Isc) was recorded by an Ussing chamber system. Data were subjected to one-way ANOVA using the GLM procedure of SAS, version 8.02. Means were separated by Fisher’s least significant difference multiple-range test. Addition of APS downregulated the expression of TNF-α, IL-1β and IL-8 (P < 0.05) and the Isc levels (P < 0.05) of LPS-stimulated Caco2 cells for all 3 administration treatments. The minimum anti-inflammatory concentration of APS was 50, 100, and 100 μg/mL for pre-, post-, and simultaneous additions of APS, respectively. The mRNA expression of ZO-1 and occludin was upregulated for post- and pre-additions of APS, respectively (P < 0.05). Results suggested that APS had context-dependent anti-inflammatory and tight junction protective properties for LPS-stimulated Caco2 cells, and may be used as a preventative treatment against LPS stimulation for intestine cells.

Key Words: astragalus polysaccharide, inflammatory response, tight junction