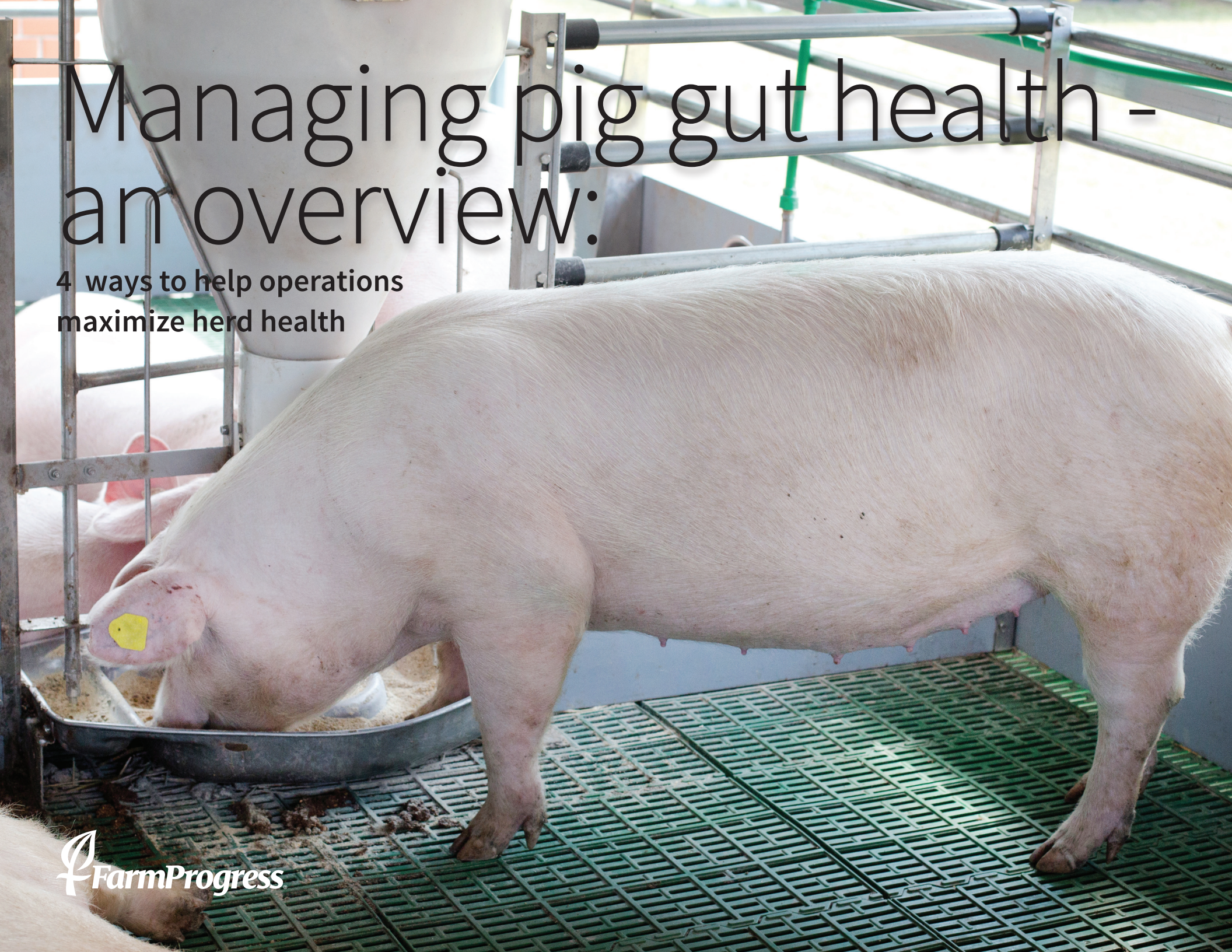
Managing pig gut health - an overview:

4 ways to help operations maximize herd health



FarmProgress

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Raising pigs is a bit like rearing babies: a cornerstone of the health of the whole animal or the whole person is their gut health. Scientific investigations into hot gut health are at the early stages, but important information has already come to light that will help producers raise healthier animals that perform better and do so with greater feed efficiency. Better gut health practices mean healthier herds.

This paper outlines four aspects of gut health science that can help the operator maximize herd health: weaning-age, antibiotic use, inclusion of fermentable fiber, as well as the aim of current research, to develop a clearer picture of the overall microbiome in the pig gut, with an increasing understanding of which gut flora and fauna perform what role in digestion, immune response and other functions.

Recent research demonstrates how weaning age and sex (i.e., being female or male) influence gut development and health throughout the lifetime of the pig. Early weaning creates stress that can have a lifelong impact. Waiting a little longer to wean can foster better health into the later stages of the pig's life. Also, early-weaned gilts show more negative effects to their gut-health than early-weaned barrows. And though these gilts have more symptoms of gut stress, they also have lower mortality than early-weaned barrows, showing that the stress may have an adaptive function in survival. In any case, waiting until the 28-30 day period to wean seems to provide benefits compared to weaning at the age of 16 or 17 days.

Another line of inquiry shows that antibiotics have powerful effects on the composition and structure of the pig's gut microbiome. These changes are likely linked to changes in the health of both the intestine locally and the host systemically.

Research is beginning to show how differences in microbial composition of the GI tract may be correlated with a pig's health and performance. Eventually, the goal is to evaluate and validate cause-and-effect relationships between these correlations.

Fiber has become an increasingly important topic in swine nutrition, specifically how does fiber impact other components of the diet. Fiber can be a positive factor in the diet because it can improve gut health. Much of the benefit accrues from fiber that is easily fermented rather than fiber that is poorly fermented. We have data showing that pig performance may be improved when exposed to a pathogenic *E. coli* challenge when fermentable soluble fiber is included in the diet, much more so than when poorly fermented fiber is present. More research is required in this area, but it is clear that the health of the young pig can be improved by increasing the levels of fermentable fiber in the diet.

Gender and stress matter in pig gut health

By Adam Moeser, M.S., DVM, Ph.D., Matilda R. Wilson Endowed Chair, Michigan State University Department of Large Animal Clinical Sciences' Gastrointestinal Stress Biology Laboratory

As the U.S. swine industry transitions away from antimicrobial growth promotants, an increased focus has been placed on developing alternative methods to preserve gut health and animal performance. However, the underlying biological basis of many gut health disorders in pigs remains poorly understood, and thus well-defined and predictable biomarkers and biological targets for modifying gut health are lacking.

This represents a large gap in knowledge, with profound health, welfare and economic implications to the U.S. swine industry.

Therefore, there remains a critical need for fundamental research investigating important factors (e.g., environmental and biological) that impact gut development and disease risk in pigs, which will be essential for the discovery of targeted, effective gut health therapeutics and the identification of potentially new management practices. This article will provide an overview of gut health and highlight recent research demonstrating how wean age and sex (i.e., being female or male) influence gut development and health throughout the lifetime of the pig.

