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Lunch and Refreshments at Breaks

Mayberry Cafe, Danville, IN; Brad and Christine Born

Pre-Conference BBQ Dinner

Shoup's Catering

Schedule of Presentations

8:15 Registration

9:00 Welcome – *Dennis Liptrap, Ralco Nutrition*

9:05 Early Life Nutrition in Pigs in Relation to Immune Function and Disease. *Daniel Columbus, University of Saskatchewan, Canada*

9:50 Microbiota-Gut-Brain Axis. *Sheila Jacobi, The Ohio State University*

10:20 Break

10:50 Technology to Make Nutrition Implementation Easier: Continuous, Real-Time Assessment of Swine Physical Condition. *Isabella Condotta, University of Illinois*

11:25 Fundamentals, Limitations, and Pitfalls on the Development and Application of Sustainable Pig Precision Nutrition. *Aline Remus, Sherbrooke R&D Centre, Quebec, Canada*

12:00 Lunch

1:10 Normal Vitamin Status of Pigs at Different Life Stages. *Laura Greiner, Iowa State University*

1:55 Vitamin D and Vitamin D Metabolites Impact on Calcium and Phosphorus Balance in Gestating Sows. *Hans Stein, University of Illinois*

2:30 Break

2:50 Injecting Different Amounts of Iron - Effects on Blood Parameters During Late Nursery-Early Grower Phase. *Merlin Lindemann, University of Kentucky*

3:25 Effects and Functions of Copper on Nutrient Utilization in Growing Pigs. *Charmaine Espinosa, University of Illinois*

4:00 Closing Remarks

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Early Life Nutrition in Pigs in Relation to Immune Function and Disease

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Summary

Current practice in diet formulation is to match diet nutrient content to requirements to optimize growth performance and profitability of pork production. While this has been a largely successful strategy, with recent societal pressure and legislation to reduce or eliminate antimicrobial use in livestock production, there is increased need to determine the interaction between diet and animal robustness. In particular, this includes a re-evaluation of nutrient requirements not only for growth but also for supporting proper gastrointestinal development and maintenance and immune status and response during disease challenge. While a number of strategies have been investigated to support health, this paper will focus on dietary nutrient content and supply in relation to immunity and robustness, specifically for protein, fibre, and amino acids.

Introduction

A number of factors contribute to the gap observed between performance potential and actual performance achieved under commercial conditions, with immune status likely playing a significant role. Pigs are continuously exposed to microbial pathogens and immune-stimulatory antigens that negatively impact animal productivity. This decrease in performance can have a substantial impact on profitability of producers.

Impact of disease challenge on performance and nutrient metabolism

A primary result of immune challenge is a reduction in feed intake, with subsequent reduction in growth performance of pigs. While feed intake generally explains a majority of this reduced growth, recent meta-analyses have demonstrated that both reduction in nutrient supply (i.e., feed intake) and nutrient utilization (i.e., maintenance requirements) are contributing factors. The proportion with which these factors impact performance is dependent on the specific challenge type, with reduced growth during enteric bacterial infections being more a result of changes in nutrient utilization while the negative effects of mycotoxins and respiratory disease are more due to decreased feed intake (Pastorelli et al., 2012; Rodrigues et al., 2021a).

The overall magnitude of response and recovery period is also dependent on the sex and age at which the pig is exposed to the immune challenge. Rodrigues et al. (2021a) found that female pigs showed a greater negative response in performance compared to males and that the reduction in male pigs appears to be more related to alterations in nutrient utilization than the reduction in female pigs. It was also shown that younger pigs (e.g., nursery) are more affected by immune challenge than older pigs, however, older pigs take longer to recover to pre-challenge performance levels.

Regardless of the type of immune challenge, stimulation of the immune system alters protein and amino acid metabolism and utilization, with amino acids redirected from growth towards supporting the immune response. As feed intake also decreases during immune stimulation, the increase in amino acid supply to support the immune response is met, in part, through a reduction in protein synthesis and increase in protein catabolism in the muscle, which represents the largest pool of amino acids. However, the amino acid profile of muscle protein differs significantly from that of protein involved in the immune response (Reeds et al., 1994), resulting in an amino acid imbalance and a disproportionate use of some amino acids during immune challenge and obligatorily lead to an increase in whole-body amino acid catabolism and reduction in body protein growth. For example, based on amino acid profiles of skeletal muscle protein and acute phase proteins, 6 g of muscle protein would be required to meet the demands for cysteine for every 1 g of albumin (Rakhshandeh, 2011).

Overall, while it is true that immune challenge will result in a reduction in feed intake and, therefore, reduce growth and nutrient requirements for growth, it is likely that amino acid requirements (i.e., amino acid ratios to lysine) need to be adjusted during times of immune challenge and requirements determined in healthy pigs are, most likely, inappropriate.

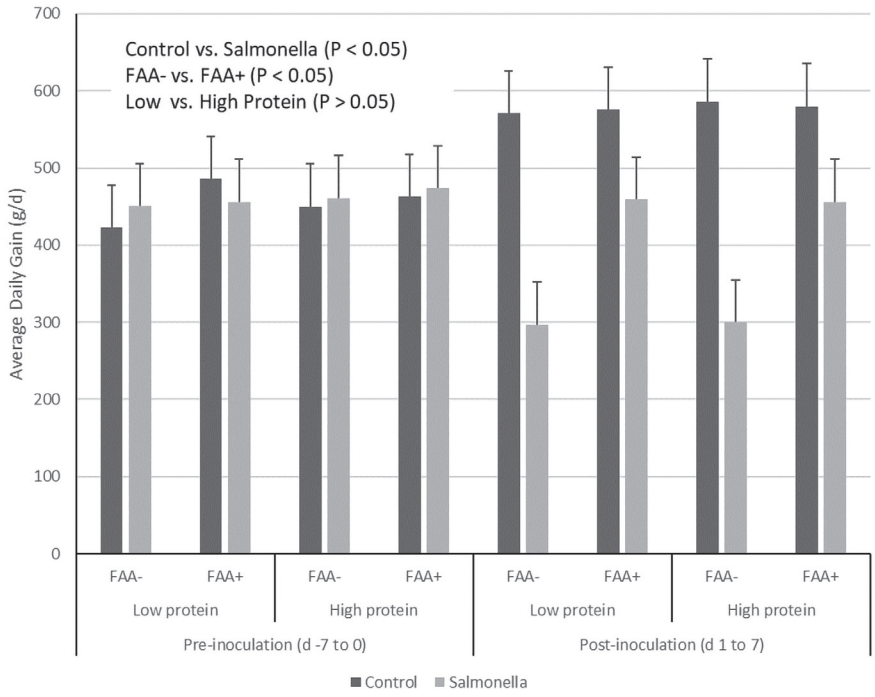

Figure 1. Growth performance of weaned pigs pre- and post-inoculation with saline (Control) or *Salmonella* and fed diets containing low or high protein (16.6 vs. 19.5%) with (FAA+) or without (FAA-) a functional amino acid blend supplying methionine, threonine, and tryptophan at 120% of NRC (2012) requirements for growth (n=8 pigs/treatment) Adapted from Rodrigues et al. (2021b).

Nutrient effects on gut health and immunity

A number of dietary interventions have been assessed for their potential to improve gut health and overall health in pigs and as potential replacements to antibiotics, including organic acids, phytochemicals, enzymes, and probiotics, and many reviews have been published on their use in swine. Here, the focus will be on dietary nutrient content, including dietary protein, fibre, and amino acids and their role in promoting health in the pig.

Dietary protein and fibre content can have significant impacts on both animal performance and health, largely through effects within the gastrointestinal tract (Jha and Berrocoso, 2016). The impact of protein and fibre on animal health is increasingly important to understand with the increased use of co-products and other novel feedstuffs which generally have a greater fibre content and variable protein content and protein availability (Pieper et al., 2012a; Zijlstra and Beltranena, 2013), especially when such ingredients have undergone heat processing. Dietary protein, while necessary to meet requirements for growth in pigs, can have a detrimental effect on gastrointestinal health if provided in excessive amounts. Dietary indigestible protein content (i.e., protein not absorbed in the small intestine) is available for microbial fermentation and may have detrimental effects on gut health. Metabolites of protein fermentation, including branched-chain fatty acids, ammonia, biogenic amines, hydrogen sulfide, and phenolic and indolic compounds (Pieper et al., 2012a; Yao et al., 2015; Jha and Berrocoso 2016)), have been associated with toxic and pro-inflammatory effects on the gut epithelium, including compromised colonic epithelial cell structure and metabolic functions, thinning of the mucus barrier, and increased colonic permeability (Gaskins, 2001; Hughes et al., 2008; Yao et al., 2015). Therefore, it has been suggested that high protein diets increase susceptibility to enteric pathogens and are a predisposing factor in the development of post-weaning diarrhea. Further, it has been suggested that feeding a low protein diet will reduce the amount of substrate available for the proliferation of pathogenic bacteria and thus minimizes production of associated toxic metabolites, which in turn will improve gut health and function in piglets (Nyachoti et al., 2006). However, while there is a general trend for reduced incidence of diarrhea with decreasing dietary protein content, this is not consistent across studies when examining the same protein content, suggesting a factor other than simply total dietary protein content (e.g., indigestible content, protein type) is involved. For example, while increased dietary protein content had no impact on pig response to a Salmonella challenge in one study (Fig. 1; Rodrigues et al., 2021b), there was a significant difference in response to a subsequent challenge when pigs were fed either plant-based or animal-based protein sources in nursery diets (Fig. 2; Rodrigues et al., 2022). This suggests that digestibility, which is greater in animal-based products, anti-nutritional factors, which are greater in plant-based products, and/or bioactive compounds present in animal-based products contribute to the observed response to dietary protein content and post-weaning diarrhea and susceptibility to enteric pathogens.

Unlike with protein, fermentation of fibre is generally considered to result in production of beneficial metabolites, such as short-chain fatty acids, that promote gut health and limit pathogen growth (Pieper et al., 2012ab; Jeaurond et al., 2008). As with protein, the impact of dietary inclusion of fibre on nursery pig performance and intestinal health has been inconsistent, likely due to differences in the physicochemical properties and fermentability of different fibre sources. Inclusion of a non-structural/soluble fibre source may provide intestinal bacteria an alternative substrate for fermentation (Pieper et al, 2012ab) whereas inclusion of

structural/insoluble source of dietary fibre may reduce the impact of indigestible protein through increased digesta flow through the gut and reduced adhesion of pathogens (Molist, 2020). Two studies examining the impact of a blend of soluble and insoluble fibres (i.e., wheat bran and sugar beet pulp) demonstrated the potential benefits, but also the variability in response, of dietary fibre inclusion to reduce effects of high indigestible protein content. Pieper et al. (2012a) showed that while fermentable protein increased production of harmful metabolites and altered the mucosal response in weaned pigs, this was not impacted by inclusion of fibre, although higher fibre inclusion did beneficially impact the microbial environment. Alternately, in grower pigs, Wellington et al. (2020a) demonstrated a negative impact of indigestible protein content on metabolite production and mucosal response, which was partially attenuated (i.e., improved short-chain fatty acid/branched-chain fatty acid production and intestinal barrier gene expression) with inclusion of the fibre blend (i.e., sugar beet pulp and wheat bran). The benefit of dietary fibre inclusion on gut health has also been demonstrated by Wellington et al. (2020b), who observed improved barrier function in pigs challenged with either *E. coli* lipopolysaccharide or Salmonella through increased mucin production and production capacity (i.e., goblet cell number).

