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Midwest Swine Nutrition Conference

Schedule of Presentations

8:15 am	Registration
9:00	Welcome and Introductions *Merlin Lindemann, University of Kentucky*
9:05	Microbial Endocrinology and Nutrition: Why the Intersection of Microbiology and Neurobiology Matters to Animal Health *Mark Lyte, Iowa State University*
9:50	Environmental Influences on Gastrointestinal Development, Function, and Disease Resistance *Adam J. Moeser, Michigan State University*
10:20	Break
11:00	Low Crude Protein Diet and its Effect on Diarrhea *Martin Nyachoti, University of Manitoba*
11:30	Nutritional and Management Strategies to Alleviate the Impact of Stress on Swine Health and Productivity *Jay S. Johnson, USDA-ARS Livestock Behavior Research Unit*
12:00	Lunch
1:00 pm	Peroxidized Lipids in Nursery Pig Diets – Why and When Should We be Concerned? *Eric van Heugten, North Carolina State University*
1:45	Implications of Dietary Oxidized Oils for Fresh and Further Processed Pork Quality *Anna C. Dilger, University of Illinois*
2:15	Break
2:55	Phytase Supplementation – The Response to Phytase Beyond Phosphorus *Merlin D. Lindemann, University of Kentucky*
3:25	Calcium Digestibility and Requirements for Digestible Calcium by Growing Pigs *Hans H. Stein, University of Illinois*
4:00	Adjourn

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Donald C. Mahan

May 28, 1938 - August 15, 2016

Dr. Donald C. Mahan, an outstanding researcher, teacher, and renowned swine nutritionist, died at the age of 78 at his home, surrounded by his family. He is survived by his wife (Amy Jo), three daughters (Melanie, Jean, and Laurie), 10 grandchildren, and his brother (Gene).

Don was the son of Clarence and Irene Mahan and was born in East Chicago, Indiana. After living in Hessville for a few years, his family moved to a farm near Lowell where he graduated from high school in 1956. He then obtained his BS degree in Agricultural Education from Purdue University before working as a County Youth Agent in Sullivan, Indiana, where he met and married Amy Jo Osburn before returning to Purdue for graduate study. After completing his MS degree at Purdue and his PhD in Animal Nutrition at the University of Illinois, he began his professional career as an Assistant Professor at The Ohio State University in 1969.

Don Mahan greatly impacted the swine and feed industries in diverse areas over his career. Although well known nationally and internationally for his Se and vitamin E research, he was also recognized for his research in other areas. His multi-parity sow research studies demonstrated the requirement for Ca and P, and that organic Se resulted in more and healthier pigs, less sow parturition problems, and greater milk Se for multiple parities. He identified the need for vitamin C, high quality dried whey, lactose, and chloride for the early weaned pig. His research was the basis for FDA's approval for both organic and inorganic Se. Body composition research studies with sows and growing-finishing pigs helped to establish the mineral needs of swine. These are just a few of the research areas in which Don Mahan helped nutritionists better understand the nutrition of pigs.

Dr. Mahan received several ASAS awards during his career including the AFIA Nutrition Research Award, Gustav Bohstedt Award for Mineral Research, and an ASAS Fellow Award in Research. Most recently, he received the 2016 FASS-AFIA New Frontiers in Animal Nutrition Award given for his lifetime production of innovative research that significantly benefitted the livestock and feed industries. He mentored many graduate students at Ohio State, and authored or co-authored more than 175 refereed journal articles and over 400 other publications. He was an invited speaker at 213 conferences in several states and 50 countries.

Don was one of the organizers of the Midwest Swine Nutrition Conference and served on the planning committee for its entirety. He made presentations at this conference numerous times during the past 16 years. His last presentation was in 2014.

Don had a delightfully unique sense of humor which was enjoyed by all who knew him. He will be greatly missed by his many friends and colleagues in academia and the feed industry.

Microbial Endocrinology and Nutrition: Why the Intersection of Microbiology and Neurobiology Matters to Animal Health

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Summary

The union of microbiology and neurobiology, which has been termed microbial endocrinology, has led to increased understanding of the mechanisms by which the microbiome may influence human and animal health. The symbiotic microorganisms which inhabit multiple niches in mammalian hosts, such as the intestinal tract and skin, serve and influence many important physiological functions. The use of a microbial endocrinology-based evolutionary approach in which commonly shared neurochemicals serve as the mechanistic bridge between host and microbiome has important implications for many areas of biology. In this paper advances in the field of microbial endocrinology which may hold relevance for the swine industry are examined.

Introduction

Microorganisms communicate with, influence and are affected by their host and their neighboring microbes. Systems of host-microbe communication appear across a wide range of organisms from plants to animals. Some of these interactions are undoubtedly critically involved in determining the health of the hosting organism. The realization that the microbiome and host communicate with one another through the endocrine and nervous systems led to the creation of the multidisciplinary field known as microbial endocrinology (Lyte, 2016).

Microbial endocrinology represents the union of the fields of microbiology and neurobiology. It is defined as the study of the ability of microorganisms to produce and recognize neurochemicals that originate either within the microorganisms themselves or within the host they inhabit. While the term originated in 1993, evidence supporting some of the principle tenants of this field can be traced to studies going back as far as 1930. For example, *Clostridium perfringens,* an agent with a subtype known to cause highly fatal, necrohemorrhagic enteritis in piglets, also turns out to be among the earliest documented cases of bacterial growth being influenced by a host released neurochemical. The infective potential of *C. perfringens* changes following exposure to stress-related neurochemicals, such as the catecholamine epinephrine. Epinephrine was shown decades ago to increase the growth rate of *C. perfringens* to such a degree that the infective dose needed to cause infection decreased by up to one-million fold. Cases related to this phenomenon were reported throughout the 20th century (Lyte, 2016).

The potential for neurochemicals to influence virulence is not solely limited to *Clostridium.* The growth of the respiratory pathogen *Bordetella bronchiseptica* is greatly increased by the presence of norepinephrine *in vitro*. The stress neurochemical norepinephrine has also been shown to stimulate the growth and pathogenicity of other organisms including *Escherichia coli* and *Campylobacter jejuni*. The release of catecholamines from injured enteric neurons is associated with a rapid alteration of the microbiota community from one dominated by Gram-positive taxa to one dominated by a single Gram-negative bacterial species, *E. coli*. The overgrowth of *E. coli* has been proposed to be a possible contributory factor in trauma-induced sepsis. Conversely, while catecholamines like dopamine may increase microbial growth, dopaminergic antagonists (agents which bind the dopamine receptor in a way that prevents its response to a signal) have been demonstrated to block catecholamine-induced growth in *Escherichia coli* O157:H7, *Salmonella enterica,* and *Yersinia enterocolitica* (Lyte, 2016).

Though neurochemical influences on the growth rate of pathogens are of obvious importance to infectious disease, it should be noted that the mechanisms and physiological changes by which neurochemicals influence microorganisms are quite varied. For example,

concentrations of norepinephrine as low as 1.2 x 10^{-5} M can significantly influence the aerotolerance of organisms such as *Clostridium jejuni* and *Spirillum volutsans.* To achieve these diverse outcomes, many microorganisms, like their hosts, have the ability to detect the nuanced differences between various catecholamines or other neurochemicals. The bacterial pathogen *Actinobacillus pleuropneumoniae*, for example, has been shown to display differential expression of 158 and 105 genes, respectively, when exposed to epinephrine or norepinephrine. Though both chemicals are catecholamines, only 18 of the differentially expressed genes were common to both epinephrine and norepinephrine exposure. This suggests that organisms like *A. pleuropneumoniae* may possess multiple responsive systems for individual catecholamines (Li et al., 2012).

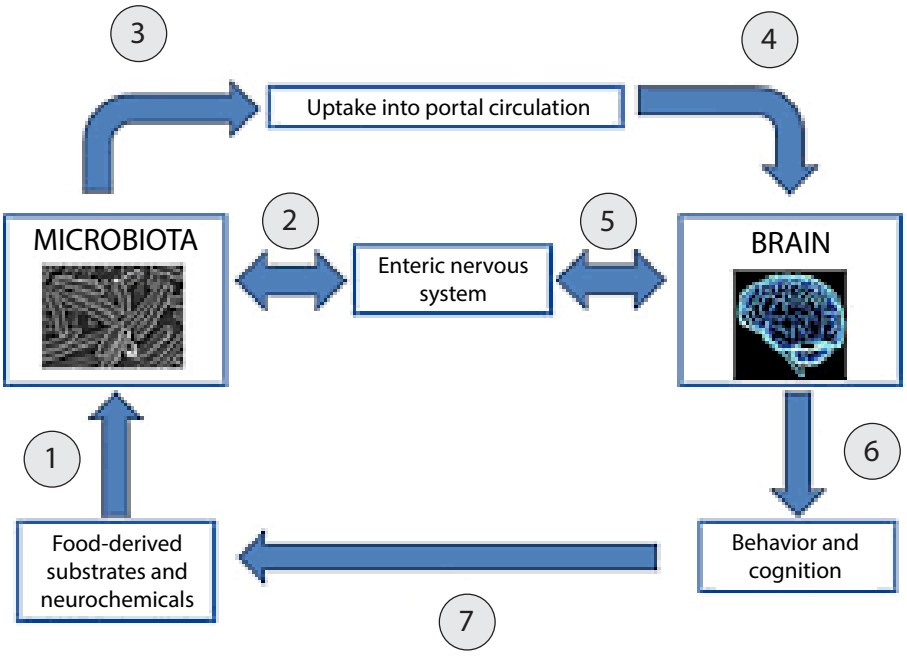


Figure 1. Food ingested by the host contains substrates needed for neurochemical production as well as fully functional neuroactive components (1). The microbiota may synthesize neurochemicals from these substrates; respond to the neuroactive food components themselves; or respond to neurochemicals secreted into the gut by components of the host enteric nervous system (2). Neurochemicals produced by the microbiota can be taken into the bloodstream through the portal circulation (3) or directly interact with the enteric nervous system (2). Through the bloodstream neurochemicals may influence distant systems and ultimately the brain (4). Microbiota-derived neurochemicals can also influence the enteric nervous system which may indirectly influence the brain through enteric system-central nervous system communication (5). The result of either pathway (4) or (5) on the brain may result in an alteration of behavior or cognition (6) as well as food preferences and appetite (7).

With multiple distinct classes of catecholamine receptors ranging from dopaminergic to adrenergic each with their own subtypes, it is not surprising to learn that the range of neurochemicals and the variety of microorganisms in which they have been identified is quite extensive. The examples acetylcholine, histamine, serotonin, agmatine and catecholamines can all be found in the literature (Lyte, 2016). To successfully develop the strong and reliable approaches needed to modify *in vivo* systems, a more evolutionary-based approach must be utilized to explore the mechanisms through which microorganisms can influence their host. This includes viewing these neurochemicals from the point of view of the microorganism as well as the host.

The Microbiota-Gut-Brain Axis and Similarities between Humans and Swine

Among several other known mechanisms, gastrointestinal microbes can influence their host through interactions with the endocrine and enteric nervous systems in addition to their effects on the composition of digested material. A physiological model drawn from our understanding of these interactions has been developed. This model is termed the microbiota-gut-brain axis (Figure 1). Food ingested by the host can be metabolized by the microbiota in ways that generate or release neuroactive components. These neuroactive molecules can be taken up into the circulation or interact with the host locally at the level of the enteric nervous system. Some signals may be transduced to the brain where they can influence cognition and behavior whereas other signals may result in changes in the enteric system which feedback on the microbiota in a bidirectional way (Lyte, 2016).

Similarities Between Swine and Humans

It should be noted that to date, much of the research establishing the microbiota-gut-brain axis has been done in species other than pigs with a focus on trying to understand human physiology. However, the same similarities which allow us to infer new knowledge about humans from studying pigs also allow us to do the reverse to understand porcine systems better.

Structurally, developmentally and functionally, humans and pigs share many similarities in their gastro-

intestinal tract (Guilloteau et al., 2010). In particular, these similarities include renal, cardiovascular, and gastrointestinal anatomy and physiology. There is also close functional overlap since both species have an analogous distribution of ducts draining the pancreas as well as comparable cholecystokinin receptors and neuro-hormonal mechanisms controlling pancreatic juice secretion. Throughout early gastrointestinal development, porcine digestive enzymes closely mirror those found in humans. Though differences do arise over the course of later development, the enzymes expressed in a pig closely resemble those found in humans. Developmental exposures for the fetal and neonatal gastrointestinal tracks of each species are also similar. *In utero,* both species rely on some degree of enteral nutrition through ingestion of amniotic fluid. During early development both species naturally have a period of reliance on their mothers for nursing. This is significant because some of these exposures may be deterministic to the ultimate microbiome expressed in the organism.

Significant similarities have also been noted in the development of neural structures. (Conrad et al., 2012). Gross anatomical features including gyral pattern and distribution of grey and white matter of the neonatal porcine brain are similar to that of human infants. Like humans, porcine brain development contains a period of rapid postnatal development. (In humans, the brain can increase from 25% of adult volume to 85% of adult volume within the first two years of life. Pigs have a corresponding period of rapid development from 4-12 weeks.) Of particular interest for furthering understanding of the microbiota-gut-brain axis would be comparisons of the limbic system which is a region of the brain associated with motivation and emotion. The limbic system can exert influence through the endocrine system as well as autonomic system and is a crucial part of transducing messages between the central nervous system and gut. Although these structures are grossly alike, at the time of this review detailed comparisons of these particular structures remained elusive.

Though there are many parallels between humans and swine which justify the usage of the microbiota-gut-brain axis model in swine, one difference bears mentioning. Unlike humans, the porcine lower small intestine has a much higher microbial density than humans and this allows pigs to degrade some materials to a higher degree than humans (Guilloteau et al., 2010). This observation does not however minimalize the importance of the microbiota-gut-brain axis. Rather the increased microbial density in a swine would suggest an increased importance of the microbiome and its contribution to the gut brain axis in pigs.

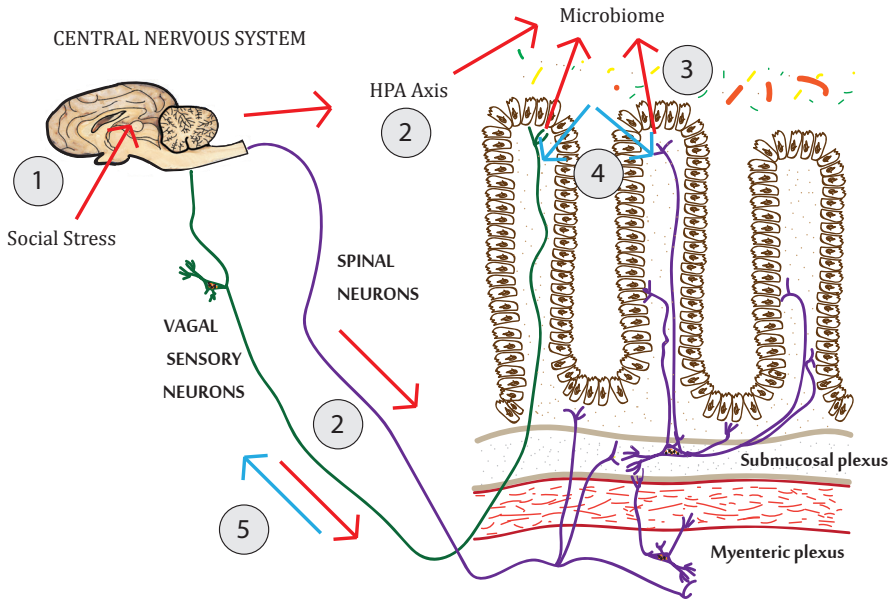
Implications of Microbial Endocrinology for the Swine Industry

Relevance to Stress

Stress has been tied to multiple issues of potential cost to a producer ranging from decreases in meat quality (Grandin, 1980) to aggression and the potential injury of handlers and other livestock. Aggressive animals may dominate weaker animals, denying them access to sufficient food and thus retarding herd growth rates. Some have even gone so far as to suggest that aggression among pigs is "one of the most important health, welfare, and economic problems in intensive farming" (Lee et al., 2016).

The link between stress and the neuroendocrine system is well established if not completely understood (Figure 2). Stress can act through the central nervous

Figure 2. Social stress activates the central nervous system. (1) The stress signal may be transduced by neurons of the autonomic nervous system as well as the HPA axis. (2) Chemicals released by these systems can act on the microbiome. (3) Changes in host microbiome and the production of metabolites may then be detected by the host through the gut-brain axis (4) and transmitted by vagal sensory neurons back to the central nervous system (CNS) or otherwise effect changes in the host. (5)



system and effects changes to the microbiome through the sympathoadrenal and hypothalamic-pituitary-adrenal (HPA) axes. These neurochemical axes release chemicals which can act on and change the microbiome. Changes in the host's microbiome may then feedback onto the host through the gut-brain axis to effect further changes. Simply stated, the microbiome and host are intimately linked in a bi-directional way.

Relevance to Infectious Colonization

Under normal (healthy) conditions a common mammalian microbiome is dominated by Firmicutes (65.7% by sequencing), Bacteroidetes (16.3%), Proteobacteria (8.8%) and Actinobacteria (4.7%) (Ley et al., 2008). Numerous studies have demonstrated that stress can affect the gut's microbial composition, influence microbiota-gut-brain communication, and result in behavioral alteration. Physical and psychosocial stress, as well as alteration of the circadian rhythm, can alter the microbial community structure within the gut. The microbial community structure can rapidly change as a response to the influx of host stress-related neurochemicals in the lumen.

The fact that microbial diversity is lost with exposure to stress conditions is important because rich microbial diversity appears to be associated with better host health whereas decreased diversity has been associated with health problems ranging from recurrent *C. difficile* infection to metabolic disturbances (Chang et al., 2008; Claesson et al., 2012). Further, what determines whether an organism behaves in a benign or invasive manner often depends on how the organism perceives its environment. If pathogenic microorganisms perceive a stressed environment, this can lead to increased pathogen colonization which in turn can lead to more highly contaminated food and a greater risk of foodborne infection. A perfect example of this is the human pathogen *Y. enterocolitica* which is among the foodborne pathogens most important to the swine industry. Undercooked pork and pig products are reported as the main source of human infections and pigs are considered the major reservoir of the pathogen (Virtanen et al., 2012).

In rodents, investigations have shown that social conflict stress leaves the animal more susceptible to infection. In 1991-1992 a series of experiments by Lyte et al. were conducted with animals stressed by social conflict (Lyte, 2016). These stressed animals were then challenged with the oral pathogen *Y. enterocolitica.* After 14 days, the stressed population showed over 100% greater mortality. Cumulative survival rates of 80% for non-stressed infected animals but less than 40% survival of stressed animals. Crucially, it was demonstrated that a mechanistic link existed between stress and the pathogenesis of bacteria such as *Y. enterocolitica* (Lyte, 2016). Catecholamines, the key chemical modulators of stress responses also induce growth changes in *Y. enterocolitica*. Those catecholamines which are found in the GI tract and are released as a consequence of stress, such as dopamine and norepinephrine, increased the growth of *Y. enterocolitica* over a million-fold.

Interestingly *Y. enterocolitica* contamination is closely associated with activities leading up to slaughter with the prevalence of pathogenic *Y. enterocolitica* high in pigs at slaughter (Fredriksson-Ahomaa et al., 2000). It has also been established that the time before slaughter is widely considered to be a stressful time in a pig's experience (transport, introduction to new animals, enclosed spaces environment) (Grandin, 1980). As such, it may be suggested that utilization of microbial endocrinology as a mechanistic link between these two phenomena may provide an explanation of how stress could be a contributing factor in *Y. enterocolitica* colonization.

Impact of Nutrition on the Microbiome

As was stated earlier, the behavior of a microorganism often depends on how the organism perceives its environment. Obviously, a great deal of a gut microbe's environment will depend on the diet of the host organism and indeed, diet is one of the most important modifiers of the intestinal microbiome with the capacity to influence its composition and functional metabolism of the microbiome. (Albenberg and Wu, 2014; David et al., 2014). This is not limited to digestible material. Dietary components like fiber, xenobiotics, neurochemicals, and their precursors can all have an influence.

The diet can profoundly impact the levels of certain neurochemicals in a host. For example, it has been demonstrated that the rates of synthesis for serotonin and catecholamines in the brain are sensitive to local substrate concentrations. (Fernstrom and Fernstrom, 2007) Physiologic factors (particularly diet) which influence the pool of neurochemical precursors in the brain also influence the synthetic rate of neurochemical products. This finding has functional consequences. Consider, the dietary consumption of tyrosine, the catecholamine precursor, increases anger in subjects exposed to psychological stress (Lieberman et al., 2015).

Just as a host organism can synthesize more neurochemicals with an abundance of precursors, so can microbes. Microbes which produce neurochemicals often use the same conserved synthetic pathways as their eukaryotic host. Therefore, it would not be surprising

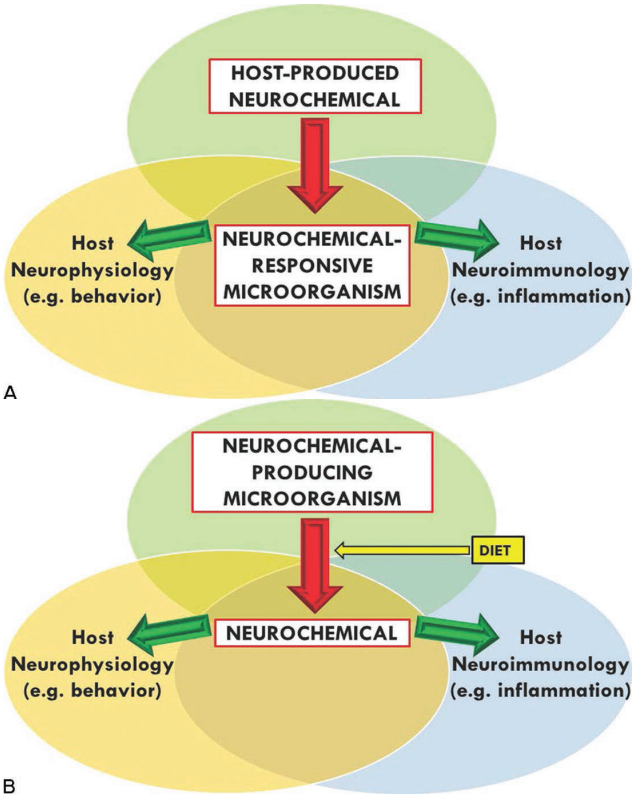


Figure 3. Bi-directional nature of microbial endocrinology in which neurochemicals produced by the host can influence the microbiota (A) and the very same neurochemicals produced by the microbiota can influence the host (B). The evolutionary-based neurochemical signaling pathway between microbiota and host means that a neurochemical(s) produced by the host can influence the microbiota (A) and at the same time a neurochemical(s) produced by the microbiota can, in turn, influence the host (B).

to learn that increased dietary consumption of food substrates which are neurochemical precursors, could also lead to an abundance of neurochemicals in the gut. Once produced, these neurochemicals could influence the microbiome and the host which would in turn influence each other bi-directionally (Figure 3).

Conclusions

The influence of the microbiome on host physiology is an area of increasing interest as new generations of molecular techniques revolutionize the way we approach the biological sciences. It is proposed that observations and research undertaken from the perspective of microbial endocrinology could lead to useful applications such as the design of a microbiome to influence behavior through modulation of the bi-directional neurochemical signaling that occurs within the microbiota-gut-brain axis. Additionally, there may be other potential benefits to swine health that may be realized by selecting for specific microbiomes based on microbial endocrinology-based approaches. Some microbiomes may prove advantageous to swine growth by improving the uptake of nutrients and thereby improving feed efficiency. Ultimately, the benefits that can be realistically obtained can only be determined with more research. Through a rigorous study of the bi-directional microbial endocrinology-based mechanisms by which microbiomes influence their host, as well as hosts influencing the microbiome, we may develop new tools that will help address the problems facing the swine industry today.