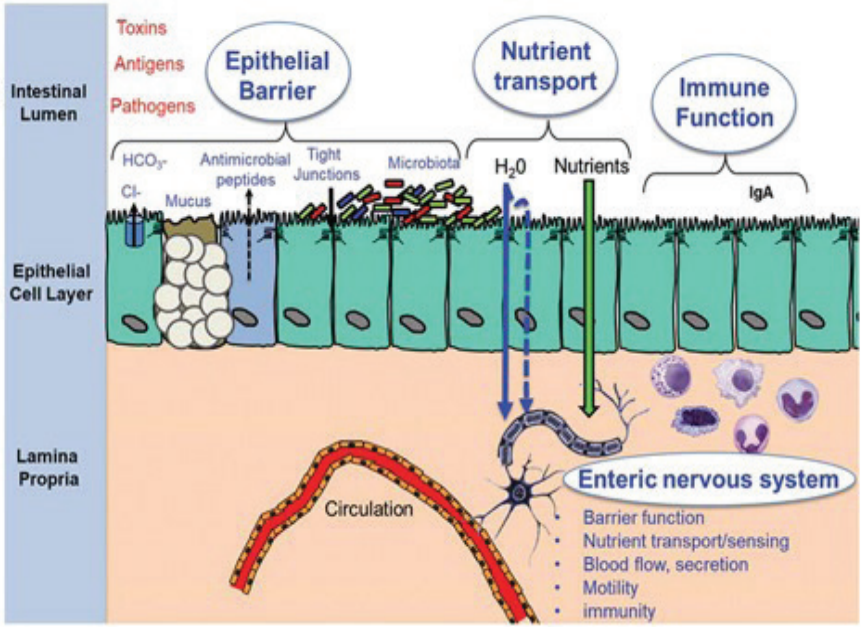


Figure 1: Major components of gut health

What is gut health, and how is it measured?

This topic has been subject to much debate as we continue to search for true biomarkers that reflect gut health. Gut health is best defined in terms relative to the critical functions the gut performs on a daily basis. First, the gut must provide a selective permeability barrier (aka barrier function) to limit or tightly regulate the exposure of environmental antigens (e.g., feed antigens), toxins and microorganisms to the gut mucosal immune system. Excessive intestinal permeability causes intestinal inflammation, and in severe cases, sepsis and death. Second, the gut is an important immune barrier that includes the ability to initiate a rapid and robust immune response required for clearance of pathogens. The gut immune system also functions to establish an immunologically tolerant environment to educate, develop and prevent overwhelming inflammatory responses in the highly antigenic gut lumen. Third, the gut must facilitate nutrient uptake (e.g., nutrient digestion, absorption and metabolism) to support overall organ system function and growth (Figure 1).

While seemingly distinct, there is considerable overlap in gut functions. For example, in addition to optimal growth, efficient nutrient absorption is required during the time of stress and disease challenges to support critical functions of the immune system.

Growth performance and feed efficiency measurements have been classically used as indexes of gut health. However, this measure alone does not always reflect optimal gut health.

Under specific environmental conditions such as high health status or concurrent use of antibiotics, pigs can exhibit good performance and be disease-free despite subtle defects in underlying gut dysfunction (e.g., increased gut permeability or leaky gut, poorly developed or suppressed gut immune function, etc.). The impact of gut dysfunction on performance and disease may not be revealed unless the pig is faced with production stressors or an infectious challenge, both common in swine production.

Stress impacts gut health

In swine production, pigs are exposed to numerous stressors including weaning, temperature fluctuations (cold and heat stress), mixing and transport — which is known to erode gut health and performance and increase the vulnerability to infectious pathogens. The ability of the pigs to respond and resolve the stress, i.e., their stress resilience, is a major determinant of whether they will exhibit compromised gut health and disease resistance.

On the one hand, the pig must be able to initiate a robust physiologic response to stress which involves activation of the fight-or-flight response — a conserved mechanism critical for survival. The stress response is mediated largely by activation of the hypothalamic pituitary adrenal axis (aka fight-or-flight response), which is critical for mobilization of body resources (e.g. blood flow, nutrients), enhancement of immune function, and defense and initiation of behaviors that help to cope with the stress and return to homeostasis once the stressor has been dealt with or adapted to.

Alternatively, the stress response can often become detrimental, such as in situations where the stressors are overwhelming (e.g., multiple concurrent stressors or a high stress load) or chronic in nature, which can exhaust coping mechanisms and prevent the return to homeostasis.

The timing of stress matters. Early-life stress induces long-term detrimental effects on gut health.

Weaning is the most stressful period in pig production. During the weaning period, the young pig is exposed to multiple concurrent stressors including maternal separation, separation of littermates and disruption of social hierarchy, abrupt changes in diet and environment and often long transportation times. Importantly, weaning stress occurs during a major postnatal development window, where many biological systems in the pigs are undergoing extensive development and maturation (Reviewed by Pohl et al., 2016; Moeser et al., 2018).

For example, in the first three to four months of postnatal life, there is extensive development and programming of the HPA axis stress response system, intestinal barrier function, and gut immune and nervous systems.

It is therefore not surprising that the length of postnatal gut development coincides with the natural weaning process in wild pigs, which is gradual and occurs at three to four months of age. Because environmental influences play a key role in shaping postnatal development of the gut (and other systems), significant stress or trauma can alter the programming of a gut function, having potentially lasting detrimental impacts.

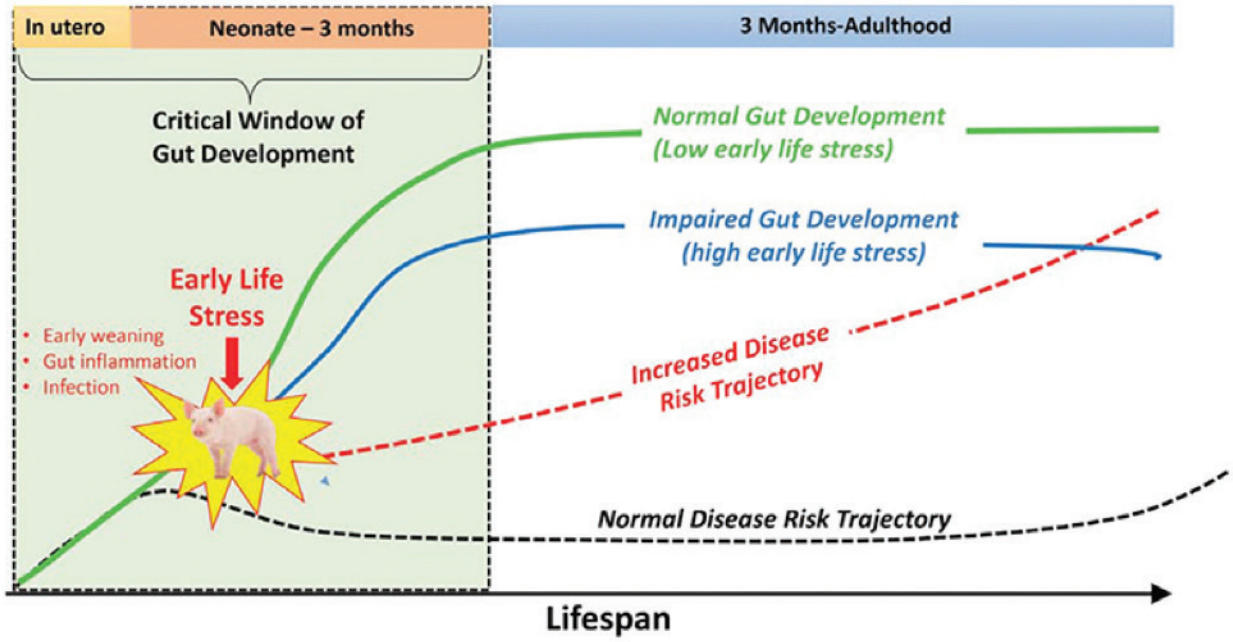


Figure 2: Early life stress changes the trajectory of gut development and increases lifetime disease risk. Michigan State University

Our previous work and that of others demonstrate that early weaning in production induces significant gut barrier injury, characterized by increased intestinal permeability and intestinal inflammation. Further, we demonstrated that nursery-age pigs that were previously early-weaned (<23 days of age) exhibit increased intestinal permeability (compromised barrier function) that persists into adulthood (Pohl et al., 2017) and exhibit more severe disease and performance reductions, compared with later-weaned pigs, in response to a challenge with postweaning F18 E. coli (McLamb et al., 2010).