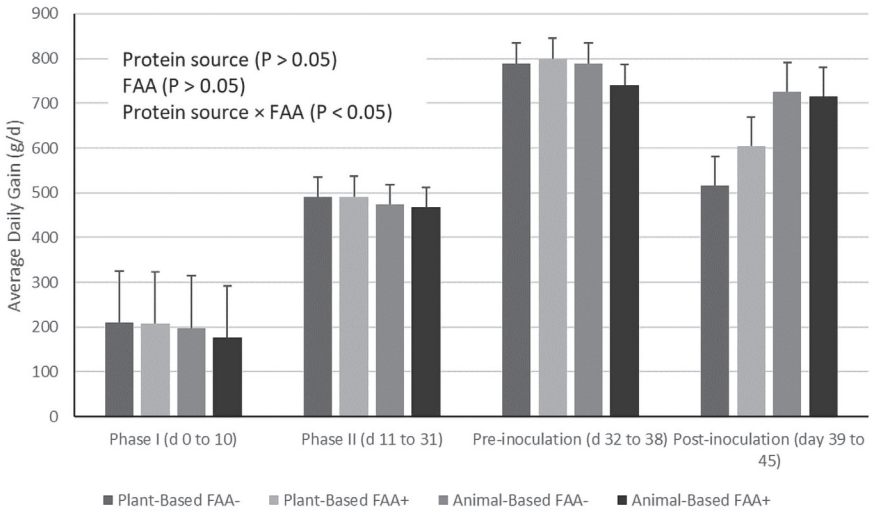

Figure 2. Growth performance of weaned pigs fed nursery diets containing either plant-based or animal-based protein sources and with (FAA+) or without (FAA-) a functional amino acid blend supplying methionine, threonine, and tryptophan at 120% of NRC (2012) requirements for growth. On d 32, piglets were placed on a common grower diet and inoculated with Salmonella Typhimurium on d 39 (n=8 pigs/treatment) (Rodrigues et al., unpublished).

Functional amino acids and pig health

Of the amino acids, glutamine, arginine, threonine, and aromatic and sulfur amino acids are of particular importance during immune challenge (Reeds and Jahoor, 2001) and provision of these amino acids in excess of requirements for growth during immune challenge may be of benefit in mitigating the effect of immune challenge on growth performance. Previous studies have demonstrated an increased requirement for growth for methionine and cysteine (Rakhshandeh et al., 2010; Litvak et al., 2013), threonine (Jayaraman et al., 2015; Wellington et al., 2018; McGilvray et al., 2019), and tryptophan (De Ridder et al., 2012) in response to immune stimulation.

As indicated above, while past research has indicated alterations in nutrient metabolism, generally, and amino acid requirements, specifically, only recently has the effect of supplementation with amino acids for pig robustness received significant attention. The term 'functional amino acids' has been used to describe those amino acids supplemented in the diet for their roles beyond those for protein synthesis (i.e., lean gain). In the context of health, these include amino acids with significant roles in gastrointestinal health (e.g., barrier function) and immune status (e.g., antioxidant balance, acute phase response). Supplementation of individual functional amino acids has been shown to improve growth performance of pigs under immune challenge. For example, Wellington et al. (2019) showed that supplementation with threonine resulted in improved growth performance of growing pigs fed high fibre diets during Salmonella challenge most likely through support of improved mucin production, as indicated by increased fecal mucin output. Likewise, Koo et al. (2020) observed improved gut integrity (i.e., goblet cell density, tight junction gene expression) in pigs fed a 'simple' diet which induced an intestinal inflammatory response. Jayaraman et al. (2015) and Trevisi et al. (2015) observed improved growth performance in pigs housed in unsanitary conditions and challenged with E. coli, respectively, when fed diets containing supplemental threonine. In another study, Koo et al. (2021) observed improved immune status in pigs fed supplemental valine and Jayaraman et al. (2015) and Le Floc'h et al. (2009) observed improved growth performance in weaned pigs provided supplemental tryptophan when housed in unsanitary conditions.

Recent work has examined the use of a blend of functional amino acids on growth performance and immune status in weaned pigs. Rodrigues et al. (2021b) fed diets containing either a standard amino acid profile (NRC, 2012) or one in which threonine, methionine, and tryptophan were supplemented at 120% of the requirements for growth. When challenged with Salmonella, those pigs that

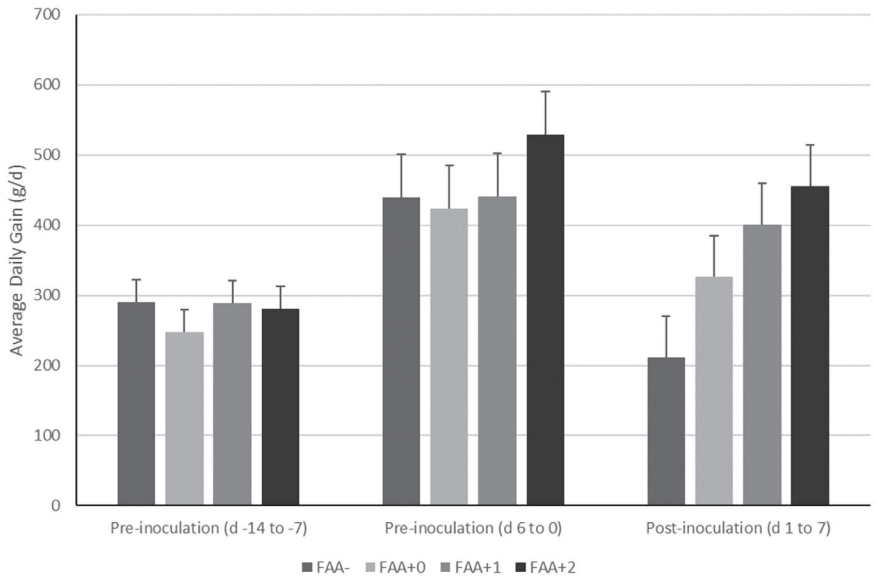

Figure 3. Growth performance of weaned pig pre- and post-inoculation with Salmonella and fed diets without (FAA-) or with a functional amino acid blend supplying methionine, threonine, and tryptophan at 120% of NRC (2012) requirements for growth during the post-inoculation period (FAA+0), for 1 week pre- and post-inoculation (FAA+1), or for 2 weeks pre- and 1 week post-inoculation (FAA+2) (n=8 pigs/treatment). Adapted from Rodrigues et al. (2021c).

had been provided the supplemented amino acid profile had improved growth performance and immune status (i.e., acute phase response, antioxidant balance) than those that had received the basal profile. Supplemental amino acids also resulted in a decrease in fecal myeloperoxidase, an indicator of intestinal damage, indicating a role of these amino acids in supporting gut health during an enteric pathogen challenge. The effectiveness of supplementation with this amino acid blend may be dependent on specific conditions, as van der Meer et al. (2016) showed improved immune status but limited effect on growth performance from wean to finish when pigs were given the same amino acid blend and supplementation level but under low or high sanitary conditions.

The effectiveness of dietary interventions for improved pig performance during health challenge may be dependent on a number of factors. The timing of functional amino acid supplementation may be important with respect to their effectiveness at improving performance and immune status. For example, Rodrigues et al. (2021c) demonstrated a further increase in growth performance in pigs provided the blend of functional amino acids when the adaptation period prior to Salmonella infection was increased from 0 to 2 weeks (Fig. 3). However, the response to functional amino acids was variable with respect to immune status in these pigs, with acute phase response responding positively to a longer adaptation time while antioxidant balance did not. While there was no overall effect of amino acid supplementation, van der Meer et al. (2016) observed improved average daily gain in the nursery period and improved feed efficiency in the finisher period in pigs housed in unsanitary conditions. Moreover, the response to sanitary conditions was greater during the nursery period, indicating that supplementation may be more beneficial in the immediate post-weaned period. Schweer et al. (2019) and Jasper et al. (2020a) were able to improve growth performance in pigs inoculated with PRRS virus by adjusting overall dietary nutrient content (i.e., increasing lysine:energy) at the time of challenge, however, no improvement was observed if the adjusted diet was provided post-challenge (Gabler, 2021). Functional amino acids may provide more long-term benefits, as Rodrigues et al. (2022) demonstrated improved growth in pigs that had received a supplemental functional amino acid blend in the nursery period but were fed a common grower diet at the time of Salmonella challenge. This blend of amino acids, however, was only partially able to attenuate the negative effect of disease challenge in low-birth weight pigs during the subsequent disease challenge. Other components in the diet may also affect functional amino acid effectiveness, as supplementation with the same blend of functional amino acids was able to improve performance of nursery pigs that had received a plant-based diet, however, provided no further benefit to those pigs that had received animal-based protein sources in the nursery (Fig. 2; Rodrigues et al., unpublished).

The appropriate intervention may be dependent on the type of challenge experienced, and is likely related to the specific immune response to different challenge types and the proportion of the decrease in performance that is due to either reduced feed intake or alterations in nutrient utilization (Rodrigues et al., 2021a, Pastorelli et al., 2012). For example, while nutrient adjustment was effective in PRRS infected pigs, this adjustment was not effective in pigs challenged with Mycoplasma hyopneumoniae (Jasper et al., 2020b). This may also explain why the blend of functional amino acids was effective during an enteric pathogen challenge (Rodrigues et al., 2021bc) but had reduced effectiveness during a sanitary challenge (van der Meer et al., 2016).

Conclusions

Disease challenge can have a substantial negative effect on productivity and profitability of swine production, however, there are a number of dietary strategies that can be used to improve pig robustness. Dietary protein and fibre can be

adjusted to promote a healthy gut environment. Recently, functional amino acids have been investigated for their role during disease challenge and ability to support both the immune response and growth performance. While these have been shown to be effective under some circumstances (i.e., normal birth weight, enteric challenge), this is not always the case. Overall, dietary nutrient content is another tool that can be incorporated into pig health programs. Dietary strategies to improve pig health should, however, be based on the specific circumstances and goals of the production unit.

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Microbiota-Gut-Brain Axis

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Summary

Between the gut and the brain, there are complex bidirectional communication pathways called the microbiota-gut-brain (MGB) axis. The gut and the brain are constantly communicating and influencing each other through neural, endocrine, and immune signals. The communication is integral to physiological homeostasis within an animal. The gut microbiota has been linked to the homeostasis of gut health and efficiency of digestion and absorption of nutrients. More recently the gut microbiota and microbial endocrinology have become a major focus of research in human and animal health and disease because of the many links to the host well-being. Whereby, a diverse and stable gut microbiota has been correlated with improvements in immunological, physiological, and neurological states across life span. The composition of the gut microbiota is closely associated with nutrition, environment, and microbial genomics. Microbial derived neurochemicals such as serotonin, gamma-aminobutyric acid (GABA), dopamine, norepinephrine, and acetylcholine are implicated in regulation of appetite, metabolism, and behavior. In fact, the gut and its microbiota are a major source of neurochemicals which links it to neural function. Additionally, gut hormones that regulate appetite regulation in the brain have been shown to be modulated by microbiota-derived hormones that regulate feed intake in rodents. The food animal production industry has used probiotics to improve gut health as an alternative to in-feed antibiotics to help maintain gut health and performance. While many defined probiotics are available for use, we still have inconsistent results of probiotics depending on the health status or the environment in which they are used. Therefore, defining probiotic mechanisms could be advantageous in modulation of the MGB axis to improve animal performance and well-being.