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Environmental Influences on Gastrointestinal Development, Function, and Disease Resistance

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Summary

During the first 4-6 weeks of life, the pig gastrointestinal (GI) tract undergoes extensive development and maturation as the epithelial, immune, and nervous systems acquire critical functions to allow the animal to survive and thrive in the extra-uterine environment. The key developmental events which occur during this critical period shape the developmental trajectory and long-term function and health of the GI system. In nature, this is a protected time for the neonate, both socially and immunologically, which is essential for optimal GI development. Unfortunately in modern swine production, it is during this critical period when some of the most profound stressors occur. Therefore, early life stressors in production could have long-last effects on development and lifelong health of the pig. Understanding how early life factors impact GI development has significant implications to lifelong gut health including performance, nutrient utilization and disease resistance. This presentation will focus on weaning stress and its impact on gut function and implications for long-term health and performance of pigs.

Gut Health and Functions

Gut health is of central importance to health and productivity in swine and thus has become a central topic in the swine industry. Gut health in pig production can be defined as the health of the gastrointestinal (**GI**) system as measured by the critical functions is performs to support efficient growth and defense and survival of the host. Therefore, compromised gut health can range from suboptimal nutrient utilization and feed efficiency to clinical enteric disease.

A primary function of the GI system is to digest and absorb nutrients from dietary and endogenous origin and transport massive amounts of water and electrolytes. This is accomplished primarily through a variety of enzymatic processes which break down intact carbohydrates, proteins, and lipids via digestive enzymes such us pancreatic amylases, lipases, and proteases yielding breakdown products that are further broken down by intestinal epithelial brush border enzymes including aminopeptidases and diasaccharidases. The resulting products (e.g., simple sugars, di/tripeptides and amino acids, and free fatty acids and monoglycerides) resulting from the digestive processes are then transported across the intestinal epithelium and into the body through a variety of highly selective, energy-dependent, nutrient transporters. Electrolyte pumps, channels, and transporters for Na^+, K^+, Cl^-, etc., are critical as the generative the electrochemical gradient needed for nutrient absorption while also fluid water absorption and secretion. Therefore, any impairment or inefficiencies in these processes can have significant GI and systemic health and thus economic consequences for pigs in production.

Another critical function of the GI system is the barrier function. At the same time the gut epithelium is facilitating massive absorption of nutrients, it must selectively prevent entry of potentially fatal intestinal contents (e.g., bacteria, antigens, and toxins) from getting across the across the epithelium and into the body. This function is critical for health and productivity of the host as breaches in the epithelial barrier or "leaky gut" induces over-stimulation of the GI immune system leading to inflammation and potentially death. The permeability of the gut epithelium can be adversely affected by a number of stressors in pig production including social stress, environmental temperature changes, and pathogen challenges (Moeser et al., 2007ab; McLamb et al., 2013; Pearce et al., 2014; Schweer et al., 2016). A leaky gut may also contribute to impaired nutrient transporter function either through loss of the transepithelial electrochemical gradients or indirectly via inflammation. Inflammatory products in the gut have been show to directly impair nutrient transport activity which could have a direct consequence on animal performance and disease resistance.

In addition to the epithelial barrier properties, an equally important component of the GI barrier is the GI mucosal immune system. The intestinal tract harbors the largest collection of immune cells in the body and therefore GI immune function and activity have a tremendous influence on the overall health of the animal. The GI immune system operates under a delicate balance between sufficient activation to promote immune tolerance and defense against pathogenic challenges and regulatory control to prevent excessive immune activation and chronic inflammatory diseases. There are a variety of mechanisms that achieve this balance including the innate immune cells (e.g., mast cells, neutrophils, macrophages, epithelial cells, and more) which must rapidly respond to any disruption in barrier function or pathogen challenge to prevent spread of the infection and promote pathogen clearance. In addition, interactions of antigen presenting cells and antigens and subsequent trafficking to secondary lymphoid organs are critical in the establishment of tolerance and adaptive protective immunity, either from natural exposure or vaccinations.

In summary, the GI system performs numerous functions critical function and therefore, a healthy, well-developed GI system is essential to achieve optimal animal health and performance efficiency.

Importance of Early GI Development and Lifelong GI Health

During the first 4-6 weeks of postnatal life, the GI tract in the pig undergoes extensive development and maturation. During this critical period, many GI system functions are acquired and take shape. While certain postnatal changes are thought to be genetically "hard wired", many changes are driven or influenced by environmental, microbial, and endocrine cues. The specific processes of gut development has been recently reviewed (Pohl et al., 2015) and therefore will not be covered in detail in this paper. Some major events that occur simultaneously and in concert during this time are development of the GI epithelial barrier and epithelial functions, colonization of the microbiota, immune cell proliferation and functional differentiation, and enteric nervous system expansion and pruning. While the early life developmental changes occurring in these systems allow the host to survive and thrive in the extra-uterine environment, perturbations in normal developmental processes during these plasticity periods can lead to a deviation in long-term function of the GI system and increased disease susceptibility. Therefore, as nature intended, it is essential that this period remains relatively undisturbed to achieve optimal GI development and long-term health.

Weaning Stress and its Impact on Gut Health in Pigs

In nature, weaning in pigs is a gradual process and completed by ~ 12 weeks of age. However, in modern swine production weaning is abrupt and occurs between 17-24 d of age in most production systems. Weaning is also compounded with multiple stressors including maternal and littermate separation, dietary changes, transport and commingling stress, and increased pathogen exposure. As a result, weaned pigs exhibit impaired growth performance and feed efficiency and increased susceptibility to infectious enteric pathogens. Most important to note is that early weaning practices in swine production occur during the most critical stages of GI development. This likely has major impact on the development and long-term function of the GI system in pigs; however, the long-term consequences have not been investigated extensively. On the other hand, the short-term effects of weaning stress on GI health have been extensively studied.

It is known that weaning is marked by profound changes in the structure and function of the gut. These include impairment in barrier function or increased permeability (Boudry et al., 2004; Moeser et al., 2007a,b). altered nutrient and electrolyte transport (Boudry et al., 2004, Moeser et al., 2007a), villous atrophy, and inflammation (Kelly et al., 1990; McCracken, 1999). These studies have focused on the short-term (1-2 weeks post-weaning) effects on physiological and structural changes in the gut of the weaned pigs.

Impacts of Weaning Age on Short and Long-term GI Health

Weaning age is determined by many factors in production including lactation space, economics, herd health status, etc.). In previous work, we investigated whether weaning at different ages impacted the weaning-induced GI barrier function injury. Our hypothesis was that by delaying weaning age to time when the GI system was more mature, less GI injury would result. These studies demonstrated that weaning induces intestinal barrier injury and inflammation regardless of age at weaning; however, as the weaning age decreased, the GI barrier injury and epithelial secretory activity became significantly greater (Moeser et al., 2007b; Smith et al., 2010) (Figure 1). Interestingly, when measuring serum cortisol levels 24 h post-weaning, we observed that both early weaned pigs (18 d weaning) and late weaned pigs (28 d weaning) both exhibited a robust elevation in serum cortisol concentrations, compare with age-matched unweaned controls, with late weaned pigs

having slightly higher levels that early weaned pigs (Moeser et al., 2007b). This suggests that while the weaning event was equally as stressful to early and late weaned pigs, the GI response was profoundly different.

Given our knowledge of the early developmental periods in the gut, we next investigated whether early weaning impacted the long-term development of the GI system. We showed that grower pigs (4 weeks post-weaning), that were previously subjected to early weaning at between 17-19 d of age, exhibited elevated intestinal permeability compared with late weaned counterparts (Smith et al., 2010). This indicated that there were long-term deleterious changes in the intestinal barrier in early weaned pigs. Recently, we demonstrated that disturbances in intestinal permeability, enteric nervous system development, and neural-evoked secretory activity were observed at 2 and 5.5 months post-weaning (Medland et al., 2016), confirming that early weaning has long-term effects on GI development and function.

We also investigated whether piglets subjected to early weaning (16 d weaning age) influenced intestinal injury and clinical disease in response to a subsequent infectious challenge. In this study, we demonstrated that pigs weaned at 16 d of age and then challenged with F18 enterotoxigenic *E. coli* (**ETEC**) at 35 d of age, exhibited exacerbated clinical disease responses and more severe intestinal injury (impaired barrier function and histological damage) compared with pigs weaned at 22 d of age (McLamb et al., 2013) (Figure 2). Interestingly, we found that heightened disease in early weaned pigs coincided with a markedly diminished ileal IL-6 response (by 70%), and IL-8 response (by 57%) compared with late weaned pigs (Figure 2). Reductions in the cytokine response correlated with reduced ileal neutrophil infiltration (by 85%). In line with the suppressed immune response against ETEC challenge, significantly higher numbers of *E. coli* organisms were found adhered to the intestinal epithelium of the early weaned pigs.

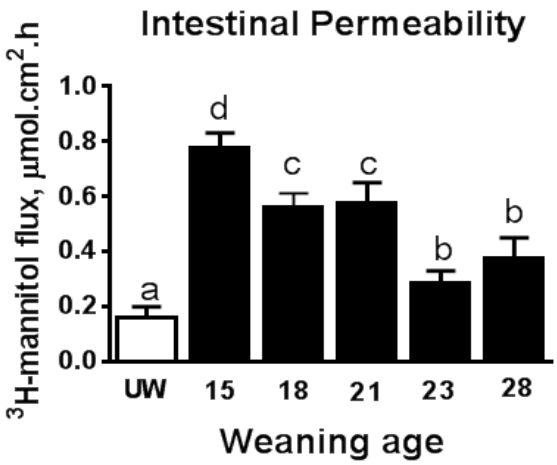
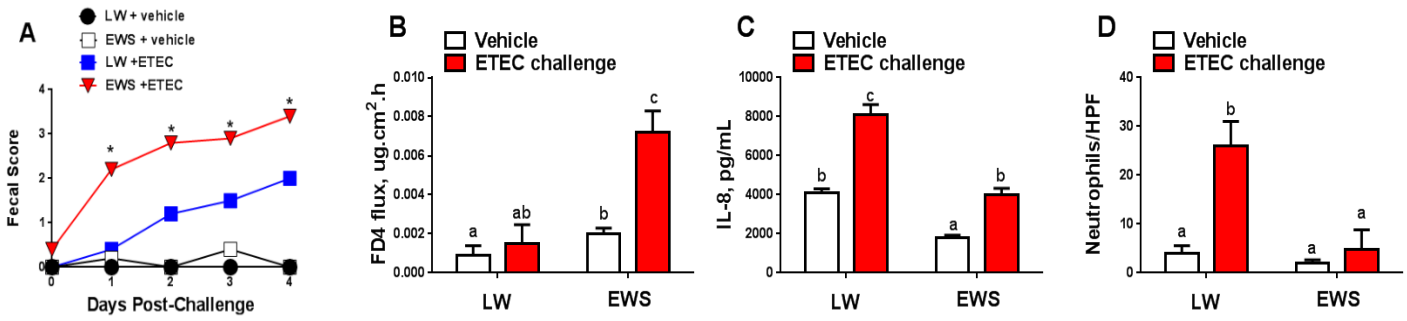


Figure 1. Impact of weaning age on post-weaning intestinal permeability. Intestinal permeability (ileum), measured as mucosal-to-serosal permeability flux rates of 3H-mannitol on Ussing chambers was measured in unweaned pigs (UW; 28 d of age) and pigs weaned at different ages.
[a,b,c,d] Different letter differ by $P<0.05$; 1-Way ANOVA. n = 6-10 pigs/wean age group.

As previously mentioned, a robust innate immune response (including pro-inflammatory cytokine release and neutrophil recruitment) is required for pathogen clearance and disease resolution which may have contributed to increased disease severity. More studies are required to understand the long-term effects of current weaning practices on immune develop; however, these data suggest that an important mechanism by which early weaning predisposes pigs to subsequent infectious challenges may be due in part to lasting defects in GI immune function.

Figure 2. Early weaned (EWS) pigs exhibit heightened clinical disease and gut barrier disruption in response to ETEC challenge with suppressed mucosal immune responses. **(A)** EWS pigs (weaned at 15-16 d of age) exhibited a more rapid onset and severity of diarrhea (indicated by blinded fecal scores), compared with late-weaned pigs (LW; weaned at 20-22 d of age). **(B)** Intestinal permeability was greater in EWS pigs compared with LW pigs measured at 4 d post-post-challenge measured as FD4 flux in Ussing chambers. Mucosal IL-8 concentrations **(C)** and neutrophil infiltration **(D)** following ETEC challenge were lower in EWS pigs compared with LW pigs. [a,b,c,d] Different letters differ by $P<0.05$; 2-Way ANOVA. n = 6-8 animals/wean age group.



Mechanisms of Weaning-induced Gut Dysfunction

Our group has begun to define the mechanisms of early weaning-induced intestinal GI dysfunction. To date, we have demonstrated critical role for an upregulated intestinal corticotropin releasing factor system and mast cell activation in driving post-weaning gut barrier injury (Moeser et al., 2007a,b). Our specific focus has been on how mast cells specifically contribute to GI barrier injury. Mast cells are hematopoetic-derived innate immune cells that reside in essentially all organ systems. While most well-known for their pathologic role in allergy/anaphylaxis and asthma (Galli et al., 2008), mast cells also play crucial, beneficial roles in pathogen defense (Abraham and St John, 2010), orchestrating immune responses (Shelburne and Abraham, 2011), and wound healing. We have shown that mast cells become rapidly activated in response to early weaning stress which is a central mechanism driving increased intestinal permeability (Moeser et al., 2007b). We have also showed that early activation of mast cells is followed intestinal mast cell proliferation observed several months after weaning and that increased mast cell activity contributed to persistent increased in intestinal permeability in early weaned pigs (Smith et al., 2010). Furthermore, our group showed that specific mast cell products including mast cell proteases (tryptase, chymase) and TNFα cause increases in intestinal permeability and disruption of the tight junction protein occludin (Overman et al., 2012).

Conclusions

Taken together, our studies demonstrate that current weaning practices (i.e., early weaning) induce immediate and long-lasting deleterious changes in intestinal development and disease susceptibility. The specific early life factors contributing to long-term gut health remain poorly understood. This is a significant gap in knowledge that has major implications to pig lifetime health and efficiency. Future research is required to gain a fundamental understanding of how early life stressors such as weaning impact long-term GI health. With this knowledge, it will then be possible to design targeted, early life intervention strategies that will support optimal development and long-term GI health, while at the same time maintaining or enhancing production efficiency.

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Low Crude Protein Diet and its Effect on Diarrhea

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Summary

Post-weaning diarrhea is a major challenge in the nutritional management of weaned piglets. Various nutritional interventions have been suggested as possible tools to mitigate this disease which cause significant economic losses to the swine industry. In this regard, feeding a low crude protein diet supplemented with crystalline amino acids has shown potential to support optimal gut health in piglets. Specifically, various studies have shown that feeding a low protein diet reduces the proliferation of pathogenic bacteria in the gut and favors those with potential gut health benefits. Also, feeding a low protein diet minimizes the attachment of enterotoxigenic Escherichia coli to the intestinal mucosa, which is an important step in its ability to cause disease. Some existing evidence suggests that a low protein diet may be used in combination with other dietary interventions to further enhance gut health outcomes in piglets. On the basis of available data, a minimum reduction of dietary protein by 4 percentage units coupled with supplementation of the most limiting amino acids (lysine, methionine, threonine, and tryptophan) is necessary to effectively utilize a low crude protein diet strategy to control piglet diarrhea.

Introduction

The immediate post-weaning period poses major challenges in the nutritional management of the piglet that has direct consequences on growth performance in the nursery through to market weight. Furthermore, this period is characterized by poor feed intake which, in concert with immature digestive and immune systems, predisposes the piglet to intestinal disturbances, especially proliferation of enteric pathogens such as enterotoxigenic *Escherichia coli* (**ETEC**) K88 (Pluske et al., 2007). To minimize the effects of weaning, baby pigs are fed complex diets often fortified with subtherapeutic levels of antimicrobial growth promoters (**AGP**). However, long-term use of AGP has been linked to the potential problem of increasing transferable resistance of bacteria to antimicrobial drugs and has been banned in many jurisdictions. Generally, piglets raised on feeds without AGP experience higher incidences of intestinal health problems and prolonged period of an immunological challenge. Therefore, a major challenge for the pig industry is to formulate starter diets that primarily fit the digestive capacity, maintain gut health, and promote growth.

Various feed additives and dietary manipulation strategies have been suggested as possible nutritional means to minimize and/or replace the use of AGP in swine diets. In this regards, it has been suggested that the use of low-crude protein (**LCP**) diets supplemented with crystalline amino acids (**AA**) may be used as part of the overall strategy for the nutritional management of early-weaned pigs, especially in antibiotic-free feeding programs. This is because pig starter diets usually contain high levels of crude protein (**CP**), which may encourage proliferation of pathogenic bacteria in the gastrointestinal tract thus leading to increased incidences of post-weaning diarrhea in weaned pigs and poor performance (Ball and Aherne, 1987; Pluske et al., 2002). It is known that most enteric pathogens preferentially ferment the proteins (Rist et al., 2013).

This review examines the concept of using LCP diets as a nutritional means of preventing incidences of post-weaning diarrhea in newly weaned pigs. The proposed mode of action is presented with supporting evidence from the literature as is the major challenge with the application of this strategy in commercial pork production.

Low Crude Protein Diets

Weaned piglets are fed high CP diets because of their low capacity for feed intake and a high potential for protein accretion. Due to incomplete digestibility, a large proportion of undigested dietary CP enters the large intestine and is subject to bacterial fermentation. However, it has been shown that the efficiency of nitrogen (**N**) utilization efficiency can be improved and pig performance maintained when feeding LCP diets supplemented with crystalline AA to meet requirements. This concept has been applied to nursery pigs to minimize the amount of fermentable protein that

enters the lower gut and the associated enteric problems (Nyachoti et al., 2006; Wellock et al., 2006; Heo et al., 2008) Although there is no general consensus on the content of CP in LCP diets to optimize gut health in piglets, a reduction by 4 percentage units coupled with fortification with the most limiting AA (Lys, Thr, Met and Trp) has been suggested (Gloaguen et al., 2014).

Proposed Mode of Action of LCP Diets

The mechanisms by which dietary CP level modulates gut health outcomes in weaned pigs are mediated mainly through alterations in microbial population and activities (Figure 1). Feeding LCP diet reduces the amount of substrate available for the proliferation of pathogenic bacteria and thus minimizes proteolytic fermentation and the production of associated toxic metabolites (Opapeju et al., 2008; Figure 2). Various studies have shown that feeding LCP diets to nursery pigs reduced intestinal concentrations of ammonia N, branched chain volatile fatty acids, putrescine and cadaverine (Bikker et al., 2006; Nyachoti et al., 2006; Htoo et a1., 2007). These metabolites of proteolytic fermentation are considered to be toxic to intestinal cells and might predispose piglets to post-weaning diarrhea. A high dietary CP level could support the proliferation of pathogenic bacteria such as ETEC by increasing the pH of the gut through its high buffering capacity (Prohaszka and Baron, 1980).

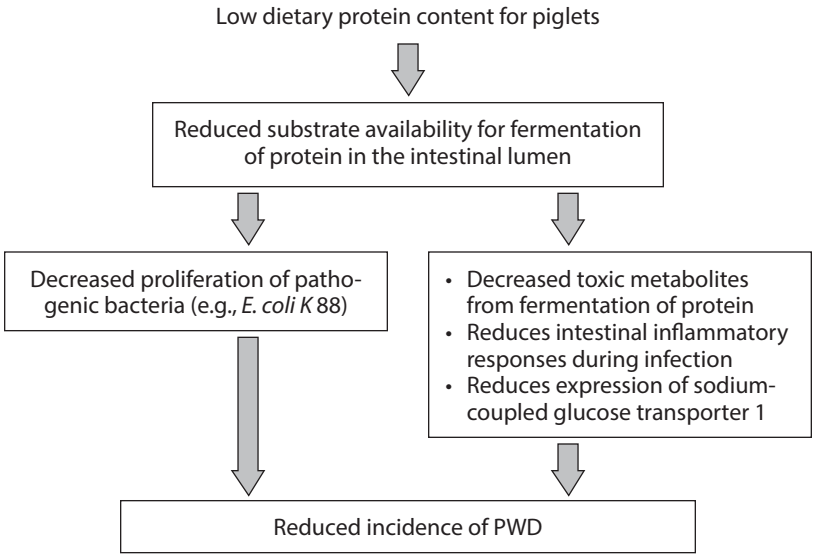

Figure 1. Proposed mechanism of action of low dietary protein level as a means to control post-weaning diarrhea. *Adapted from Opapeju et al. (2009).*

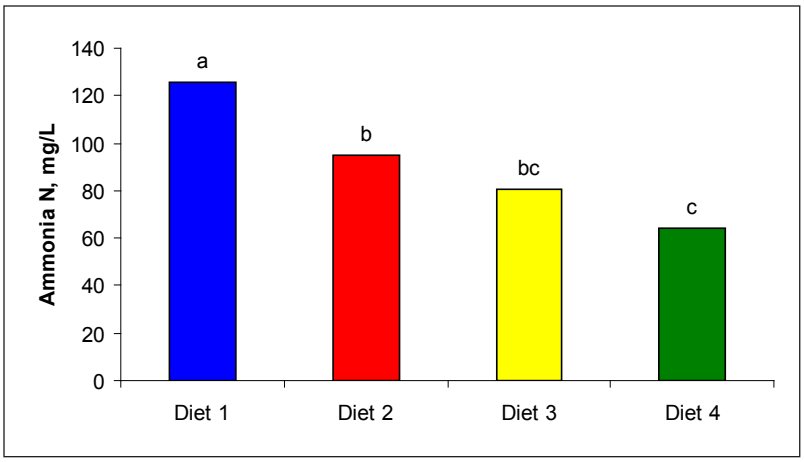

Figure 2. Ammonia nitrogen content in cecal digesta of piglets fed diets containing graded crude protein content. Diet 1 = 21% CP; Diet 2 = 19% CP, Ile deficient; Diet 3 = 19% CP plus crystalline Ile; Diet 4 = 17% CP plus crystalline Ile and Val. *Adapted from Nyachoti et al. (2006).*

In addition, reducing dietary CP from 23% to 13% in piglet diets resulted in reduced faecal water content and lower counts of pathogenic bacteria, such as coliforms, and a greater ratio of lactobacilli to coliforms, indicative of a lowered risk of incidence of post-weaning diarrhea (Wellock et al., 2008).

Effects of LCP-AA Supplemented Diets on Gut Health in Piglets

Although the term gut health is not well-defined, several indices, including those that relate to gut structure and function, and microbial population, incidences of diarrhea have been used to describe gut health outcomes. As mentioned previously, pathogenic bacteria preferentially ferments protein-producing toxic metabolites with potential to damage the gut and cause diarrhea. Indeed, several studies reported reduced incidences of diarrhea when fed LCP diets compared with diet containing high CP content to weaned pigs (Heo et al., 2008, 2009; Garcia et al., 2014; Reynoso et al., 2014; Table 1) while supporting adequate performance. Figure 3, clearly shows that there is a positive correlation between protein intake and incidences of diarrhea in piglets (Heo, 2010).

