Nonruminant Nutrition: Gastrointestinal Physiology

T201 Intestinal short-chain fatty acid sensors, FFA2 and FFA3, and control of food intake. M. Al-Rammahi*, K. Daly, A. Moran, and S. Shirazi-Beechey, *University of Liverpool, Liverpool, UK.*

Dietary fiber and resistant starch are fermented by colonic microbiota to short-chain fatty acids (SCFA), acetate, propionate and butyrate. SCFA are known to have variety of physiological effects on gastrointestinal function. However, until recently little was known about the mechanism by which lumenal SCFA are sensed by colonic epithelial cells. Two orphan human G protein-coupled receptors, free fatty acid receptor 3 (FFA3; formerly GPR41) and free fatty acid receptor 2 (FFA2; formerly GPR43), have been cloned and demonstrated to be sensors for SCFA. It has been reported that, in response to dietary fiber, the large intestine secretes gut hormones such as glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), which control appetite and food intake, and SCFA have been implicated in this effect. Here we report, using qPCR, that FFA3 and FFA2 are expressed in human and pig colon with FFA2 having higher expression. Using immunohistochemistry, we show that FFA2 and FFA3 proteins are solely expressed in enteroendocrine L- and enterochromaffin cells of pig and human colon and not on the luminal membrane of colonic absorptive epithelial cells or intra-cellularly as shown by Karaki et al. (2006, 2008). Furthermore we show that FFA2 and FFA3 are co-expressed in endocrine cells containing, GLP1, PYY and serotonin. In addition, using GluTag cells, a murine enteroendocrine L-cell model, we show that these cells express FFA2 and FFA3 and that they secrete GLP-1 in response to either 10 mM butyrate or 10 μM chloro-α-(1-methylethyl)-N-2-thiazolylbenzeneacetamide (4-CMTB), a specific activator of FFA2.

Key words: dietary fiber, food intake, nutrient sensing

T202 Gene expression of the L-amino acid–sensing receptor T1R1/T1R3 changes in gut tissues of pigs in response to dietary protein. G. Tedo[1], E. Roura[1,3], I. Ipharraguerre*[1], and X. Manteca[2], *[1]Lucta SA, Feed Additives Division, Montornes del Vallés, Barcelona, Spain, [2]Autonomous University of Barcelona, Bellaterra, Barcelona, Spain, [3]Current address: University of Queensland, Brisbane, Australia.*

We have shown that the porcine umami taste receptor T1R1/T1R3 is present in pig′s gut and its mRNA abundance increases in the small intestine after weaning. The aim of this study was to determine if the expression of the pT1r1/pT1r3 genes in taste and gut tissues changes in response to variation in the content of dietary CP and essential AA (EAA). Forty-eight Pietrain x Landrace piglets were used from weaning (26 d of age) to 20 d after weaning. Piglets were allotted to 3 dietary treatments (16 piglets/treatment): high CP diet (HCP, 24%CP, 15 g/kg of Lys), low CP diet (LCP, 17%CP, 9 g/kg of Lys) and LCP diet supplemented with all EAA (SAA, 17%CP, 15 g/kg of Lys). Four animals per treatment were sacrificed on d 20 after weaning to collect tissue samples of fungiform and circumvallate papillae (TC), stomach (S), liver (L), duodenum (D) and ileum (I). Remaining piglets (12 pigs/treament) were used to monitor animal performance. The relative abundance of mRNA of the pT1r1 and pT1r3 genes was quantified via real-time PCR using the tata box binding protein as housekeeping gene. Real-time data were analyzed using the GEE model with an exchangeable correlation structure and the GENMOD procedure of SAS. Fold change estimations were performed for sex, diet, tissue and their interaction relative to liver and the HCP group. The expression of the pT1r1 gene was upregulated ($P < 0.05$) in S (3-fold), D (22.6-fold) and I (7.9-fold) of the LCP group, whereas the expression of the pT1r3 gene was upregulated ($P < 0.05$) in TC (1.7-fold), S (2.8-fold) and D (1.6-fold) of the SAA group and tended ($P < 0.06$) to increase in D (2-fold) and I (3.2-fold) of the LCP group. In summary, the expression of the porcine umami taste receptor genes mainly responded to changes in CP intake. Interestingly, supplementing the LCP diet with essential AA to meet piglet′s requirement tended to prevent such a response in the pT1r1 gene. Taken together, these observations suggest that the porcine umami taste receptor plays a role in sensing the enteral supply of protein.