Original research by Roger Main et al. (2004) and more recently by David Rosero and Dean Boyd (Hanor Farms, Proceedings from the Allen D. Leman Swine Conference, 2016) showed increased disease risk and performance reductions associated with early wean ages in field conditions. Together, basic and applied research support the concept that trauma or stress during critical developmental windows in early life alters the gut developmental trajectories toward impaired gut function and increases disease risk throughout the life span (Figure 2).

Sex and gut health

Biological sex (i.e., being male or female) has long been known as a significant risk factor for many diseases in people. For example, irritable bowel syndrome, a functional bowel disorder that affects up to 25% of the population, is two to four times more common in women compared to men. Similar sex difference trends are found for gastrointestinal parasite-related diseases, while inflammatory bowel disease and GI cancer are often found in higher rates in men.

Do sex differences exist in gut health disorders in pigs? The effects of sex on growth performance and feed efficiency has been extensively studied. It is also well-established that preweaning and postweaning mortality rates (resulting from many causes) are higher in male pigs compared with gilts. Straw et al. (2001) reported in one study that barrows exhibited a higher death loss from hemorrhagic bowel syndrome during summer months.

The authors also reported that deaths from other causes were 1.7 times higher in barrows, compared with gilts. While the studies above certainly support that sex plays an important role in disease and mortality in pigs, whether sex plays a role in gut health has not been extensively investigated.

Study objective: To determine how biological sex and early weaning impacts long-term gut development and function in pigs

Study design: Gilts and castrated barrows (Yorkshire crossbred) were randomly divided into early-weaning (16- to 17-day wean age) or late-weaning (28- to 30-day wean age) experimental groups. Weaned pigs were co-housed in the same nursery according to standard guidelines for stocking density. Pigs were fed the same phase feeding program and identical diets formulated to meet or exceed nutrient requirements for the pig (NRC Swine, 2012). Pigs were allowed ad libitum access to feed and water.

At late-nursery (60 days of age) and finisher (170 days of age) stages, gut health and function were assessed by conducting intestinal function assays (intestinal permeability, nervous system and secretory activity) and histological and biochemical assays. Also, fecal scores were performed throughout the study by evaluators, blinded to experimental groups, as a clinical index of gut function. Full details of the experimental procedures are described in Medland et al. (2016) and Pohl et al. (2017).

Results

Early-weaned gilts exhibit more severe and persistent intestinal barrier defects and greater incidence and severity of diarrhea compared with barrows.

Intestinal permeability, a sensitive index of gut barrier injury, was measured, and fecal scores were recorded as a general clinical index of function in nursery and finisher stages. In late-weaned pigs, intestinal permeability and fecal scores were similar between gilts and barrows. However, in early-weaned pigs, significant sex differences were revealed, with gilts exhibiting greater intestinal permeability (by about 1.5-fold) and more time exhibiting loose stools (58.8% vs. 29.9%, $p=0.0016$), in nursery and finisher stages.

Early-weaned gilts develop a hypersensitive gut nervous system and increased mast cell activity.

The GI tract contains an abundance of neurons, equaling that of the spinal cord. The gut nervous system plays a role in essentially all aspects of gut health including epithelial nutrient transport and barrier function, secretion and absorption, immunity and motility. In pig gut health disorders associated with stress, infectious pathogens (e.g., Salmonella typhimurium, rotavirus, *E. coli*, etc.) cause hyperactivation of nerves in the gut, which drives hypersecretion of fluid, and hypermotility, which results in diarrhea.

We, therefore, examined whether wean age and biological sex influenced the development of the gut nervous system. Similar to intestinal permeability and fecal scores, late-weaned gilts and barrows had similar numbers of gut neurons and activity (determined by measuring secretory function in response pharmacologic nerve activation).

However, in early-weaned pigs, gut neurons were higher in number and displayed enhanced sensitivity to nerve-induced secretory response, reflective of a hyperreactive gut nervous system. Further, early-weaned pigs expressed a different profile of neurotransmitters within their gut neurons characterized by increased expression of acetylcholine — the predominant neurotransmitter system regulating fluid secretion, motility and immune function in the gut.

While early-weaned barrows and gilts both exhibited increased numbers of gut neurons in the late nursery and finisher phases, early-weaned gilts had enhanced sensitivity to nerve-induced secretory function compared with early-weaned barrows.

Mast cells are innate immune cells that play critical roles in host defense against environmental allergens and pathogens. In the gut, mast cells are located near blood vessels, neurons and gut epithelial cells. When activated, they rapidly release mediators (e.g., histamine, proteases, serotonin and cytokines) that induce rapid and profound effects on gut blood supply, permeability and immune responses.

Our previous work showed that mast cell activation in pigs is enhanced in response to early weaning, which was shown to be a major pathway causing increased intestinal permeability in early-weaned pigs (Moeser et al., 2007; Smith et al., 2009). Given the central importance of gut mast cells in gut health, we examined whether sex differences exist in mast cell activity, and how this is influenced by early weaning.

These investigations revealed that early-weaned gilts and barrows exhibited higher numbers of intestinal mast cells throughout their GI tracts, compared with late-weaned pigs; however, no sex differences were observed in mast cell numbers between the sexes.

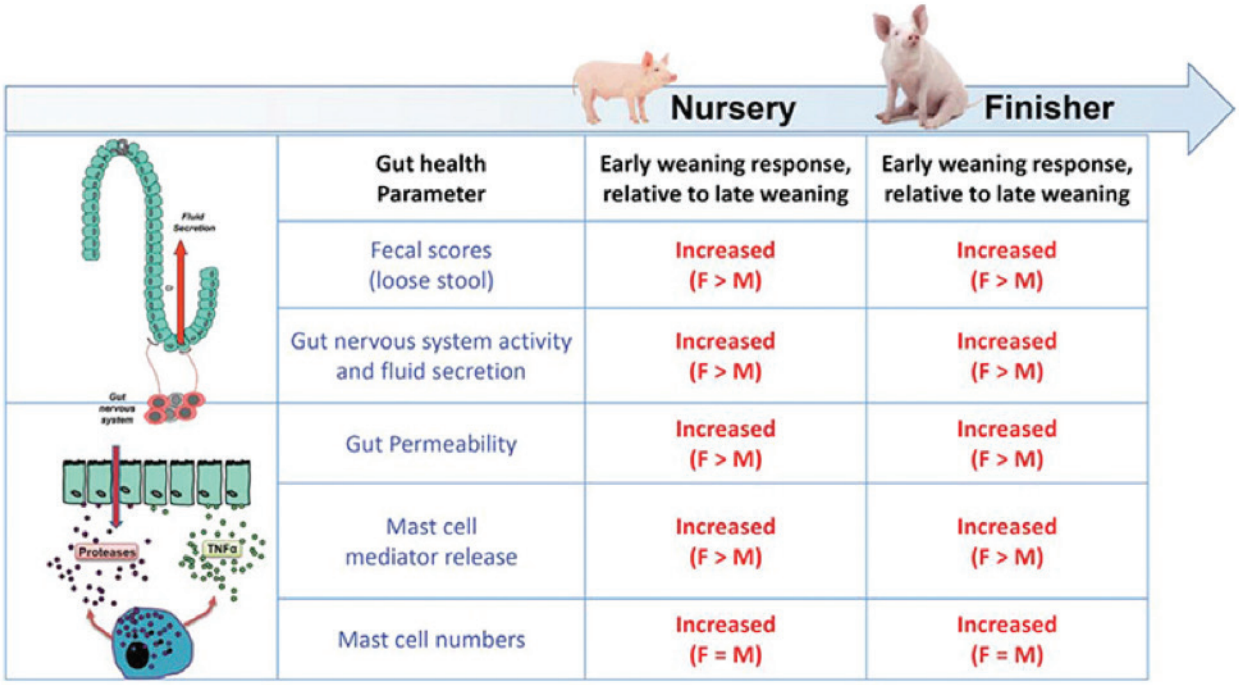
However, when measuring mast cell mediator release, significant sex differences were found, with early-weaned gilts exhibiting greater amounts of mast cell protease release, compared with barrows. This sex effect in

mast cells was also shown in other animal models of stress (Mackey et al., 2016). A summary of sex differences is provided in Figure 3.