Studies by Opapeju et al. (2009) and Bhandari et al. (2010; Table 2) demonstrated that feeding a LCP diet to weaned piglets that were challenged with *E. coli* K88 significantly reduced the population of *E. coli* K88 in the jenunal and ileal digesta and mucosa-associated ETEC

Table 1. Effects of dietary protein level on performance and fecal consistency score in piglets

Reference	Dietary CP levels %	Key findings
Heo et al., 2008	17.3, 24.3%	Reduced incidence of PWD
Heo et al., 2009	25.6, 17.5%	Reduced the incidence of diarrhea after *Escherichia coli* K88 challenge. Growth performance was not affected.
Yue and Xiao, 2008	23.1%, 17.2%	Poor ADG Fecal consistency score improved.
Le Bellego and Noblet, 2002	22.4%, 16.9%	No effect on growth performance.
Gloaguen et al., 2014	19.8%, 12.7% 17.6%, 11.8%	Reduced ADG at 11.8% CP.
Bikker et al., 2006	22.5%, 15% (Fermentable carbohydrate 13.4% and 7.5%)	Altered the microflora and fermentation patterns in the GIT of weaned pigs.
Hermes et al., 2009	20.0%, 16.0% (High dietary fiber 7.15% and low dietary fiber 5.3%)	Piglets fed 16% CP supplemented with dietary fiber reduced fecal score and increased antibiotic interventions.
Luo et al., 2015	20%, 14%	Low-CP diet reduced protein and carbohydrate fermentation metabolites and altered microbial communities in the cecal digesta of piglets.
Garcia et al., 2014	20% (with or without AGP), 16% (with probiotics)	No difference in growth performance. High incidence of diarrhea – 20% CP (without AGP).
Reynoso et al., 2004	21.2%, 18.4%	Growth performance was not affected. Reduced diarrhea in piglets fed the LCP diet.
Nyachoti et al., 2006	23%, 17%	Reduced ADFI, ADG, and PUN.

compared with those fed the high CP diets. These observations suggest that dietary CP content not only influences ETEC population but also affects its ability to cause diarrhea. Furthermore, based on a review by Rist et al. (2013) and results of Opapeju et al. (2009) it is suggested that LCP diets may improve gut health outcomes by suppressing the proliferation of pathogenic bacteria while promoting those with beneficial effects. Also, Wellock et al. (2006) observed higher incidence of diarrhea and more pathogenic bacteria (coliforms) in feces in piglets fed a 23% CP diet. However, it is important to note that other studies have failed to demonstrate an effect on coliform and lactobacilli counts in colon and jejunum of weaned piglets fed LCP diets (Bikker et al., 2006, 2007; Pieper et al., 2012; Jeaurond et al., 2008). In any case there is sufficient evidence to support the argument that the benefits of feeding a LCP diet to piglets are likely to be more obvious during stress conditions such as pathogenic infection.

Effects of LCP-AA Supplemented Diets on Gut Structure and Function

Clearly, dietary protein content influences the composition and activities of the gut microbiome, which in turn has potential to affect gut structure and function. To this end, a few studies have examined the effect of dietary protein content on gut structure (represented by morphological measurements) and function. For example, Bikker et al. (2006) and Opapeju et al. (2009) reported that feeding piglets a LCP diet had no effect on the activities of intestinal enzymes (e.g., sucrose, lactase, maltase, leucine aminopeptidase, etc.). These findings suggest that feeding a LCP-AA diet to weaned piglets has no negative effect on the development of intestinal brush-border enzymes and gut maturation (Yue and Qiao, 2008). Although limited data are available, a study by Opapeju et al. (2015) reported that

Figure 3. Relationship between average daily N intake and the incidence of postweaning diarrhea in weaned pigs. HP = pigs fed high protein diet; HP+E = pigs fed high protein with ETEC infection, LP = pigs fed low protein diet, LP+E = pigs fed low protein with ETEC. *Adapted from Heo, 2010.*

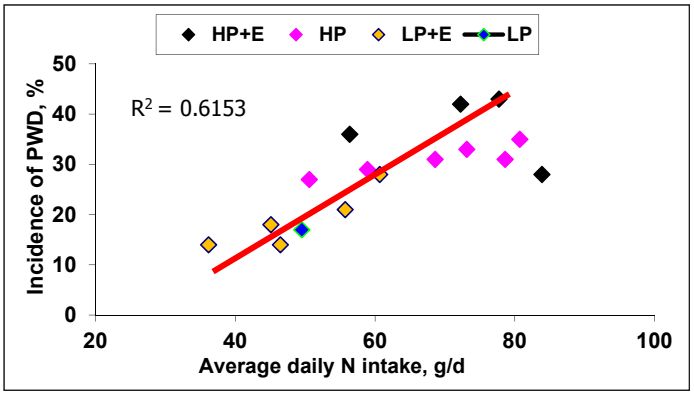

Table 2. Effect of different protein levels on performance, fecal consistency score and *Escherichia coli* K88 counts from ileum mucosa in early-weaned pigs. *Adapted from Bhandari et al. (2010).*

	Diets[a]									
	High protein			Low protein				P-value[c]		
Item	NA	AB	PRO	NA	AB	PRO	SEM[b]	A	B	A*B
Daily gain, g										
Pre-infection, d 0 to d 6	73	94	79	121	101	179	8.41	<0.001	<0.001	<0.001
Post-infection, d 7 to d 12	176	176	200	201	219	249	16.33	0.007	0.086	0.757
Daily feed intake, g										
Pre-infection, d 0 to d 6	123	140	122	187	128	238	41.56	0.111	0.48	0.363
Post-infection, d 7 to d 12	288	261	287	299	281	326	34.11	0.384	0.624	0.917
Fecal consistency score, 72 h post-infection[d]	2.38	1.55	1.42	1.75	1.05	1.17	0.086	<0.001	<0.001	0.103
ETEC K88 count in mucosa of ileum, log cfu/g	5.25	2.96	2.72	3.9	2.47	2.51	0.235	0.002	<0.001	0.951

[a] NA = no additive; AB = in-feed antibiotics; PRO = probiotics (two probiotic strains of *E. coli* named UM2 and UM7)
[b] Pooled SEM; n = 8/diet
[c] A = factor A-two levels of protein (high protein and low protein); B = factor B-three additive (NA, AB and PRO)
[d] Fecal consistency score: 0, normal; 1, soft feces; 2, mild diarrhea; and 3, severe diarrhea.

high CP diet increases the expression of SGLT-1 compared to a LCP diet in jejunum of weaned piglets which could be an adaptive response to onset of diarrhea in response to ETEC infection.

For optimal digestive and absorptive function of the small intestine, longer villi height is desirable (Pluske et al., 1997). In this regard, Opapeju et al. (2009) demonstrated that piglets fed a high CP diet (22.2%) and those fed a LCP-AA diet (17.3%) had similar villi height in the small intestine. Similarly, Heo et al. (2010) observed no differences in small intestinal morphology in piglets fed a LCP-AA diet (19%) compared to a high CP diet (23.9%). Hypertrophy of crypt is an indication of injury to the intestine, which could be due to pathogen colonization, toxic metabolites, or feed antigens. In a study by Opapeju et al. (2008), piglets fed high a CP diet (21.0%) had significantly deeper crypt depth than those fed a LCP-AA diet (17.0%). The outcomes of these studies indicate that a LCP diet fed to piglets reduced crypt depth but did not affect villi height.

The effect of feeding a LCP diet to piglets on the immune response has been examined only in limited number of studies. In a study with weaned piglets challenged with ETEC, feeding a LCP-AA diet was shown to reduce inflammatory responses (Heo et al., 2008; Opapeju et al., 2010). Specifically, piglets fed a LCP-AA diet (17.6%) had significantly lower concentrations of serum interleukin-1β and haptoglobin compared to those fed a high CP diet (22.5%) during the post-challenge period (Opapeju et al., 2010). Similarly, Houdijk et al. (2007) observed an increase in plasma haptoglobin concentrations in piglets fed high CP diets compared to those fed a LCP diet. Collectively, these findings suggest that feeding a LCP diet could modulation inflammatory responses in piglets subjected to disease challenge conditions.

Considerations for Using LCP Diets to Control Piglet Diarrhea

Reducing dietary CP content inevitably leads to a reduction in dietary AA acid supply, which can compromise animal performance. In an attempt to overcome this, LCP diets are fortified with crystalline AA, which have been shown in several studies to control gut health without compromising growth performance of weaned pigs compared to high-CP diets. However, in other studies feeding LCP diets to weaned piglets has not always supported performance to levels similar to those of pigs fed a high CP diet. For example, feeding piglets a 17.6% diets supplemented with essential AA reduced growth performance (Opapeju et al., 2008); an observation that was attributed to a deficiencies of nonessential AA in the LCP diet. Similarly, Nyachoti et al. (2006) concluded that weaned piglets fed a diet with 19% or less CP reduced growth performance. Weaned pigs fed a diet with 17.2% CP had poorer growth rates compared to those fed a 23.1% CP diet (Yue and Xiao, 2008). A similar observation was made in a study with piglets fed a diet with 11.8% CP compared to controls (Gloaguen et al., 2014). These observations emphasize the need to carefully formulate LCP diets to ensure that adequate levels of all AA are provided in the diet in appropriate proportions.

Another important consideration is how a LCP diet can be utilized along with the available interventions to manage diarrhea in piglets. In this regard, few studies have examined synergistic effects of a LCP diet with other feed additives or formulation strategies. Nonetheless, a study by Bhandari et al. (2010) utilizing an ETEC challenge model reported that a LCP diet could act synergistically with probiotics to support growth performance in piglets similar to that piglets fed a con-

trol diet containing an AGP (Table 2). The authors suggested that these beneficial effects were possibly mediated through different mechanisms by the two factors. Because addition of fermentable fiber to swine diets has been shown to reduce protein fermentation in the gut (Konstantinov et al., 2004; Awati et al., 2006; Rist et al., 2013), it is possible that combining additional fermentable fiber in a LCP diet may further enhance gut health outcomes in piglets. However, these concepts need to be developed further before they can be effectively and routinely applied in managing diarrhea in piglets.

Conclusions

High dietary protein is an important predisposing factor for the occurrence of diarrhea in piglets because it encourages the proliferation of pathogenic bacteria with significant implications on gut health outcomes. Thus, feeding a LCP diet (i.e., a minimum reduction of 4 percentage units) has been suggested as a strategy to mitigate diarrhea disease in weaned piglets as several studies have shown that it not only reduces the proliferation of pathogenic bacteria but also their ability to cause infection. However, feeding a LCP diet may lead to reduced performance due to inadequate supply of AA, thus such a diet must be carefully fortified with crystalline AA (i.e., those that are likely to be most limiting) to meet requirements. Combining a LCP diet with other feed additives may further enhance gut health outcomes in piglets.

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Nutritional and Management Strategies to Alleviate the Impact of Stress on Swine Health and Productivity

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Summary

Although pigs are subjected to multiple stressful events throughout their life cycle they are often able to adapt and maintain homeostasis. However, when exposed to multiple stressors such as thermal stress, weaning stress, and transport stress, distress may occur and this has the potential to increase disease states, reduce welfare, decrease productivity, and in severe cases cause morbidity and mortality. In order to prevent or reduce the likelihood that pigs will succumb to stress, several management and nutritional strategies have been developed to alleviate the impact of stress on swine health and productivity. Specifically, targeting gut health during times of stress may be an effective strategy to improve the health and well-being of swine.

Introduction

Animal stress is a multi-factorial problem that negatively impacts pig performance and is closely linked with animal welfare. Although stress is a normal experience for swine and not necessarily harmful when animals are able to cope, there is a potential for distress, and therefore compromised animal welfare when animals are exposed to multiple stressors and are unable to maintain homeostasis. Unfortunately, in modern production systems, pigs are frequently exposed to multiple stressors (e.g., mixing, weaning, transport, handling, isolation, thermal stress) that have the potential to increase incidence of disease, pathogen shedding, and in severe cases, animal mortality. Furthermore, as public concern for farm animal welfare and husbandry practices increase, so will the need for research demonstrating production practices that not only increase animal performance, but also improve animal welfare and promote stress recovery.

Stress in Swine Production

Thermal Stress

Heat stress (**HS**) can negatively impact animal health, and extreme cases may result in reduced animal welfare and mortality depending on the severity of the heat load (as reviewed by Johnson et al., 2015). Livestock primarily use non-evaporative cooling (radiation, convection, and conduction) to exchange heat with their environment. However, when the ambient temperature rises to an animal's upper critical limit, non-evaporative cooling is no longer efficient and animals rely more on evaporative cooling from skin (e.g., sweating) and upper respiratory tract (e.g., panting; Johnson et al., 2015). Unfortunately, swine do not possess functional sweat glands and their lungs are relatively small, thus increasing pigs' susceptibility to HS (Johnson et al., 2015). Heat stress-induced reductions in growth rate and carcass value, increased health care cost, and mortality are a substantial cost to the swine industry and it is likely that climate change will have a greater effect on HS-related losses in the future. In addition to its well-documented postnatal effects, prenatal HS can cause long-lasting phenotypic changes in offspring that can negatively affect the future productivity and well-being of pigs (Johnson et al., 2015). Recent studies (Johnson et al., 2015) have indicated that *in utero* HS may reduce growth performance and efficiency, change postnatal nutrient partitioning priorities, cause teratogenicity, affect post absorptive metabolism, and alter thermoregulation in pigs during postnatal life. Although not currently quantified, it is likely that these changes would increase production losses during postnatal life resulting in greater economic losses for the producer.

In addition to the effects of HS, swine can be impacted by cold stress (**CS**); however, CS susceptibility depends on age and the physiological state since swine become more resistant as they grow. A neonatal pig's

lower critical temperature is approximately 30°C compared to 20°C for growing pigs, and 10°C for pregnant sows (Young, 1981), and this has implications for how pigs at different ages are managed during times of CS. Neonatal pigs are particularly susceptible to the effects of CS due to a lack of subcutaneous fat, brown adipose tissue, and a high surface area-to-mass ratio (Le Dividich and Noblet, 1983). As a result, CS is reported to be the leading cause of death among newborn piglets (Le Dividich and Noblet, 1983). In addition to the risk of CS after birth, weanling pigs transported during times of CS may have an increased risk of morbidity and mortality since trailers are not climate-controlled and piglets are transported year round. Therefore, it is important to maintain ambient temperature at the appropriate level based on the production stage and avoid rapid temperature fluctuations to improve productivity and health of swine.

Weaning and Transport Stress

Weaning piglets at 21 d has been broadly adopted by the U.S. swine industry to increase the number of litters per sow and increase overall productivity. Although this is a widely adopted practice that is necessary for efficient production, weaning in modern production systems is considered a stressful process for piglets due to sow separation, piglet mixing, and the abrupt change from milk to dry feed. As a result, weaning leads to depressed growth rates, increased intestinal permeability, and greater disease susceptibility (Smith et al., 2010), putting piglets at a higher infection risk. In addition, although essential in commercial swine production to reduce the vertical transfer of disease and to improve post-weaning growth and productivity, newly weaned pigs are often transported for long periods of time to growing facilities and this can reduce pigs' welfare and increase mortality. Transport of livestock is one of the most stressful and injurious stage in the chain of operations, and contributes significantly to poor animal welfare and production losses. Average transport times can often last up to 24 h, and during this time, loss of body condition can occur due to dehydration and feed restriction, which can have detrimental effects on production (i.e., reduced weight gain and feed intake) that are exacerbated by disease states (i.e., diarrhea) and weaning. Furthermore, possible temperature fluctuations within a wide range during different seasons may exacerbate weaning stress. Taken together, weaning and transport stress can increase the incidence of infections, morbidity, and can reduce welfare and the overall productivity of pigs.

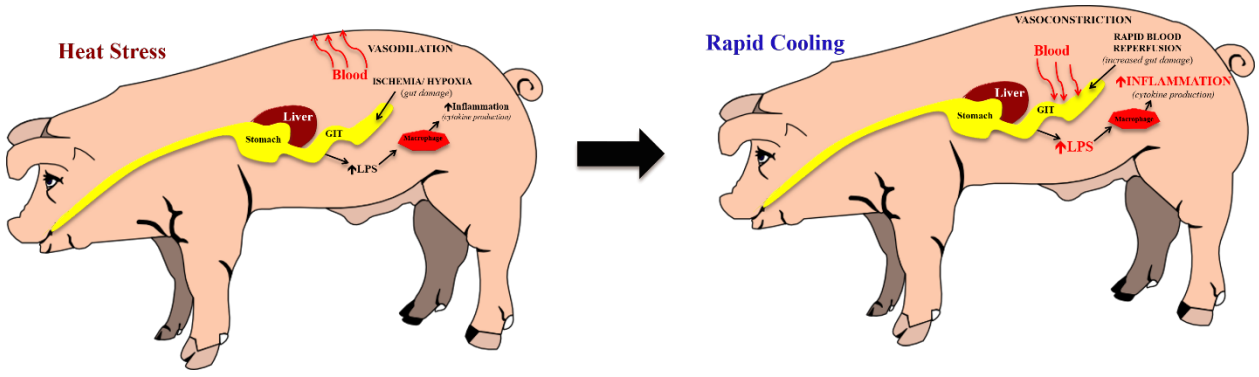
Stress and Gut Health

Thermal Stress

Exposure to high ambient temperatures can impose considerable health and physiological stress-related problems in swine, and the gastrointestinal tract is one of the primary organs affected. Heat stress repartitions blood to the periphery which decreases intestinal blood flow and may lead to hypoxia, ATP depletion, and apoptosis (Johnson et al., 2015). The reduced blood flow and resulting hypoxia at the intestinal epithelium can alter intestinal morphology and may compromise the ability of tight junctions to maintain an effective barrier increasing the probability of bacterial translocation into the blood stream (Johnson et al., 2015), and the subsequent endotoxemia/septicemia and inflammation may be partially responsible for the negative effects of HS on productivity. Furthermore, HS-induced changes on villi morphology may decrease nutrient digestibility and absorption in heat-stressed animals (Johnson et al., 2015).

Hyperthermia negatively affects swine and the management of individual pigs after a heat event may cause additional harm depending on the method used in cooling. Rapidly cooling pigs after acute HS results in a pathological condition characterized by skin vasoconstriction, intestinal damage, and a whole-body septic

Figure 1. Physiological impact of rapid cooling on pigs. *Adapted from Johnson et al., 2015, 2016a.*



response in pigs (Johnson et al., 2016a; Fig. 1). Rapidly cooled pigs experience an immediate reduction in skin and rectal temperature; however, despite the apparent increase in thermal gradient, heat dissipation capacity is reduced and a sustained elevation in gastrointestinal tract temperature can occur. These data indicate that rapid cooling procedures cause peripheral vasoconstriction, which in turn may stimulate a rapid reperfusion of the gut with heated blood (Johnson et al., 2016a). As a result, rapid cooling after HS appears to exacerbate intestinal morphological changes as indicated by a reduction in villus height and the villus height-to-crypt depth ratio compared with gradually-cooled pigs, likely contributing to a reduction in intestinal integrity (Johnson et al., 2016a).

Weaning and Transport Stress

Weaning increases piglet disease vulnerability, especially since the associated stress can compromise intestinal integrity. Deterioration of the intestinal barrier is primarily due to a combination of chronically elevated stress hormones (Smith et al., 2010) and decreased feed intake, which is illustrated by reduced villous surface area, increased intestinal permeability, and mucosal inflammation (Smith et al., 2010). Unfortunately, the negative effects of weaning on intestinal function may have long-term implications for swine health and welfare because stress (physical and psychological) in early life is associated with the development of chronic persistent gastrointestinal tract disorders and disease in adulthood (O'Mahony et al., 2009), likely mediated by permanent defects in intestinal barrier properties. Since the intestinal epithelial barrier is the first line of defense against a hostile environment within the intestinal lumen, disruption can lead to translocation of luminal antigens and bacteria across the epithelium, triggering mucosal inflammation and whole-body sepsis, as well as immunological responses and an increased susceptibility to infections (Lallés et al., 2004). When combined with the immature immune system of newly weaned piglets and a sudden cessation of passive immunity from the milk of the sow, the effects of weaning on piglet health and long-term growth rate can be devastating if not appropriately managed, especially when combined with other external stressors such as transport stress.

Stress Mitigation Strategies

Thermal Stress

Although significant genetic, reproductive, and nutritional improvements have been made to combat HS in swine, appropriate management strategies still represent the main approach (Johnson et al., 2015). Providing

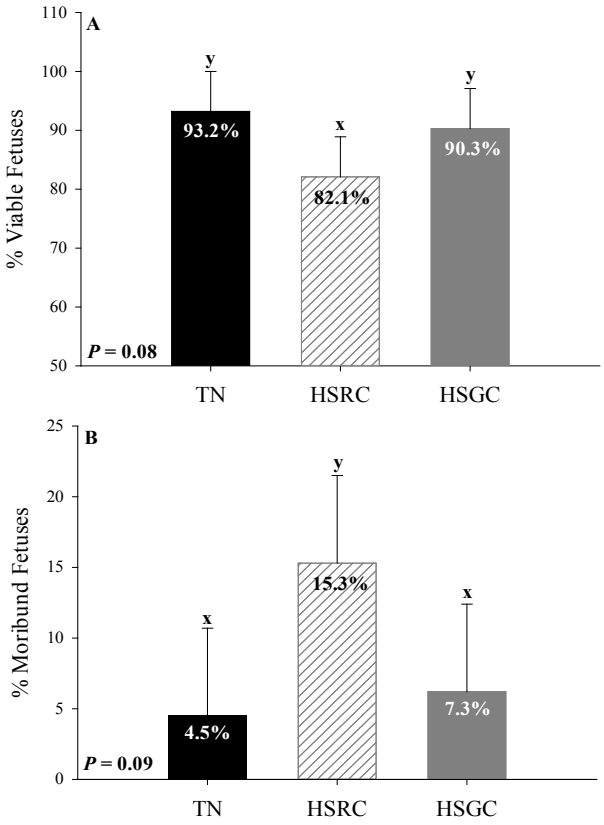


Figure 2. Effects of rapid temperature fluctuations on (A) fetal viability, and (B) fetal moribundity. Abbreviations are: thermoneutral (**TN**), heat stress and rapid cooling (**HSRC**), and heat stress and gradual cooling (**HSGC**) recovery treatment. Error bars indicate ± 1 SEM. Letters (x < y) indicate tendencies (0.05 < *P* < 0.10) between recovery treatments. *Johnson et al., 2016b.*

proper ventilation, air circulating fans, and evaporative cooling can greatly reduce the heat load and help pigs maintain euthermia. During hotter parts of the day, stressors such as vaccinations and restraint should be avoided as handling stress can exacerbate the negative effects of HS and social stressors such as mixing can reduce feed intake and growth during times of HS (Johnson et al., 2015). Finally, building design can significantly influence the health and well-being of pigs during times of thermal stress and must take into account animal heat and moisture production.

In addition to the aforementioned management strategies, controlling for rapid temperature fluctuations may reduce the incidence of seasonal infertility and can improve reproductive efficiency in sows. Rapidly cooling pigs after acute HS results in a pathological condition characterized by skin vasoconstriction, maintenance of intestinal damage, and a whole-body septic response in pigs (Johnson et al., 2016a; Fig. 1). Consequently, this may be one mechanism by which rapid temperature fluctuations can contribute to repro-

ductive losses in sows, especially when low night-time temperatures are combined with evaporative cooling technology during times of HS (Johnson et al., 2016b). After subjecting sows to either TN conditions, HS and rapid cooling (**HSRC**), or HS and gradual cooling (**HSGC**) 8 ± 0.3 d prior to estrus and breeding, it was determined that rapid cooling tends to reduce viable fetus count and increase moribund fetus count when measured ~30 d post-breeding (Fig. 2; Johnson et al., 2016b). Similar to previous observations (Johnson et al., 2016a), rapid cooling directly prevented effective heat dissipation through the skin, and caused an increase in TNFα production, which likely contributed to the observed increase in insulin resistance since TNFα can inhibit downstream insulin signaling from the insulin receptor (Marik and Raghavan, 2012). Therefore, because insulin is a pleiotrophic hormone and is a crucial component in normal oocyte development and function (Ou et al., 2012), greater insulin resistance may have decreased oocyte quality and could explain why fetal viability was reduced in HSRC pigs despite the fact that insulin resistance was detected 8 ± 0.3 d prior to breeding. Furthermore, reduced fetal viability and increased moribundity has obvious implications towards future farrowing rates and litter size and assuming that only viable fetuses would have survived to term, HSRC pigs would have farrowed 1.1 less piglets per litter (Johnson et al., 2016b).

