

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

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## COMPARATIVE GUT PHYSIOLOGY SYMPOSIUM

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### 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

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### 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 $\alpha$ , IL-1 $\beta$ , 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

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### 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

environment changes, our metabolism adapts to maintain homeostasis within an optimal metabolic space, and so do our microbiota. So how does this interplay result in an optimal metabolic state? And how can this be measured? Nutrimetabonomics is a useful tool to assess the metabolic state of the host in response to environmental perturbations. Here, we will illustrate how it was used to gain new understanding of the metabolic disruptions triggered by *Brachyspira pilosicoli*-induced spirochaetosis, a common condition in poultry farms. We will discuss how a better knowledge of the host metabolic response to the pathogen, and to the antibiotic treatment, can help design new therapeutic alternatives to antibiotics.

**Key Words:** gut microbiota, nutrimetabonomics, host-pathogen interaction

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#### 0444 Effects of dietary fibers on obesity related physiological parameters in C57BL/6 mice.

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Obesity, a metabolic disease resulting from an imbalance between caloric intake and expenditure, is a global concern. Studies suggest that the intake of dietary fiber improves metabolic health; however, the amount of dietary fiber and the fiber type that contribute to this improvement is unclear. This completely randomized study investigated the effect of 1.25, 2.5, and 5.0% (w/w) glucomannan or oat  $\beta$ -glucan in the diet versus a control diet on metabolism. Obesity related variables such as liver steatosis, and short chain fatty acid (SCFA) production was evaluated in diet-induced obese male C57BL/6 mice. Six-wk-old mice ( $n = 84$ ) were fed one of 7 diets for 12 wk. On d 84, whole blood was collected and serum metabolites were analyzed. Small liver lobe portions were used to examine steatosis severity and cecum samples were analyzed for SCFA concentration. The glucomannan diets had an interaction between fiber and their inclusion levels for relative liver weight ( $P < 0.05$ ) and percent steatosis ( $P < 0.001$ ). The oat  $\beta$ -glucan diet resulted in lower serum triglyceride concentrations ( $P < 0.05$ ), whereas including glucomannan in the diet resulted in higher acetate and propionate levels ( $P < 0.05$ ) in comparison to the other dietary treatments. In the liver, the inclusion of 2.5 and 5% of fiber caused a decrease in microvesicular fat in comparison to the inclusion of 1.25% of fiber. This study highlights that the inclusion of glucomannan and oat  $\beta$ -glucan fiber in the diet at specific inclusion levels is capable of having significant effects on relative liver weight, percent steatosis, and serum triglycerides in obese mice. Glucomannan decreased the severity of mediovesicular fat, while both fibers decreased severity of macrovesicular fat. Thus, supplementing a diet with an adequate amount of specific dietary fiber may be a strategic method to reduce obesity in animals, and this may eventually be translated toward treating

human obesity to reduce obesity related health issues.

**Key Words:** fiber, obesity, mice

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#### 0445 The gut microbiome as a regulator of physiology, brain and behavior: Implications for the treatment of stress-related disorders.

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It has become increasingly clear that multiple aspects of host physiology are heavily influenced by the gut microbiome. Included in this remit is not just host metabolism and body composition but also a marked influence on the stress response via the hypothalamic-pituitary-adrenal axis. This is clear from studies in microbiota-deficient germ-free animals who display exaggerated responses to acute stressors that can be normalized by monocolonization with certain bacterial species including *Bifidobacterium infantis*. Also coming into focus is microbial regulation of the metabolism of tryptophan, an essential amino acid and precursor to serotonin, a key neurotransmitter within both the enteric and central nervous systems. The gut microbiota may thus be a tractable target for treating or preventing stress-related microbiome-gut-brain axis disorders and metabolic diseases. Moreover, the implications of these findings need to be considered in the context of new control points for endocrine-immune-metabolic targeting in farm and domestic animal physiology and behavior.

**Key Words:** gut microbiome, stress, tryptophan

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#### 446 The microbiota-gut-brain axis: A key regulator of neural function across the life span.

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The microbiota-gut-brain axis is emerging as a research area of increasing interest for those investigating the biological and physiological basis of neurodevelopmental, age-related and neurodegenerative disorders. The routes of communication between the gut and brain include the vagus nerve, the immune system, tryptophan metabolism, via the enteric nervous system or by way of microbial metabolites such as short chain fatty acids. Studies in animal models have shown that the development of an appropriate stress response is dependent on the microbiota. Developmentally, a variety of factors can impact the microbiota in early life, including mode of birth delivery, antibiotic exposure, mode of nutritional provision, infection, stress as well as host genetics. At the other extreme of life, individuals who age with considerable ill health tend to show narrowing in microbial diversity and a proinflammatory phenotype. Stress can significantly impact the microbiota-gut-brain axis at all stages across the life span. Recently, the gut microbiota has been implicated in a variety of conditions including autism, schizophrenia and Parkinson's disease. Moreover, fundamental brain processes from adult

hippocampal neurogenesis to prefrontal cortex myelination to microglia activation have been recently shown to be regulated by the microbiome. Further studies will focus on understanding the mechanisms underlying such brain effects.

