
COMPARATIVE GUT PHYSIOLOGY

0438 β -hydroxybutyrate and glucose concentrations in the blood of dairy calves. F. X. Suarez-Mena*, W. Hu, T. S. Dennis, T. M. Hill, J. D. Quigley, R. L. Schlotterbeck, *Provimi, Brookville, OH.*

This research was conducted to determine how blood β -hydroxybutyrate (BHBA) and glucose are impacted by age, time of day, voluntary starter intake, stress, weaning, and intake restriction in 1- to 9-wk-old calves and to see if either is an acceptable proxy for starter intake. Male Holstein calves were fed a 27% CP, 17% fat milk replacer at 660 g DM daily to weaning on d 42, along with free-choice starter (20% CP, 41% starch) and water. Jugular blood was sampled at 0800, 1200, and 1600 h and within 5 min of sampling BHBA and glucose concentrations were estimated using test strips (Nova Max® Plus meter, Nova Biomedical). Age effects were estimated by sampling blood weekly (d 6, 13, 20, 27, 34, 41, and 48). To determine vaccination stress, a *Pasteurella* vaccine was administered after blood sampling at 0800 h on d 36. Effect of voluntary starter intake was tested by selecting calves for low and high intakes (d 35 to 39) and sampling on d 40, 41, 43, and 44. Starter intake restriction was tested by restricting intake in 6 of 12 calves and sampling on d 60 and 61. Data were analyzed using several mixed model procedures (repeated measures, regression, etc.) in SAS. Time of day did not impact blood BHBA or glucose to wk 6, but did in wk 7 ($P < 0.05$). Blood glucose was greater ($P < 0.05$) in the first 5 wk compared with wk 6 and 7. Blood BHBA increased ($P < 0.02$; $R^2 = 0.28$) and glucose decreased ($P < 0.02$; $R^2 = 0.23$) with increasing starter intake. Blood BHBA declined ($P < 0.05$) due to vaccination but glucose was unaffected. Starter intake restriction reduced BHBA for 3 d ($P < 0.05$) and glucose for 2 d ($P < 0.05$) after intake restriction. Around weaning (d 40 to 44), BHBA and glucose increased ($P < 0.05$) with increasing starter intake. Blood BHBA was positively and glucose negatively related with starter intake; however, relationships were weak, variable, and impacted by time of day, stress, and intake restriction. Over 30% of calves tested ≤ 0.2 mmol/L BHBA when consuming > 1250 g/d of starter, and test strip increments were 0.1 mmol/L which represented $> 25\%$ of the mean blood BHBA concentration. In this study, neither blood BHBA nor glucose were an acceptable proxy for estimating starter intake.

Key Words: blood β -hydroxybutyrate, dairy calves, intake

0439 Comparison of intestinal goblet cell staining methods in turkey poults. S. O. Osho*, T. Wang, N. L. Horn, and O. Adeola, *Department of Animal Sciences, Purdue University, West Lafayette, IN.*

This study compared the intestinal goblet cell density of turkey poults at two different ages using Alcian blue-Periodic acid shiff (AB-PAS) and Mucicarmine stains. Neutral mucins are stained with PAS while acidic mucins are stained with AB. Mucicarmine is specific to the mucins of epithelial origin and it is currently used for human samples, Mucicarmine may have advantages for use in animals as a result of the methodological simplicity of staining as compared with AB-PAS. Jejunum samples were taken from 80 turkey poults at 21 and 28 d, and were assigned to two treatments which consisted of AB-PAS and Mucicarmine stains in a completely randomized design. A mid-section of jejunum from each bird was taken and placed in 10% buffered formalin for 48 h, dehydrated with ethanol, cleared with Sub-X and placed in a paraffin, prepared on two slides, and then tissues were briefly cleared and hydrated. Each slide was stained with either AB-PAS reagents or Mucicarmine reagents. Goblet cell counts were taken from four villi per slide and the villi height was measured and averaged. There was no difference in the goblet cell density between the staining methods AB-PAS and Mucicarmine at 21 or 28 d post-hatching. These results show that both staining methods are viable for assessment of goblet cell density in turkey poults.

Key Words: goblet cells, jejunum, stain

0440 The development of a cecum-cannulated gnotobiotic piglet model to study the human gut microbiota. N. D. Aluthge*¹, W. Tom², T. E. Burkley², D. E. Hostetler², K. D. Heath², C. Kreikemeier², and S. C. Fernando², ¹*University of Nebraska, Lincoln*, ²*University of Nebraska, Lincoln.*