Introduction

Probiotics are defined as a living microorganism that when supplemented in adequate amounts confers a health benefit to the host (Butel, 2014). Through interacting with the host microbiota and intestinal epithelium, probiotics have been shown to exert a wide range of effects upon host health, with various strains improving metabolism, immunity, endocrine function, and intestinal health (Salami, 2021). Perhaps the most intriguing effects of probiotics on the host is their modulation of brain physiology and behavior. Considerable research over the last decade has documented how probiotics can influence various central neuronal processes such as neurotransmission, neurogenesis, expression of neuropeptides, and neuroinflammation, which affect appetite, mood, and behavior (Yong et al., 2020). In human research, the term psychobiotics are now defined as microbiota-targeted interventions such as probiotics that influence bacteria-brain relationships or the MGB axis (Yong et al., 2020). As the evidence to support the effects of psychobiotics on brain and behavior continues to develop (Yong et al., 2020), the field is now turning to mechanistic studies to elucidate the biological underpinnings of the MGB axis.

In livestock, work by Cheng et al. (2019) in poultry investigated the effects of *Bacillus subtilis* supplementation in aggressive hens and was able to show a reduction in feather pecking and kicking through intestinal modulation of serotonin synthesis and signaling. Further, others have shown microbial neurochemicals associated with probiotic supplementation regulate gut hormones that modulate brain feed intake centers in weaning pigs (Sun et al., 2021). Therefore, a novel focus in enteric microbial research has shifted towards neurophysiology and immune modulatory effects of the gut microbiome, and much of this interaction revolves around the MGB axis. The intersection of microbiology and neurophysiology, known as microbial endocrinology, holds that disruptions to the gut microbiome and intestinal health can influence neural physiology, cognition, behavior, mood, and feed intake (Rogers et al., 2016). Microbial endocrinology-based mechanisms mediate the ability of bacteria and host to interact with each other in a bidirectional manner, ultimately influencing host

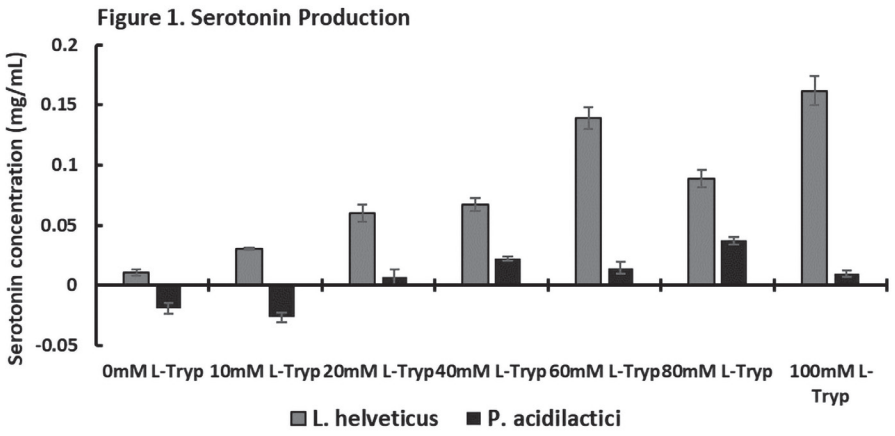

Figure 1. Lactobacillus helveticus OSU-PECh-4A serotonin secretion. We assessed Pediococcus acidilactici OSU-PECh-3A and Lactobacillus helveticus OSU-PECh-4A ability to secrete serotonin in the presence of increasing L-tryptophan.

physiology ranging from susceptibility to infectious disease and behavior to overall host performance (Lyte and Lyte, 2019). We know dietary probiotics alter composition and modulate global metabolic function of the intestinal microbiome (Plaza-Diaz et al., 2019). What we do not completely understand are the bio/neurochemical mechanisms through which probiotics modulate the MGB axis. The psychophysiological response to stress, which affects many aspects of an animal's physiology and ultimately performance of the animal, should be of primary interest to the food animal industry because in addition to performance it is linked to overall animal well-being. The objective of this work is to understand the mechanisms by which two genome-sequenced probiotics improve intestinal health, and therefore could impact overall weaning pig performance. The first phase of this work was to determine probiotic mechanisms *in vitro* on intestinal barrier function using pig intestinal cells and phase two is to investigate the MGB in weaning pigs.

Methods

Probiotics. Two novel probiotic strains *Pediococcus acidilactici OSU-PECh-3A* and *Lactobacillus helveticus OSU-PECh-4A* were isolated and genome-sequenced at the Molecular and Cellular Imaging Center in Wooster, OH in collaboration with Dr. Rafael Jimenez-Flores of the Department of Food Science and Technology at The Ohio State University.

Cell culture. The intestinal porcine epithelial jejunal (**IPEC-J2**) cell line was a kind gift from Dr. Doug Burrin of USDA-ARS Childrens' Nutrition Research Center. The IPEC-J2 cells used in this study are non-transformed, polarized- porcine jejunal epithelial cells and were originally isolated from a neonatal, unsuckled piglet (Vergauwen, 2015). Cells were cultured at 37°C at 5% CO_2 in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 media (Sigma-Aldrich, Saint Louis, MO) with 5% fetal bovine serum (Atlanta Biologicals - Flowery Branch, GA), 5 µg/mL Insulin, Transferrin and Selenium (Corning, Sigma-Aldrich, Saint Louis, MO), 5 µg/mL Epidermal Growth Factor (Corning, Sigma-Aldrich- Saint Louis, MO) and Penicillin and Streptomycin (Gibco, Gaithersburg, MD) until challenge.

Bacteria for challenge assay. Enterotoxigenic Escherichia coli (**ETEC**) strain 3030-2: K88ac, LT, STb was kindly provided by Dr. Philip Hardwidge of Kansas State University. The strain was grown at 37°C overnight and then diluted 1:10 in Luria Broth, grown for 90 minutes and then bacterial populations were estimated by spectrophotometry at 600 nm optical density. ETEC was added to the media in the apical compartment at the multiplicity of infection (**MOI**) of 2:1 for 3 hours.

Probiotic adherence. IPEC-J2 cells were seeded at a concentration of 1×10^5 cells/insert and grown for 4 d. Probiotic cultures were grown, washed, and diluted to appropriate concentrations. Cells were treated with either *Pediococcus acidilactici* or *Lactobacillus helveticus* for 3h at concentrations of 10^8, 10^9, 10^{10}, and 10^{11} colony forming units (**CFU**)/mL. After treatment with probiotics, the cells were washed with Dulbecco's phosphate-buffered saline then plated on De Man, Rogosa and Sharpe (**MRS**) agar plates to determine CFU of probiotics attached to epithelial cells.

Probiotic inhibition of ETEC attachment. IPEC-J2 cells were seeded on 12-well plates (Corning; Sigma-Aldrich, Saint Louis, MO) at a density of 1×10^5 cells/well. Cells reached confluency on day three. Cells were washed and treated with either *Pediococcus acidilactici* or *Lactobacillus helveticus* for 1 h at concentrations of 10^8, 10^9, 10^{10}, and 10^{11} CFU/mL before ETEC challenge. ETEC solution was added at an MOI of 2 for 2h. Bacterial adhesion was counted according to the procedures of Qiao et al. (2020) with modifications. Briefly, IPEC-J2 cells of different treatments were washed three times with DPBS to remove non-adherent ETEC. 300 uL of 0.5% Triton X-100 (Sigma-Aldrich, Saint Louis, MO) was added to lyse the cells in the 37 °C incubator. The lysates were serially diluted and plated on Luria-Bertani agar dishes overnight for growth and then ETEC colonies were counted.

Cell toxicity. Lactate dehydrogenase (LDH) is a cytoplastic enzyme measured to determine damage to the cell plasma membrane. Cellular cytotoxicity and membrane integrity were assessed by measurement of LDH activity in

the IPEC-J2 cell media at an optical density of 492 nm using the LDH Cytotoxicity Assay Kit (Cayman Chemical, Ann arbor, MI) according to the manufacturer's protocol.

Neurotransmitter concentration. Levels of neurotransmitters serotonin and gamma-aminobutyric acid and serotonin from media of IPEC-J2 cells treated with probiotics was determined using ELISA kits (Fisher Scientific, Waltham, MA) according to the manufacturer's instructions.

Statistical Analyses. Probiotic adhesion, ETEC adhesion, LDH and neurotransmitters data were analyzed as a randomized complete design using the PROC MIXED procedure of SAS version 9.4. Individual wells served as the experimental unit. The model included the fixed effects of bacterial treatment, challenge, and interactions. Differences were considered significant if $P < 0.05$.

Results

Genome Sequencing of Pediococcus acidilactici OSU-PECh-3A and *Lactobacillus helveticus OSU-PECh-4A.* In our research group, we have isolated a particular *P. acidilactici OSU-PECh-3A* strain from Gouda cheese. This strain stood out over 137 other LABs, in terms of lipolytic, proteolytic, and neurochemical synthesis (García-Cano et al., 2019; in preparation). In terms of genetic analysis, the strain has a genome sequence of 1,947,223 bp and its guanine-cytosine content is 42.19%. According to gene prediction and functional annotation by PROKKA v1.14.5, this assembly has 1,956 genes, 1 copy of 16S rRNA, and 55 genes encoding tRNAs. Using this approach, we have elucidated neurotransmitters production such as GABA by L-Glutamate via glutamate decarboxylase activity. As well, serotonin production via L-Tryptophan metabolism using a tryptophan hydroxylate to produce L-5-hydroxytryptophan (**5'HT**) and an aromatic L-amino acid decarboxylase to generate serotonin. Also, we have elucidated norepinephrine (**NE**) production and dopamine (**DA**) production via Phenylalanine/Tyrosine metabolism, using a tyrosine oxidase and an aromatic L-amino acid decarboxylase (García-Cano et al, in preparation).

Lactobacillus helveticus OSU-PECh-4A is a lactic acid bacterium. We have sequenced the complete genome and it showed 1,834,843 bp in its genome and 36.69% of guanine-cytosine content. Using InterProScan v5.50-84.0 software, four genes with putative function of β-galactosidase were found that are important in lactose metabolism (García-Cano et al, 2022). We further performed a bioinformatic analysis and elucidated the mechanism of production of neurotransmitters 5-HT, NE and DA, through gene sequences associated with the same metabolic pathways described previously for *Pediococcus acidilactici*. Interestingly, *Lactobacillus helveticus OSU-PECh-4A* does not seem to

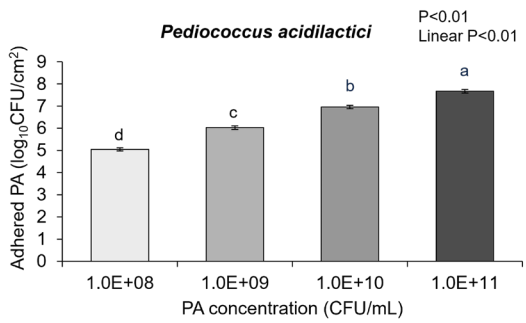

Figure 2a

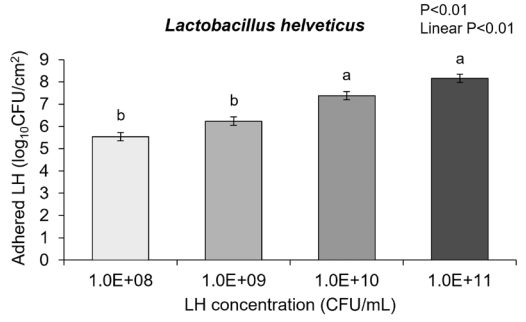

Figure 2b

Figure 2. Adhesion of *Pediococcus acidilactici OSU-PECh-3A* (a) and *Lactobacillus helveticus OSU-PECh-4A* (b) to pig intestinal epithelial cell increases linearly from 10^8 to 10^{11} CFU/mL. We have created growth curves for the probiotic strains of *Pediococcus acidilactici OSU-PECh-3A* and *Lactobacillus helveticus OSU-PECh-4A* to allow calculation of CFU/mL of each bacterium. Then, we have examined the effects of increasing dose of the probiotic on attachment to the porcine intestinal epithelial cell line, IPEC-J2. The data show a linear increase in probiotic attachment. These data demonstrate *Pediococcus acidilactici OSU-PECh-3A* and *Lactobacillus helveticus OSU-PECh-4A* display intestinal adhesion properties that can have an important role in probiotic functional mechanisms associated with maintaining intestinal barrier function and MGB communication. Bars with different letters indicate a significant difference at $P < 0.01$.

code for the gene glutamate decarboxylase for synthesis of GABA from L-glutamate.