**Key Words:** myelin, neurodevelopment, psychobiotic, stress

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**0447 Microbial modulation of the neonatal immune system: Lessons from infants and piglets.**

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Studies from germ-free and gnotobiotic animals clearly demonstrate that basic developmental features of the mammalian immune system depend on interactions with the microbiome. The objective of this presentation is to review how early life nutrition and the microbiome influence immune development and function in the neonate. Comparative aspects between different forms of nutrition (mother-fed versus artificially reared) on systemic and mucosal immunity and findings across species (human versus piglet) will be highlighted. Briefly, our laboratory has shown that the T cell and natural killer cell repertoire and cytokine secretion profiles differ by mode of nutrition in both species. In addition, although the composition of the microbiota differs between human infants, being bifidobacteria-predominant, and piglets, where lactobacilli predominate, the microbiome composition of both species responds to mode of nutrition and the addition of prebiotics to formula. Data from our group on the impact of transfaunation of breast-fed infant microbiome into piglets on piglet gut gene expression will be presented. Lastly, findings from our laboratory showing cross-talk between the bacterial metagenome and the intestinal epithelial transcriptome of human infants using shed epithelial cells will be described. Supported by NIH grant no. R01 HD061929 and Hatch ILLU-698-311.

**Key Words:** microbiota, human, swine, immunity, nutrition

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**0448 The growing importance of defining gut “health” in animal nutrition and health.** P. Celi<sup>\*1</sup>,

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Optimal gastrointestinal health (effective immune status, normal and stable microbiota, absence of inflammatory state) and functionality (digestion and absorption of feed) are essential for sustainable animal production (growth, milk yield, meat

and egg quality). However, while gut health is an increasingly important topic in animal nutrition, a clear scientific definition is still lacking although it has been used repeatedly in animal health. A clear definition of gut health and how it can be measured is required to monitor animal health and to evaluate the effects of any nutritional intervention on animal performance. While in human medicine gut health is often associated with the “absence of clinical diseases,” this definition cannot be applied to farm animals as it is well known that animal performance can be impaired without any clinical signs of disease. Perhaps a more comprehensive definition of gut health would be “a steady state where the microbiome and the intestinal tract exist in symbiotic equilibrium and where the welfare and performance of the animal is not constrained by intestinal dysfunction.” This definition combines the principal components of gut health, namely diet, effective structure and function of the gastrointestinal (GIT) barrier and normal and stable microbiota, with effective digestion and absorption of feed and effective immune status. All these components play a critical role in GIT physiology, animal health, welfare and performance. Clarity of understanding of gut health will require the characterization of the interactions between all of these components. The development of biomarkers of gut health is imperative to gain clarity of understanding of the pathophysiological events that influence the intestinal barrier, its functionality and the ecology of the GIT microbiota. While there is considerable knowledge in biomarkers that are indicative of the GIT ability to digest, absorb, transport and secrete major macro and micronutrients, a large gap in the literature exists in relation to biomarkers of GIT permeability, GIT barrier function, or biomarkers that are indicative of the functional presence of beneficial microbiota or their metabolites. Therefore, future research should focus on the establishment of a reference panel of biomarkers of gut health to be used in farm animals and address the issue of standardization of techniques and methodologies to study gut health.

**Key Words:** biomarkers, gut health, microbiome

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**0449 The microbiome and animal health.**

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For monogastrics, the linkage between the microbiome and animal health has been established, and it is known that colonization of the gastrointestinal tract (GIT) stimulates development of the immune system. In ruminants, the microbiome has largely been evaluated to assess the potential contribution