Conclusions

Here are the take-home messages from the study:

- Gut health is best defined and measured by assessing the critical functions it performs, including barrier function, nervous and immune function and nutrient uptake.
- Early life stressors (e.g., weaning) have long-lasting, deleterious impacts on gut health and disease risk, and they compromise performance throughout the production life span.
- Biological sex plays a major role in gut health. Gilts exhibit heightened nervous and immune activation, increased intestinal permeability and increased diarrhea. However, the fact that gilts exhibit lower mortality rates than barrows or boars suggests that the heightened gut reactivity in females might confer an advantage over males for survival.
- More basic fundamental and applied research is needed to determine the mechanisms of sex differences between gilts and barrows in gut health disorders, which would be expected to uncover novel targets and strategies to optimize gut health in both sexes.



Gut health Parameter	Early weaning response, relative to late weaning	Early weaning response, relative to late weaning
Fecal scores (loose stool)	Increased (F > M)	Increased (F > M)
Gut nervous system activity and fluid secretion	Increased (F > M)	Increased (F > M)
Gut Permeability	Increased (F > M)	Increased (F > M)
Mast cell mediator release	Increased (F > M)	Increased (F > M)
Mast cell numbers	Increased (F = M)	Increased (F = M)

Figure 3: Impact of early-weaning stress and sex on gut health throughout the life span. Michigan State University

How do antibiotics impact gut health?

By James Lowe, University of Illinois College of Veterinary Medicine i-Learning Center director

The benefit of antibiotics to animal and human health is both profound and unquestioned.

From our historical "pathogen-centric" view of disease, where specific microbes caused specific diseases, antibiotics were the unquestioned king of managing infectious disease. As technology has improved, so has our understanding of how microbes interact with both humans and animals, which has led to a more sophisticated view of infectious disease.

We have learned that microbes (bacteria, viruses and protozoa), commonly referred to as the microbiota, along with macroparasites (worms), have extensive communication with each other and with the host they reside on and in. This "cross talk" is beneficial to the host, as it helps control the pathogens both by direct challenge (microbe-microbe) and by enhancing the host local immune response.

In this "host-centric" view of disease, it is a lack of balance in the microbe-host interaction that results in disease. Pathogens can only succeed in the host-microbiota ecosystem when the stability of the ecosystem is disrupted from stress, diet changes, the addition of new microbes or other environmental factors. Exposure to the pathogen alone is not enough to cause disease; it takes a failure of the host-microbiota ecosystem to maintain homeostasis.

Gut health is a good example of this complex interaction. The normal development of the intestine relies on complex interactions with the microbiota that colonizes in the intestine. This exposure begins at birth and continues throughout the lifetime of the individual.

While less is known in pigs, the process has been studied extensively in humans. Babies born via caesarean section have very different gut microbiomes than do vaginally delivered babies.[1,2] Information remains limited regarding the source of these microbes.

We investigated whether specific strains of bifidobacteria in the maternal intestinal flora are transmitted to the infant's intestine. Fecal samples were collected from 17 healthy mother-and-infant pairs (vaginal delivery: 12; C-section delivery: 5). This "colonizing" microbiome has long-term consequences for health in humans, including asthma[3] and Type 1 diabetes.[4,5] In addition, extended antibiotic treatment in the perinatal period results in a higher risk of late-onset Group B Streptococcus sepsis, necrotizing enterocolitis and overall mortality in the neonatal period.[4,6]

While the impact of antibiotics during the neonatal period is significant in humans, less is known about pigs. Our group has attempted to understand the factors that drive the development of the microbiome of the pig's intestine though a series of experiments.

In our first experiment, we employed a cross-fostering strategy to determine which sources of microbiota were the most important in establishing the piglet's gut microbiome, and if different microbiomes

resulted in different levels of “gut health” in piglets. Piglets were given high-quality colostrum from their birth dam or a foster dam upon birth. Twenty-four piglets from two litters (12 pigs per litter) were randomly assigned to one of three treatment groups according to the source of colostrum and postcolostral milk feeding for the subsequent 21 days.

Treatment 1 (T1; n=8) received colostrum and postcolostral milk feeding from their own dam. Treatment 2 (T2; n=8) were litter exchanged at birth to receive colostrum from a foster dam for 24 hours and then returned to their own dam for postcolostral milk feeding the subsequent days. Treatment 3 (T3; n=8) were litter exchanged at birth to receive colostrum and postcolostral milk feeding from a foster dam, and they remained with the foster dam for the subsequent days.

Each piglet was allowed to suck colostrum for equivalent times. The piglets were observed to exhibit vigorous teat sucking and subsequent satiation. All sows were clinically healthy with no history of receiving any antibiotics prior to farrowing. None of the piglets was administered antibiotics during the experimental period. All piglets were weighed directly after birth and before being euthanized at Day 21.

At farrowing, a single colostrum sample, fecal and vaginal swab were collected from each sow. Fecal swabs were collected from each piglet on Day 0 and Day 21. At Day 21, a group of 12 piglets was humanely euthanized. Samples of the luminal contents, tissue and mucosa were collected along the gastrointestinal tract. To determine the microbiome of each sample, high-throughput sequencing was used on the Illumina MiSeq platform. To determine the function of the gut wall, quantitative real-time polymerase chain reaction analysis was also performed to quantify the expression of toll-like receptors 2, TLR 4, TLR 10, tumor necrosis factor alpha, interferon gamma, and interleukin 4 and IL 10.

We found that microbial communities varied according to the GI biogeographical location, with the colon being the most diverse section. Bacterial communities in both maternal colostrum and vaginal samples were significantly associated with those present in the fecal samples of piglets, suggesting that, like in humans, the mother is an important source of the initial colonizing microbiome in pigs.

Cross-fostering did not affect bacterial communities present in the piglet GI tract. The mRNA expression of TLR and inflammatory cytokines changed ($P<0.05$) with biogeographical location in the GI tract. Higher mRNA expression of TLR and inflammatory cytokines were observed in ileum and ileum-associated lymph tissues.

This study suggests an impact of colostrum and maternal microbial communities on the microbiota development and mucosal immune gene expression in the newly born piglet that closely mimics the process that has been described in humans. So, what about the impact of antibiotics in neonatal piglets?

In a second experiment, we investigated the impact of five different antibiotics given at birth on growth, microbiome development and the prevalence of antimicrobial resistance genes in suckling pigs. We used

a randomized complete block design. Forty-eight litters were blocked to one of six treatments (N=8) by farrowing day, dam parity group and litters with a minimum of nine pigs. Within the litter, all pigs received the same treatment. Pigs were weighed, and treatments were administered within 24 hours of age, after litters were balanced for size.

Treatments were as follows: control (saline 1 cc), tulathromycin (2.5 mg/kg IM), ceftiofur crystalline free acid (5 mg/kg IM), ceftiofur hydrochloride (5 mg/kg IM), oxytetracycline (22 mg/kg IM) and procaine penicillin G (33,000 units/kg IM). Two pigs per litter were individually identified, and weights and deep fecal swabs were collected at days 0 (pretreatment), 5, 10, 15 and 20 (see Figure 1).

As in the first study, samples were subjected to high-throughput sequencing used on the Illumina MiSeq platform to determine the microbiome and the prevalence of 7 ARG in each sample. Antimicrobial treatment had no effect on individual weight gain, litter weight gain or mortality. Unlike the data from prolonged use in neonatal humans, antibiotics had no effect on the fecal microbiome composition or diversity during the neonatal period (see Figure 2).