Management strategies can be complemented by nutritional interventions during times of HS. Insulin action, the heat of nutrient processing, and gut integrity can be significantly impacted during HS, and can benefit from changes in diet composition or the addition of nutritional supplements (as reviewed by Rhoads et al., 2013). Improving insulin sensitivity through the addition of supplements (e.g., chromium, lipoic acid, and thiazolidinediones) can be an effective tactic to improve animal performance during HS (Rhoads et al., 2013), since insulin action is thought to be a key component of successfully adapting to and surviving a heat load. Increasing energy density by replacing fiber with fat sources can also reduce the impact of HS on growing pigs and increase the amount of energy received per kg of feed intake, which is important considering that a reduction in feed intake is a widely reported consequence of HS in pigs (Johnson et al., 2015). Finally, the gastrointestinal tract is highly susceptible to the effects of HS and may be manipulated by nutritional interventions such as the inclusion of glutamine, zinc, and betaine.

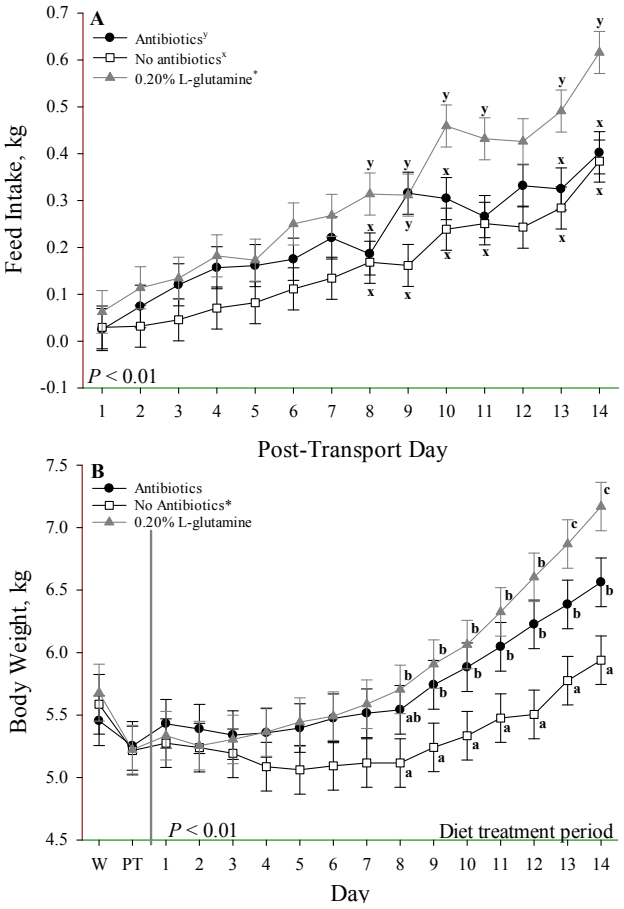


Figure 3. Effects of post-transport dietary supplementation of antibiotics (CTC + Denagard), no antibiotics, and 0.20% L-glutamine by post-transport day of treatment on (A) feed intake and (B) body weight in pigs. Error bars indicate ± 1 SEM. Letters (a < b) and an asterisk (*) indicate significance and letters (x < y) indicate tendencies (0.05 < *P* < 0.10) between dietary treatments (unpublished data, USDA-ARS).

Weaning and Transport Stress

To combat the negative effects of weaning and transport stress on pig health and well-being, current management practices call for environmental, social, and nutritional management strategies to improve pig health and performance (Chiba, 2010). Piglets are traditionally weaned at 21 d of age in U.S. commercial swine operations and possibly earlier (12-14 d of age) depending on individual farm practices and available facilities and often immediately transported to finishing facilities, and this has the potential to induce further stress in piglets. While weaning piglets past 21 d of age or waiting to transport piglets until post-weaning stress has subsided may not be an economically viable or a practical option, steps can be taken on an individual farm basis to reduce stress resulting from weaning and transport. For example, split weaning can be utilized to assist smaller pigs, farms can keep littermates intact and prevent mixing with new pigs which will reduce

fighting (Chiba, 2010). Additionally, group sizes can be kept to a minimum (≤ 20 pigs/pen), which can help to reduce the effects of weaning time on piglet health and performance (Chiba, 2010). Environmental accommodations such as daily cleaning of nursery pens can reduce pathogenic organisms. Furthermore, maintaining ambient temperature at levels that are within the zone of thermal comfort based on piglet size, reducing drafts in nursery pens and in trailers during seasonal cold stress, and providing adequate space for weaned piglets in pens (0.6-0.8 m^2 per piglet) and trailers (0.06-0.07 m^2 per piglet) are all management procedures that can be made to improve the welfare and productivity of piglets.

In addition to altering management practices, nutritional strategies can be employed to improve piglet welfare and performance during weaning and transport stress. The primary reason for post-weaning stress and performance loss is a drastic reduction in energy intake following weaning due to a radical change in diet (Chiba, 2010), which can result in nutritional, psychological, and immunological disruptions. In order to stimulate feed intake and improve piglet health and growth performance, nutritional practices such as hand feeding several times per day and adding water or milk replacer to starter diets may be used to improve feed intake (Chiba, 2010). Phase feeding based in piglet age and weight can also stimulate feed intake and can be used to gradually adapt the gastrointestinal tract from digesting highly digestible and complex sow milk to less digestible and simple starch-based diets (Chiba, 2010).

Inclusion of therapeutic or sub-therapeutic antibiotics to reduce pathogen load and promote growth in pigs recovering from weaning and transport stress is a common practice; however, due to the possible contribution of in-feed antibiotics to the development of antibiotic resistant strains of bacteria (Smith et al., 2010), and increasing consumer pressure towards reducing therapeutic antibiotic use, nutraceutical supplements may be beneficial in reducing pathogen load and improving piglet health. Including dietary protein supplements, as well as probiotics, and organic acids can also serve to reduce pathogen load and improve gut health. Specifically, adding protein sources such as milk products, fish meal, dried whey, and dried plasma proteins to weaned pig diets can improve stomach barrier function (Halas et al., 2007), which is essential for eliminating ingested bacterial pathogens.

Probiotics are live microorganisms that may confer a health benefit to the host when administered in adequate amounts and may be used to physically exclude pathogens, for production of natural antibiotics and antifungals, and for maintenance of gut barrier function and promotion of the anti-inflammatory response (Roselli et al., 2007). However, their usefulness in swine production as a tool to promote improved health and productivity has yet to be determined as results from studies looking at the appropriate organism, dose of viable organisms, and life cycle stage of the recipient animal are often varied. Weak organic acids can improve nutrient digestibility in newly weaned pigs by reducing

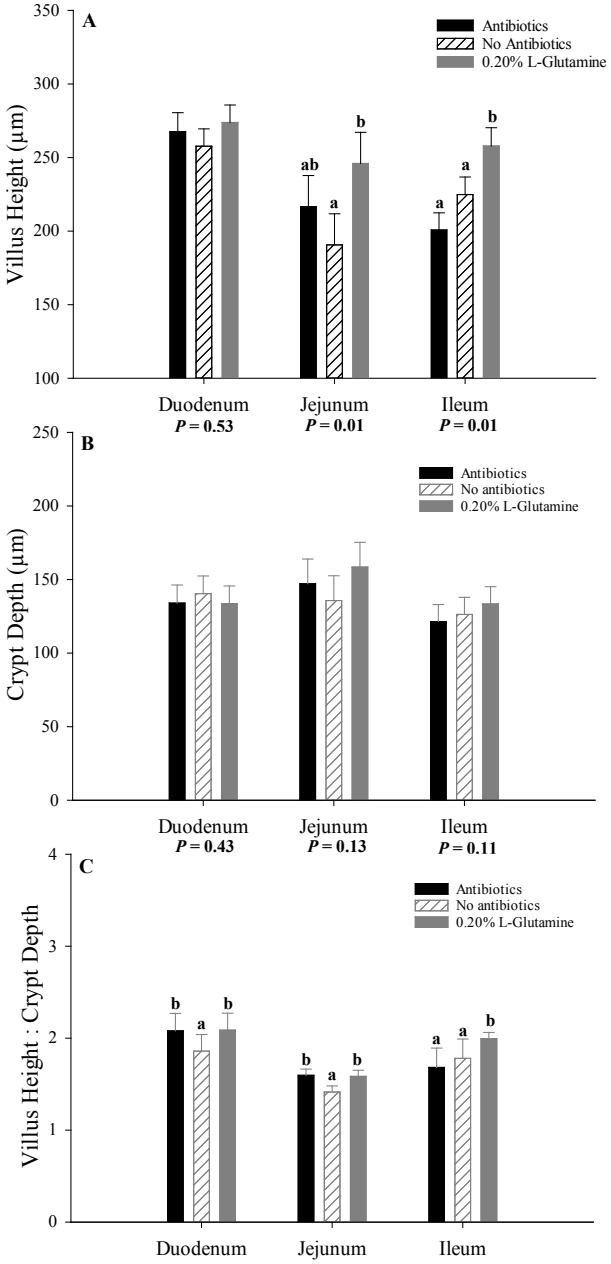


Figure 4. Effects of post-transport dietary supplementation of antibiotics (CTC + Denagard), no antibiotics, and 0.20% L-glutamine by post-transport day of treatment on (A) villus height, (B) crypt depth, and (C) the villus height to crypt depth ratio in pigs. Error bars indicate ± 1 SEM. Letters (a < b) and indicate significance and letters (x < between dietary treatments (unpublished data, USDA-ARS).

gastric pH which can increase the conversion of pepsinogen to active pepsin for effective protein hydrolysis, as well as enhance apparent total tract digestibility and growth performance (Suiryanrayna and Ramana, 2015). Additionally, the pH reducing function of organic acids such as lactic acid can delay the multiplication of enterogenic *E. coli* (Suiryanrayna and Ramana, 2015), and therefore decrease pathogen load in pigs stressed from the weaning and transport process.

In addition to the aforementioned nutraceutical agents, we recently determined that replacing therapeutic antibiotics (CTC + Denagard) in swine starter diets with 0.20% L-glutamine (cost-competitive compared to therapeutic antibiotics) can improve piglet health and productivity after weaning and simulated transport (unpublished data, USDA-ARS). In this study, feed intake (Fig. 3A) and body weight gain (Fig. 3B) were greater in newly weaned piglets that were supplemented with 0.20% L-glutamine for 14 d after a simulated transport compared to those provided therapeutic antibiotics (CTC + Denagard) or no therapeutic antibiotics (unpublished data, USDA-ARS), and this increase in productivity was likely due to improved intestinal health (Fig. 4). By increasing feed intake and intestinal health, incidences of illness were reduced in pigs supplemented with 0.20% L-glutamine compared to those given no therapeutic antibiotics. Therefore, dietary supplementation with 0.20% L-glutamine may be a viable alternative to therapeutic antibiotics to improve piglet health and productivity following weaning and transport; however, more research must be done to evaluate its effectiveness in a production environment.

Conclusions

Stress can have a devastating impact on the health and well-being of swine if inappropriately managed. However, research to improve management practices and develop nutritional strategies have provided producers with tools to reduce or promote recovery from stress and improve the overall health and productivity of swine. Despite these advances, pigs still succumb to the negative effects of stress and this can reduce animal welfare, and cause economic losses for the producer. Therefore, there is an urgent need for research to enhance swine stress resilience while improving both productivity and animal welfare.

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Peroxidized Lipids in Nursery Pig Diets – Why and When Should We be Concerned?

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Summary

Lipid peroxidation products can induce oxidative stress, compromise immunity, decrease intestinal function, and reduce growth performance. Therefore, it is important to characterize the oxidative status of lipids that are used in swine diets. However, the impact of the several quantifiable oxidative products that collectively describe peroxidation status on production performance has not been experimentally determined. We demonstrated that forced lipid peroxidation of soybean oil, using heat and oxygen perfusion, progressively increased markers of peroxidation with increasing time of exposure, resulting in decreased feed intake (by up to 7%) and growth rate (by up to 9%) when fed to nursery pigs. In addition, digestibility, absorptive capacity, and morphology of the intestine were compromised with increasing peroxidation, and these effects were related with disruption of the redox environment of the intestinal mucosa. The response of pigs to peroxidation products appears to be progressive, and a minimum threshold for lipid peroxidation above which detrimental effects are detectable likely exists. We further demonstrated that peroxidation reduced serum vitamin E concentrations indicating increased utilization of antioxidant components in pigs, even in pigs where growth performance was not significantly affected. Concurrently, feeding a synthetic antioxidant blend exerted a sparing effect as evidenced by increased serum concentrations of vitamin E. Although the impact of peroxidized lipids on growth performance may appear to be subtle, we clearly demonstrated in a large field study that increasing the level of peroxidation significantly increased mortality, number of pigs medicated, and number of pigs that were excessively light, resulting in reduced total pig weight produced at the end of the nursery. Although growth of pigs that remained in test pens was not affected, total pen gain was decidedly harmed because it takes into account pig mortality and removal for extensive medical treatment. Thus, quantification of peroxidation level of lipid sources for swine is critically important to design quality control programs for oil and fat sources and to increase profitability of pork production, especially for weaned pigs that are expected to be the most vulnerable to poor lipid quality.

Introduction

Lipids that are supplemented to diets for swine are derived from a variety of sources and may include rendered animal fats, restaurant grease, vegetable oils, including oils from DDGS, and blended fats and oils. Therefore, the composition and quality of lipids vary considerably (van Kempen and McComas, 2002; Shurson et al., 2015). The oxidative quality of lipids may be compromised and can be impacted by a variety of factors (Kerr et al., 2015). Sebastian et al. (2014) determined that frying oils that were in use in restaurants in Canada were highly peroxidized and that the degree of peroxidation of discarded lipids was even greater. Discarded lipids are commonly recycled into livestock diets. Peroxidized lipids can reduce production performance, induce oxidative stress, impair the antioxidant defense system, and impair intestinal integrity and function as reviewed by Kerr et al. (2015) and Shurson et al. (2015). However, effects of peroxidized lipids have not been consistent, which may be related to the extent and dose of peroxidized lipid consumed.

Lipid Peroxidation

Lipid peroxidation initiates with the formation of free lipid radicals and hydroperoxides, which can subsequently react with other unsaturated fatty acids to form additional hydroperoxides (propagation step). Hydroperoxides are subsequently decomposed into secondary and tertiary oxidation products (termination). During lipid peroxidation, there is a rapid increase in hydroperoxides as fatty acid oxidation initiates, followed by a decline in hydroperoxides and increase in secondary and tertiary oxidation products (Figure 1).

Most decomposed products of hydroperoxides, such as alcohols, aldehydes, furans, hydrocarbons, ketones, and acids, are responsible for undesirable odors and fla-

vors in peroxidized oils (Kim and Min, 2008). Saturated lipids are less prone to peroxidation, and peroxidation rates increase with increasing degree of unsaturation of lipids. In addition, increasing temperature, irradiation, and oxygen pressure stimulate the rate of peroxidation. Because many compounds are produced during lipid peroxidation, a variety of analytical tests must be used to measure peroxidation. Preferentially, these tests should be combined to better determine the peroxidation status of the lipid (NRC, 2012) and measurements of the primary (i.e., hydroperoxides) and secondary (i.e., aldehydes) peroxidation products are needed to truly determine the peroxidation status of a lipid source. Nonetheless, the degree of lipid peroxidation is very difficult to characterize because the compounds that are produced are unstable and decompose as peroxidation progresses.

Measurements of Lipid Peroxidation

The most common indicative assays of peroxidation include peroxide value, anisidine value, and thiobarbituric acid reactive substances (**TBARS**) (NRC, 2012; Shurson et al., 2015; Kerr et al., 2015). Peroxide value measures primary oxidation products (hydroperoxides and peroxides), while anisidine value and TBARS measure formation of aldehydes in the propagation step. The reaction of TBARS with malondialdehyde (**MDA**) produces a conjugated-double-bond compound which is measured to indicate oxidation in the TBARS assay. Specific aldehydes (4-hydroxynonenal and 2,4-decadienal) produced in the termination step can also be determined (Kerr et al., 2015). Furthermore, predictive tests such as the active oxygen method and oxidative stability index are used to quantify the oxidative stability of lipid and involve the exposure of samples to increased temperatures and oxygen pressure, accelerating the peroxidation process. Nonetheless, all of these and other assays have limitations (NRC, 2012; Liu et al., 2014a; Shurson et al., 2015), which further complicates the estimation of peroxidation and predicates the need for novel approaches.

Biological Response to Lipid Peroxidation

Absorption and metabolism of peroxides in the intestine is related to the redox status of the gut. Generally, the proportion of reducible glutathione (**GSH**) to its oxidized product, glutathione disulfide (**GSSG**), is a useful indicator of redox status in enterocytes (Circu and Aw, 2011). Glutathione concentration in the intestine determines peroxide accumulation in the lumen, in that increased glutathione concentration decreases peroxide concentrations in the lumen (LeGrand and Aw, 2001), as supported by Aw et al. (1992a,b). As peroxides enter the cell, reduction is achieved by glutathione peroxidase, producing GSSG and hydroxide. GSSG can be reduced back to GSH by GSSG reductase, in a NADPH dependent reaction (LeGrand and Aw, 2001). Moreover, the cysteine/cystine concentration contributes to the GSH/GSSG redox couple, and it is important in maintaining extracellular and luminal redox state (Circu and Aw, 2011).

Kanazawa and Ashida (1998) reported that dietary oxidized lipids decompose to aldehydes and ketones in the gastric lumen where most of them are absorbed and incorporated into gastric tissue, while remaining aldehydes pass to the small intestine where they are absorbed and finally accumulate in the liver. Toxicity of dietary lipid peroxides has been reported in rats. Kanazawa et al. (1985) found secondary products of oxidation to cause enlarged livers and increased serum transaminase activity. Tsunada et al. (2003) found increased peroxide content in tissues, along with a decreased GSH and increased GSSG concentration at 2 weeks of feeding, after which redox status was comparable to controls, suggesting an adaptive response to prolonged feeding. Moreover, GSH peroxidase and GSSG reductase activities were increased in the jejunum after 8 weeks of feeding. Peroxidized oil also depressed enterocyte proliferation and apoptosis in the small intestine, but not in the colon. A plethora of studies have demonstrated that dietary lipid peroxidation sub-products can induce cellular oxidative stress (Tsunada et al., 2003; Ringseis et al., 2007; Rosero et al., 2015). This is defined as the disturbance of biological redox signaling events and appears to compromise immunity, fertility and other important biological events (Sordillo and Aitken, 2009). Feeding of peroxidized lipids has been reported to decrease growth performance of pigs (DeRouchey et al., 2004; Harrell et al., 2010; Liu et al., 2014b; Rosero et al., 2015), although others failed to detect differences (Hanson et al., 2015). The response of animals to peroxidation products is likely progressive and a minimum dose of lipid peroxidation, above which detrimental effects are manifested, is expected.

Degree of Lipid Peroxidation and Intestinal Function in Pigs

We conducted a study with 216 crossbred barrows and gilts (6.5 kg body weight) to determine the effect of increasing degrees (dose) of lipid peroxidation on growth response, intestinal morphology, and function of the small intestine in young pigs (Rosero et al., 2015). Treatments included a control diet without added lipid, and diets supplemented with 6% soybean oil that was exposed to heat (80°C) and constant oxygen flow (1 L/min) for 0, 6, 9 and 12 d. Peroxidative exposure for in-

creasing lengths of time resulted in increasing p-anisidine values and MDA concentrations (Table 1). Initial peroxide value of soybean oil increased until d 9 and remained relatively similar on d 12. No effects on moisture content, free fatty acids, insoluble impurities, or unsaponifiable matter of soybean oil were observed.

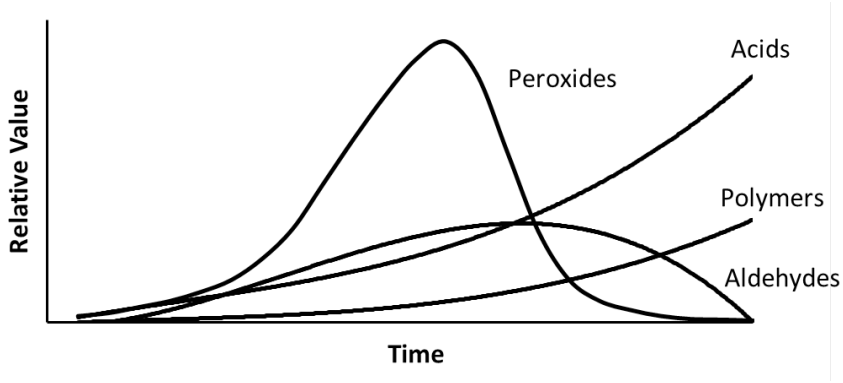


Figure 1. Production and degradation of products of lipid peroxidation over time (Adapted from Fitch Haumann, 1993).

Increasing lipid peroxidation linearly reduced (P<0.05) feed intake and body weight gain (Figure 2). We observed a 9% reduction in body weight gain (when comparing 0 d and 12 d of peroxidation), which was largely the result of reduced feed intake (7% reduction when comparing 0 d and 12 d). Apparent total tract digestibility of gross energy and fat decreased linearly with increasing lipid peroxidation (Figure 3). Absorption of mannitol and D-xylose were used to assess the absorptive capacity of the small intestine and tended to decrease (P < 0.10) progressively as peroxidation level increased (Figure 3). Increasing peroxidation also resulted in increased villi height (linear, P<0.001) and crypt depth (quadratic, P=0.005) in the jejunum. Malondialdehyde concentrations (quadratic, P=0.035) increased and total antioxidant capacity decreased (linear, P=0.044) with increasing peroxidation when measured in the jejunal mucosa. This study showed that increasing degrees of lipid peroxidation progressively diminished growth performance and modified function and morphology of the small intestine of nursery pigs. Thus, the extent to which measures are affected must be stated in relation to the dose of peroxidation products.

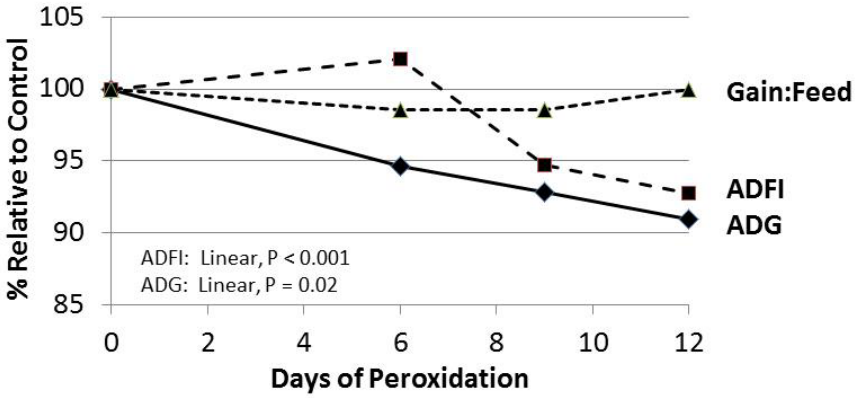
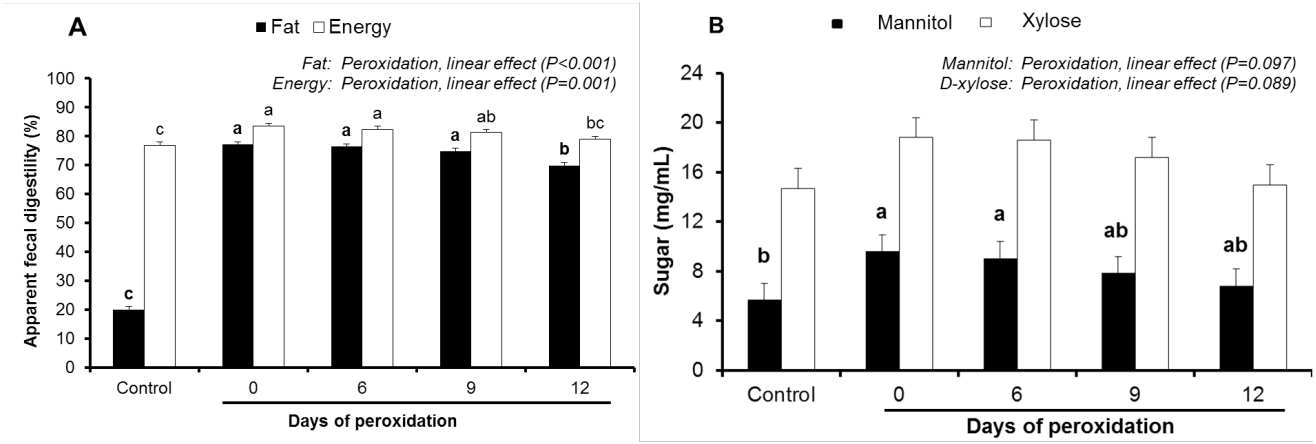


Figure 2. Effects of peroxidized dietary soybean oil on growth performance of nursery pigs.