toward feed digestion and adaptive responses as a consequence of dietary change. For example, previous research has shown a positive relationship between the Firmicutes:Bacteroidetes ratio and milk fat yield, and that the abundance of Clostridiales (family VIII) is negatively associated with feed conversion. A positive association for the systemic acute phase response caused by a severe form of subacute ruminal acidosis and the prevalence of *Escherichia coli* in the rumen has also been reported. Separating cause and effect continue to be a challenge with this area of research. The ruminal microbiome is responsive to diet and changes are particularly evident when comparing high-forage and high-grain feeding scenarios. That said, there is evidence to suggest that the microbial community is relatively resistant to change and can revert back to a composition similar to the original community structure after being disturbed. The microbiome robustness presents a challenge when modifications to the community structure may be desired. Moreover, differences between the digesta associated versus mucosa and epithelia associated communities are present and these communities change throughout the GIT. While understanding the rumen microbiome is important, more distal regions of the GIT have not been thoroughly examined. The change in microbial community structure along the GIT may not be that surprising given changes in retention time and substrate availability. In addition, microbial-host crosstalk mechanisms may differ among regions helping to explain why the microbial community structure differs. Understanding the regulation of the microbial-host communication may provide the necessary information to develop practical strategies to modulate the microbial community structure. Accordingly, evaluating strategies to manipulate microbial colonization and succession in pre-ruminants appears to be a logical intervention strategy. In addition to the core microbiome, diversity of the microbiome appears to be a critical aspect and calves that develop scours have been reported to have lower diversity when evaluating the fecal microbiome. Thus, a systematic approach to improve our understanding of the relationship between microbiome, or at least key species, is needed to advance this area. Such research will require an in depth understanding of both the microbiome and host gastrointestinal physiology.

**Key Words:** gastrointestinal tract, microbiome, cattle

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**0450 In vitro fermentation characteristics of agricultural products and coproducts and its effect on the large intestinal microbiota of swine.** U. P. Tiwari<sup>\*1</sup>, S. Mattus<sup>1</sup>, K. Neupane<sup>2</sup>, and R. Jha<sup>1</sup>, <sup>1</sup>University of Hawaii at Manoa, Honolulu, <sup>2</sup>University of Hawaii, Leeward Community College, Pearl City.

Dietary fibers and resistant starches are fermented in the gastrointestinal tract (GIT) and alter the microbial community. Specific microbes in the GIT are found to promote host health, the microbial population is also dependent on the type

of fermentation substrates available in the GIT. Alternative feedstuffs are explored and evaluated to contribute in reducing feed costs of swine. These feedstuffs are typically rich in fiber and/or resistant starches which may provide prebiotic effects for the pigs. Six alternative feedstuffs were evaluated for their fermentation characteristics and effect on the microbiota of the large intestine of swine using an in vitro model. Three fibrous (macadamia nut cake, MNC; barley brewers grain, BBG; wheat millrun, WMR) and three starchy (Okinawan sweet potato, OSP; yam, and taro) feedstuffs along with inulin and blank as a positive and negative control, respectively were used in this study. After two-step enzymatic digestion assay, residues were fermented using fresh pig feces as microbial inoculum and gas production were recorded periodically. The residue after 72 h of microbial fermentation was used for genomic DNA isolation. The V3 region of the 16S rDNA of the genome was amplified using bacterial primers and the product used to generate banding profiles via temperature gradient gel electrophoresis (TGGE). The unique profile created by each sample was analyzed, and compared with determine similarities between samples. The fibrous feedstuffs (MNC, BBG and WMR) were most closely related to each other, and to inulin, indicating they may cause a health-promoting shift in the microbial community as inulin. The starchy feedstuffs (OSP, yam and taro) also showed similarities to each other, but were less related to inulin, with the exception of OSP, which had a similar profile to inulin. The MNC was least similar to the starchy feedstuffs. Total gas production of OSP (298), inulin (291) and taro (276) were significantly higher ( $P < 0.01$ ) than MNC (87) and BBG (75 mL/g sample). In conclusion, some of the alternative feedstuffs tested may exert comparable prebiotic effects to inulin, thus may be included in swine diets to favorably impact the GIT microbiota.

**Key Words:** coproducts, fermentation, gut microbiota

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**0451 Analysis of the gut microbiome in beef cattle and its association with feed intake, growth, and efficiency.** P. R. Myer<sup>\*1</sup>, J. E. Wells<sup>2</sup>, T. P. L. Smith<sup>2</sup>, L. A. Kuehn<sup>2</sup>, and H. C. Freetly<sup>2</sup>, <sup>1</sup>University of Tennessee Institute of Agriculture, Knoxville, <sup>2</sup>USDA-ARS, U.S. Meat Animal Research Center, Clay Center, NE.