Interestingly, there were also no major increases in ARG with antibiotic admiration at birth. Only one antibiotic, TUL, resulted in any increase in ARG during the entire neonatal period (see Figure 3). Of the seven ARG investigated, only four were elevated in samples from the TUL-treated pigs.

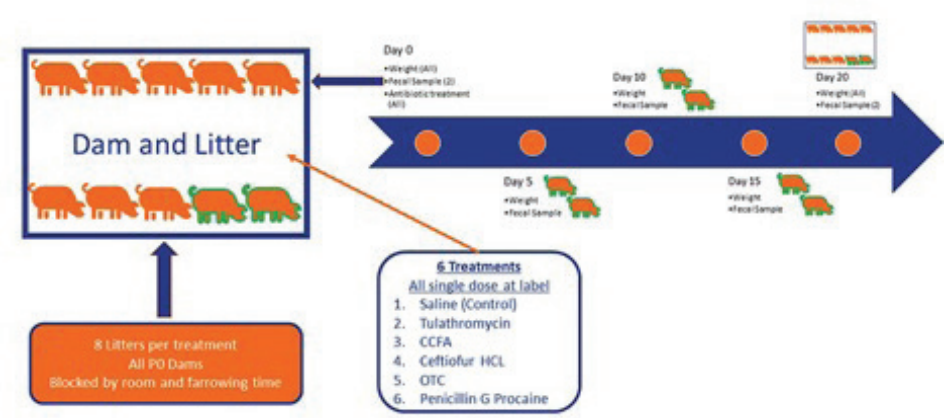


Figure 1: Experimental design to determine the impact of giving antibiotics to piglets at birth on their growth, their fecal microbiome composition and the development of antibiotic resistance. University of Illinois

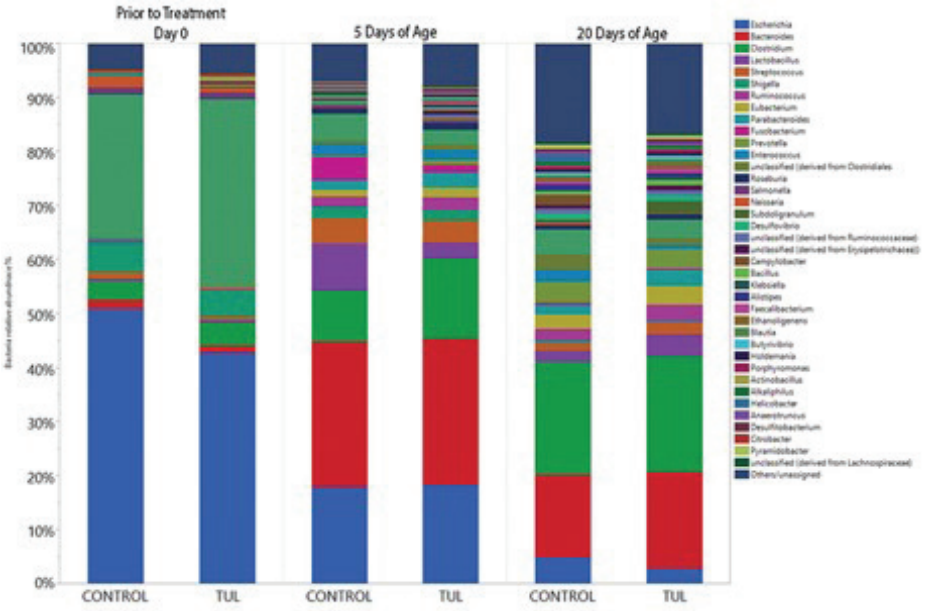


Figure 2: Antibiotic impact on fecal microbiome composition 20 days after administration in neonatal pigs. Pigs were sampled at birth and then treated with tulathromycin or saline (control). Pigs had fecal samples collected on days 5 and 20. Colors represent different bacterial genus. There were no significant differences between treatments, but the composition of the fecal microbiome changed over time. University of Illinois

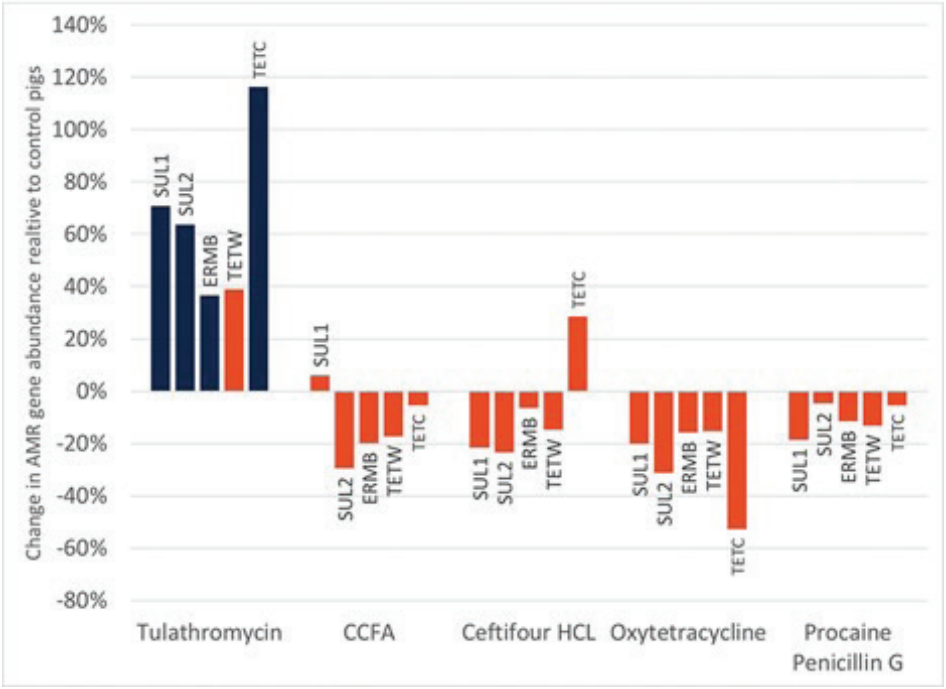


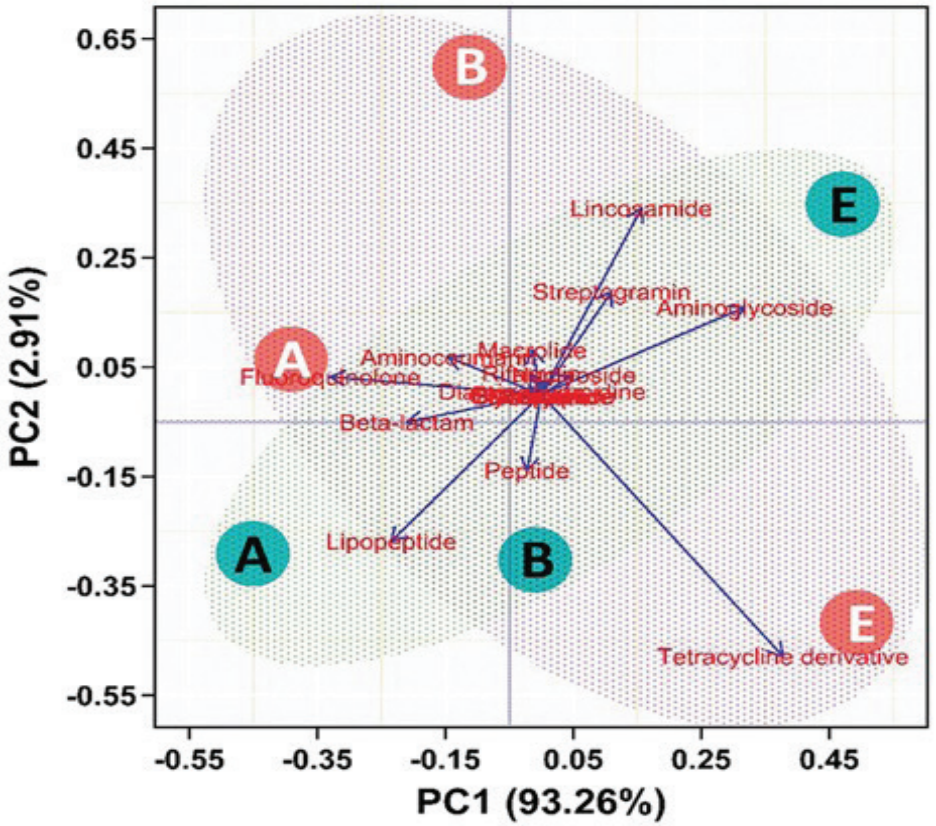
Figure 3: Relative abundance of antimicrobial resistance genes relative to control samples in piglets given various antibiotics at birth. Only one antibiotic had a significant impact (blue bars) on the relative abundance of ARG during the neonatal period. University of Illinois