Neurotransmitter secretion. Growth of *Lactobacillus helveticus OSU-PECh-4A* with increasing concentration of L-tryptophan in the culture media showed increased secretion of serotonin by the probiotic (Figure 1). *Pediococcus acidilactici OSU-PECh-3A* did not increase serotonin secretion in the presence of increasing concentration of L-tryptophan. Glutamate supplementation into the probiotic growth media preliminarily suggest *Pediococcus acidilactici* increases GABA secretion, but *Lactobacillus helveticus* under the same conditions does not increase GABA secretion (data not shown).

Probiotic adhesion to IPEC-J2 cells. Neonatal pig intestinal epithelial cells were co-cultured for increasing doses of *Pediococcus acidilactici OSU-PECh-3A* and *Lactobacillus helveticus OSU-PECh-4A* and both probi-

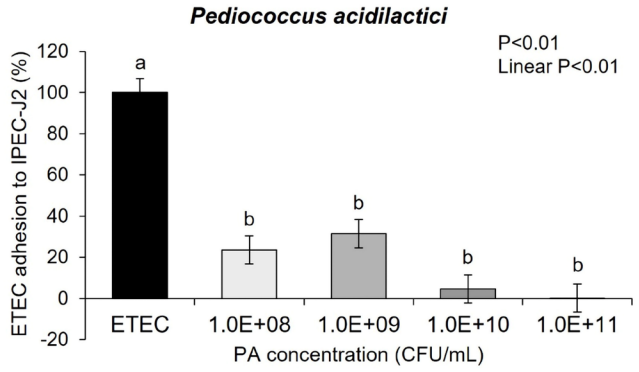

Figure 3a

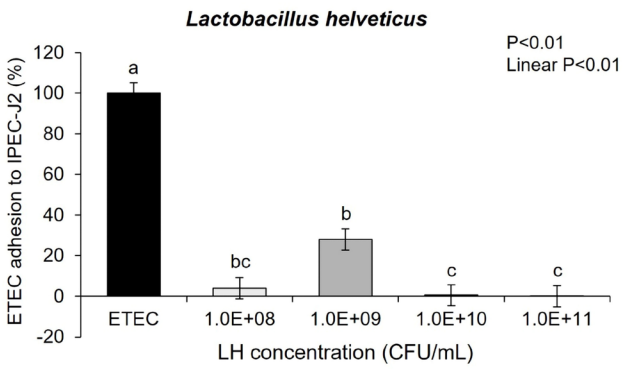

Figure 3b

Figure 3. Inhibitory effects of *Pediococcus acidilactici OSU-PECh-3A* (a) and *Lactobacillus helveticus OSU-PECh-4A* (b) on adhesion of porcine Enterotoxigenic *E. coli* to pig intestinal epithelial cell. We have examined if the probiotic bacteria, *Pediococcus acidilactici OSU-PECh-3A (a)* and *Lactobacillus helveticus OSU-PECh-4A (b)* could inhibit the adhesion of pathogenic enterotoxigenic *E. coli* (ETEC at 10^8CFU/mL) to the pig intestinal cell surface. Both *Pediococcus acidilactici OSU-PECh-3A* and *Lactobacillus helveticus OSU-PECh-4A* from 10^8 to 10^{11} CFU/mL inclusion demonstrated a linear decline in ETEC attachment to the pig intestinal epithelia. Bars with different letters indicate a significant difference at $P < 0.01$.

otics showed in linear increase in adhesion of the probiotic to the intestinal epithelial cells surface (Figure 2a and 2b; linear $P<0.01$).

Probiotic inhibition of ETEC adhesion to IPEC-J2 cells. Pediococcus acidilactici OSU-PECh-3A and *Lactobacillus helveticus OSU-PECh-4A* were cultured with IPEC-J2 pig intestinal cells in the presence of ETEC for 3 hours. Both probiotics linearly decreased adhesion of ETEC to the IPEC-J2 cells across the probiotic doses of 10^8, 10^9, 10^{10}, and 10^{11} CFU/mL (Figure 3a and 3b; linear $P<0.01$).

Cell cytotoxicity. To identify whether ETEC K88 caused cell membrane damage, LDH in IPEC-J2 cell culture medium was detected. Challenge with ETEC K88 MOI of 2 for 3 hours increased LDH secretion from IPEC-J2 cells into culture media indicating increased cell membrane damage (Figure 4a and 4b; $P<0.01$). As expected, *P. acidilactici OSU-PECh-3A* and *L. helveticus OSU-PECh-4A* in co-culture with ETEC inhibited pathogen induced IPEC-J2 release of LDH into cell media (Figure 4a and 4b). Further, the probiotics alone cultured with the IPEC-J2 cells had no

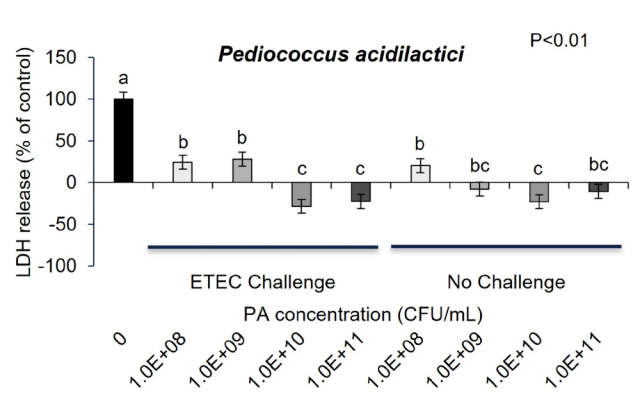

Figure 4a

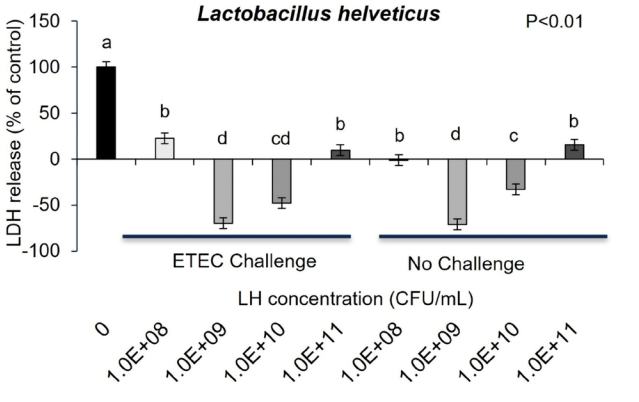

Figure 4b

Figure 4. *Pediococcus acidilactici OSU-PECh-3A* (a) and *Lactobacillus helveticus OSU-PECh-4A* (b) decrease lactate dehydrogenase (LDH) secretion from IPEC-J2 cells co-culture with ETEC. We assessed cell membrane integrity and cytotoxic effects of the probiotics alone (no challenge) or the probiotic and ETEC challenge by measuring LDH, a marker of cell membrane integrity, into the culture medium. The data demonstrated the probiotics have no negative effects on cell membrane integrity from 10^8 to 10^{11} CFU/mL and they have protective effects on cell membrane integrity when co-supplemented with ETEC. In this study, we demonstrate that *Pediococcus acidilactici OSU-PECh-3A* (a) and *Lactobacillus helveticus OSU-PECh-4A* (b) protect cell membrane integrity likely linked to improved intestinal barrier function. Bars with different letters indicate a significant difference at $P < 0.01$.

effect on cell cytotoxicity as measured by LDH release into cell culture media.

Conclusions

Probiotics have been used to improve gut health in livestock species as well as humans. However, probiotic supplementation as a replacement for in-feed antibiotics in livestock does not produce consistent results across animal studies. Given the role of microbial endocrinology on the MGB axis, a deeper understanding of multiple probiotic mechanisms could be beneficial to understand how probiotics affect gut function and the bi-directional communication between gut and the brain. Results from the probiotic genome sequencing indicate *Pediococcus acidilactici OSU-PECh-3A* and *Lactobacillus helveticus OSU-PECh-4A* both have the genes necessary to synthesize and secrete neurotransmitters associated with regulation of the MGB axis. However, *Lactobacillus helveticus OSU-PECh-4A* did not code for glutamate decarboxylase for synthesis of GABA from L-glutamate. These genomic data provide important information about how this probiotic can communicate with other microbes in the lumen, host epithelial cells, and neuroendocrine circuits. Additionally, both selected probiotics have genes expressed to improve digestibility of lipids and carbohydrates that may be beneficial for neonatal pigs (García-Cano et al., 2019). These attributes could be useful in supplementation pre-weaning to improve nutrient utilization during weaning stress. Further, data provide evidence in the presence of L-tryptophan there is a dose dependent response of serotonin secretion by *Lactobacillus helveticus OSU-PECh-4A* (Figure 1). However, *Pediococcus acidilactici OSU-PECh-3A* did not increase serotonin secretion in culture with increasing L-tryptophan. This could be related to different applications of these probiotics as the mechanisms are further developed. Research on other *Pediococcus* probiotic strains show this genus of bacteria is more likely to synthesize and secrete GABA (Anggraini et al., 2019). Preliminary data from our lab would suggest the *Pediococcus acidilactici OSU-PECh-3A* in the presence of glutamate increase GABA secretion (data not shown).

Mechanistically, data indicate *Pediococcus acidilactici OSU-PECh-3A* and *Lactobacillus helveticus OSU-PECh-4A* both seem to function in similar manners with adhesion to pig intestinal epithelial cells and inhibition of pathogen, ETEC K88, adhesion (Figure 2 and 3). A fundamental mechanism of probiotics is competitive exclusion of pathogens from the gut environment through multiple mechanisms. Although, it is not fully described if the bacteria in the lumen of the gut or those adhered to the mucosal epithelium have different functions, it would seem logical that probiotic exclusion of pathogen adhesion to the mucosal cell surface would be beneficial to gut health. In fact, the cell cytotoxicity data supports this conclusion showing that ETEC K88 pathogen induces increased cell membrane permeability while *Pediococcus acidilactici OSU-PECh-3A* and *Lactobacillus helveticus OSU-PECh-4A* did not adversely affect IPEC-J2 cell membrane permeability. Further, when ETEC K88 and *Pediococcus acidilactici OSU-PECh-3A* or *Lactobacillus helveticus OSU-PECh-4A* were co-cultured with the pig intestinal cells the probiotics prevented the ETEC from inducing cell membrane cytotoxicity. Although there is linear dose response in *Pediococcus acidilactici OSU-PECh-3A* and *Lactobacillus helveticus OSU-PECh-4A* from 10^8 to 10^{11} CFU/mL in the adhesion of the probiotics to the IPEC-J2 cells, the dose response in inhibition of ETEC adherence or improving cell cytotoxicity did not seem to have as significant of impact on those mechanisms. Further, *in vitro* and animal trials are ongoing to understand the mechanisms on the gut barrier, microbial endocrine function, and regulation of feed intake centers in the brain.