Figure 3. Impact of lipid peroxidation on: 1) Apparent fecal digestibility of dietary fat and gross energy in nursery pigs [Panel A]; and 2) Intestinal absorptive capacity measured using mannitol and D-xylose [Panel B]. Control diets contained no supplemented soybean oil, whereas treatment diets were supplemented with 6% soybean oil exposed to peroxidation for 0 to 12 d. Adapted from Rosero et al. (2015).



Lipid Peroxidation and Antioxidants

To prevent peroxidation of lipids, antioxidants can be added to diets. Various natural and synthetic compounds display antioxidant properties, including natural carotenoids, flavonoids, tocopherols and citric acid, and synthetic ethoxyquin, butylated hydroxytoluene (**BHT**), butylated hydroxyanisole (**BHA**) and propyl gallate (Wanasundara and Shahidi, 2005; Jacela et al., 2010).

Among the synthetic antioxidants, the most commonly used in swine diets include ethoxyquin, BHA, BHT, and propyl gallate. Ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline) is a yellow liquid that when added to oils acts as a free radical terminator. BHA and BHT are most effective in animal fats and are susceptible to volatilization during frying (Reische et al., 2008). BHA and BHT can work synergistically: BHA reaction with a peroxide radical forms a BHA phenoxy radical, which then abstracts a hydrogen from the hydroxyl group of BHT. BHA is regenerated and the newly formed BHT phenoxy radical can react with a peroxide radical and act as a chain terminator (Belitz et al., 2009). Propyl gallate is more effective in vegetable oils than BHA and BHT, but also degrades at high temperatures and should be used together with metal chelators to avoid formation of dark-colored complexes (Reische et al., 2008).

Supplementation of commercial antioxidant blends has been studied in pigs. Positive effects of antioxidants on nursery pig performance were reported by Harrell et al. (2010), but antioxidant supplementation was not able to correct reduced growth performance in pigs fed oxidized corn oil or DDGS. In contrast, Lu et al. (2014a) fed weaned pigs 5% oxidized soybean oil diets with an antioxidant blend and vitamin E and observed that treatments with added antioxidant blend restored the reduction in pig growth performance associated with feeding peroxidized lipids. Increases in liver weight, plasma aspartate transaminase, and bilirubin indicated liver damage in pigs fed peroxidized oil. Moreover, addition of antioxidant blend, but not vitamin E alone, protected against oxidative stress, which was indicated by elevated TBARS and protein carbonyl in plasma and liver of pigs fed peroxidized oil. In the same study, inferior carcass characteristics (weight, backfat thickness, lean mass, and loin eye area), induced by dietary peroxidized oil, were prevented by addition of antioxidant blend (Lu et al., 2014b). Similarly, Boler et al. (2012) fed growing pigs 5% of peroxidized corn oil and a commercial antioxidant blend (tert-butylhydroquinone and ethoxyquin) and found that inclusion of antioxidant decreased protein carbonyl in plasma and improved shelf life of pork.

To evaluate the impact of peroxidized corn oil with or without the addition of a synthetic antioxidant blend on growth performance, oxidative stress markers, and response to vaccination, we conducted a study using a total of 176 nursery pigs (initial body weight, 9.11 ± 0.4 kg) (Chang and van Heugten, 2016). Pigs were housed in pens (4 pigs per pen) and assigned to 4 dietary treatments. Treatments consisted of a corn-soybean meal basal mix that was supplemented with 6% of either control corn oil or oxidized corn oil with (0.1%) or without addition of an antioxidant blend containing ethoxyquin (min 3%), BHT, and BHA. Peroxidized corn oil was obtained by exposing the control corn oil to heat (80°C) with constant oxygen supply (15.4 mL/min per kg of lipid) for 12 d. Following peroxidation, a liquid antioxidant containing tertiary butyl hydroquinone (**TBHQ**) was added to both lipid sources to prevent further peroxidation.

Pigs were fed a 2-phase nursery diet program with Phase 1 diets fed for 14 d and Phase 2 diets fed for 16 d. Pigs were vaccinated with porcine circovirus type 2 (**PCV2**) and Mycoplasma hyopneumoniae (**Mhyo**) killed vaccine (Circumvent PCV M, Intervet Inc.) at d 3 and 17 of the study to determine the impact of peroxidized lipids on the immune response. Blood samples were collected from 2 pigs per pen prior to vaccination on d 3, on d 17 (prior to the booster) and at the end of the study (d 31) to determine antibody titers to vaccinations. Serum concentrations of MDA and vitamin E were also determined.

The measures of peroxidation achieved by forcing peroxidation of the corn oil, by using heat and oxygen exposure (Table 2), are comparable to other recent publications (Boler et al., 2012; Liu et al., 2014a; Rosero et al., 2015); demonstrating a high degree of peroxidation of the oil. Nonetheless, growth performance was not impacted by peroxidation, antioxidants, or their interaction. Pigs fed peroxidized oil had a final body weight of 24.1 kg (vs. 24.65; P = 0.15), ADG of 487 g/d (vs. 475; P = 0.59), ADFI of 789 g/d (vs. 763; P = 0.40), and gain:feed of 656 g/kg (vs. 654; P = 0.70) compared to pigs fed control oil. Similarly, no impact of antioxidant supplementation was detected for final body weight (24.2 vs. 24.6 kg for control vs. antioxidant, respectively; P = 0.30), ADG (478 vs. 485 g/d; P = 0.74), ADFI (761 vs. 790 g/d; P = 0.35), or gain:feed (659 vs. 652 g/kg; P = 0.25). Antibody titers to Mhyo and PCV2 increased following vaccination, as expected, but there were no differences due to dietary treatments. Dietary treatments did not impact TBARS; however, serum vitamin E concentrations decreased in pigs fed peroxidized oil by 29% when measured on d 17 (0.79 vs. 1.11 μg/mL for pigs fed peroxidized oil vs. con-

trol, respectively) and 36% (1.06 vs. 1.65 μg/mL) when measured on d 30 (Figure 4). Supplementation of antioxidant increased serum vitamin E concentration ($P < 0.001$; 1.28 vs 1.01 μg/mL) and this effect tended to be greater in pigs fed control oil.

Vitamin E is a chain-breaking antioxidant with protective effects against membrane damage (Wiseman, 1996). Despite the lack of differences in TBARS, the reduction in serum vitamin E concentrations indicates that peroxidized oil disturbed the antioxidant systems in pigs, but not to the extent where performance was affected. Concurrently, feeding antioxidant exerted a sparing effect as evidenced by increased serum concentrations of vitamin E.

This study indicates that pig performance and response to vaccine were not affected by peroxidized corn oil or supplementation of antioxidant. However, serum vitamin E status was reduced by the consumption of peroxidized oils and increased with the addition of antioxidant. We propose that a longer feeding period of peroxidized lipids may be necessary to demonstrate adverse effects on growth performance, while a decline in vitamin E status may be an early indicator of induced oxidative stress in pigs fed peroxidized lipids.

Lipid Peroxidation and Pig Viability

Previous research clearly demonstrated that lipid peroxidation negatively affects cell integrity and increases oxidative stress, which can compromise health status. Nevertheless, no information exists with regard to the impact of lipid peroxidation on health, viability, and mortality of pigs housed under the rigors of commercial conditions, such as population density and greater immune stress than a laboratory environment. We previously observed numerically greater death losses in nursery pigs fed acidulated poultry fat (which was peroxidized and contained high levels of free fatty acids) compared to regular poultry fat, although the number of pigs used in that study was too small to provide conclusive evidence (Mendoza and van Heugten, 2014). Accordingly, we conducted a study to determine the impact of lipid peroxidation in nursery pigs housed under commercial conditions on growth performance, morbidity, and mortality (Smith et al., 2016).

Table 1. Peroxidation analysis of soybean oil used to evaluate the impact of progressive peroxidation of lipids on pig performance and intestinal function.

	Days of peroxidative exposure[1]			
Item	**0**	**6**	**9**	**12**
Peroxidation parameters				
p-anisidine value[2]	4.0	19.0	25.0	39.0
MDA[3] (mmol/L oil)	1.1	4.5	5.6	6.9
Peroxide value (mEq O_2/kg)				
Initial	1.0	46.0	58.0	52.0
4 h AOM[4]	11.0	90.0	94.0	84.0
20 h AOM	409.0	568.0	508.0	517.0

1 Soybean oil was peroxidized for 0, 6, 9 and 12 d by exposing to heat (80°C) and constant oxygen flow (1 L/min).
2 p-Anisidine value is a relative measure used to determine aldehyde content of peroxidized oils.
3 Malondialdehyde.
4 Active oxygen method.

Table 2. Peroxidation analysis of corn oil used to evaluate the impact of lipid peroxidation and antioxidants on performance and oxidative stress in nursery pigs.

Item	**Control**	**Peroxidized**
Peroxidation parameters		
p-anisidine value[1]	2.2	164.4
Peroxide value (mEq O_2/kg)		
Initial	0.4	146.0
4 h AOM[2]	3.0	290.0
20 h AOM	443.0	539.0
Oxidative Stability Index (OSI), hours	20.63	2.95
Hexanal, ppm	<10.0	345.0
2,4-Decadienal, ppm	7.0	1,622.0

1 p-Anisidine value is a relative measure used to determine aldehyde content of peroxidized oils.
2 Active oxygen method.

Table 3. Peroxidation analysis of corn oil used to evaluate the impact of peroxidation of lipids on performance and survivability of pigs housed under commercial conditions.

Item	**Control**	**Peroxidized**
Peroxidation parameters		
p-anisidine value[1]	2.5	30.4
Peroxide value (mEq O_2/kg)		
Initial	4.1	158.5
4 h AOM[2]	4.6	148.0
20 h AOM	6.8	442.0
Oxidative Stability Index (OSI), hours	47.8	0.78
Hexanal, ppm	<5.0	61.0
2,4-Decadienal, ppm	15.0	1,080.0
TBHQ, ppm[3]	196.8	17.6

1 Anisidine value is a relative measure used to determine aldehyde content of peroxidized oils.
2 Active oxygen method.
3 Tertiary butyl hydroquinone was added to control oil and peroxidized oil after peroxidation was stopped.

The study was conducted at a commercial research facility located in Illinois owned and operated by Hanor Company A total of 2,200 gilts and castrates (Camborough derivative x PIC TR-4 sire; 5.95 ± 0.2 kg) were placed in 100 pens (22 pigs/pen). Pigs were blocked by sex and body weight and randomly allotted within blocks to 1 of 5 dietary treatments (20 pens per dietary treatment). Dietary treatments consisted of 5 degrees of peroxidation to present a dose response challenge of

increasing peroxidation: no peroxidation, low, medium-low, medium-high, and high peroxidation. Treatments were administered for the duration of the nursery phase (43 d). Peroxidation was accomplished by exposing a restaurant-grade control corn oil to heat (65°C) while bubbling air through the oil at a constant rate of 20 L/min for 12 d. Lipids were stabilized with liquid antioxidant containing TBHQ after peroxidation was stopped. Peroxidation of the oil by using heat and air exposure was clearly achieved as shown by increased peroxidative parameters (Table 3). In addition, the concentration of TBHQ was much lower in peroxidized oil compared to control oil, suggesting it was destroyed in its role as an antioxidant after peroxidation. Control corn oil was added to experimental diets at 5% and represented the no peroxidation treatment, while peroxidized corn oil was added to diets at 5% representing the high peroxidation level. Intermediate diets were blended on the farm using a FeedLogic feeding system that blends, weighs, and records feed delivered to individual pens. Upon arrival at the facility, all pigs received 0.23 kg of a complex nursery starter diet (Phase 1). Subsequently, experimental diets were provided during the remaining nursery period using a feed budget of 1.8 kg/pig of the Phase 2 diet, 5.4 kg/pig of the Phase 3 diet, followed by the Phase 4 diet fed until pigs reached the final nursery body weight.

All pigs and feeders were weighed at placement (d 0), when switching diet phases on d 7 and d 20, and at the end of the study on d 43 for growth performance calculations. Calculations of ADG and ADFI accounted for dead pig weights and the number of days they were present in the pen. Strict protocols were in place for individual pig medication within pens and removal of pigs to medical pens for concentrated medical treatment. The decision to remove pigs to a medical pen was based on a format devised by licensed veterinarians that considered a serious decline in body condition, lameness, injury or noticeable respiratory distress. Pulled pigs were tagged with individual identification numbers and placed in medical pens that received the same treatment diet that their cohorts were receiving. Pigs weighing less than or equal to 13.6 kg at the end of the study were considered in the non-full-value category (this body weight was approximately 4 SD removed from the mean, which was possible because of a long left tail of the distribution curve).

Pigs were vaccinated with PRRS-MLV mixed with Mhyo vaccine at placement and a PCV2 and Mhyo combination vaccine at approximately 9 weeks of age. Blood samples were collected from 20 pigs per treatment (10 light- and 10 heavy-weight pigs) prior to (on d 33) and 14 d after the second vaccine dose to determine antibody titers to vaccination. Samples collected on d 33 were used to assess oxidative status (total antioxidant capacity, TAC, MDA, DNA damage, protein carbonyl) and vitamin E concentrations in serum.

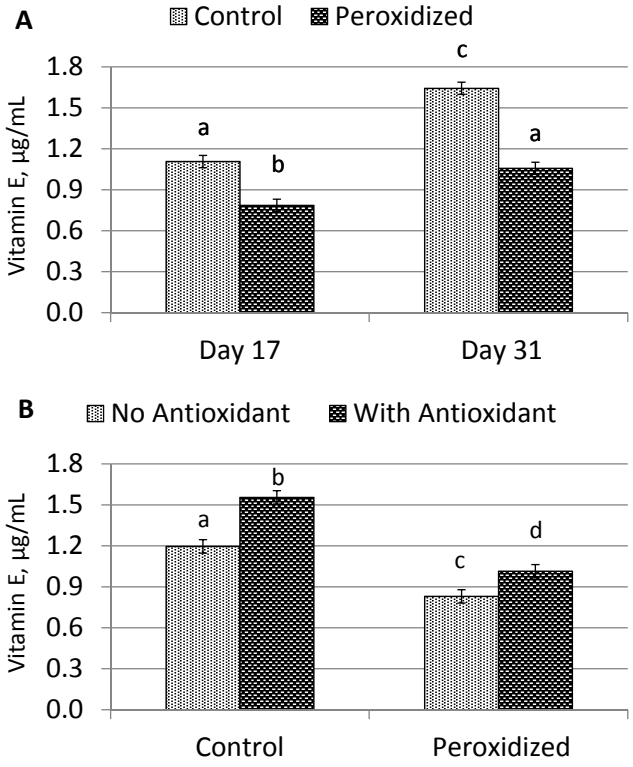


Figure 4. Serum vitamin E concentrations in pigs fed diets containing either 6% control oil or 6% peroxidized oil supplemented either without or with antioxidant at 0.1%. Peroxidation decreased serum vitamin E concentrations and this effect was greater on d 31 than d 17 of the study (Panel A). Antioxidant supplementation increased vitamin E concentrations and this effect tended to be greater in pigs fed the diet with control oil (Panel B; Interaction, $P < 0.06$).[abcd] $P < 0.05$.

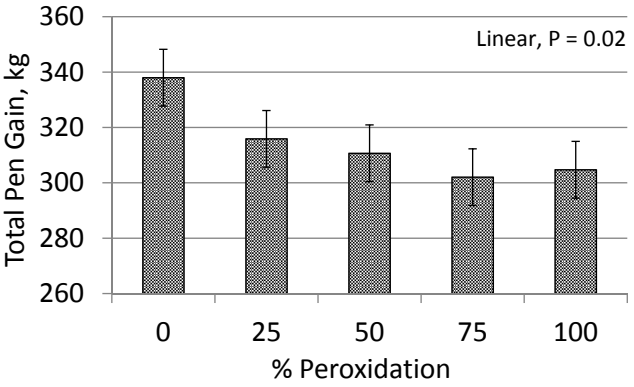


Figure 5. Impact of lipid peroxidation on total gain of pens of pigs during the 43-d nursery period. Each value represents the mean of 20 pens with 22 pigs per pen (at the start of the study). Pen gain was calculated as the sum of weights of pigs present at the end of the study minus the sum of the weights of pigs at the start of the study.

Lipid peroxidation did not impact pig ADG, ADFI, or feed efficiency. However, average gain of the pen of pigs was reduced linearly when pigs were fed diets with increasing levels of peroxidation (Figure 5). Pen gain was calculated by difference of the total weight of pigs in the pen at the end of the study and the total weight of pigs in the pen at the start of the study. Thus, this measure considers only the pigs that finished the study and gives no value to pigs that died or were culled, which is practically relevant. Indeed, peroxidation increased (linear, P = 0.03) death losses and the number of pigs that were culled (less than 13.6 kg at the end of the nursery) and decreased (P = 0.007) the number of full-value pigs available to enter the finisher (Figure 6). The reduction in pen gain was primarily due to death losses and increased number of small (no-value) pigs, indicating that pigs that survive and are successfully treated have the ability to gain similarly, regardless of peroxidation level of the diet. The number of pigs treated with injectable medication and the number of pigs pulled increased linearly (P < 0.007) with increasing levels of peroxidation (Figure 7).

Antibody titers to PCV2 and Mhyo were not impacted by lipid peroxidation. Peroxidation decreased serum vitamin E concentration in a linear fashion and decreased total antioxidant capacity quadratically, with the lowest value observed at the 75% oxidation level (Figure 8). Markers of oxidative stress were not impacted by level of dietary peroxidation, suggesting that antioxidant capacity may have been sufficient to maintain oxidative stress to levels similar to the control treatment. It is interesting to note that antioxidant capacity appeared to be reduced to the largest extent at the 75% peroxidation level, which was also the level with the greatest reduction in pen gain and morbidity. It is not clear why the negative effects of peroxidation did not continue with the 100% peroxidized lipid diet. Diets were mixed using a food-grade corn oil as control diet and the peroxidized oil to create the 100% peroxidized diet. Intermediate diets were prepared by on-farm blending of the appropriate proportions of the control and the 100% peroxidized diets. Thus, primary and secondary products of peroxidation would be expected to progressively increase with increasing levels of the 100% peroxidized diet in the dietary treatment mix, rather than changes in the composition of the peroxidation products. Nonetheless, data clearly indicate the negative impact of peroxidation on pig productivity and survival. To our knowledge, this is the first study that quantified peroxidation level of supplemental lipids in relation to the negative impact on viability of pigs, and presents population responses to peroxidation, including pulled pigs, excessively light pigs, mortality, and medical treatments, that are otherwise often ignored. It is possible that there will be variation in responsiveness to peroxidation dose with the age at weaning and robustness of the genetic lines involved.

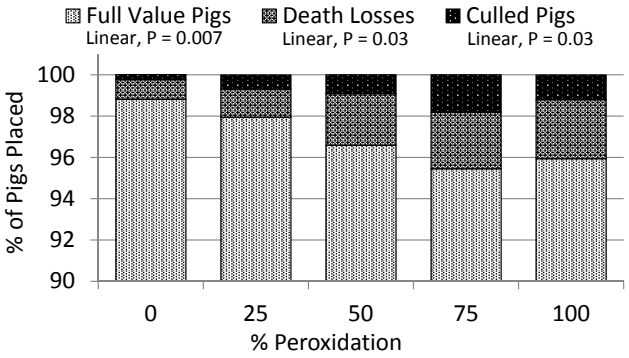


Figure 6. Impact of lipid peroxidation on death losses, culled pigs, and full value pigs produced.

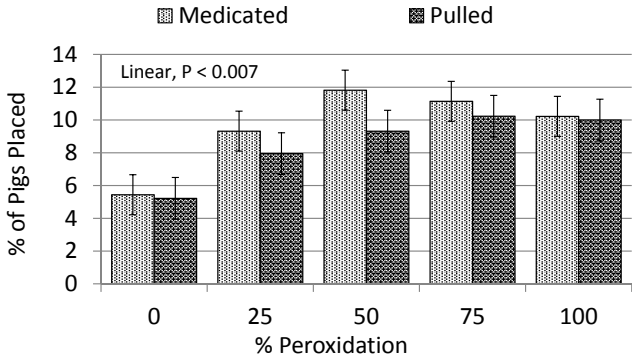


Figure 7. Impact of lipid peroxidation on pigs pulled due to slow growth, injury, or respiratory problems and percentage of pigs treated with injectable medications. Means designated as "Medicated" represent pigs that were medicated in the experimental pen and not removed. Means designated as "Pulled" represent pigs removed from test pens to a medical pen for repetitive treatment as directed by licensed veterinarians.

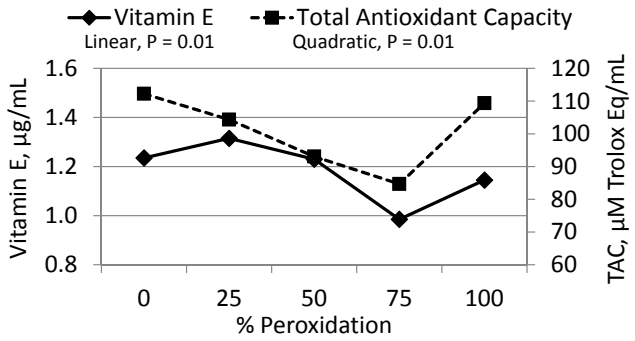


Figure 8. Impact of lipid peroxidation on serum concentrations of vitamin E and total antioxidant capacity.

Conclusions

Lipid sources that are added to diets for swine are derived from a variety of sources and are prone to peroxidation, especially unsaturated lipids. Lipid peroxidation is a complex process and the degree of lipid peroxidation is very difficult to characterize because the compounds that are produced are unstable and decompose as peroxidation progresses. A complete characterization of lipid peroxidation should, at least, quantify primary and secondary peroxidation products. However, the impact of several quantifiable oxidative products that collectively describe peroxidation status on production performance has not been experimentally determined. Dietary lipid peroxidation products impose oxidative stress, and can compromise immunity, decrease intestinal function, and reduce growth performance. The response of animals to peroxidation products is likely progressive and a minimum threshold for lipid peroxidation above which detrimental effects are detectable likely exists. Although the impact of peroxidized lipids on growth performance may appear to be subtle, we demonstrated, under field conditions, that increasing the level of peroxidation resulted in a dose-related increase in mortality, number of pigs medicated, and number of pigs that were excessively light. This resulted in a reduced number of pigs and a lower total pig weight by the end of the nursery. The financial value of retaining viable pigs is a powerful driver of return over investment. This proof of a dose related effect of peroxidation on viability is novel. The challenge that remains is to define the acceptable levels of the several oxidation markers of a lipid quality control program for oil and fat sources. A maximum threshold or dose might, for example, involve anisidine and the sum of the two aldehydes, hexanal and 2,4-decadienal. This application is probably most important for weaned pigs as compared to older growing pigs and sows, and responsiveness may vary with age at weaning and robustness of genetic lines used..