While this is interesting, remember that antimicrobial resistance is a complex process, and increases in single genes may not reflect the entire picture of host health. To further investigate the TUL-treated pigs and determine the significance of the increased prevalence of the ARG, we subjected the TUL and control samples to whole genome sequencing on the Illumina MiSeq platform to amplify and determine all of the genetic elements responsible for antimicrobial resistance.

In this second, more robust (and more expensive, which is why there were limited samples analyzed) analysis pipeline, we determined that while the resistance to different antibiotics changed over time, TUL administration had only a minor, not significant, influence on the development of antibiotic resistance (see Figure 4).

This more-robust analysis points out the fact that with these advanced, complicated approaches to analysis, how we conduct the analysis has a material impact on our findings. While we are not completely sure

Figure 4: Principal component analysis of whole genome sequencing of swine fecal samples after administration in neonatal pigs. Resistance factors for classes of antibiotics were assessed for change over time (A=birth, B=5 days, C=20 days) and treatment (green=TUL, orange=control). The horizontal axis represents 93.3% of the total variation. The composition of the resistance factors changed with time (A, B and C are in same relative place on horizontal axis) but not treatment. University of Illinois



and further studies are needed, these data suggest that early antibiotic therapy in pigs has little impact on improved performance in the neonatal period or harm in producing more antimicrobial resistance. These results should be interpreted with caution, however, as these studies were terminated at weaning, and there may be lifetime benefit or harm that could not be measured due to our approach.

How the microbiome develops over time has lasting impacts on the health of both humans and animals. If the development of the microbiome in people is shifted, it can result in altered digestive function, which can result in either malnutrition or obesity. These changes can be shaped by the diet (calorie restriction or high-calorie, low-quality diet),[7] exposure to disease (diarrhea or excessive hygiene resulting in low bacterial exposure)[8] or antibiotics.[9]

In cases of human malnutrition, a weeklong course of antibiotics has become the standard of care.[10] The response to antibiotics in human malnutrition mimics the growth response seen in animals when we use oral antibiotics.[11]

There has been widespread focus on the role of antibiotics in the emergence of antibiotics resistance among bacteria in the host,[11,12] due to medical use as well as use in farm animals and crops. Microbiome composition can be rapidly altered by exposure to antibiotics, with potential immediate effects on health — for instance, through the selection of resistant opportunistic pathogens that can cause acute disease.

Microbiome alterations induced by antibiotics can also indirectly affect long-term health. The mutualistic microbes in the human body interact with many physiological processes, and participate in the regulation of immune and metabolic homeostasis. Therefore, antibiotic exposure can alter many basic physiological equilibria, promoting long-term disease. In addition, excessive antibiotic use fosters bacterial resistance, and the overly exposed human microbiome has become a significant reservoir of resistance genes, contributing to the increasing difficulty in controlling bacterial infections.

Here, the complex relationships between antibiotics and the human microbiome are reviewed, with focus on the intestinal microbiota. Another important potential consequence of antimicrobial use is a negative impact on the structure and composition of the host microbiota.

It is important to understand that the gastrointestinal microbiota is not simply a transient population of microbes involved in nutrient metabolism, but that many microbial taxa coexist in a coordinated, complex mucosal ecosystem that contributes to host gastrointestinal and immunological development, particularly in the growing animal.[13]

If a healthy and stable microbiome is an important element of host health and development, then it is important to understand how common management practices, such as antimicrobial administration, might impact this complex host ecosystem in animals raised in intensive production systems.

There are multiple studies that investigate the use of oral antibiotics and their impact on the fecal microbiome in pigs. ASP 250 has been demonstrated to increase the relative abundance of *E. coli* in pig feces while increasing the expression of genes related to energy production and conversion.[14] In another study, ASP 250 feeding in the immediate postweaning period resulted in no change in growth, but improved species richness and a lower prevalence of potential pathogens in the fecal material.[15]

There is a need of developing antibiotic growth promoter alternatives; however, the mechanisms by which AGP enhances livestock growth performance are not clearly understood. In this study, we fed 3-week-old swine for nine weeks with and without AGP containing chlortetracycline, sulfathiazole and penicillin to investigate the effects of AGPs on swine gut microbiota. Microbial community analysis was done based on bacterial 16S rRNA genes using MiSeq.

The use of AGP showed no growth-promoting effect, but inhibited the growth of potential pathogens during the early growth stage. Our results showed the significant increase in species richness after the stabilization of gut microbiota during the postweaning period (4-week-old).

When tylosin was fed to growing pigs over a 19-week period, there were significant shifts in the composition of the gut microbiome community that were not observed relative to controls in pigs fed chlortetracycline.[16]

Taken in total, these data suggest there are variable impacts on gut microbial community composition based on antibiotic class, but little is known about how these changes impact gut and host health.

Conversely, there is less known about the impact of injectable antibiotics on the swine gut microbiome. We conducted a study to characterize the impact of parenteral antibiotics administration on the composition and diversity of the resident fecal microbiota in growing pigs. Five antimicrobial treatment groups, each consisting of four 8-week-old piglets, were administered one of the antimicrobials — ceftiofur crystalline free acid, ceftiofur hydrochloride, oxytetracycline, procaine penicillin G and tulathromycin — at label dose and route. Individual fecal

swabs were collected immediately before antimicrobial administration (control=Day 0), and again on days 1, 3, 7 and 14 after dosing. Genomic DNA was extracted, and the V1-V3 hypervariable region of 16S rRNA gene was amplified and sequenced using Illumina MiSeq-based sequencing.

Across all groups, the most abundant phyla were Firmicutes, Bacteroidetes and Proteobacteria. Linear discriminant analysis and stacked area graphs showed a pronounced, antimicrobial-dependent shift in the composition of fecal microbiota over time from Day 0.

By Day 14, the fecal microbial compositions of the groups receiving CHC and TUL had returned to a distribution that closely resembled that observed on Day 0, but differences were still evident.

In contrast, animals that received PPG, OTC and CCFA showed a tendency toward a balanced homeostatic microbiota structure on Day 7, but appeared to deviate away from the Day 0 composition by Day 14.

In conclusion, parenteral antimicrobials administration showed significant shifts in the composition and diversity of fecal microbiota in growing pigs. The observed changes in fecal microbiota structure showed antimicrobial-specific variations in both duration and extent. None of the groups exhibited a full return to preadministration fecal microbial community structure by Day 14 post-treatment.

In total, these data suggest there are likely both benefits and dangers for gut health when we give injectable antibiotics. As we gain a deeper understanding of what these effects are, we will be able to design treatment regimens focused on optimizing the health of the host by targeting both pathogens and optimizing the structure of the host microbiome.