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Technology to Make Nutrition Implementation Easier: Continuous, Real-Time Assessment of Swine Physical Condition

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Summary

Observation, control, and maintenance of pigs' physical condition at acceptable levels are critical to maintaining animal welfare and production standards. Early recognition of animals with atypical physical conditions is essential to guide management practices, such as nutrition, and prevent production losses. Animals' body weight variation could potentially be used as an indicator of their physical condition, assist in subjective assessment methods such as body condition scores, and also provide crucial information about animals' overall health. Automating the collection of weights is a way of accessing this information continuously and in real-time. A method of estimating grow-finishing pigs' weights from body volume acquired through cameras was developed and validated; the average error associated with this method was 4.6%. While this error is acceptable, a smaller error would be ideal. Furthermore, testing if the methodology would work with other animals, such as sows, is advantageous. We summarize the development and test of an improved model that can predict grow-finish pigs' and sows' body weight from images. This method could be used to develop systems for continuous, real-time, and individual assessment of animal weight in the production system environment.

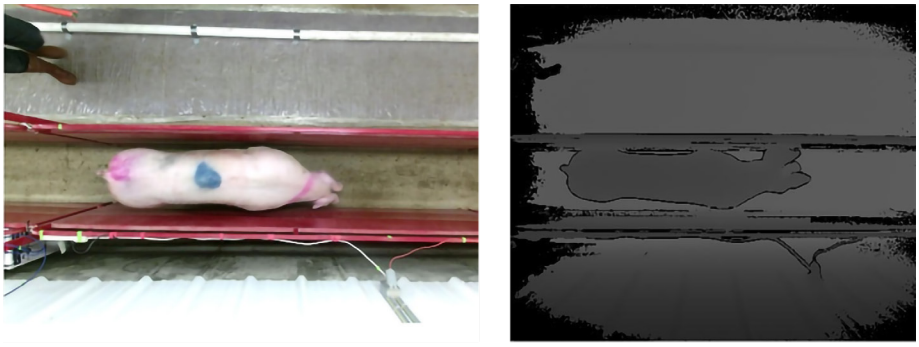
Introduction

Observing, controlling, and maintaining pigs' physical condition at acceptable levels are critical to maintaining animal welfare and production standards. It is known that, during pregnancy, sows should receive specific diets based, among other factors, on their body condition. Larger, older, or skinnier sows, for example, should receive larger amounts of feed to meet their nutritional needs. Furthermore, underweight animals are more likely to have nutritional deficiencies and give birth to fewer piglets that are more likely to also present nutritional deficiencies and smaller body mass at birth (Eissen et al., 2000). On the other hand, overweight sows are usually larger than the space provided in the pen, which leads to stress for the animal and may cause a higher incidence of piglets' crushing. These sows can have abnormal development of mammary glands, reducing the amount of milk produced during lactation. Additionally, sows that are obese during pregnancy tend to reduce the amount of food ingested during lactation, which negatively impacts milk production (Eissen et al., 2000). These factors can result in economic losses.

Therefore, the early recognition of animals that present physical conditions outside the standards is essential to prevent production losses. Body condition assessment through visual scores is a method that has been broadly used over the years (Patience & Thacker, 1989; Charette et al., 1996; Maes et al., 2004; Knauer et al., 2007; Knauer et al., 2012; Salak-Johnson et al., 2014; Knauer & Batinger, 2015). Due to the subjectiveness of such scoring systems, various alternative methods have been proposed to obtain a more objective assessment of animals' physical condition.

At the end of the '90s, for example, it was noted (Charette et al., 1996) the need for evaluation of the body condition of sows using not only subjective methods of classification but also more objective values, such as parity, body mass, backfat thickness, and dimensions of the animal. This type of classification, covering a larger number of variables, was adopted more recently (Sell-Kubiak et al., 2013) and expanded, using the duration of the sow's gestation as another factor to be considered.

Body mass is an important variable that can not only assist in classifying an animal's body condition but also provide crucial information about its overall health. The knowledge of the daily variation of the animals' weight in real time would allow producers to improve the animals' well-being and production. It would be possible to use this information to optimize the space provided per animal, improve nutritional management practices, predict and control the mass for the slaughter, and, potentially, serve as disease outbreaks monitor (Brandl and Jørgensen, 1996; Kashiha et al., 2014).



(a) (b)

Figure 1. Example of (a) color and (b) depth frames acquired.

In contrast, weighing is a time-consuming and stressful practice and represents an ergonomic risk (Brandl and Jørgensen, 1996). Over the years, two approaches to automating animal weighing have been evaluated: (1) automated weighing systems combined with individual animal identification equipment and (2) indirect determination of body mass through the animals' dimensions. In general, automatic weighing systems involve direct contact with the animal. They can be used in the form of semi-automatic scales (Smith and Turner, 1974) or automatic feeders with automatic scales (Slader and Gregory, 1988; Ramaekers et al., 1995; Schofield et al., 2002), significantly reducing the time of weighing. Problems with this approach involve the presence of more than one animal and/or other material on the scale during weighing and material under the feeder, which could generate measures that are not accurate.

The significant correlation between mass and pigs' dimensions has led many authors to study the possibility of estimating body mass using this relationship (Brandl and Jorgensen, 1996). Several authors (Schofield, 1990; Frost et al., 1997; Schofield, 1999; Whittemore and Schofield, 2000; Wang et al., 2008; Kashiha et al., 2014) developed techniques for obtaining the animals' dimensions through digital images for its non-invasiveness and speed. In general, the obstacle with this method is that the pig's color must be different from the color of the environment in order to perform accurate image segmentation and, therefore, dimensions acquisition.

A new approach was proposed by Kongsro (2014) to use a commercial depth sensor (Microsoft Kinect® v.1) to obtain depth images, which contain three-dimensional information. The volume of the animal obtained through these images was correlated with the mass of Landrace and Duroc boars. The system could acquire the mass of the pigs with an error of 4 to 5%. These depth images tend to be easier to segment because they rely less on the light condition of the environment as object heights and not colors are used to perform segmentation.

A similar approach was used (Condotta et al., 2018) to extract grow-finish pigs' weight data from depth images for three commercial sire-lines (Duroc, Landrace, and Yorkshire) and two sexes (gilts and barrows). A significant correlation between body volume and body mass was found, showing that the weight can be predicted from body volume with an average error of 4.6%, or 2.2 kg, when using a simple linear regression to correlate those two variables. And no significant influence of either sex or sire-line on the model was found. This study left some questions about whether and how the weight prediction could be improved.

This presentation aims to summarize the development and test of an improved model that can predict grow-finish pigs' and sows' body weight from images.

Experimental Procedures

The experiment was conducted in a grow-finish building of the U.S. Meat Animal Research Center, from the Agriculture Research Service-ARS of the United States Department of Agriculture – USDA (-98.13° W, 42.52° N). Animal body mass and digital and depth images were collected on a population of grow-finish pigs at four distinct time-points through the grow-finish period. Furthermore, animal digital and depth images were collected on a population of sows at four parities and at two different time-points: moving to the farrowing building and moving from the farrowing building. All animal procedures were performed in compliance with federal and institutional regulations regarding proper animal care practices (FASS, 2010).

Animal specifics

Two hundred and thirty-four grow-finish pigs (equal number of barrows and gilts) were sampled at each of 4 approximate ages: 8-, 12-, 16- and 21-weeks old. The maternal line was a mix of Landrace x Yorkshire. Pigs were housed in a standard grow-finish type arrangement, 39 pigs pen^{-1} (0.93 m^2 pig^{-1}), and had *ad libitum* access to feed and water through the growing period.

Furthermore, 114 sows from a rotational Landrace and Yorkshire cross were sampled at four different parities (1, 2, 3, and 4), weighing between 130 and 260 kg. The animals were allocated in a farrowing building and were assessed when moving back to the gestation building. Animals had *ad libitum* access to feed and water and were housed in individual crates before data collection. Diets for all animals were a mix of corn and soybean meal formulated to meet

or exceed National Research Council recommendations (NRC, 2012).

Data acquisition

Seven hundred and six top-view depth images from grow-finish pigs were collected with a commercially available depth camera (Microsoft Kinect®) while pigs were individually weighed and analyzed. Additionally, Microsoft's Kinect Studio program was used to acquire digital color (RGB) and depth videos from sows. The camera was positioned above the hallway of the building, mounted to the ceiling to take both dorsal color (1920 x 1080 pixels per frame, **Figure 1a**) and depth videos (512 x 424 pixels per frame, **Figure 1b**) of the animals while being moved from the farrowing building to the gestating building, at approximately 30 frames per second. Frames were extracted as individual images for further analysis.

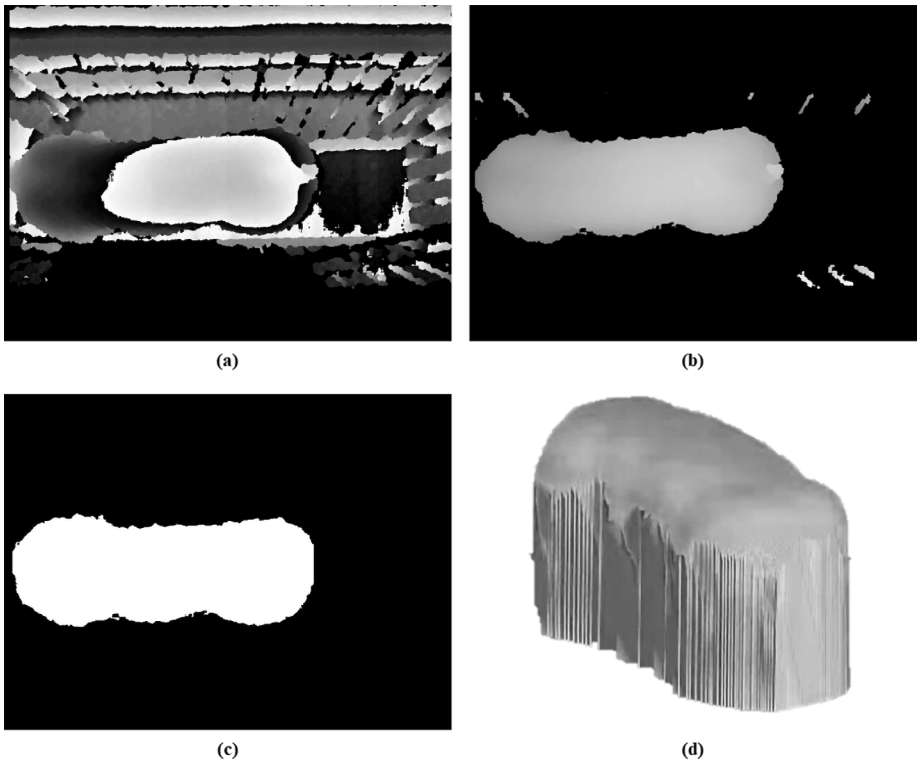


Figure 2. Depth images collected while the animals were being weighed. (a) Raw depth image acquired, (b) pre-processed depth image, (c) binary image of pig's body region without head and tail, and (d) projected volume of pig's body.

Each image contained one animal. Image processing steps were taken to segment the animal from the background (**Figures 2a** and **2b**) and eliminate the animal's head and tail regions (**Figure 2c**) for better correlation with its body weight (Schofield, 1990). From the selected part of the animals, the following variables were acquired: projected body volume (cm^3) (**Figure 2d**), dorsal area (cm^2), length from neck to rump (cm), width at shoulders (cm), width at the hip (cm), with at last rib (cm), and average and maximum heights (cm) (**Figure 3**). Condotta et al. (2018) describe further details of the image processing methodology. Animals were weighed using a Rice Lake Weighing Systems digital weighing scale that was calibrated by the company and regularly checked with a 50 lb. weight.