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Implications of Dietary Oxidized Oils for Fresh and Further Processed Pork Quality

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Summary

The quality of the lipids fed to growing-finishing pigs has long been recognized as having profound effects on pork quality. Much effort has been put forth to understand lipid nutrition as it affects meat quality, with most research focusing on the fatty acid profile of lipids and their influence on fat deposition. Recently, there has been renewed interest in other areas of dietary lipid quality; namely, the oxidative status of lipids. Human nutrition and medicine have long recognized that consumption of oxidized lipids have deleterious effects on the circulatory system and there has also been more recent research focusing on the effects of oxidized lipids on gut health, immune response, and digestibility in the field of swine nutrition. Although research has been conducted to investigate the effects of oxidized dietary lipids on pork quality, results have been inconclusive. Still, with the continued use of ingredients such as DDGS and various plant oils in swine diets, it is of importance to understand the potential impacts the oxidative quality of these ingredients has on pork quality.

Introduction

Increased use of corn co-products such as DDGS and oils in swine diets have increased concerns over the effects of dietary lipid composition on the quality and shelf-life of fresh and processed pork products. Numerous studies have been conducted to investigate the effects of the use of DDGS in diets on the quality and shelf-life of both fresh (Leick et al., 2010) and processed (Bumsted et al., 2015) pork products. The majority of pork quality and shelf-life research on the effects of DDGS and other ingredients that serve as a source of dietary lipids has revolved around the manipulation of the fatty acid composition of adipose tissue with the objective of reducing the proportion of oxidatively-labile polyunsaturated fatty acids. This increased interest in the role of dietary lipid composition has also drawn attention to dietary lipid quality, particularly the oxidative status of the lipids. There is a growing body of evidence to indicate that consumption of oxidized lipids and their secondary reaction products have implications for health, growth, development, and metabolism that are of consequence to the pork industry. In addition to the ramifications of dietary oxidized lipids on pig performance, there are potential implications for the quality and shelf-life of pork products.

It is the objective of this presentation to review the body of literature relating to the effects of oxidized lipids in growing-finishing swine diets on the quality and shelf-life of fresh and processed pork, with an emphasis on the relationship between the oxidative state of tissues and measures of shelf-life of meat products.

Absorption and Metabolism of Dietary Oxidized Lipids and Lipid Oxidation Products

The implications of dietary oxidized lipids for pork quality and shelf-life begins with the effects that oxidized fats and oils have on nutrient digestibility, intestinal function, and growth performance; these topics have been reviewed elsewhere (Shurson et al., 2015). Along with reductions in feed intake and growth rate of finishing pigs fed oxidized corn oil, concomitant reductions in hot carcass weight and 10th rib fat thickness have also been reported (Fernández-Dueñas, 2009). Although these effects on carcass composition are likely explained by the reduced growth performance, the implications of dietary oxidized lipids on pork shelf-life are more likely to lie within the realm of metabolism of those oxidized lipids and their secondary oxidation products. Therefore, to fully understand how dietary oxidized lipids affect the functionality of pork products and their oxidative stability during storage, it is important to understand the metabolism of those products in the living animal.

Dietary lipids are absorbed from the intestine where they are packaged into chylomicrons in enterocytes, released into the lymphatic system and enter the blood stream, and are ultimately transported to muscle, adi-

pose tissue, or the liver to be metabolized. Along with unoxidized lipids, oxidized lipids and many lipid oxidation products, such as 4-hydroxynonenal (**HNE**) and other α, β-unsaturated hydroxyaldehydes, are readily absorbed, with lipid oxidation products being of particular concern due to their cytotoxicity (Esterbauer et al., 1991; Grootveld et al., 1998). Furthermore, oxidized lipids have been demonstrated to be present in postprandial chylomicrons in both humans (Staprans et al., 1994) and rats (Staprans et al., 1996). It is likely that the increased concentrations of plasma thiobarbituric acid reactive substances (**TBARS**), a measure of lipid oxidation, observed in pigs fed diets containing oxidized oils (Fernández-Dueñas, 2009) was due to the presence of lipid oxidation products in chylomicrons. After absorption, chylomicrons containing the oxidized lipids, along with secondary oxidation products, are transported to the liver to be metabolized (Pignitter and Somoza, 2015), accounting for the increased concentration of TBARS in hepatic tissue in pigs fed diets containing oxidized oils (Fernández-Dueñas, 2009). Chylomicrons can also transport lipids to muscle or adipose tissue; however, it is yet uncertain if oxidized lipids and secondary oxidation products are also transported to, and potentially incorporated into, these tissues.

The liver serves as the primary point of detoxification in the body; thus, it is especially susceptible to the effects of lipid oxidation products. The toxicity of secondary lipid oxidation products results in an increase in the synthesis of enzymes to aid in detoxification (Huang et al., 1988) as well as increased proliferation of hepatocytes (Dibner et al., 1996). This proliferation may underlie the increased liver weight observed in pigs (Liu et al., 2014), as well as rats (Eder, 1999) fed oxidized oils. Additionally, feeding oxidized lipids increases expression of peroxisome-proliferator activated receptor alpha (**PPARα**) and other genes associated with lipid metabolism; however, the exact mechanisms, nor the regulatory role, of PPARα in lipid metabolism are fully understood (Shurson et al., 2015). Despite the livers capacity to neutralize and excrete cytotoxic lipid oxidation products, it may not be completely effective. Winter et al. (1987) injected radioactively labeled hexenal (**HHE**), a major secondary lipid oxidation product, which itself is a catalyst of oxidation, into the hepatic portal vein of rats, and although 77-83% of the administered radioactivity was excreted in urine, approximately 9% was recovered from skeletal muscle. However, it is unclear if this was indicative of HHE being deposited in muscle or if the labelled carbons were incorporated into other compounds, which were then transported to skeletal muscle.

Moreover, carbons from radioactively marked linoleic acid administered to rats (Strapans et al., 1996) and pigs (Suomela et al., 2005) in the form of chylomicrons were present in very low density lipoprotein (**VLDL**), indicating that oxidized fatty acids are metabolized and released into circulation similarly to the non-oxidized form. The same marked carbons were also detected in extrahepatic tissues, including skeletal muscle. However, it is unclear whether the marked carbons were still part of linoleic acid, or if they had been donated to other compounds. There is, in fact, evidence that oxidized lipids from the diet are not only absorbed and metabolized, but also interact with cellular structures. Buckley et al. (1989) reported the mitochondrial membranes of pigs fed oxidized corn oil (peroxide value = 9 meq/kg feed) for 10 weeks were less oxidatively stable than those of pigs not fed oxidized oil. They hypothesized that the oxidized lipids in the diet were a source of free radicals, thus destabilizing the membranes. Therefore, feeding pigs diets containing oxidized lipids increases the presence of oxidized lipids in both circulation and liver, and these oxidized lipids and their secondary oxidation products can also be deposited in tissues. It is the deposition of oxidized lipids and secondary oxidation products in muscle and adipose tissue that are of the most concern for pork quality and shelf-life.

Implications for Pork Quality and Shelf-life

Lipid oxidation, as it pertains to the development of rancidity, occurs during storage of meat products, even at refrigeration and frozen temperatures. In short, lipid oxidation is initiated with the removal of a hydrogen atom from a carbon adjacent to a double bond of an unsaturated fatty acid to form a free radical. This free radical then combines with oxygen to form a peroxyradical, which in turn interacts with another fatty acid to form a hydroperoxide and another free radical, which then leads to the propagation of the autocatalytic oxidation process (Ladikos and Lougovois, 1990).

Lipid oxidation in meat ultimately results in oxidative rancidity and the development of associated undesirable odors and flavors. Moreover, lipid oxidation is typically accompanied with deterioration of meat color from a desirable red to an undesirable brown color, due to the oxidation of oxymyoglobin to metmyoglobin. This reaction is catalyzed not only by the radicals produced by lipid oxidation, but also accelerated by secondary lipid oxidation products including members of the α, β-unsaturated hydroxyaldehydes, such as HNE and HHE (Faustman et al., 1999). The final result of lipid and myoglobin oxidation is a meat product that has an unacceptable color accompanied with objectionable

odors and flavors that is unlikely to be purchased or consumed.

Shelf-life of pork products is dependent upon many factors, including the lipid content of the product, the use of preservative ingredients, packaging, storage method, and lighting. The detection threshold for rancidity in fresh pork is 0.5 to 1.0 mg-TBARS/kg of tissue (Wood et al., 2008), however it is possible that deterioration of color will occur before the threshold for rancidity is reached. Stored in typical retail display conditions in oxygen permeable packaging, the shelf-life, as determined by the aforementioned TBARS threshold, of fresh pork loin chops may be 7 to 14 days (Sheard et al., 2000; Fernández-Dueñas, 2009;); whereas, fresh seasoned pork patties may only have a shelf-life of 5 to 7 days (Fernández-Dueñas, 2009; Qin et al., 2013). Cured meat products typically have a much longer shelf-life, due to the preservative roles of several curing ingredients, with a typical shelf-life of refrigerated vacuum-packaged sliced bacon being in excess of 90 days (Lowell et al., 2016). The premature end of shelf-life has major economic implications for the entire food industry, with the loss of meat and poultry products being the most severe. In 2010, it was estimated that 8.6 billion pounds of meat were lost at the retail and consumer points of the U.S. supply chain due to spoilage, representing an economic loss of $23.2 billion (Buzby et al., 2014).

Much of the recent improvements in prolonging shelf-life of meat products has focused on the use of novel packaging systems, such as modified atmosphere packaging (McMillin, 2008) and antioxidant-active packaging films (Gómez-Estaca et al., 2014), as well as novel display lighting sources (Steele et al., 2016). Despite the improvements in packaging and display technology, the fact that diet influences the shelf-life of meat products, remains. For example, the inclusion of DDGS in growing-finishing pig diets increased the deposition of oxidatively-labile polyunsaturated fatty acids in adipose tissue and decreased shelf-life of blade chops (Leick et al., 2010); whereas, the supplementation of antioxidants can increase or spare stores of endogenous antioxidants, thereby improving shelf-life (Boler et al., 2012). Given these results, several researchers have investigated whether absorption and deposition of dietary oxidized lipids and secondary oxidation products in muscle and adipose tissues may reduce the shelf-life and negatively affect the quality of meat products. However, the results of such experiments have been mixed (Table 1).

An early study by Buckley et al. (1989) reported that both loin chops and ground pork patties of pigs fed oxidized corn oil displayed substantially greater rates of lipid oxidation than did those from pigs fed fresh oil at all stages of either fresh or frozen storage. Furthermore, feeding rancid rice bran (15.6% free fatty acids) increased the rate of lipid oxidation in fresh and cooked pork loin chops (Chae and Lee, 2002). Monahan et al. (1992) reported that loin chops from pigs fed oxidized corn oil tended to have greater TBARS concentrations than did chops from pigs fed fresh oil; however, the per-

Table 1. Summary of results of studies reporting the effect of dietary oxidized lipids on pork quality and shelf-life

Study	Fat source	Fat source PV[1], mEq/kg	Diet PV[1], mEq/kg	Product	Results
Lu et al. (2014)	soybean oil	180	9	Fresh loin muscle	Oxidized oil decreased lean redness, but did not affect TBARS[2] of fresh loin muscle
Boler et al. (2012)	corn oil	150	7.5	Fresh loin chops, fresh ground pork	No effect of oxidized oil on color stability or TBARS during retail display of loin chops or ground pork. Oxidized oil reduced sensory tenderness of chops at 14 d of storage (15 d postmortem).
Chae and Lee (2002)	rice bran	NR[3]	NR[3]	Fresh ground pork, cooked ground pork	Oxidized rice bran increased TBARS during storage of fresh ground pork, but had no effect on cooked pork
Monahan et al. (1994)	corn oil	150	4.5	Fresh loin muscle	No effect of oxidized oil on discoloration or TBARS during refrigerated display
Monahan et al. (1992)	corn oil	150	4.5	Fresh loin chops, frozen/thawed loin chops, cooked ground pork	Oxidized oil tended to increase TBARS in fresh chops and cooked ground pork
Buckley et al. (1989)	corn oil	300	9	Fresh loin chops, frozen loin chops, fresh ground pork, salted ground pork	Oxidized oil rapidly increased TBARS during storage of both fresh and frozen loin chops and fresh and salted ground pork, destabilized microsomal and mitochondrial membranes

1 Peroxide value
2 Thiobarbituric acid reactive substances
3 Not reported. Chae and Lee (2002) reported free fatty acid (FFA) concentrations of rice bran. Rancid rice bran = 15.6% FFA, fresh rice bran = 8.2% FFA; fed at 20% inclusion in the diet, as fed basis.

oxide value of the oil used in their study was half that of the earlier work of Buckley et al. (1989). In contrast to this earlier research, more recent studies have reported that although feeding oxidized corn oil induced markers of oxidative stress in both liver and plasma, there was no effect of oil quality on TBARS of loin chops (Boler et al., 2012). However, chops from pigs fed oxidized oil were rated as less tender after 14 days of simulated retail display than those from pigs fed fresh oil. This may have resulted from oxidation of myofibrillar proteins. Like the oxidation of lipids, protein oxidation occurs via a free radical chain reaction. The oxidation of myosin can result in cross-linking of myosins; thereby, altering the structure of the contractile unit in such a way that decreases tenderness as well as water holding capacity, and protein solubility (Lund et al., 2011). There is some evidence that protein and lipid oxidation work in tandem, with the propagation of one initiating the other, yet the exact mechanism by which this may occur is not well defined (Lund et al., 2011). Lu et al. (2014) reported that although a* (redness) values were reduced (became less red) by feeding oxidized soybean oil, suggesting oxidation of myoglobin to brown metmyoglobin, TBARS of fresh loin muscle were not different than those from pigs fed fresh oil. In this study, however, it should be noted that the loin samples were not subjected to simulated retail storage and therefore were not truly indicative of the shelf-life of the meat. Additionally, the inclusion of 3% oxidized corn oil (150 meq/kg oil) did not affect either lipid oxidation or color stability of loin chops compared to chops from pigs fed fresh corn oil under simulated retail display conditions (Monahan et al., 1994). The inconsistency of these results of feeding oxidized oils to pigs and subsequent effects on lipid oxidation in the product can be attributed to several factors including the particular level of oxidation in the diets, antioxidant supplementation levels, fat level in the pork products evaluated, and storage time and conditions prior to evaluation. It is also important to consider that bacon and sausage make up 37.9% of all in-home pork consumption in the U.S (Pork Check Off, 2009). The greater fat content of these products make them more susceptible to the effects of oxidation than lean cuts, such as loin chops, which have been the focus of much of the research on the effects of dietary oxidized lipids on pork quality and shelf-life. Therefore, the lack of data regarding the effects of dietary oxidized oil inclusion on the shelf-life and quality of these products is troubling.

In contrast to the mixed results reported in pork studies, studies in broilers have routinely reported that oxidized oils in diets resulted in increased rate of lipid oxidation in meat during storage (Lin et al., 1989; Jensen et al., 1997; Tavárez et al., 2011; Zhang et al., 2011). This disparity in response is likely due to differences in the endogenous antioxidant systems between poultry and pigs, which are primarily driven by the difference in muscle fiber types and their preferred metabolic pathways. Oxidative fibers make up a greater proportion of pork muscle than in chickens, which have a greater proportion of glycolytic fibers. The preference of oxidative muscle fibers to utilize fatty acid oxidation to produce energy results in greater concentrations of free radicals and secondary oxidation products. Thus, oxidative muscle fibers have more robust antioxidant systems to handle these by-products of oxidative metabolism. Therefore, pigs in contrast to chickens may be more suited to combat increased oxidative stress from the consumption of oxidized lipids.

The ability to combat oxidation in tissues lies with antioxidant enzyme systems. Many of these antioxidant enzymes are used as markers of oxidative stress. Glutathione peroxidase (**GSHPx**) is one such enzyme that is routinely measured. Glutathione peroxidases are found in several tissues, with the greatest concentrations in the liver and plasma of both pigs (Daun and Åkesson, 2004; Boler et al., 2012) and chickens (Tappel et al., 1982), but with significantly less activity in skeletal muscle of either species (Yamauchi et al., 1984). Zhou and Decker (1999) suggested that though GSHPx was a powerful antioxidant, concentrations were not sufficient in skeletal muscle to be the primary antioxidant in the system. In fact, the same authors demonstrated that at concentrations present in skeletal muscle, carnosine was more effective at neutralizing lipid oxidation product than GSHPx. Overall, chicken and pork muscle have comparable concentrations of GSHPx, with chickens having slightly greater levels of activity than pigs (Yamauchi et al., 1984). However, pork contains far greater concentrations of carnosine, regardless of muscle location, than chickens (Tian et al., 2007; Mora et al., 2008). In addition to carnosine, the antioxidant enzymes catalase (Rhee et al., 1996; Pradhan et al., 2000) and superoxide dismutase (Avanzo et al., 2001; Hernández et al., 2004) are present in greater concentrations in pork than in chicken. With GSHPx activities being similar across tissues between species, the greater concentration of carnosine, catalase, and superoxide dismutase in pork may account for the disparity in results of shelf-life studies between the two species.

Conclusions

The inclusion of fats and oils in growing-finishing pig diets can, under some scenarios, offer cost savings in diet formulation and improve some carcass characteristics. However, not all dietary lipids are created equal, as is clearly demonstrated when comparing the fatty acid composition of tallow against corn oil. Much consideration has been given to evaluating the levels of polyunsaturated, monounsaturated, and saturated fatty acids in dietary lipids from various sources, but less has been given to the oxidative status of those lipids. Certainly, a growing body of data are available in the literature in regards to the effects of oxidized oils and fats on nutrient digestibility, growth performance, and immune function at all stages of pig growth and development, but data regarding the implications of feeding oxidized oils on meat quality and shelf-life are comparatively limited. The data that are available are somewhat contradictory, and this is possibly due to the complex nature of not just the chemical composition of the dietary lipids, but also their metabolism. As researchers continue to investigate the roles and effects of lipids from various sources in pig diets, the oxidative stability and status of those ingredients should also garner consideration. Finally, future research conducted in this area should first focus on identifying whether dietary oxidized oils have any meaningful effects on the quality and shelf-life on fattier pork products (e.g., sausage, bacon) that are at a greater risk for oxidation, then determining interventions to mitigate those quality issues.

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Phytase Supplementation – The Response to Phytase Beyond Phosphorus

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Summary

Phytase has a clear effect of improving P availability in swine diets. Recently, super-dosing phytase supplementation has been investigated to evaluate the responses to degrading all of the phytate in the diets beyond the P that is released by the phytase. Studies were conducted at the University of Kentucky with growing-finishing pigs and lactating sows fed diets containing adequate P to which super-dose levels of phytase were supplemented. In a study with growing-finishing pigs using high fiber diets, growth rate, apparent total tract digestibility (ATTD) of multiple nutrients and carcass leanness increased with increasing phytase supplementation levels. A supplementation level over 1,000 FTU/kg phytase maximized these responses. In a study with lactating sows fed a corn-SBM based lactation diet, weaning to estrus interval was shortened by super-dosing phytase (3,000 FTU/kg). These results indicate that super-dosing phytase to swine diets may have beneficial effects beyond the P release on not only ATTD of certain nutrients, but also carcass leanness of pigs and some aspects of sow reproduction.

Introduction

Phytate is a storage form of P in cereal grains and oil seeds, and has been known as an anti-nutritional factor in swine and poultry diets (Selle and Ravindran, 2008). Phytate binds not only to dietary cations such as Cu, Zn, Ca, Fe, Mg and Mn, but also protein, fat, and vitamins, and thereby results in potential reductions in availability of these nutrients (Bohn et al., 2008). Because pigs poorly utilize the phytate P in most diets due to the lack of endogenous enzyme secretion (Selle and Ravindran, 2008), supplementation of exogenous phytase may be a useful means to enhance utilization of these non-P-nutrients by degrading the phytate. Liberation of Ca, Zn, and other nutrients such as fat and proteins resulting in increased availability of these non-P-nutrients along with P has been demonstrated by Dersjant-Li et al. (2015).

Most of the phytase research for pigs has used low P or P-deficient basal diets to confirm the responses to phytase and measured how much P is released from phytate by the phytase addition to satisfy the P need of animals. However, using low P or P-deficient diets does not allow investigators the ability to determine the extent to which any benefits might be from the P release or from removing any possible anti-nutrient effects of the phytate.

Recently, the response to phytase beyond P release and super-dosing phytase supplementation have been investigated in swine nutrition (Zeng et al., 2014; Wealleans et al., 2015; Holloway et al., 2016). Regarding the response to phytase beyond P release, further responses to phytase supplementation of the diets beyond P release are called "extra-phosphoric effects" such as enhanced macro- and trace-mineral availability and enhanced digestibility of amino acid and energy (Selle and Ravindran, 2008). These extra-phosphoric effects are usually associated with phytase supplementation at "super-dosing" levels which are defined as the use of unconventional high doses of phytase to degrade all, or the majority of, phytate and remove its anti-nutritional effects (Cowieson et al., 2011). Because super-dosing phytase supplementation is a concept to remove all 6 phosphate molecules by full destruction of phytate, it improves availability of not only the P but other nutrients (protein, fat, starch, vitamins and minerals), ultimately producing inositol which can act as an antioxidant (Cowieson et al., 2011). In addition to the effect of releasing multiple bound nutrients and producing inositol, super-dosing phytase supplementation could remove such potential anti-nutrient effects of phytate as: 1) inhibiting endogenous enzymes such as trypsin and amylase, 2) increasing endogenous losses of amino acids and minerals, and 3) increasing endogenous secretions in the gastrointestinal tract that may increase the maintenance protein and energy costs of pigs (Dersjant-Li et al., 2015). Therefore, the improvements in performance with super-dosing phytase supplementation may result from a multitude of possible effects within the gastrointestinal tract.

Effects of Super-Dosing Phytase to Pigs

In nursery pigs, high levels of phytase supplementation uniquely improved nutrient utilization, growth rate and feed efficiency in pigs fed a low P diet (Zeng et al., 2014). Kies et al. (2006) also reported that phytase supplementation up to 15,000 FTU/kg of a P-deficient weaning pig diet improved growth performance and digestibility of minerals with 85% of phytate-P digestibility at 15,000 FTU/kg compared with 14% in the control diet. But Walk et al. (2013) reported that supplementation of 2,500 FTU/kg phytase to a nutritionally adequate diet for weanling pigs enhanced overall growth rate.

In growing-finishing pigs, phytase supplementation up to 2,000 FTU/kg of a low Ca and low P grower diet (corn-SBM based) improved ADG and G:F ratio compared with an adequate Ca and P diet (Santos et al., 2014). Holloway et al. (2016) reported that super-dosing phytase supplementation (1,000, 1,750 and 2,500 FTU/kg) tended to improve feed efficiency of growing pigs fed a low lysine and energy diet. However, in other studies supplementation of 2,000 FTU/kg of phytase from 3 different phytase sources, did not affect growth rate, feed intake and feed conversion ratio as well as carcass characteristics in finishing pigs fed a corn-SBM based diet containing adequate P (Langbein et al., 2013). Another study with finishing pigs fed increasing dietary phytase up to 2,000 FTU/kg in a diet containing 15% bakery meal and DDGS that contained adequate P did not observe improvements in carcass measurements although feed efficiency was increased by phytase addition up to 500 FTU/kg (Flohr et al., 2014).

In sows, Swiatkiewicz and Hanczakowska (2008) reported that super-dosing phytase supplementation up to 10,000 FTU/kg had no significant effects on reproductive and litter performance. However, Wealleans et al. (2015) reported that phytase supplementation reduced sow body weight (**BW**) loss during lactation with an increasing benefit as the dosage of phytase supplementation was increased from 250 to 2,000 FTU/kg in a low Ca and P diet (corn or wheat based); the benefits were nonexistent in first parity females and most pronounced in sows greater than parity 5.