In summary, antibiotics have powerful effects on the composition and structure of the pig's gut microbiome. These changes are likely linked to changes in the health of both the intestine locally and the host systemically.

As we continue to apply advanced genomic techniques under controlled experimental conditions, we will gain a much deeper understanding of the intricate interplay between the host and its microbiome. This understanding will continue to shape how we think about optimizing host health as we shift from a pathogen-centric to a host-centric view of disease. This shift will be critical if we want to optimize the efficiency of animal-based food systems and feed 9 billion people by 2020.

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What does 'gut health' mean and how should it be evaluated in pigs?

By Milena Saqui-Salces, University of Minnesota Department of Animal Science assistant professor, Integrated Animal Systems Biology Team

Gut health is difficult to define because it is a concept that involves numerous aspects of the gastrointestinal tract function that promotes or results in animal well-being.

I discourage the use of the term "gut health" because I find many of the measurements reported in the scientific and commercial literature are inadequate to support the conclusions of GI function improvement in response to feed additives and nutritional interventions. Many studies overstate their findings by assuming physiological benefits from the changes observed, often failing to demonstrate improved function. This results in conflicting studies and widespread confusion in the pork industry when interventions that claimed to provide benefits to the pig do not show their effects on the farm.

With our current knowledge, the best we can aim for is to identify interventions that improve animal performance in specific conditions, and accept that there is not, and probably will not be, a single product that will work on all pigs, under all conditions.

The purpose of this article is to help pork producers, nutritionists and veterinarians understand the common gut health measurements reported in the literature and how to interpret their relevance for achieving improved performance in pigs. Understanding the "what" and how function is measured is very important to identify when determining if a claim of "gut health" promotion is supported or not.

The GI tract has four basic functions: digestion, absorption, motility and secretion. The gut also has a significant role in immune response because of its direct exposure to everything an animal consumes, including pathogens, antinutritional factors and toxins, as well as nutrients and beneficial bacteria.

The majority of studies reported in scientific literature involving pigs assess gut health status by analyzing some of the characteristics of the GI tract, which include maintenance of barrier function, intestinal morphology, immune response and the changes in microbiome composition. These properties affect the function of the GI tract and the whole animal, but none — on their own or in combination — guarantees improved pig performance. Defining the physiological characteristics and parameters of a "healthy gut" is difficult, but identifying an unhealthy intestine is relatively easy.

Most scientists evaluate either loss of function or the absence of disease as a measurement of gut health. The obvious indicator of disease or inadequate intestinal function is diarrhea. However, when attempting to improve animal performance, we need to focus on measuring indicators of subclinical conditions that compromise optimal growth.

In general, there are three ways to investigate GI function: in vivo, ex vivo and in vitro. Everything measured in a living animal, or from a sample that comes from a living animal, is considered to be an in vivo response. Ex vivo are all those measurements conducted using a sample obtained from a living animal but tested in the laboratory. When doing in vivo studies, sometimes animals are kept alive, but often they need to be euthanized to obtain the samples. Ex vivo studies almost always require the animals to be euthanized.

In vitro studies do not use tissues or samples obtained from animals, but use cells grown in the laboratory. In vitro studies are not adequate to prove function, but are a way to identify mechanisms of action and evaluate potential interventions.

From all the in vitro models, enteroids best represent the structure, function and dynamics of the intestine. Enteroids are derived from stem cells of the pig's intestine grown in special culture medium. They can be used to evaluate the physiological effects of various compounds, and even to model intestinal diseases, without the complexity, time and expense of feeding these compounds to animals.

In vivo, the function of the GI tract can be evaluated with digestibility studies, evaluation of intestinal permeability, mucosal structure if animals are euthanized and systemic immune responses.

The most common example of ex vivo studies is the use of Ussing chambers. These chambers are devices with two sides connected by a small opening where a piece of intestinal mucosa collected from an animal immediately after euthanasia is securely sealed on both sides. As the only way that compounds can move from one side to the other is through this piece of intestine, Ussing chambers allow measuring the capacity of the intestine to transport nutrients, and determining whether the intestine is leaky or not.

"Leaky gut" is a term often used to describe a loss of intestinal barrier function or increased intestinal permeability. Intestinal permeability is the capacity of the intestine to regulate the transport of compounds that enter and leave the body. Two major conditions compromise the intestinal barrier: loss of tight junctions between epithelial cells (per-

meability) or damage to the epithelium (major structure changes) that results in partial or complete loss of the barrier.

Changes associated with tight junctions are more commonly used to make claims about gut health in the literature. However, as we will discuss, unless the function is tested, most measurements involving tight junctions in the literature are uninformative of gut health.

Tight junctions are the structures that keep the intestinal epithelial cells together and block the passage of many substances into the body, while allowing the transfer of others. Tight junctions are comprised of different proteins that work together: claudins and occludins that are located in the junction, and zona occludens proteins that anchor claudins and occludins to the cell.

ZO proteins are not exclusive to tight junctions; they also form adherens junctions that help keep the cells together but have only minor roles regulating transport of nutrients and other compounds. Because of these reasons, analyzing ZO-1 or ZO-2, although commonly done in many scientific studies, is not informative for assessing the status of tight junctions. Instead, studies should focus on analyzing maintenance of the function (with Ussing chambers) or on location of claudins and occludins when evaluating tight junctions.

That said, reports of changes in gene expression of tight junction proteins or protein quantification in the tissue do not provide evidence of changes in tight junctions or intestinal permeability.

Currently, the gold standard for assessing intestinal permeability is the use of Ussing chambers to measure trans-epithelial resistance and permeability. TER is the measure of the electric resistance to current (ion flow) of the tissue. An intestine that has strong tight junctions will have higher TER than a leaky intestine.

Another approach used to measure intestinal permeability includes feeding molecules that would normally not be absorbed in the intestine (e.g. lactulose; polyethylene glycol; labeled dextran; mannitol; or a combination of lactulose, cellobiose, mannitol and L-ramnose) to measure their concentrations in urine or blood as indicators of increased permeability.

Despite the possible confounding factors of bacterial processing, and liver and kidney function, this approach is the best tool available at this time to assess intestinal permeability in vivo. Some researchers measure circulating bacterial endotoxins, endotoxin core antibodies (anti-lipid A antibodies) and D-lactate. The presence of these compounds in the blood is considered an indicator of loss of barrier function, but none is specific to the intestine, and can result from loss of barrier function in the respiratory or reproductive tract, skin or other organs.

Another common assessment of GI tract health is a histological evaluation of mucosal structure. Mucosal structure refers to the general organization of the intestinal tissue, including the length of the villi, depth of the crypt, number of villi-crypt units by unit of length of the intestine, the presence of inflammation, and overall weight and size of the organs. Mucosal structure is highly informative when major changes are observed; for example, the villi in pigs infected with pathogens are usually blunted and half or less than half the length of the villi of a healthy pig, plus they show cellular damage.

However, when changes are not that drastic, like under stress or as a result of dietary interventions, interpreting changes of the mucosa becomes a challenge.

In the animal science field, it is widely accepted that longer villi are associated with greater absorptive capacity, and thus are interpreted as an improvement in gut health. However, not a single published study shows that intestines with longer villi indeed absorb more nutrients. Moreover, greater intestinal villi height and deeper crypts mean the GI tract may be heavier, which is undesirable relative to pig growth performance.

Several studies have shown increases in intestinal villi and crypt lengths in pigs — a result of feeding low-nutrient-dense diets. Authors suggest that is a normal response of the intestine to increase absorptive capacity to better use those nutrients. Following that thought, it would be even more useful to demonstrate more villi per length of intestine, which implies even larger absorptive capacity even if villi do not change in height; but this measurement has rarely been reported in scientific literature.