Key words: gut sensing, amino acids, umami taste receptors

T203 Gene expression of the porcine sweet taste receptor in tongue and gut tissues changes after weaning. G. Tedo[1], X. Manteca[2], I. Ipharraguerre*[1], M. Reina[3], D. Torrallardona[4], and E. Roura[1,5], *[1]Lucta SA, Feed Additives Division, Montornes del Valles, Barcelona, Spain, [2]Autonomous University of Barcelona, Veterinary School, Bellaterra, Barcelona, Spain, [3]University of Barcelona Cell Biology Dpt., Celltec-UB, Barcelona, Spain, [4]IRTA -Mas de Bover, Constanti, Tarragona, Spain, [5]Current address: University of Queensland, Brisbane, Australia.*

Sugars and artificial sweeteners are sensed by the sweet taste receptor T1R2/T1R3 present in taste buds. Recent studies in rodents showed that this receptor is also present in the gut forming part of the mucosal chemosensing system by which luminal glucose and other chemicals are sensed to trigger gut physiological responses. The aim of this study was to investigate the expression and changes during development of the porcine T1r2 and T1r3 genes in taste and gastrointestinal tissues of pigs. Fifty-six Pietrain x Landrace piglets were selected at birth and fed standard diets from weaning until 46 d of age. On d 0 (birth), 26 (weaning), 28 (48h after weaning), and 46 from birth, 4 piglets (2 of each sex) were sacrificed to collect tissue samples of fungiform and circumvallate papillae (TC), stomach (S), liver (L), duodenum (D), jejunum (J), and ileum (I). Remaining piglets (40) were used to monitor animal performance. The relative abundance of mRNA of the pT1r2 and pT1r3 genes was quantified via real-time PCR using the tata box binding protein as housekeeping gene. Real–time data (Ct values) were analyzed using the GEE model with an exchangeable correlation structure and the GENMOD procedure of SAS. Fold change estimations ($2^{-\Delta\Delta Ct}$) were performed for sex, age, tissue and their interaction relative to liver and the weaning group (d 26). Both genes were expressed ($P < 0.05$) in all tissues at all ages and the interaction between age and tissue for the expression of the pT1r2 gene tended to be significant ($P < 0.07$). On d 46, the expression of pT1r2 was upregulated ($P < 0.05$) in TC (4.7-fold), D (11.1-fold), and I (17.4-fold), but downregulated in L (0.14-fold). In conclusion, the receptor T1R2/T1R3 is present in taste buds and gastrointestinal tract of pigs and its expression changes remarkably after weaning.

Key words: sweet taste receptor, pig, weaning

T204 Evaluation of seaweed-derived polysaccharides on indices of gastrointestinal fermentation and selected populations of microbiota in newly weaned pigs challenged with *Salmonella* Typhimurium. S. Dillon[1], J. Fanning[2], T. Sweeney[1], J. Egan[2], C. J. O'Shea[1], M. Gutierrez[2], C. Mannion[2], F. Leonard[1], and J. V. O'Doherty*[1], *[1]University College Dublin, Dublin, Ireland, [2]Cen-*