Collectively, super-dosing phytase supplementation has demonstrated beneficial effects on growth performance and reducing sow BW loss in lactation. However, the effects are still inconsistent depending on the basal diet composition and basal P content, and there is limited information about the effect of super-dosing phytase supplementation of pig diets containing a high amount of substrate (i.e., phytate) as would occur by using high amounts of byproducts as will occur with increasing incidence in the future.

Experimental Procedures

Experiment 1

The objective of this study was to evaluate the effect of graded supplementation of phytase in conjunction with xylanase supplementation on growth performance, carcass characteristics, and apparent total tract digestibility (**ATTD**) in corn-based diets containing high fiber byproducts such as corn DDGS, wheat middlings, and corn germ meal.

A total of 45 crossbred growing pigs [27 barrows (3 replicates) and 18 gilts (2 replicates); mean initial BW = 26.4 ± 0.2 kg] were blocked by sex and BW, and randomly assigned to 9 dietary treatments (a 1 + 2 × 4 factorial arrangement). The pigs were housed in individual pens in an environmentally controlled research facility. Feed and water were consumed on an ad libitum basis. Dietary treatments were: 1) increasing phytase [6-phytase (EC 3.1.3.26); Quantum Blue; AB Vista Feed Ingredients, Marlborough, UK] supplementation levels (0, 500, 1,000 and 2,000 FTU phytase/kg diet) and 2) inclusion of xylanase (24,000 BXU/kg diet; endo-1,4-beta xylanase [EC 3.2.1.8]; Econase XT; AB Vista Feed Ingredients, Marlborough, UK) in a basal control (**NC**) diet along with a higher energy positive control (**PC**) diet as follows:1) PC: a corn-SBM based diet with 15% corn DDGS, 15% wheat middlings and 13% corn germ meal, 2) NC: ME was reduced by 103 kcal/kg from the PC diet by replacement of fat with corn starch, 3) NP500: NC + phytase (500 FTU/kg diet), 4) NP1000: NC + phytase (1,000 FTU/kg diet), 5) NP2000: NC + phytase (2,000 FTU/kg diet), 6) NX: NC + xylanase (24,000 BXU/kg diet), 7) NXP500: NC + phytase (500 FTU/kg diet) + xylanase (24,000 BXU/kg diet), 8) NXP1000: NC + phytase (1,000 FTU/kg diet) + xylanase (24,000 BXU/kg diet), and 9) NXP2000: NC + phytase (2,000 FTU/kg diet) + xylanase (24,000 BXU/kg diet). The basal diet met or exceeded all NRC (2012) nutrient requirement estimates including P. Diets were formulated in 4 dietary phases based on BW (Phase 1, 25-50 kg; Phase 2, 50-75 kg; Phase 3, 75-100 kg; Phase 4, 100-125 kg, respectively), and 0.30% TiO_2 was added in the Phase 4 diets as an indigestible marker for ATTD determinations. The summit diet mixing concept was applied wherein a single batch of the basal diet for all 9 treatments was mixed to prevent differences in non-treatment components of the diets.

Body weight and feed consumption were recorded for calculation of ADG, ADFI and G:F ratio. For ATTD estimation, fecal collection was performed for 3 consecutive days during Phase 4 after pigs had received the treatment diets for at least 10 days. All feed and fecal samples were analyzed for DM, GE, N, ether extract

(**EE**), NDF, ADF, Ca, and P following AOAC (1990; 2006) methods and for Ti concentration as described by Myers et al. (2004). The ATTD was calculated by the indirect method using TiO_2 as an indicator. For ATTD calculation, because of using the summit mixing concept wherein a single basal diet was mixed, averaged values of nutrients from all diets were used except for GE and EE in which averaged values from all NC associated diets were used for all of the lower energy diets separately from the PC diet. At a BW of about 120 kg, pigs were scanned by real-time ultrasound (Animal Ultrasound Services, Ithaca, NY) by an experienced technician, and backfat thickness and longissimus muscle (**LM**) depth were measured. Longissimus muscle area was estimated from the LM depth for each pig. The percent of carcass lean was estimated from backfat thickness and LM depth using the equations provided by the User's Manual for AUSKey System (AUSKey System v2.0) adapted to metric units. Carcass daily lean gain was estimated by subtracting the kilograms of initial lean for each pig from the kilograms of final lean, and then dividing by the number of days on test. The weights of initial and final lean were calculated from NPPC (2000) equations. Following calculation of the daily lean gain during the overall period, the ADFI during the overall period was compared to the lean gain to create a lean gain to feed efficiency ratio.

Data on growth performance, carcass characteristics, and ATTD were subjected to ANOVA using the GLM procedure in SAS (Statistical Analysis System, Cary, NC) with a randomized complete block design. The individual pig served as the experimental unit. A single degree of freedom contrast was performed for the comparison between PC and NC treatments to validate the energy-associated aspect of the design. Apart from the PC treatment, the treatment structure was a 2 × 4 factorial arrangement with the main factors of xylanase and phytase supplementation, and the model included the effects of replication, xylanase, phytase, and xylanase × phytase interaction. Orthogonal polynomial contrasts were performed to evaluate linear and quadratic effects of phytase supplementation levels. For the analysis of carcass characteristics, the BW at real-time ultrasound scan was used as a covariate. Least squares mean separations were conducted using the PDIFF option of SAS. The final BW and feed consumption were analyzed on both a common weight and a common age basis to assess potential differences between data using the same feeding duration (common age) and using a common end weight (different days on test but analogous to feeding to a common market weight).

Experiment 2

This was a proof of concept study to evaluate superdosing phytase effect in lactating sows fed an adequate Ca/P diet on standard lactation performance measurements, ATTD, sow milk composition, and bone characteristics of their pigs. Data were only partially available at the time of development of this paper.

A total of 17 crossbred sows around farrowing (from day 5 prepartum to day 3 postpartum) were assigned to the 2 dietary treatments (9 and 8 sows for Treatment 1 and 2, respectively) based on genetic background, parity and BW of the sows. Treatments were: 1) control, no phytase using a conventional corn-SBM lactation diet containing adequate Ca (0.75%) and total P (0.60%) levels based on NRC (1998), and 2) control diet plus 3,000 FTU/kg of phytase. All diets contained 0.3% TiO_2 for ATTD measurements. The sows were fed the experimental diets until weaning. The number of piglets for each lactating sow was equalized by cross-fostering (target 10 piglets per sow). Sow BW at initial, farrowing and weaning, lactation feed intake, litter size and individual piglet weight at birth and weaning, and weaning-to-estrus interval (**WEI**) were recorded. Milk samples were collected in early lactation (d 7-11 of lactation) and late lactation (d 16-19 of lactation) and analyzed for milk composition (solids, protein, lactose, and fat) using Foss Milkoscan™ FT 120 (FOSS Electric, Eden Prairie, MN, USA). Fecal samples were collected on the same day of milk collection for ATTD measurements. Sow blood samples were collected in late lactation at the same day of milk collection. At weaning, 2 average-weight pigs (1 barrow and 1gilt) from each sow were slaughtered to collect liver, metacarpal bones and femurs. Mineral content (Ca, P, Zn, and Mg) in milk and bone of piglets with bone breaking strength and ash, and ATTD of sows are still being conducted. Data on sow BW, reproductive and litter performance, and milk composition were subjected to ANOVA using the GLM procedure in SAS with a completely randomized design. The individual sow or litter served as the experimental unit. Least squares mean separations were conducted using the PDIFF option of SAS.

Results and Discussion

Experiment 1

A variety of differing high fiber and phytate-including ingredients (i.e., corn germ meal, corn DDGS, and wheat middlings) were used to provide enough substrate for possible xylanase and phytase responses. While there was a high level of phytate that would yield P from phytase, the released P was not needed as actual daily standardized total tract digestible P intake in the current study was greater than the daily P requirement

estimates of NRC (2012). Therefore, the responses to the graded increase of phytase supplementation levels are beyond the P-releasing action of the phytase. There was no xylanase effect ($P > 0.12$) nor interaction ($P > 0.14$) between xylanase and phytase supplementation in this experiment.

In evaluating the main effect of phytase supplementation for growth performance (Table 1), there were quadratic increases with increasing phytase supplementation levels for ADG in Phase 1 ($P = 0.06$), 2, 4, and overall ($P < 0.01$), tendencies for an increase in ADFI in Phase 3 ($P = 0.07$) and 4 ($P = 0.09$), and linear increases in G:F ratio for Phase 2 ($P = 0.095$) and overall ($P < 0.05$ and $P = 0.06$ for the common weight and age basis, respectively). These results demonstrated that phytase supplementation clearly improved weight gain and feed efficiency, with 1,000 FTU/kg of phytase supplementation to the diets providing the greatest growth rate during the entire experimental period. Braña et al. (2006) reported that there were linear increases in ADG and G:F ratio as phytase supplementation levels increased up to 1,000 FTU/kg in low-P diets for growing pigs. Santos et al. (2014) reported that phytase supplementation at 2,000 FTU/kg in a low-P diet improved feed efficiency in growing pigs. While those previous studies demonstrated that high level of phytase supplementation in low-P or P-deficient diets for growing-finishing pigs improved growth rate and feed efficiency with increased P digestibility, they could not separate any extra-phosphoric effects of super-dosing phytase supplementation from its effect on P release. However, because all pigs in the current study consumed P above their daily requirement estimates (NRC, 2012), the effect of phytase supplementation on growth performance reflects its effect beyond the P requirement of the pigs.

Table 1. Growth performance for pigs fed high by-products diets with xylanase and phytase supplementation (main effects)[1,2]

			XYL, BXU/kg			PHY, FTU/kg					P-values[3]			
Item	PC	NC	0	24,000	SEM	0	500	1,000	2,000	SEM	PC vs. NC	XYL	PHY-L	PHY-Q
Body weight, kg														
Initial	26.27	26.27	26.34	26.43	0.17	26.32	26.45	26.55	26.23	0.24	1.00	0.71	0.73	0.35
End of trial	123.27	117.82	121.30	122.14	1.17	118.68	122.77	123.95	121.45	1.66	0.098	0.62	0.37	0.04
ADG, kg/d														
Phase 1	0.831	0.828	0.860	0.845	0.012	0.843	0.849	0.883	0.834	0.016	0.92	0.38	0.79	0.06
Phase 2	0.899	0.851	0.923	0.912	0.014	0.869	0.955	0.935	0.911	0.020	0.22	0.58	0.44	0.01
Phase 3	0.981	0.864	0.903	0.942	0.020	0.884	0.929	0.929	0.948	0.028	0.05	0.17	0.15	0.53
Phase 4	0.955	0.828	0.900	0.907	0.028	0.800	0.944	0.963	0.906	0.040	0.11	0.85	0.16	0.01
Overall common weight	0.913	0.840	0.894	0.901	0.013	0.847	0.917	0.928	0.897	0.018	0.05	0.71	0.15	<0.01
Overall common age	0.914	0.842	0.895	0.904	0.012	0.853	0.917	0.928	0.900	0.017	0.04	0.60	0.15	<0.01
ADFI, kg/d														
Phase 1	1.660	1.741	1.761	1.710	0.034	1.753	1.699	1.776	1.715	0.048	0.39	0.29	0.78	0.79
Phase 2	2.210	2.484	2.519	2.475	0.065	2.585	2.482	2.544	2.377	0.092	0.14	0.63	0.15	0.79
Phase 3	2.748	2.796	2.944	2.918	0.060	2.818	3.084	2.961	2.861	0.085	0.78	0.77	0.79	0.07
Phase 4	3.130	3.060	3.273	3.239	0.096	3.057	3.447	3.319	3.200	0.135	0.79	0.80	0.81	0.09
Overall common weight	2.410	2.509	2.592	2.557	0.047	2.543	2.632	2.616	2.506	0.066	0.44	0.60	0.51	0.18
Overall common age	2.400	2.487	2.585	2.550	0.048	2.522	2.632	2.616	2.500	0.067	0.50	0.61	0.59	0.13
G:F ratio														
Phase 1	0.502	0.476	0.490	0.496	0.008	0.481	0.502	0.501	0.487	0.011	0.24	0.59	0.94	0.13
Phase 2	0.407	0.353	0.373	0.374	0.009	0.346	0.386	0.377	0.384	0.012	0.04	0.93	0.095	0.18
Phase 3	0.356	0.310	0.309	0.324	0.006	0.315	0.303	0.317	0.330	0.009	0.02	0.12	0.11	0.35
Phase 4	0.304	0.271	0.276	0.279	0.006	0.262	0.277	0.290	0.282	0.009	0.06	0.72	0.13	0.12
Overall common weight	0.379	0.336	0.346	0.353	0.005	0.334	0.350	0.357	0.358	0.007	<0.01	0.35	0.04	0.18
Overall common age	0.381	0.340	0.348	0.356	0.005	0.340	0.350	0.357	0.360	0.007	<0.01	0.29	0.06	0.40

[1] FTU = phytase (PHY) units; BXU = xylanase (XYL) unit.

[2] Least squares means (n = 5 per treatment).

[3] *P*-values are for the single degree of freedom contrast between positive control (PC) and negative control (NC) treatments, the main effect of XYL and linear (PHY-L) and quadratic (PHY-Q) responses based on PHY supplementation levels. There was no interaction between XYL and PHY supplementation ($P > 0.22$).

In evaluating the main effect of phytase supplementation for carcass characteristics (Table 2), carcass lean percentage ($P < 0.05$), final lean weight ($P < 0.05$), daily lean gain ($P < 0.05$), and lean gain to feed ratio ($P < 0.05$) increased linearly on the common age basis as phytase supplementation increased. Backfat thickness, on the common age basis, tended to decrease linearly as phytase supplementation levels increased ($P = 0.098$). It has been demonstrated that an increased P deposition is associated with an increased N deposition of pigs (NRC, 2012; Pettey et al., 2015). Therefore, phytase supplementation can increase carcass leanness of pigs provided other nutrients are available and that genotypic lean deposition has not been reached.

In the ATTD results taken in Phase 4 (Table 3), the ATTD of DM, EE, NDF and hemicellulose ($P \leq 0.050$) increased quadratically as phytase supplementation increased. The ATTD of P increased both linearly ($P < 0.01$) and quadratically ($P < 0.05$) as phytase supplementation levels increased. It has been reported that the efficacy of phytase supplementation on P digestibility could be diminished when phytase was supplemented to diets containing P close to or above the requirement (Kemme et al., 1997; Jang et al., 2014). While this is obvious and logical, P digestibility did still increase in this study as phytase supplementation levels increased. This study further observed quadratic increases in digestibility of DM, NDF and hemicellulose as phytase supplementation levels increased and consequently increases in energy digestibility also. Therefore, the improvements in growth rate, feed efficiency and carcass measures can be attributed to phytase supplementation effects beyond P release.

Table 2. Carcass characteristics for pigs fed high by-products diets with xylanase and phytase supplementation with scan weight as a covariate (main effects)[1,2]

			XYL, BXU/kg			PHY, FTU/kg					P-values[3]			
Item	**PC**	**NC**	**0**	**24,000**	**SEM**	**0**	**500**	**1,000**	**2,000**	**SEM**	**PC vs. NC**	**XYL**	**PHY-L**	**PHY-Q**
Common weight basis														
Scan weight[4], kg	123.27	117.82	121.30	122.14	1.17	118.68	122.77	123.95	121.45	1.66	0.10	0.62	0.37	0.04
Backfat thickness, mm	13.74	15.59	15.62	15.63	0.55	16.76	15.74	14.58	15.41	0.79	0.25	0.99	0.24	0.17
Longissimus muscle depth, mm	62.61	60.35	62.81	62.14	1.40	62.06	60.98	62.06	64.80	2.02	0.60	0.74	0.25	0.50
Longissimus area, cm^2	41.66	40.30	41.78	41.38	0.84	41.33	40.68	41.33	42.97	1.21	0.60	0.74	0.25	0.50
Carcass lean, %	57.61	56.23	56.50	56.42	0.40	55.73	56.21	57.04	56.86	0.58	0.25	0.89	0.15	0.39
Initial lean, kg	9.31	9.35	9.35	9.39	0.07	9.36	9.39	9.42	9.31	0.10	0.85	0.75	0.66	0.53
Final lean, kg	47.85	46.79	47.29	47.14	0.39	46.78	46.83	47.42	47.81	0.57	0.37	0.78	0.16	0.98
Lean gain, g/d	362.06	345.16	357.09	354.92	3.67	344.28	356.20	361.08	362.48	5.30	0.14	0.68	0.03	0.20
Lean gain/feed	0.152	0.134	0.138	0.140	0.003	0.133	0.137	0.142	0.145	0.004	0.06	0.65	0.06	0.60
Common age basis														
Scan weight[4], kg	122.27	114.64	120.32	121.36	1.30	115.91	122.77	123.95	120.73	1.83	0.04	0.57	0.17	<0.01
Backfat thickness, mm	13.88	15.59	15.35	15.35	0.52	16.61	15.60	14.38	14.81	0.77	0.28	0.99	0.098	0.23
Longissimus muscle depth, mm	61.97	59.15	62.29	60.46	1.17	59.49	60.85	61.86	63.31	1.74	0.46	0.28	0.12	0.82
Longissimus area, cm^2	41.28	39.59	41.47	40.37	0.70	39.79	40.60	41.21	42.08	1.04	0.46	0.28	0.12	0.82
Carcass lean, %	57.45	56.09	56.60	56.39	0.36	55.52	56.28	57.14	57.05	0.53	0.23	0.68	0.046	0.26
Initial lean, kg	9.31	9.37	9.36	9.39	0.07	9.38	9.38	9.41	9.30	0.11	0.78	0.77	0.61	0.65
Final lean, kg	47.40	46.25	47.00	46.58	0.33	45.98	46.59	47.18	47.39	0.49	0.28	0.38	0.04	0.45
Lean gain, g/d	362.76	351.24	358.48	354.22	3.19	348.60	354.37	359.73	362.70	4.71	0.27	0.35	0.04	0.52
Lean gain/feed	0.152	0.136	0.139	0.140	0.003	0.134	0.137	0.142	0.145	0.004	0.06	0.76	0.046	0.64

1 FTU = phytase (PHY) units; BXU = xylanase (XYL) unit.
2 Least squares means (n = 5 per treatment).
3 *P*-values are for the single degree of freedom contrast between positive control (PC) and negative control (NC) treatments, the main effect of XYL, and linear (PHY-L) and quadratic (PHY-Q) responses based on PHY supplementation levels. There was no interaction between XYL and PHY supplementation ($P > 0.34$).
4 Because scan weight on both a weight and an age basis was significantly different with phytase supplementation, scan weight was used as a covariate.

Table 3. Apparent total tract digestibility (%) for pigs fed high by-products diets with xylanase and phytase supplementation in Phase 4 (main effects)[1,2]

			XYL, BXU/kg			PHY, FTU/kg					P-values[3]			
Item	PC	NC	0	24,000	SEM	0	500	1,000	2,000	SEM	PC vs. NC	XYL	PHY-L	PHY-Q
DM	76.84	77.74	78.58	78.54	0.24	77.57	79.12	78.82	78.71	0.34	0.20	0.91	0.10	0.02
GE	76.84	77.38	78.01	77.87	0.28	77.11[b]	78.56[a]	77.94[ab]	78.15[a]	0.40	0.50	0.73	0.22	0.15
N	74.12	75.24	76.24	76.21	0.46	75.05	76.91	76.44	76.51	0.66	0.40	0.96	0.26	0.18
Ether extract	76.64	66.21	71.61	70.60	1.48	65.01	73.28	74.79	70.28	2.09	0.02	0.46	0.20	<0.01
ADF	50.07	49.52	51.93	50.70	0.91	49.81	51.89	51.92	51.64	1.28	0.83	0.35	0.42	0.32
NDF	49.33	50.38	53.12	52.67	0.56	50.35	54.56	53.26	53.41	0.79	0.52	0.58	0.07	0.02
Hemicellulose	48.80	50.99	53.96	54.09	0.87	50.74	56.47	54.22	54.67	1.23	0.41	0.92	0.15	0.05
Ca	44.25	46.75	45.64	47.83	1.52	46.83	44.23	48.38	47.50	2.15	0.55	0.32	0.55	0.96
P	35.52	42.66	47.84	46.94	1.05	41.53	46.22	50.34	51.48	1.49	0.02	0.55	<.001	0.04

[a,b]Means within the same row without a common superscript differ ($P < 0.10$).
[1] FTU = phytase (PHY) units; BXU = xylanase (XYL) unit.
[2] Least squares means (n = 5 per treatment).
[3] *P*-values are for the single degree of freedom contrast between positive control (PC) and negative control (NC) treatments, the main effect of XYL and linear (PHY-L) and quadratic (PHY-Q) responses based on PHY supplementation levels. There was no interaction between XYL and PHY supplementation ($P > 0.14$).

The energy uplift by phytase supplementation was 44 kcal/kg.

It is well-known that phytase supplementation increases P digestibility in pigs (Kerr et al., 2010). In the current study, P digestibility increased quadratically where the ATTD of P was maximized when phytase was supplemented at 1,000 FTU/kg which agrees with Almeida et al. (2013) who reported that the ATTD of P in growing pigs plateaued when phytase was added at 801 FTU/kg in low-P diets. In this study, the increases of DM and energy digestibility, which can be attributed to the increases of NDF and hemicellulose digestibility, agrees with Adedokun et al. (2015) who reported that increasing level of phytase supplementation up to 2,000 FTU/kg for pigs weighing 22 to 30 kg BW and fed diets containing wheat middlings and corn DDGS with marginally deficient nonphytate P resulted in increased ATTD of DM and energy. However, the effects of phytase supplementation on fibrous components are rather limited and still inconsistent. Johnston et al. (2004) reported that ileal NDF digestibility increased when 500 FTU/kg of phytase was supplemented in diets with adequate levels of Ca and P for 52 kg BW pigs but there was no phytase effect in the Ca/P-deficient diets. The microbial fibrolytic activity in the large intestine largely depends on the P availability in pigs because P is essential for fiber degradation by microbes, and P availability for microbes in the large intestine could potentially be reduced by increased P absorption mediated by the phytase response in the small intestine (Metzler and Mosenthin, 2008).

In conclusion, high levels of phytase supplementation to the diets containing adequate P content with high amounts of fibrous ingredients improved growth performance, carcass leanness, and digestibility of fat and fibrous components as well as P. However, the synergistic effect of combined supplementation of both xylanase and phytase was not confirmed.

Experiment 2

In Experiment 1 (a super-dosing phytase study for growing-finishing pigs), phytase supplementation up to 1,000 FTU/kg diet to pigs fed an adequate P diet had a

Table 4. Effect of super-dosing phytase supplementation of lactation diet on sow and litter performance

	Phytase, FTU/kg			
Items	0	3,000	SEM	P-values
No. of sows	9	8		
Sow BW, kg				
At farrowing	227.58	233.13	8.70	0.66
At weaning	230.61	234.49	7.45	0.72
BW change (farrow to wean)	3.03	1.36	5.82	0.84
Weaning age, d	20.33	19.50	0.45	0.21
Weaning to estrus interval, d	5.75	4.00	0.46	0.02
Lactation feed intake, kg/d	5.76	5.71	0.41	0.93
Litter size, no. pigs				
Total born	11.78	11.25	0.92	0.69
Live born	10.11	9.38	0.90	0.57
After cross-fostering	9.78	9.88	0.54	0.90
Weaned	9.00	9.50	0.52	0.50
Piglet BW, kg				
Total born	1.49	1.49	0.11	0.97
Live born	1.54	1.54	0.11	0.98
After cross-fostering	1.55	1.49	0.11	0.75
Weaned	6.20	5.58	0.39	0.28
Litter BW, kg				
Total born	17.53	16.11	1.26	0.44
Live born	15.52	13.88	1.31	0.39
After cross-fostering	15.15	14.61	1.27	0.77
Weaned	56.55	52.70	5.13	0.60

positive effect to increase not only nutrient digestibility (DM, fat, NDF, hemicellulose, and P) but also carcass leanness as shown by increased carcass lean percentage and daily lean gain, and decreased backfat thickness, as phytase supplementation level increased. Additionally, in a multi-university study (Jang et al., 2014) with phytase supplementation to gestation and lactation diets, weaning weight of piglets increased as phytase supplementation level increased and bone integrity of piglets at weaning was improved when phytase was supplemented to the sow diet. Collectively, these results may suggest that super-dosing phytase supplementation of sow diets, particularly in lactation, may reduce weight loss of sows during lactation, and improve litter performance and bone integrity of their progeny with potentially increased milk P content.