Next-generation sequencing has taken a central role in studies of microbial ecology, especially with regard to culture-independent methods based on molecular phylogenies of the small-subunit ribosomal RNA gene (16S rRNA gene). The ability to relate trends at the species or genus level to host/environmental parameters using 16S profiling has proven powerful. Within the rumen and lower gastrointestinal tract (GIT), the diverse microbial ecosystems present are essential for the host to digest plant material and regulate nutrient uptake and utilization. Their examination utilizing next-generation technologies has been instrumental to aid in the understanding of

the microbial-associated interactions throughout the gut with intake, growth, and feed efficiency. Using a feed efficiency design in which steers were selected from two contemporary groups and were ranked based on their standardized distance from the bivariate mean (ADG and ADFI), four steers with the greatest deviation within each Cartesian quadrant were sampled ( $n = 16/\text{group}$ ; 2 groups) to examine the association of the microbiome throughout the gut with ADG, average daily DMI (ADFI), and feed efficiency. In addition, phylogenetic analyses of the ruminal bacterial community were compared based on varying sequencing technologies, 16S variable region selection, and short read 16S amplicons, near full-length 16S amplicons, and metagenomic sequence. In all studies, although no differences in bacterial diversity and richness metrics were revealed among the quadrants, finer changes in the relative abundance of microbial populations and operational taxonomic units did reveal differences between feed efficiency groups ( $P < 0.05$ ), suggesting throughout the GIT, the microbial communities differ at the 16S level in cattle that vary in ADG, ADFI, and feed efficiency. However, additional phylogenetic analyses on the rumen bacterial community demonstrated that utilizing near full-length 16S reads may be useful in conducting a more thorough study, or for developing a niche-specific database to utilize in analyzing data from shorter read technologies when budgetary constraints preclude use of near-full length 16S sequencing. Partially funded by National Institute of Food and Agriculture Grant no. 2011-68004-30214, National Program for Genetic Improvement of Feed Efficiency in Beef Cattle.

**Key Words:** feed efficiency, microbiome, 16S rRNA

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## CONTEMPORARY AND EMERGING ISSUES SYMPOSIUM: COMMUNICATING ANIMAL SCIENCES EFFECTIVELY

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**0452 Public perceptions of animal-sourced genetically modified food products.** W. K. Hallman\*, C. L. Cuite, and X. K. Morin, *Rutgers University, New Brunswick, NJ.*

The success of agricultural biotechnology depends as much on consumer acceptance of Genetically Modified (GM) products as it does on the ability to create them. To explore public perceptions of GM food products, we surveyed a nationally representative sample of 1148 American adults during October 23–27, 2013. The data was collected by GfK Knowledge Networks from an internet panel recruited using proportional random sampling. The data was weighted to project to the U.S. population, and has a margin of error of  $\pm 3\%$ . The results show that despite the ongoing controversy over GM foods, 50% of Americans report having heard or read little or nothing about them, 55% report that they know very little or nothing at all about them, and two-thirds (66%) say they have

never discussed the issue of GM foods with anyone. Estimates are that 75% of processed foods in the U.S. contain ingredients derived from GM crops. However, only 43% of Americans say that they believe that there are foods containing GM ingredients in supermarkets right now, while 4% say there are no such foods in U.S. supermarkets, and 51% say they don't know. Many of those who believe that there are GM foods in the supermarket are confused about which products are available. For example, while 75% correctly believe that there are products in U.S. supermarkets containing GM corn, and 59% correctly believe that there are products containing GM soy, nearly as many (56%) believe that GM tomatoes, GM Wheat (55%), and GM Chicken (50%) products are available and 35% believe that GM salmon are currently for sale. Moreover, even though GM food products have been on the market in the U.S. for more than two decades, only 26% of Americans believe that they have ever eaten a food containing GM ingredients. Yet, while most Americans say they have heard and read little about GM foods, know little about them, have never had a conversation about them, don't believe they are currently in the supermarket, and don't believe they have ever eaten them, most are willing to express an opinion about the acceptability of GM food products. When asked directly, only 10% of consumers say they approve of GM animal-sourced food products, 44% say they disapprove of them, and 43% neither approve nor disapprove of them, or are unsure. However, there is much greater public acceptance expressed when specific product benefits are described.

**Key Words:** public perceptions, genetically modified, animal-sourced foods

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**0453 What is the science of science communication for? And why should animal scientists care?** D. Kahan\*, *Yale Law College, New Haven, CT.*

The source of nearly every science-communication misadventure can be traced to a single mistake: the confusion of the processes that make science valid for the ones that vouch for the validity of it. The scientific knowledge that individuals rely on in the course of their everyday lives is far too voluminous, far too specialized for any—including a scientist—to comprehend or verify for herself. So how do people manage to pull it off? What are social cues they rely to distinguish the currency of scientific knowledge from the myriad counterfeit alternatives to it? What processes generate those cues? What are the cognitive faculties that determine how proficiently individuals are able to recognize and interpret them? These questions not only admit of scientific inquiry; they demand it. Unless we understand how ordinary members of the public ordinarily do manage to converge on the best available evidence, we will never fully understand why they occasionally do not, and what can be done to combat these noxious sources of ignorance. I will discuss these basic themes and relate them to the stake that the animal science community has in the