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Growing pigs encounter multiple stressors in the immediate post-weaning period, and become vulnerable to infection by microbial pathogens such as *Salmonella*. An experiment was conducted to investigate the effects of offering diets containing seaweed-derived laminarin or fucoidan on numbers of *Salmonella* Typhimurium in the distal gastrointestinal tract (GIT), in select tissue locations, and in fecal matter of pigs experimentally challenged with *Salmonella* Typhimurium. Twenty-four individually penned entire male pigs (n = 6), weaned at 24 d (7.9 kg) were assigned (d 0 to 32) to 1 of 4 dietary treatments: T1) basal diet (control); T2) basal diet + a commercial admixture containing organic acids and herbs (positive control; 3.6g/kg); T3) basal diet + laminarin (300 mg/kg); (T4) basal diet + fucoidan (240 mg/kg). Sampling of fecal matter was carried out periodically during the experiment and GIT contents and tissue samples were collected post-sacrifice (d 32). Consumption of diets containing fucoidan increased counts of lactobacilli in the cecum ($P < 0.05$) and the molar proportion of butyric acid in the cecum ($P < 0.05$) and colon ($P < 0.05$) and decreased the molar proportion of valeric acid in the cecum ($P < 0.05$) and colon ($P < 0.01$). However, fecal counts of *Salmonella* Typhimurium increased on d 2 ($P < 0.05$) and d 14 ($P < 0.05$) post-challenge (PC) of pigs offered fucoidan, and on d 14 ($P < 0.05$) and d 20 ($P < 0.05$) PC of pigs offered laminarin ($P < 0.05$) compared with the control. Diets containing the commercial admixture increased lactobacilli ($P < 0.05$) and butyric acid ($P < 0.05$) in the cecum and decreased counts of *Salmonella* Typhimurium ($P < 0.001$) in tonsil tissue. In conclusion, consumption of diets containing fucoidan induced increases in lactobacilli in the cecum, and butyric acid in the cecum and colon, however both laminarin and fucoidan increased shedding of fecal *Salmonella* Typhimurium at select sampling periods of the experimental study.

Key words: *Salmonella*, pig

T205 Fermentation activity of colonic microbiota from piglets fed diets including alfalfa, citrus pulp or inulin. S. Brambillasca*[1], M. Hernández[1], A. Britos[1], L. Reyes[1], P. Zunino[2], and C. Cajarville[1], *[1]Departamento de Nutrición Animal, Facultad de Veterinaria, UdelaR, Montevideo, Montevideo, Uruguay, [2]Departamento de Microbiología, Instituto de Investigaciones Biológicas Clemente Estable, MEC, Montevideo, Montevideo, Uruguay.*

The effect of including alfalfa, citrus pulp or inulin in diets for piglets on the fermentation activity of the colonic microbiota was studied. Twenty 4 cross breed piglets (12.1 ± 1.7kg BW) in a randomized complete block design were housed in metabolic cages and assigned to one of 4 diets for 23d: 100% corn and soybean meal based diet (CO), 97% CO+3% inulin (IN), 95.5% CO+4.5% fresh alfalfa (AL) and 95.5% CO+4.5% fresh citrus pulp (CP) in DM basis. The last day of the experiment all animals were euthanized and colonic digesta was individually sampled. In vitro gas production was performed using individual diluted colonic digesta as inoculum and pre-digested (pepsin+pancreatin) CO as substrate (6 flasks/animal, n = 144). Gas volume was recorded between 2 and 90h post inoculation. Asymptotic gas production (A, mL/g OM), time to reach 50% of the asymptote (B, h), maximal rate of gas production (Rmax, mL/h) and time of occurrence of Rmax (Tmax, h) were determined. DM disappearance (DMD) and organic matter disappearance (OMD) were determined by drying and ashing the fermentation residues respectively. Data were analyzed by PROC MIXED considering treatment effect, and means were separated by orthogonal contrasts. Rmax was the unique parameter affected by treatments and was highest for IN ($P = 0.003$). Gas production tended to be higher with fiber inclusion and AL tended to produce a higher B and Tmax than CP, whereas DMD for CP tended to be higher than AL. Piglets receiving IN presented a microbiota adapted to ferment substrates faster than the other treatments. Acknowledgments: ANII for scholarship of the first author.

Table 1. In vitro fermentation parameters for different treatments

	A (mL/g OM)	B (h)	Rmax (mL/h)	Tmax (h)	DMD (%)	OMD (%)
CO	146.3	1.77	23.0	1.71	37.0	40.3
IN	153.3	1.75	24.8	1.62	37.7	41.1
AL	148.6	1.78	22.2	2.06	35.8	42.3
CP	153.9	1.67	22.5	1.60	40.3	39.3
SEM	8.42	0.06	1.24	0.23	1.72	2.17
P						
CO vs ADDIT	0.09	ns	ns	ns	ns	ns
IN vs AL+CP	ns	ns	0.003	ns	ns	ns
AL vs CP	ns	0.07	ns	0.06	0.06	ns

ADDIT: additives; SEM: standard error of means; P: probability of contrasts ($P \leq 0.05$).

Key words: fiber, hindgut fermentation, swine