Research conducted over the past decade using high-throughput DNA sequencing technologies have provided valuable insights into the importance of the human gut microbiota in host health and disease. Most of these studies, however, have been associative and causality of the gut microbiota in human health-associated conditions has been difficult to demonstrate due to the lack of a suitable animal model which can faithfully recapitulate the interactions between the human host and the gut microbiota. The domestic pig (*Sus scrofa*) has been used as a clinically relevant model to study various aspects of human disease and shares a high degree of anatomical, physiological, and immunological similarities with humans, thus being a potentially valuable model for human gut microbiota studies. This study was conducted with the objective of establishing human gut microbial communities in gnotobiotic piglets and to investigate the potential of using cecum cannulation as a means of obtaining microbial community samples for time series and microbial gene expression studies. Six germ-free

piglets derived using cesarean section were transferred into isolator bubbles and at 7 d of age, 3 piglets were inoculated with fecal bacteria from high body mass index (BMI > 30) human donors and the remaining 3 animals were inoculated with fecal bacteria from low BMI (BMI < 25) donors. After weaning, the piglets with the high BMI microbiota were provided a high-fat (HF) diet while the piglets with the low BMI microbiota were fed a low-fat (LF) diet. At wk 7, the high BMI microbiota piglets were cecum-cannulated and the low BMI microbiota piglets were similarly cecum-cannulated at wk 8. A cecal sample was collected from each animal immediately before surgery for use as a control for comparing cecal bacterial communities. Cecal samples were collected via the cannulae from all animals at weekly intervals until wk 10 (when the animals were euthanized). The cecal samples were sequenced using the Illumina MiSeq™ DNA sequencing platform to characterize the bacterial community composition. Comparison of the cecal bacterial communities of the cannulated piglets before surgery and at later time points revealed similar composition (PERMANOVA, $p = 0.105$), indicating no negative impact of cannulation on cecal bacterial community structure. BMI-Diet type had a significant impact on structuring cecal bacterial communities (PERMANOVA, $p < 0.001$). These results point to the potential use of cecum-cannulated humanized piglets as a model system to study the human gut microbiota.

Key Words: human microbiota, high-throughput DNA sequencing, piglet model

COMPARATIVE GUT PHYSIOLOGY SYMPOSIUM

0441 Diet, gut microbiome, brain and behavior.

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The gut microbiome consists not only of bacteria but also viruses (virome) and fungi (mycobiome). There is considerable evidence that gut bacteria influence the structure and function of both the enteric and central nervous systems and that changes in the microbiome can affect mood and cognitive functions. Dietary change alters the gut bacterial content and also the virome and these are in turn associated with changes in behavior and cognition. The pathways whereby these changes occur are multiple and interacting, and we are only just beginning to understand how these occur, but their importance to animal health is undoubted. This presentation will explore how microbes effect these changes and the pathways that may be involved in so doing from lumen to brain.

Key Words: microbiome, gut-brain axis, virome

0442 Butyrate increases tight junction protein expression and enhances tight junction integrity in porcine IPEC-J2 cells stimulated with LPS.

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The intestinal mucosal barrier is maintained by tight junctions, which are intercellular adhesion complexes and prevent the passage of pathogens and toxins through the paracellular space. Dysfunction of tight junctions induced by endotoxin and mycotoxin is highly associated with a variety of gastrointestinal disorders in pigs. Butyrate has been shown to possess immunological and metabolic modulatory effects in various cells and tissues. Therefore, we investigated protective effect of butyrate on cell integrity and tight junction protein expressions during LPS stimulation in porcine IPEC-J2 cells. We found that butyrate (1mM) and LPS (10µg/ml) significantly induced TNF α , IL-1 β , IL-6, IL-8 and MCP1 expression ($P < 0.05$) as well as IL-8 secretion. However, although LPS upregulated TLR4 expression, butyrate downregulated it ($P < 0.01$) indicating butyrate could inactivate LPS stimulation of TLR4 pathway. Barrier integrity was investigated with trans-epithelial electrical resistance (TEER) and fluorescein isothiocyanate-dextran (FITC-dextran) uptake based tests. Treatment with LPS for 24 h significantly decreased TEER ($P = 0.01$) and increased cell permeability ($P = 0.02$). On the contrary, butyrate (1 mM) significantly increased TEER ($P < 0.01$) and decreased cell permeability ($P < 0.01$), indicating that butyrate could increase cell integrity and enhance epithelial barrier against LPS-induced damage. Butyrate also induced *Claudin-1* ($P = 0.09$), *Claudin-3* ($P < 0.01$) and *Claudin-4* ($P < 0.01$) mRNA expression, and *Claudin-3* protein expression ($P < 0.05$) in a dose-dependent manner, perhaps accounting for the increase in epithelial barrier integrity induced by butyrate. Butyrate also increased ($P < 0.01$) activation of Akt by phosphorylation, whereas LPS exerted the opposite effect. Taken together, butyrate increased basal immune response and enhanced the integrity of the intestinal mucosal barrier against LPS-induced damage through an upregulation of cytokine expression and an increase in the synthesis of tight junction proteins.

Key Words: Akt, butyrate, epithelial barrier integrity, IPEC-J2 cells, tight junction protein

0443 Understanding host-microbiota interplay using nutrimentabonomics.

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Gut microbiota are now recognized as fundamental partners of the host's health. Normally, the host-microbiota symbiosis results in a healthy metabolic phenotype. But as the