Data analysis

Two sets of inputs were used for modeling. The first set was composed of the animals' body dimensions acquired from the images (**Figure 3**) and animal parameters (age, sex, and sire line for grow-finish animals and parity for sows). The second set of inputs was composed of extracted learned high-level image features from the fifth pooling layer of a pretrained convolutional neural network (ResNet101). This second set of inputs was only calculated for grow-finish animals. Models were trained, validated, and tested using a numerical computing environment (MATLAB 2022a).

Several machine learning models were trained and tested using both sets of inputs. Linear regressions, regression trees, ensemble of trees, support vector machines, Gaussian

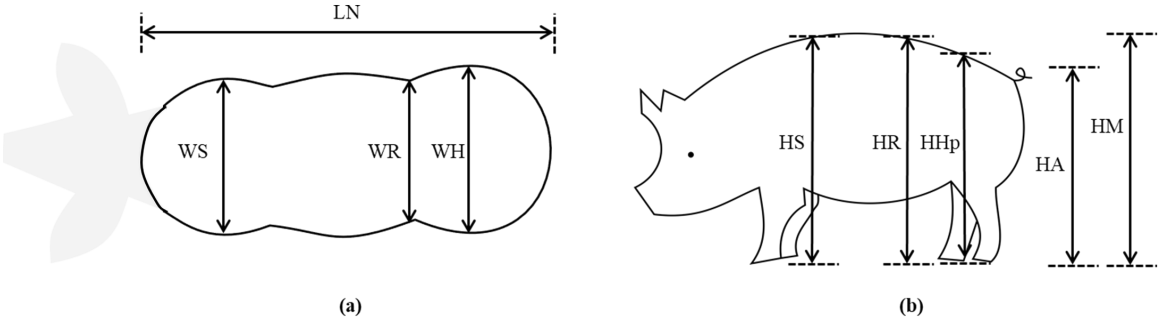


Figure 3. Dimensions acquired from top-view images after head and tail removal: (a) widths at shoulders (WS), last rib (WR), and hip (WH) and length from neck to rump (LN). (b) Heights at shoulders (HS), last rib (HR), and hip (HHp), and average height (HA) and maximum height (HM).

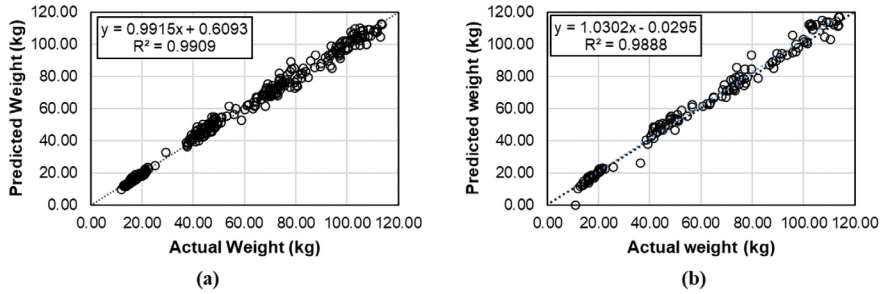


Figure 4. Correlation between measured and predicted mass (a) obtained with the original simple linear model (Condotta et al., 2018) and (b) with the simple linear model used for comparison purposes (80% of data). The circles represent individual measurements, and the dotted line represents a linear regression with $R^2 = 1.0$ (y = x).

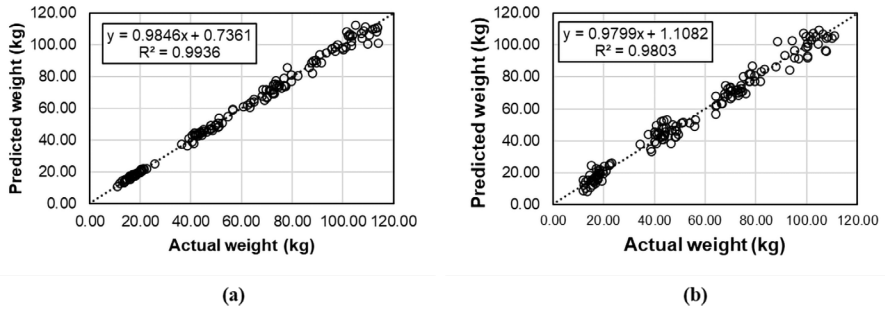


Figure 5. Correlation between measured and predicted mass obtained for the best model (Gaussian process regression). (a) Using pigs' body dimensions as inputs, and (b) using high-level features from pre-trained ResNet101 as inputs. The circles represent individual measurements, and the dotted line represents a linear regression with $R^2 = 1.0$ (y = x).

process regression, kernel approximation regression, and feed-forward neural network models were evaluated. For all models, 80% of the data were selected for training and 20% for testing. Stratified partition of the data was performed based on the age of animals to guarantee that animals of all sizes were included in both training and testing datasets. 5-fold cross-validation was chosen as the validation method. The root mean square error (**RMSE**) of the weight prediction for the test set was used to select the best model. Its performance was evaluated by comparing the predicted and measured weight using a linear regression and by its parameters - the slope, the intercept, the mean error, the RMSE, and the determination coefficient ($\mathbf{R^2}$).

Results and Discussion

Grow-finish animals

Figure 4(a) presents the original linear regression model obtained in previous work (Condotta et al., 2018), which takes only the projected volume (cm^3) as input (**eq. 1**). This model was not used for comparison with the new proposed model as it was developed with a greater number of samples. A new simple linear regression model using volume as input was developed, with 80% of the data being used for development and 20% for testing in order to follow the same partition as the other models tested (**Figure 4b**).

$$W = 6.74 \times 10^{-4} \times V - 3.75 \qquad (1)$$

where:

W = weight (kg);

V = volume (cm^3).

Feature selection was performed using an F-test feature ranking algorithm, which examines the importance of each predictor individually using an F-test and then ranks features using the p-values of the F-test statistics. Out of the 14 parameters tested (volume, dorsal area, heights [at shoulders, hip, last rib, maximum, and average], length from neck to rump, widths [at shoulders, hip, and last rib], age, sex, and sire line), all but maximum height, sex, and sire line were selected. It is important to notice that neither sire-line nor sex were selected, which points to a more robust model that could be used for weight prediction of any of the sire lines tested and arbitrary between gilts and barrows, corroborating with finds from Condotta et al. (2018).

The best machine learning model for both sets of input features (manually selected and transfer learning) was a rational quadratic Gaussian process regression model. For manual features, the RMSE for the test set was 2.58 kg. This model was able to account for 99.36% of the total variation in weight. In this case, the slope was 0.9845, and the intercept was 0.7361 (**Figure 5a**). For transfer learning features, the RMSE for the test set was 4.35 kg. This model was able to account for 98.03% of the total variation in weight. In this case, the slope was 0.9709, and the intercept was 1.1082 (**Figure 5b).**

It is possible to see that model that used known features (pig's dimensions and characteristics) as inputs had better performance than the model that used image features selected by a pre-trained network. The former model presented an improvement over the simple linear regression model (**Figure 2b**), while the latter did not.

Sows

Feature selection was also performed using an F-test feature ranking algorithm. Out of the 12 parameters tested (volume, dorsal area, heights [at shoulders, hip, last rib, maximum, and average], length from neck to rump, widths [at shoulders, hip, and last rib], and parity), volume and average height were selected for the prediction. The best machine learning model was a rational quadratic Gaussian

process regression model. The RMSE for the test set was 9.39 kg. This model was able to account for 88.54% of the total variation in weight. In this case, the slope was 0.9128, and the intercept was 15.193 (**Figure 6**). This value is larger than the value obtained by Condotta et al. (2018) (4.6%) for predicting grow-finishing pigs' weight. A simple linear regression using body volume as input was also tested. This model's performance was similar to the performance of the Gaussian process regression model (**Table 1**), which may indicate that a simpler model is preferable for sow weight prediction from cameras.

The R^2 of predicted versus actual body weight for the test set was 0.8854, which is greater than the R^2 obtained with a simple linear regression. This value is smaller than that obtained by other authors for grow-finish animals, such as Kashiha et al. (2014) for weight prediction of grow-finish pigs (R^2 of 0.92), Kongsro (2014) of boars (R^2 = 0.99), Condotta et al. (2018) of grow-finish pigs (R^2 = 0.99), and by Pezzuollo et al. (2018) for grow-finishing pigs (R^2 = 0.99). This smaller R^2 obtained probably has to do with the smaller weight range of the animals. For the greater error, it must be considered that sows have different anatomy from grow-finish pigs and boars, which can reduce the correlation between volume and weight. Overall, the proposed method showed satisfactory performance in estimating sows' weight. Table 1 summarizes all the tested models' performances.

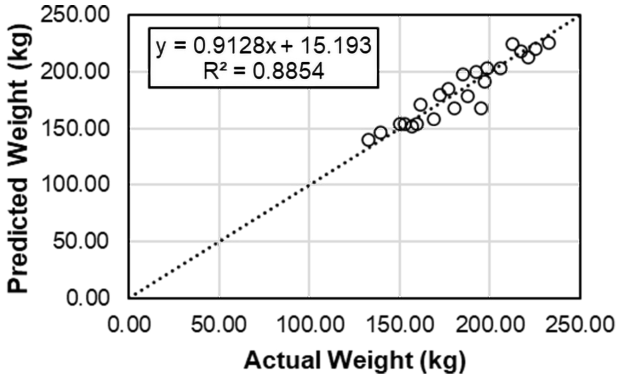


Figure 6. Correlation between measured and predicted mass obtained for the best model (Gaussian process regression) using sows' body dimensions as inputs. The circles represent individual measurements, and the dotted line represents a linear regression with $R^2 = 1.0$ (y = x).

Conclusion

A previously published method for predicting pig weight from depth images was evaluated. Its feasibility for application to animals of a different stage (sows) was assessed, and a new model based on the same dataset was proposed to reduce prediction errors. A Gaussian process regression model showed better or similar performance on swine weight prediction tasks compared with a classical linear regression-based model.

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Table 1. Statistical results for predicted weight obtained through regression models: simple linear regression (**SLR**) and Gaussian process regression (**GPR**). (d) indicates models trained using animals' body dimensions as inputs, and (h) indicates models trained using high-level features from pre-trained ResNet101 as inputs.