Contradicting the idea of longer villi happening in response to low nutrient density, it has been widely reported in many studies with nursery pigs and other animals, including humans and rodents, that under conditions of severe nutritional restriction and fasting, the absence of food in the intestine or low nutritional content results in villi becoming shorter, not longer. Therefore, interpretation of changes in villi height and crypt depth needs to be supported by functional measurements.

Currently, the optimal villus height and crypt depth, and the ideal proportion of these measures that should be in each section of a pig's intestine at different ages to support optimal growth, are unknown. Overall, unless there is clear damage to the tissue or the villi are severely blunted, changes of intestinal morphology do not provide evidence of gut health.

The intestinal barrier is essential for optimal immune function and health of pigs. Immune response is measured by quantifying the concentrations of cytokines, interleukins and other molecules in the blood by ELISA, as well as identifying the immune cells present in the tissue or blood (usually by flow cytometry.) Both cells and molecules can be measured in vivo, ex vivo or in vitro.

In general, in vitro and ex vivo studies are meant to understand mechanisms of immune response and do not accurately model responses in vivo. In vivo studies are conducted to evaluate immune responses triggered by nutritional interventions or under-stress challenges (heat or social) to determine if the immune system is being activated even without clinical manifestations.

Other studies are conducted using a stress or disease challenge to measure the efficiency and ability of animals to respond, control and recover from a challenge.

When evaluating immune responses associated with the GI tract, blood samples collected from the portal vein or intestinal tissue are necessary because changes in the local intestinal immune response may not be observed in systemic blood (samples collected from the jugular, ear veins or other than the portal vein). This is because some cytokines and immune factors may be cleared by the liver, and the systemic blood carries signals from the respiratory and reproductive tracts, skin, and other organs, in addition to the intestinal tract.

Also, comparing concentrations of cytokines from swine in different production phases (gestation, lactation, nursery, finishers) should be avoided because the concentrations vary by age. In pigs, several scientific studies have provided information on pro-inflammatory molecules, particularly IL-1β and TNFα, as well as antibody production using different models, but the information about the quantification of the impact of immune response on performance is not clear when challenges are not included in the experimental design. The lack of significant changes may be a result of a limited focus by analyzing only pro-inflammatory cytokines and overlooking other components of the immune response, such as the anti-inflammatory, regulatory and memory that are rarely studied in research involving food-producing animals.

The microbiome is unquestionably one of the more important factors affecting GI tract function, intestinal barrier and immune responses. Unfortunately, researchers are just beginning to identify changes of the gut microbiome resulting from various environmental and nutritional conditions, and to understand what the changes mean relative to pig performance and health. Most of the studies involving the modulation of the pig microbiome involve large, complex data sets generated from sequencing bacteria from feces or culture to identify different microbial species or families.

At this point, the best microbial composition for the pig's intestine has not been defined, and most statements that refer to good or bad bacteria are based on information from human or mouse studies. However, research is beginning to show how differences in microbial composition of the GI tract may be correlated with a pig's health and performance. Eventually, the goal is to evaluate and validate cause-and-effect relationships between these correlations.

Remember, the microbiome is comprised of not only bacteria, but also fungi, protozoans and viruses. Unfortunately, there is little scientific information on the role of these nonbacterial components of the microbiome in GI function and interaction with other components.

In summary, the functions of the GI tract of pigs are complex, and their study requires using different research techniques and measurements. At a minimum, these measurements should be tested for correlation with animal performance to provide meaningful interpretations of their significance for pig's health.

Furthermore, since improving animal performance involves all the systems in the body, a holistic approach needs to be used to identify genetic, phenotypic and metabolic biomarkers that identify health in pigs to be used as predictors of performance in commercial production, considering that gut health may be only one small part of the puzzle.

Focus on fiber and the impact on swine diet components

By John Patience, Iowa State University Department of Animal Science professor

Over the past decade, fiber has evolved into an increasingly important topic in swine nutrition. Prior to 2008, there was little interest in fiber because most commonly used ingredients contained very low levels. When ethanol production emerged as a major market for corn, the pork industry was forced to use the fermentation byproduct — distillers dried grains and distillers dried grains with solubles. Suddenly the neutral detergent fiber content of typical pig diets increased from 5% to 15% or even 20%.

Many questions emerged as a result of this change. How well can pigs use this fiber? Is fiber digested in the small intestine or is it fermented in the cecum and large intestine? This is important because digestion is energetically more efficient than fermentation. What can be done to improve the utilization of fiber? Does fiber alter digestion of other components of the diet?

More recently, questions arose about fiber for a different reason. Could fiber be used to improve gut health, and if so, how could it be done in a predictable manner? This coincided with the recognition that when we formulate diets for pigs, we are supplying more than nutrients like amino acids, minerals and vitamins. Different ingredients alter the functioning of the gut, and the impact can be either positive or negative.

Before delving into these questions, let's look at a few basic facts about fiber. While there are many ways to analyze a diet for fiber, none of the available assays do a really good job of explaining the physiological effect of fiber in the gut. It is widely accepted that crude fiber provides little useful information and many labs have stopped using it.

Neutral detergent fiber and acid detergent fiber are more commonly used, as they provide more useful information, but they are far from perfect. NDF essentially measures the cellulose, hemicellulose and lignin content of a diet or ingredient. ADF measures cellulose and lignin.

Hemicellulose, cellulose and lignin are all found in the cell walls of ingredients; the higher their level, the poorer will be the digestion of that ingredient. Cellulose and lignin are not digested by enzymes present in the gut, and are very poorly fermented in the cecum and large intestine. Hemicellulose is also not digested by the pig, but some breakdown by fermentation does occur.

NDF measures, in approximate terms, insoluble fiber in the diet; in most cases, insoluble fiber is poorly fermented.

Some labs measure what is called total dietary fiber; this assay is slow and laborious and thus expensive. TDF measures both insoluble and soluble fiber. The soluble fiber may be quite well fermented and thus provides some useful energy to the pig; however, to complicate things, not all soluble fiber is well fermented. It will depend on the exact composition of the fiber. Soluble fiber includes things like undigested starch, B-glucans (found largely in barley), pectins and gums.

For practical diets, the NDF assay is probably the best option. It is reasonably inexpensive and provides information on the component of fiber that is poorly fermented. To practical nutritionists, there are two main problems with the NDF assay. First, it is prone to variability, so results can vary from lab to lab. Second, it provides no information on soluble fiber that can be a problem if one is evaluating an ingredient for it prebiotic effects, as explained below. Researchers solve the problem somewhat by using the TDF assay and by measuring soluble versus insoluble fiber. They may also measure individual sugars. At the present time, these assays are too expensive to be used on a routine basis.

It is known that as fiber levels increase in the diet, the availability of certain nutrients, such as amino acids and minerals may go down, as will fat digestibility. However, this has not yet been quantified so adjusting the nutrient content of a diet due to its fiber content is currently more art than science.

It is also known that increased fermentable fiber in the diet increases the requirement for threonine. However, the increase is relatively modest in most situations.

So far, we have discussed the problems associated with elevated fiber in the diet. However, we are increasingly aware that fiber can be a positive factor in the diet because it can improve gut health. Much of the benefit accrues from fiber that is easily fermented rather than fiber that is poorly fermented. We have data showing that pig performance may be improved when exposed to a pathogenic *E. coli* challenge when fermentable soluble fiber is included in the diet, much more so than when poorly fermented fiber is present. More research is required in this area, but it is clear that the health of the young pig can be improved by increasing the levels of fermentable fiber in the diet.

I hope this very brief primer on fiber has been helpful, and I hope it has not been too confusing. It is not an easy topic to get one's head around. But because of its increasing importance, it is a topic that needs to be reasonably well understood in order to formulate diets most effectively.

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