In Experiment 2, there were no significant differences in response measures of sow BW, reproductive and litter performance, or normal milk composition measures (Table 4 and 5) except for WEI in which the sows in the phytase supplementation group had shortened WEI compared with those in the control group ($P < 0.05$). In a previous study (Wealleans et al., 2015), super-dosing phytase supplementation up to 2,000 FTU/kg in a low Ca and P diet did not affect litter size and litter performance during lactation but improved P and Ca digestibility and reduced sow BW loss during lactation. Even though there was no effect on sow BW loss in lactation by super-dosing phytase supplementation, it could be assumed that the sows consuming the super-dosing phytase supplemented diet in lactation may have improved body condition at weaning resulting in a shorter WEI compared with the control sows. In conclusion, super-dosing phytase supplementation of the lactation diet may reduce WEI but needs further investigation.

Table 5. Effect of super-dosing phytase supplementation of lactation diet on milk composition (%)

	Phytase, FTU/kg			
Items	**0**	**3,000**	**SEM**	**P-values**
No. of sows	9	8		
Milk composition				
Early lactation[1]				
Fat	5.59	6.18	0.42	0.34
Protein	4.76	4.64	0.12	0.51
Lactose	5.93	5.87	0.09	0.63
Total solids	17.31	17.75	0.46	0.51
Solids not fat	11.11	10.93	0.09	0.16
Late lactation[1]				
Fat	5.54	5.66	0.30	0.79
Protein	4.61	4.49	0.12	0.47
Lactose	6.10	6.09	0.06	0.85
Total solids	17.25	17.23	0.32	0.96
Solids not fat	11.11	10.96	0.09	0.25

[1] Average sampling days were 8.6 and 17.5 days of lactation for early and late lactation, respectively.

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Calcium Digestibility and Requirements for Digestible Calcium by Growing Pigs

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Summary

The standardized total tract digestibility (STTD) of Ca in most feed ingredients used in diets fed to pigs has been determined, and the effect of microbial phytase on STTD of Ca has also been reported. This has made it possible to formulate diets based on values for STTD of Ca and attempts to determine requirements for STTD of Ca for 11 to 25, 25 to 50, and 100 to 130 kg pigs have been made. Results of these experiments have indicated that STTD Ca provided in excess of the requirement is detrimental to pig growth performance. This is particularly true if dietary STTD P is marginal, whereas the negative effects of excess Ca is mitigated if STTD P is provided in excess of the requirement. As a consequence, effects of microbial phytase on the STTD of Ca need to be accounted for in diet formulation. Based on currently available information, it is recommended that diets for pigs less than 50 kg are formulated to have a STTD Ca:STTD P ratio between 1:1 and 1.35:1, whereas diets for pigs greater than 100 kg should have a STTD Ca:STTD P ratio that does not exceed 1.1:1.

Introduction

Historically, requirements for Ca in diets fed to pigs have been expressed on the basis of total Ca and all feed requirement tables include values for the requirement for total Ca by different categories of pigs. This is in contrast to values for P where requirements are usually expressed as values for either apparent total tract digestible (**ATTD**) P or standardized total tract digestible (**STTD**) P. In the current version of the National Research Council's *Nutrient Requirements of Swine*, requirements for total Ca were calculated by multiplying requirements for STTD P by 2.15 (NRC, 2012). However, the committee that developed "Nutrient Requirements of Swine" acknowledged that this approach was used simply because no data for the digestibility of Ca in feed ingredients were available and the committee specifically stated that *"A preferred ratio would have been a ratio between digestible Ca and digestible P, but again, because of lack of data the ratios between total Ca and STTD P are used"* (NRC, 2012). Thus, the lack of data for digestibility of Ca in feed ingredients prevented the committee from estimating requirements for STTD of Ca.

However, during the last few years, values for the digestibility of Ca in most Ca containing feed ingredients have been determined, and it has, therefore, been possible to initiate work to estimate requirements for digestible Ca in diets fed to different categories of pigs. It is the objective of the current contribution to present currently available data for digestibility of Ca in feed ingredients and preliminary data for the requirement for STTD Ca by growing-finishing pigs.

Digestibility of Ca in Feed Ingredients

Preliminary work to determine the digestibility of Ca in feed ingredients fed to pigs indicated that the basal endogenous loss of Ca from pigs is between 150 and 400 mg per kg of dry matter intake (**DMI**) (Gonzalez-Vega et al., 2013). As a consequence, values for ATTD of Ca are influenced by the inclusion level of Ca in the diets because the endogenous Ca contributes a greater proportion of Ca to the fecal output of Ca at low levels of Ca intake compared with greater levels of intake. Values for the ATTD of Ca are, therefore, not additive in mixed diets and as a consequence, digestibility values for Ca need to be corrected for the basal endogenous losses and expressed as values for the STTD of Ca.

A number of experiments have been conducted in recent years to determine the STTD of Ca in feed ingredients fed to pigs (Table 1). In general, the greatest STTD values have been obtained in feed ingredients of animal origin whereas the STTD of Ca in most inorganic sources of Ca has been observed to be less than in most animal proteins. Cereal grains and most cereal coproducts contain very little or no Ca, whereas oilseed meals provide some Ca to the diets with canola meal having the greatest concentration of Ca among oilseed

meals. However, as is the case for P, a proportion of the Ca in oilseed meals is bound to phytate with a reduced digestibility as a consequence. Therefore, inclusion of microbial phytase to diets that contain oilseed meals will result in an increased STTD of Ca (Gonzalez-Vega et al., 2013). It has, however, been demonstrated that the increase in digestibility of Ca that is observed in response to microbial phytase in diets based on corn and soybean meal is greater than what can be explained by the release of Ca bound to phytate in soybean meal (Almeida and Stein, 2013), and is was, therefore, hypothesized that dietary Ca from mineral supplements may be bound to phytate in corn-soybean meal diets. Indeed, working with corn-based diets, it was subsequently demonstrated that the STTD of Ca in calcium carbonate, but not in monocalcium phosphate or dicalcium phosphate, is increased if microbial phytase is added to the diet (Gonzalez-Vega et al., 2015a). Likewise, the STTD of Ca in fish meal included in a corn-based diet is increased by microbial phytase (Gonzalez-Vega et al., 2015b), but for other animal proteins such as meat and bone meals and poultry meals, effects of microbial phytase have been less clear (Merriman et al., 2016b). The STTD of Ca in whey permeate is also increased by microbial phytase, but that is not the case for skim milk powder and whey powder (University of Illinois, unpublished data). There is, however, no impact of particle size of calcium carbonate on the STTD of Ca (Table 2), and growth performance of pigs is not affected by the particle size of the calcium carbonate in the diets (Merriman and Stein, 2016). It has been reported that dietary fat may reduce the digestibility of Ca in humans because of formation of Ca-fat complexes in the intestinal tract (Bendsen et al., 2008), but recent data demonstrated that there are no negative effects of adding up to 7% dietary fat to diets fed to pigs (Merriman et al., 2016c).

The reason the STTD of Ca in calcium carbonate and some animal proteins is increased if microbial phytase is used is most likely that the phytate from the plant ingredients may chelate Ca from non-plant feed ingredients in the stomach of the pigs, which prevents absorption in the small intestine, but if microbial phytase reduces the chelating abilities of phytate, less Ca

Table 1. Standardized total tract digestibility (STTD) of Ca in feed ingredients, %[1]

Ingredient	STTD of Ca without phytase	STTD of Ca with phytase
Mineral supplements		
Monocalcium phosphate[1]	77	80
Dicalcium phosphate[1]	73	75
Calcium carbonate[1]	64	71
Plant feed ingredients		
Canola meal[2]	42	-
Soybean meal[2]	78	-
Animal feed ingredients		
Meat and bone meal[3]	77	82
Meat meal[3]	77	86
Fish meal[4]	76	87
Poultry meal[3]	82	76
Poultry by product meal[3]	88	87
Skim milk powder[2]	93	97
Whey powder[2]	96	93
Whey permeate[2]	58	71

[1] Gonzalez-Vega et al., 2015a.
[2] Unpublished data from the University of Illinois.
[3] Merriman et al., 2016b.
[4] Gonzalez-Vega et al., 2015b.

Table 2. Growth performance, bone ash, and Ca and P retention in 11 to 25 kg pigs fed diets varying in concentrations of standardized total tract digestible (STTD) Ca and STTD P, Exp. 1

	STTD Ca, %							
	0.32	0.40	0.48	0.56	0.64	0.72	0.72	0.72
	STTD P, %							
Item	0.36	0.36	0.36	0.36	0.36	0.36	0.33	0.40
Initial wt, kg	11.39	11.35	11.44	11.37	11.45	11.33	11.42	11.42
Final wt, kg	25.21	25.29	25.47	25.76	24.23	23.74	24.32	25.05
ADFI, g	957	961	963	1,003	927	934	970	957
ADG, g	626	631	636	652	579	565	587	622
G:F	0.65	0.66	0.66	0.65	0.62	0.61	0.61	0.65
Bone ash, g	7.8	8.5	9.8	9.5	9.0	9.1	8.1	10.2
Bone Ca, g	2.86	3.15	3.64	3.54	3.39	3.45	3.05	3.82
Bone P, g	1.41	1.53	1.77	1.71	1.62	1.62	1.45	1.84
Ca retention, g/d	2.16	2.75	3.41	3.41	3.99	3.75	3.50	4.23
P retention, g/d	2.30	2.42	2.64	2.50	2.67	2.49	2.29	2.72

Table 3. Predicted values for ADG, G:F, bone ash, and Ca retention of 25 to 50 kg pigs fed diets containing 0.15, 0.31, 0.39, or 0.47% standardized total tract digestible (STTD) P and 0.13, 0.27, 0.42, 0.57, or 0.72% STTD Ca, Exp. 2 [1]

	Dietary STTD P, %			
Item	0.15	0.31	0.39	0.47
ADG, g	0.76 (0.12)	0.87 (0.36)	0.90 (0.47)	0.92 (0.59)
G:F	0.43 (0.09)	0.46 (0.38)	0.48 (0.52)	0.50 (0.67)
Bone ash, g	14.3 (0.43)	21.3 (0.56)	23.2 (0.63)	24.0 (0.69)
Ca retention, g/d	4.8 (0.76)	7.1 (0.93)	8.4 (1.02)	9.9 (1.11)

[1] The first value in each cell is the maximum response obtained at the STTD P concentration indicated, and the value in parenthesis is the % STTD Ca that will result in this response.

will be bound to phytate, which will result in increased digestibility of Ca. However, because excess dietary Ca will reduce the digestibility of P and thereby increase P excretion in the feces (Stein et al., 2011) and reduce growth performance of pigs (Gonzalez-Vega, 2016a,b; Merriman et al., 2016a), it is important to take the increased digestibility of Ca that is a result of microbial phytase into account in formulation of diets fed to growing pigs.

Requirements for Digestible Ca by Growing Pigs

Three experiments were conducted to determine the requirements for digestible Ca by growing pigs. In the first experiment, 6 diets that contained 0.36% STTD P and 6 levels of digestible Ca from 0.32 to 0.72% were used (Gonzalez-Vega et al., 2016a). Two additional diets containing 0.72% Ca and either 0.33 or 0.40% STTD P were also formulated. The 8 diets were fed for 22 days to pigs that were 11.39 ± 1.21 kg at the start of the experiment with 4 pigs per pen and 8 replicate pens per treatment. Individual pig weights were recorded at the beginning and at the conclusion of the experiment and on the last day of the experiment, one pig per pen was sacrificed and the right femur was removed. Data for average daily gain (ADG), average daily feed intake (ADFI) and average gain to feed (G:F) ratios were calculated at the end of the experiment and the femurs from the sacrificed pigs were ashed and analyzed for Ca and P. Diets were also fed to 80 pigs that were placed individually in metabolism crates with 10 replicate pigs per diet. Feces and urine were collected from all pigs and balances for Ca and P were calculated.

Table 4. Growth performance of 100 to 130 kg pigs fed experimental diets with varying concentrations of standardized total tract digestible (STTD) Ca and P for 28 d, Exp. 3

	STTD Ca, %				
Item	**0.08**	**0.18**	**0.29**	**0.38**	**0.49**
Initial BW, kg					
0.11% STTD P	98.83	100.28	101.75	97.62	99.23
0.21 % STTD P	99.42	98.38	101.07	98.47	100.45
0.31 % STTD P	101.13	99.15	99.25	102.60	100.72
ADG, kg[1,2]					
0.11% STTD P	1.21	1.14	1.17	0.89	0.83
0.21 % STTD P	1.16	1.20	1.17	1.15	0.96
0.31 % STTD P	1.11	1.10	1.08	1.00	1.10
ADFI, kg[3,4]					
0.11% STTD P	3.70	3.29	3.27	3.16	2.88
0.21 % STTD P	3.72	3.58	3.31	3.32	3.16
0.31 % STTD P	3.46	3.32	3.27	3.16	3.16
GF, d 1-28[5,6]					
0.11% STTD P	0.33	0.35	0.37	0.28	0.29
0.21 % STTD P	0.32	0.34	0.36	0.35	0.31
0.31 % STTD P	0.33	0.33	0.34	0.32	0.35
Final BW, kg[7,8]					
0.11% STTD P	132.68	132.33	134.48	122.42	122.52
0.21 % STTD P	132.02	131.85	133.75	130.77	127.32
0.31 % STTD P	132.33	129.90	129.62	130.57	131.47

1 Results indicated that ADG from d 1 to 28 at different combinations of STTD Ca and STTD P can be described by the following model: 1.2141 – 0.6230 × STTD Ca ($P = 0.008$).
2 Standard error of the within treatment least squares means = 0.09.
3 Results indicated that ADFI from d 1 to 28 at different concentrations of STTD Ca can be described by the following model: 3.6782 – 1.2722 × STTD Ca ($P = 0.001$).
4 Standard error of the within treatment least squares means = 0.23.
5 Results indicated that G:F from d 1 to 28 could not be predicted using STTD Ca or STTD P.
6 Standard error of the within treatment least squares means = 0.02.
7 Results indicated that final BW at different combinations of STTD Ca and STTD P can be described by the following model: 140.4729 – 42.9212 × STTD Ca – 30.3919 × STTD P + 140.2884 STTD Ca × STTD P ($P = 0.006$).
8 Standard error of the within treatment least squares means = 2.87.

In the second experiment, a total of 20 diets were used. Diets contained 0.15, 0.31, 0.39, or 0.47% STTD P and 0.13, 0.27, 0.42, 0.57, or 0.72% STTD Ca. Thus, diets were formulated in a 4 × 5 factorial design. A total of 240 pigs (initial BW: 24.70 ± 1.27 kg) were allotted to the 20 diets with 2 pigs per pen and 6 pen replicates per diet (Gonzalez-Vega et al., 2016b). Pigs were fed experimental diets for 28 days and on the last day of the experiment, one pig per pen was sacrificed and the right femur was removed. Diets were also fed to 120 pigs that were placed individually in metabolism crates. All measurements in this experiment were similar to those in Exp. 1.

In the third Experiment, 90 growing pigs (initial BW: 99.89 ± 3.34 kg) were housed individually and randomly allotted to 15 diets with 6 pigs per diet (Merriman et al., 2016a). Experimental diets were formulated as a 3 × 5 factorial with 3 dietary concentrations of STTD P (0.11, 0.21, or 0.31%) and 5 concentrations of STTD Ca (0.08, 0.18, 0.29, 0.38, or 0.49%). Pigs were fed experimental diets for 28 days. Initial and final BW were recorded and daily feed allotments were recorded as well for calculation of ADG, ADFI, and average G:F for each treatment group. All pigs were sacrificed at the conclusion of the experiment and the right femur was collected from each pig and bone ash, bone Ca, and bone P were measured.

Results from Exp. 1 indicated that there were no negative effects of reducing the concentration of STTD Ca in the diets, but adding more than 0.54 or 0.50% STTD Ca to the diets resulted in a negative response

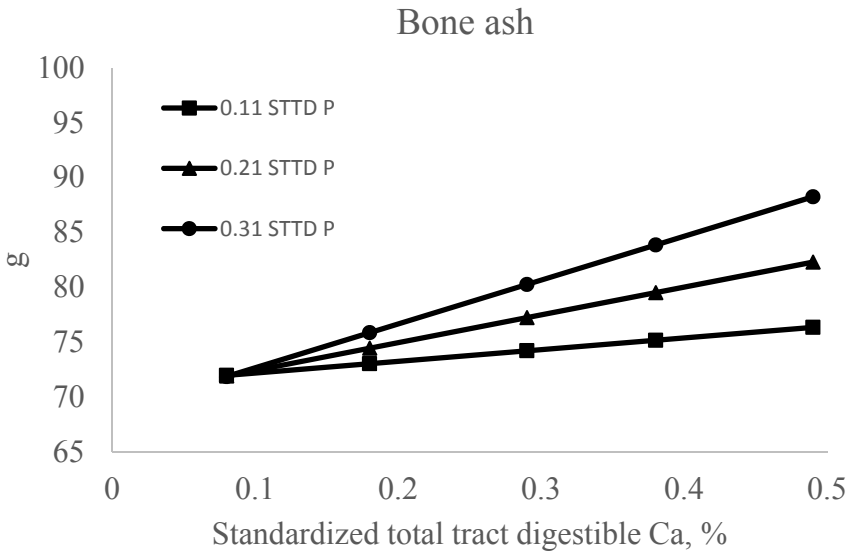

Figure 1. Predicted values, based on the interaction between STTD Ca and STTD P ($P = 0.049$), for bone ash (g) in 100 to 130 kg pigs fed diets containing from 0.08 to 0.49% standardized total tract digestible (STTD) Ca and from 0.11 to 0.31% STTD P. All responses were linear, therefore, no maximum values were estimated, Exp. 3.

to ADG and G:F, respectively (Table 2). In contrast, bone ash increased as the concentration of STTD Ca increased in the diet and the optimum amount of bone ash was achieved if STTD Ca was supplied at 0.48%. Concentrations of bone P and bone Ca were maximized at dietary concentrations of STTD Ca of 0.56 and 0.50%, respectively. Likewise, the retention of Ca and P in the body was maximized at concentrations of STTD Ca of 0.60 and 0.49%, respectively. Results of this experiment indicated that under the condition of the experiment, there were no negative effects of reducing dietary Ca to well below the expected requirement, but adding excess Ca to the diets is detrimental to ADG and G:F. Because bone ash and bone Ca were maximized at 0.48 to 0.50% STTD Ca in the diets, and because ADG and G:F declined at greater dietary concentrations of Ca, these levels should be considered the maximum concentrations of Ca that can be used in diets fed to pigs from 11 to 25 kg. These values correspond to a ratio between STTD Ca and STTD P of approximately 1.35:1 and it appears that the growth performance and bone ash are maximized in 11 to 25 kg pigs if the ratio between STTD Ca and STTD P is between 1:1 and 1.35:1, with the latter value being the absolute maximum that should be used.

Results of Exp. 2 confirmed that excess dietary Ca is detrimental to pig growth performance and this is particularly the case if dietary P is marginal or below the requirement (Table 3). However, results also confirmed that low dietary concentrations of Ca do not reduce growth performance of pigs under the conditions of this experiment. In contrast, bone ash, bone Ca, and bone P, and retention of Ca and P in the body are improved if diet concentrations of STTD Ca and STTD P are increased. The concentration of Ca that is needed to maximize Ca retention is much greater than the concentration needed to maximize Ca in bone ash, which contradicts the expectation that 96 to 99% of all Ca in the body is stored in bones (Crenshaw, 2001). Overall, results of experiment 2 indicated that ADG, G:F, and bone ash were optimized if diets were formulated to a STTD Ca to STTD P ratio between 1.16:1 and 1.43:1. Formulating diets at a greater ratio between STTD Ca and STTD P will result in reduced growth performance of pigs.

Results of Exp. 3 confirmed the negative effects of excess Ca in the diets and indicated that both ADFI and ADG is reduced as dietary Ca increases, whereas dietary STTD P concentrations had no impact on ADG and ADFI (Table 4). However, there were no consistent impacts of dietary Ca or P on G:F in this experiment. The model to describe maximum ADG was negatively impacted by STTD Ca in the diet, but not by STTD P. However, bone ash linearly increased as STTD Ca or STTD P in the diets increased and the model to describe the response on bone ash was impacted by STTD Ca as well as STTD P (Fig 1).

Conclusions

Results of 3 experiments with 11 to 25 kg pigs, 25 to 50 kg pigs, and 100 to 130 kg pigs indicate that during short term experiments, it is not possible to reduce growth performance of pigs by reducing the concentration of STTD Ca in the diets. In contrast, inclusion of excess STTD Ca in the diet will reduce ADG and G:F and this effect is more pronounced if dietary STTD P is marginal or below the requirement than if STTD P is provided in excess of the requirement. It is also clear that the amount of STTD Ca needed to maximize bone ash, bone Ca, bone P, or retention of Ca and P in the body is much greater than the amount needed to maximize growth performance. Based on these results it is recommended that the STTD Ca to STTD P ratio for pigs less than 50 kg should be between 1:1 and 1.35:1, whereas diets fed to pigs above 100 kg should have a STTD Ca to STTD P ratio that does not exceed 1.1:1. Because the STTD of Ca is increased in diets containing microbial phytase, it is important that the effect of phytase on STTD of Ca is taken into account in diet formulation.

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2015 Conference

Merlin Lindemann, University of Kentucky, welcomes the group

Terry Fleck, Center for Food Integrity, Keynote Speaker.

Dean Boyd, Hanor USA, Speaker

David Rosero, Hanor USA, Speaker

Jeremy Marchant-Forde, USDA at Purdue University, Speaker

Dustin Boler, University of Illinois, Speaker

2015 Conference

Darrin Karcher, Michigan State University, Speaker

Charles Maxwell, University of Arkansas, Speaker

Kara Stewart, Purdue University, Speaker

Thomas Burkey, University of Nebraska, Speaker

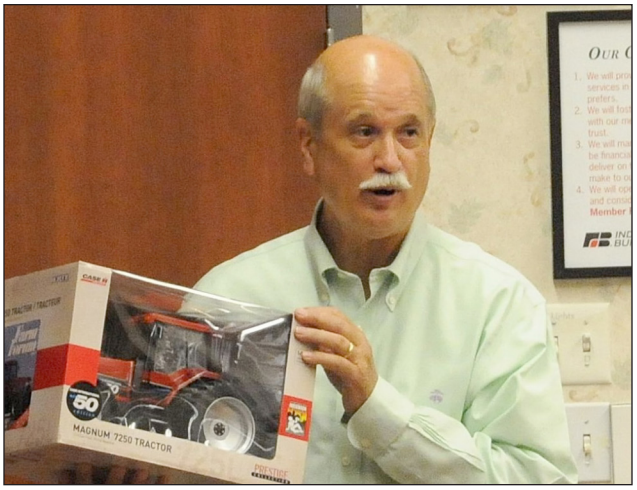
Richard Coffey, University of Kentucky, Moderator

Henry Zerby, The Ohio State University, Moderator

2015 Conference

Bob Easter, President Emeritus, University of Illinois (center), visits with Don Orr, JBS United (L); and Tip Cline, Purdue University (R).

Interaction during the break (L to R): Randy Walker, DPI Global; Tim Fakler, Gerber Milling; and Jim Pettigrew, University of Illinois.



Don Villwock, President Emeritus of Indiana Farm Bureau, was recognized for making the Farm Bureau facilities available for the conference for the past 12 years.

Attendees enjoying the roast pork loin lunch.

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