Regression Model	Stage	Mean Percentage Error (%)	Root Mean Square Error (kg)	R^2	Slope	Intercept
SLR	Grow-finish	5.71	4.25	0.9981	0.0007	-4.6943
SLR	Sow (empty)	4.33	9.54	0.8922	0.9414	13.462
GPR (d)	Grow-finish	3.00	2.58	0.9936	0.9846	0.7361
GPR (h)	Grow-finish	8.15	4.35	0.9803	0.9799	1.1082
GPR (d)	Sow (empty)	4.33	9.39	0.8854	0.9128	15.193

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Fundamentals, Limitations And Pitfalls on the Development and Application of Sustainable Pig Precision Nutrition

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Summary

*Precision feeding is the practice of feeding pigs daily with individually or group tailored diets. For this, using sensors to identify and provide the diet for each pig is needed according to a mathematical model developed for this purpose. Formulating diets for conventional phase-feeding systems requires information on previous populations and security margins are added to ensure maximal performance. Individual precision feeding (**IPF**) or daily group precision feeding (**GPF**) uses real-time data to predict nutrient requirements. The use of real-time feed intake and body weight allows for estimating the short-term requirement (current day or next day) for growth and adjusting the nutrient provision closely to requirements by decreasing nutrient concentrations over time more dynamically than the group phase feeding allows. IPF and GPF use only two feeds (high and low nutrient density) overall growth (25 -150 kg body weight), whereas phase-feeding implies the utilization of several feeds (e.g. 3, 4 or 6 feeds) to create the feeding program over time. The use of precision feeding allows decreasing feeding costs by 10%, nitrogen and phosphorus excretion by at least 30%, and greenhouse gas emissions from 3 to 32% when compared to a 3-phase conventional feeding system. Moreover, advantages related to sensor utilization include early-disease detection and a decrease in the workforce in the day-to-day operations. Limitations include the need for training to operate the system and formulate diets and the availability of the technologies at an affordable price.*

Introduction

The main challenges for the pig production sector are maximizing feed efficiency while minimizing production cost and environmental impacts. Thus, modern feeding systems should consider nutritional aspects and economic and environmental traits. Regarding environmental impacts, the issue lies mainly with nitrogen and phosphorus excretion in soil and water, with alarmingly high levels found in most intensive pig production areas (Strid Eriksson et al., 2005; Garcia-Launay et al., 2014). The high relevance of environmental impacts has forced swine producers and nutritionists worldwide to reassess the nutritional and feeding programs in use. Nutrient excretion to the environment can be reduced by providing animals with nutrients according to their requirements. This practice will also improve nutrient efficiency of utilization and reduce production costs significantly (Létourneau Montminy et al., 2005; Pomar et al., 2015; Andretta et al., 2016b).

Conventionally, pigs have their requirements estimated primarily using factorial methods (NRC, 2012). This way, pigs are fed in large groups and receive the same feed for extended periods (e.g. 21 or 28 days) throughout their production cycle, typically over three or four feeding phases. In theory, the number of feeding phases needs to be increased to avoid supplying pigs with nutrients in excess. Preferably, diets should be adjusted daily to account for the nutritional requirements of pigs more accurately and therefore improve nutrient efficiency of utilization. However, increasing the number of diets is challenging regarding industrial logistics and may increase production costs.

Aiming to solve this problem, whereas improving pigs' nutrient efficiency, precision livestock farming (**PLF**) was proposed (Hauschild et al., 2012; Pomar and Pomar, 2012a; Pomar et al., 2017). In this production system, the inter-animal variability is taken into account by feeding pigs with daily tailored diets to their individual requirements (Pomar et al., 2009; Hauschild et al., 2012; Pomar and Pomar, 2012b) based on individual body weight, feed intake, and average daily gain estimated by a real-time model (Hauschild et al., 2012). This model can predict and determine individual standardized ileal digestible (**SID**) lysine (**Lys**) requirements over time (Figure 1), with all other amino acids being

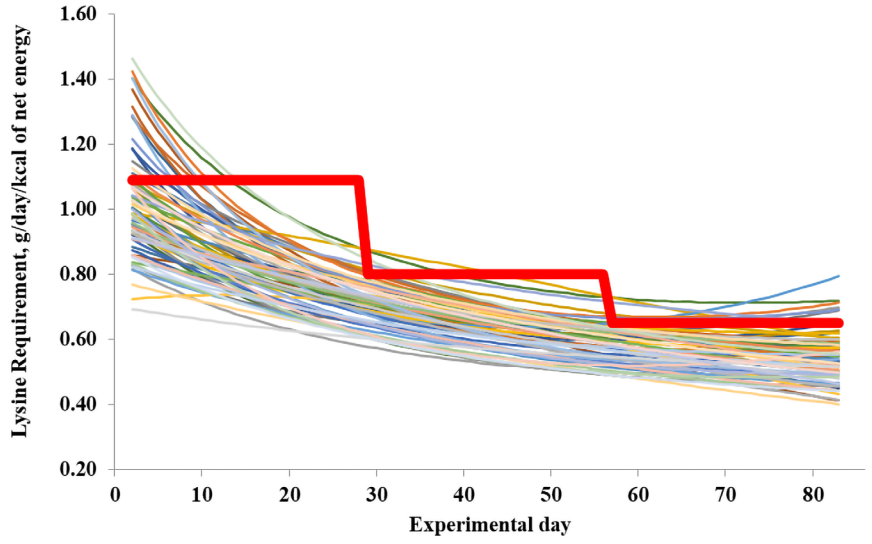

Figure 1. Estimated standardized ileal digestible lysine requirements of individual pigs (thin coloured lines) and minimal standardized ileal digestible lysine levels to be provided to pigs fed in a conventional group 3-phase-feeding system (bold red line) without affecting body weight gain according to Hauschild et al. (2010).

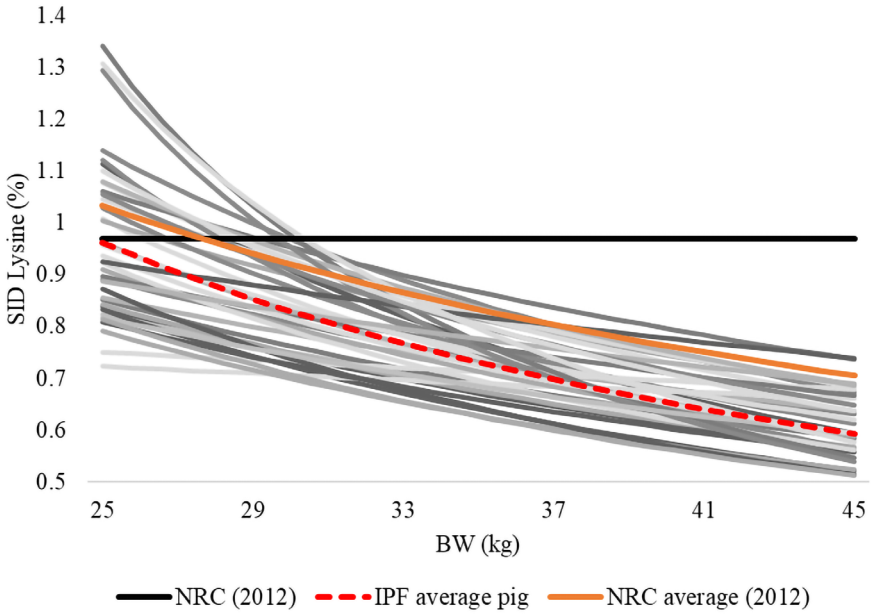

Figure 2. Daily standardized ileal digestible (SID) lysine (Lys) requirements of pigs from 25 to 50 kg body weight and optimal phase-feeding (▬) SID Lys concentrations estimated with the National Research Council (NRC) model (NRC, 2012) based on the (▬) daily NRC (2012) population requirements estimated using the average pig of the population, the daily IPF average (▬) pig estimated using the individual precision feeding (IPF) model (Hauschild et al., 2012). Each gray line represents the estimated SID Lys requirements of each pig of the herd (Remus et al., 2020b).

provided according to the ideal protein concept. This part of PLF is called precision feeding (PF) which can be applied at the population (**GPF**) or individual (**IPF**) levels.

The practical application of PLF, especially IPF, can significantly impact livestock profitability and sustainability. Feed production contributes 56–66% to the overall greenhouse gas (**GHG**) emissions in pork production (Reckmann et al., 2012). Precision feeding can be a promising technique to reduce the environmental footprint of pig production (Gerber et al., 2013). Precision feeding offers immediate and tangible benefits to the pork producer by reducing SID Lys intake by more than 25%, feeding costs by more than 8%, N and P excretion by nearly 40% (Pomar and Pomar, 2012a) and GHG emission from 6% (Andretta et al., 2018) up to 32% (Llorens et al., unpublished data) without losses in growth performance (Andretta et al., 2016b). Still, the actual on-farm application of PLF requires a better understanding of variability among individual animals in terms of their physiological, behavioural and production responses. Therefore, advanced scientific knowledge in animal sciences should be integrated with information and communication technologies to develop efficient PLF.

Principles: Conventional Group Phase Feeding vs Precision Feeding

Conventional feeding programs commonly estimate population requirements with a factorial method (e.g. NRC, 2012) and provide the same feed to the entire herd throughout feeding phases (Figure 1). In these circumstances, it is common practice to use the average pig to represent the population. Because factorial methods estimate the requirements of a particular animal at a specific point in time, they should be used with caution given that if properly calibrated, half of the population may be overfed, whereas the other half may be underfed (Hauschild et al., 2010; Brossard et al., 2014; Remus et al., 2020b), resulting in a potential performance loss for the entire pig population. Thus, these models do not consider changes during the feeding phase (Figure 2). This means that some pigs are restricted in nutrients during part of the growing period, whereas others receive excess nutrients (Remus et al., 2020b). Overall, the

pigs receive more nutrients than they need to ensure optimal herd growth performance, thus resulting in low N and P efficiency of utilization.

To obtain optimal population growth performance while minimizing production costs and environmental impacts, it is proposed in PLF systems to feed individual animals with daily personalized diets. In these systems (Figure 3), each pig is fitted with an electronic ear tag which grants access to the automatic feeding stations. A detailed description of the feeders is described later in this text and also available from previous studies (Pomar et al., 2011; Andretta et al., 2016a).

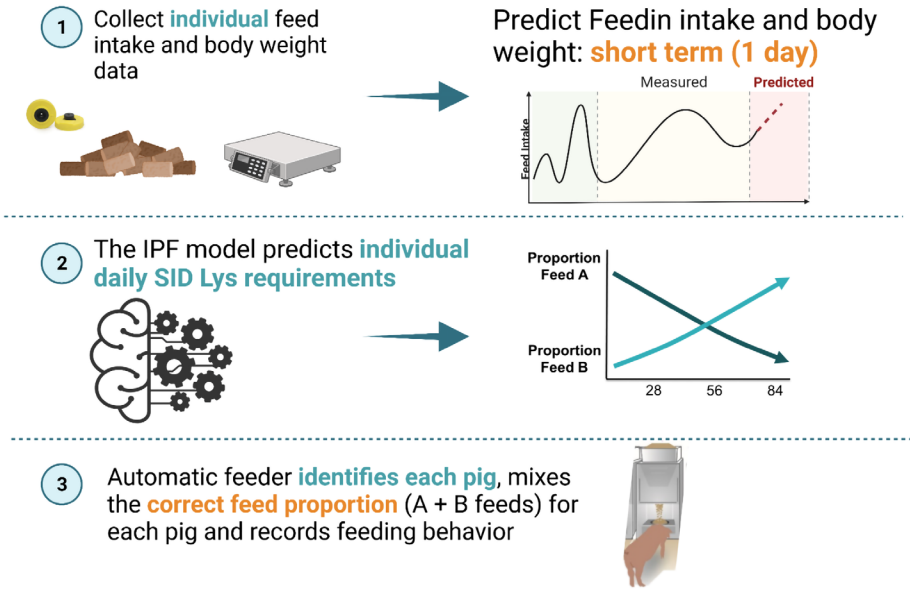

Figure 3. Scheme of the automatic precision feeding system operation using individual pig actual daily gain and daily feed intake to predict individual standardized ileal digestible (SID) lysine (Lys) requirements. Image created with BioRender.com.

With the advances in sensor technologies, many devices are today available at an affordable cost that can help making better management decisions and have enormous potential for improving production efficiency. Examples of PLF applications are the utilization of near-infrared reflectance spectroscopy (**NIRS**) to predict the digestible amino acid content of animal feeds for precision formulation (Van Kempen and Simmins, 1997), the automatic assessment of dairy cattle body condition score (Halachmi et al., 2013), the automatic counting of pigs (www.ro-main.com), automatic sows' heat detection (www.ro-main.com), automatic animal weighing with conventional load cell platforms (Turner et al., 1985), automatic activity trackers to identify and treat sick animals and animals in heat, combining video cameras with image analysis (Brandl and Jørgensen, 1996) and many others. However, introducing PLF in animal farms may involve changing several production processes and components within the system that may limit its adoption (Groot Koerkamp et al., 2007). The farmer's familiarity with these new PLF components, its ability to quantify the benefits and knowledge of how to use the information gathered with this system are important factors determining the pace of adoption of these technologies (Bewley and Russell, 2013).

Precision livestock farming allows real-time off-farm monitoring and intelligent management of feeds and animals for improved economic efficiency and significant reduction of labour requirements. Indeed, IPF will allow the swine industry to dramatically reduce the need for the periodical inspection of animals, feeds and facilities, to eliminate the manual handling of animals and feeds, to eliminate the need of the manual estimation of nutrient requirements given that these requirements will be automatically estimated in real-time for each pig of the herd, and to significantly reduce the need for veterinarian inspections given that the automatic analysis of the individual real-time feed intake patterns will allow the early and automatic identification of health and environmental disorders (Hauschild et al., 2020; Colin et al., 2021; Thomas et al., 2021). Nonetheless, this promising automation production technology must be adapted to commercial conditions and the automated decision-support systems developed to allow farmers to optimize the overall production system. Furthermore, this modern approach will allow the automatization of pig farms, allowing producers to enter the big data era and control their farms from the office while reducing animal handling.

The Implementation of Precision Feeding

Precision feeding concerns feeding techniques that provide animals with diets tailored according to the production objectives (i.e., maximum or controlled production rates), including environmental and animal welfare issues. Precision feeding is presented in this document as the practice of feeding individual animals while accounting for the changes in nutrient requirements that occur over time and for the variation in nutrient requirements that exists among animals. The accurate determination of available nutrients in feed ingredients, the precise diet formulation, and the determination of the nutrient requirements of individual animals or group of animals should be included in the development of PF (Van Kempen and Simmins, 1997; Pomar et al., 2009). The operation of IPF in commercial farms requires the integration of three types of activities: 1) automatic collection of data, 2) data processing according to the established control

Figure 4. Individual feeders allow one pig at time to request feed. Each animal is identified by the plastic button ear tags containing passive transponders, which provide pigs with diets that are tailored daily to individual requirements of each pig.

strategy and 3) actions concerning the control of the system (Aerts et al., 2003; Banhazi et al., 2012; Pomar et al., 2019) Application of PF at the individual level is only possible where measurements, data processing, and control actions can be applied to the individual animal (Wathes et al., 2008).

Data collection

Measurements on the animal, the feeds, and the environment are essential for PF; and these parameters have to be measured directly and frequently (if possible, continuously). In fact, we cannot manage and control a system without appropriate measurements. Essential measurements for PF in growing pig operations include feed intake and body weight. The availability and the rapid development of new devices and emerging sensor technologies offer to PF a great potential for other measurements (e.g., body composition, physical activity, interactions among animals) that will allow more precise estimation of requirements and real-time animal monitoring.

Data processing

Collected data has to be processed according to the farm production objectives. There are several potential control strategies available for the application of PF in swine operations. In animals offered feed ad libitum, the only way to control the nutrient intake is by varying the composition of the feed to be served. In this situation, both the between-animal and the time-dependent nutrient requirements variation can be controlled. In contrast, in animals that are offered feed restrictively the amount and the composition of the feed can be easily controlled.

Mathematical modelling is a methodology used to understand and to quantify complex biological phenomena involved in animal production and it is the basis for data processing in PF systems. Mathematical models developed for PF, however, have to be designed to operate in real-time using real-time system measurements. Therefore, they are structurally different from traditional nutrition models, which are developed to work in a retrospective manner and to simulate known production situations. The first mathematical model developed to estimate in real-time individual pigs nutrient requirements was proposed by Hauschild et al. (2012). The required daily concentration of Lys is estimated in this model using individual feed intake and body weight information. Using these data, an empirical model component estimates the expected body weight, feed intake, and weight gain for the next day, whereas a mechanistic model component uses these three estimated variables to calculate with a factorial method the optimal concentration of Lys that should be offered that day to each pig in the herd to meet its requirements. Other amino acids and nutrient requirements are assumed proportional to the Lys requirements.

Control of the system

The information collected and processed is used to control the production system. In the context of PF, automatic precision feeders are used to provide individual pigs with the right amount and composition of the feed at a given time. Ear plastic button tags containing passive transponders (**RFID**) are used for pig identification. At least two feeds (named A and B) are needed for PF. These two feeds should be formulated on the basis of net energy, standardized ileal digestible amino acids and other essential nutrients. Feed A (high nutrient density feed) is formulated for the most demanding pigs at the beginning of the growing period, whereas feed B (low nutrient density feed) is formulated for the less demanding pigs at the end of the finishing period. Blending feeds A and B at different proportions allows the feeders to provide individual pigs (i.e. IPF) or group of pigs (i.e. GPF) with the right feed. The feeders (Figure 4) consist of a single space trough in which precision Archimedes's screw conveyors deliver and blend simultaneously volumetric amounts of two feeds contained in independent feed containers. The feeder identifies each pig when their head is introduced into the feeder and the feeds are blended

and delivered upon the animal request according to the estimated optimal Lys concentration. A serving is composed of the amount of feed delivered upon each effective serving request. A time lag is imposed to ensure that pigs eat each serving before requesting a new one. Serving size is progressively increased and ranges between 15 and 25 g (Pomar et al., 2011). A meal includes all the servings delivered during each feeder visit. Pigs tend to leave the feeder trough empty or leave very small amounts of feed after each visit, thus ensuring that each pig receives the assigned amount of blended feed. Feed density needs to be measured weekly and this information used to convert feed volumes to feed weights.

A real-time modelling-control approach was used by Pomar et al. (2014) to control the time-dependent variation of group-housed pigs offered feed ad libitum. Comparing the traditional three-phase feeding system to the GPF system, these authors concluded that protein intake could be reduced by 7% while nitrogen excretion was reduced by 12%. Controlling the time-dependent and the between-animal variation, that is moving from GPF to IPF, can further help the reduction of nutrient intake and excretion. The modelling approach proposed by Hauschild et al. (2012) was used to estimate real-time nutrient requirements in individual pigs was calibrated in two animal trials (Zhang et al., 2012; Cloutier et al., 2015;), and the overall approach of estimating real-time amino acid requirements was challenged in two validation trials (Andretta et al., 2014; Andretta et al., 2016; Figure 4). The latter authors showed that daily adjustment of the diet resulted in a 27% reduction in total Lys supply, without detrimental effects on growth. This additional 20% reduction in Lys intake in relation to GPF pigs could be obtained by feeding the animals individually and thus controlling simultaneously the time-dependent and the between-animal variation (Figure 5). Although feed cost reduction depends to a great extent on feed ingredient prices, it is expected that feed cost can be reduced by 1-3% when only controlling the time-dependent variation while an 8-10% reduction can be obtained when controlling both sources of variation. Nitrogen excretion was reduced by nearly 30% when pigs were fed with daily tailored diets.

Limitations and Pitfalls

The Artificial Intelligence miracle

Over the years, PLF, and IPF within it, have generated great controversy, with comments ranging from skepticism to overoptimism. Although PLF, especially IPF, have great potential to improve pig production, some traps should be avoided. Several companies are offering the "easy-solution" package. The promise is that artificial intelligence allied to several gadgets will solve all livestock farming problems. This issue has been discussed in length (Jacobs et al., 2022), but the main take-home message to be conveyed is that without biological understanding and clear knowledge of

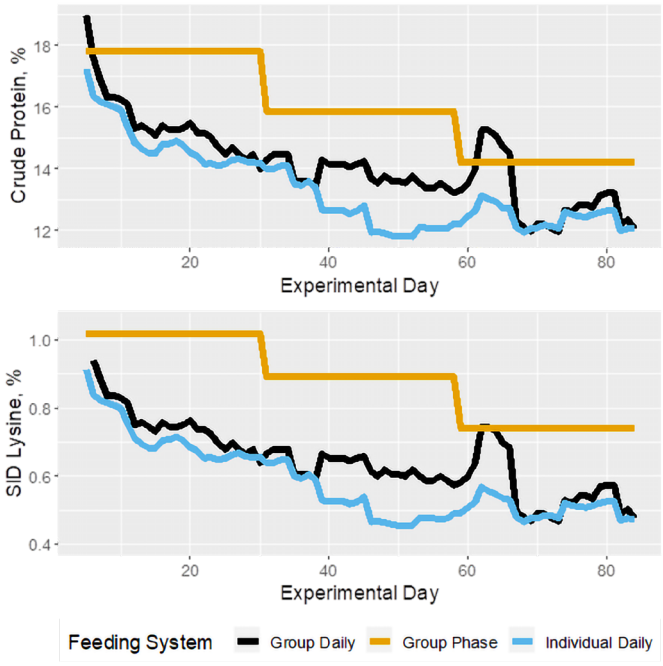

Figure 5. Changes on average crude protein intake (% of diet) and standardized ileal digestible (SID) lysine during the 84 experimental days (40 to 135 kg of body weight). Data adapted from Andretta et al., (2016).

the production system, the "big data companies" might be over-promising. The sustainable application of PLF systems relies on inverting the flow and asking the right question: "What gadgets do I need to solve the problems I have on my farm?" and not "Which gadgets can I add to my farm?". Moreover, such technologies must pass a solid scientific evaluation and have publicly available results. In other words, PLF technologies must be tested on experimental and commercial set-ups using clearly defined scientific protocols to answer the question: "does it really work?".

One size does not fit all: different farms, different diets

Another common mistake is trying to do IPF with the mindset of group phase feeding: "one size fits all". Most often, different farms with different growth performances will use the same feed. This is because the feed was formulated based on the average growth performance of the genetic line or nutritional tables (e.g. NRC or genetic line tables) without considering the herd performance. A "baby-step" transition would explore the requirements differences among the different herds. For example, use a factorial method such as the NRC or IPF model to estimate the requirements based on individual farm growth performance.

Another critical point is the choice of the reference animal to formulate the feeds. Using the average pig in the middle of the feeding phase to establish requirements might result in losses in growth performance once half of the population is overfed and the other half is underfed in terms of

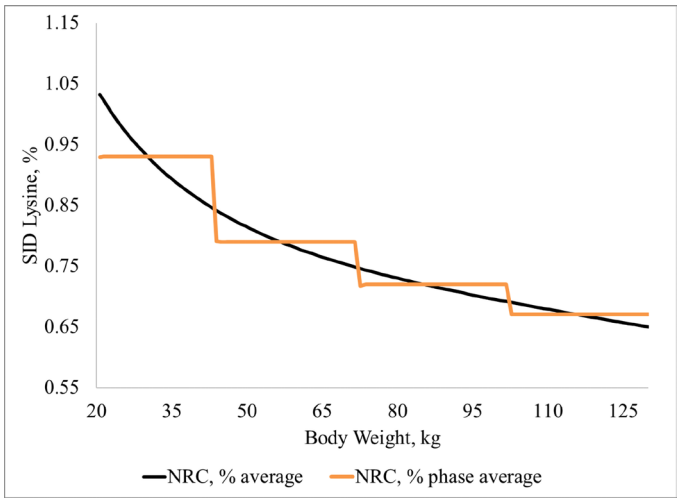

Figure 6-a.

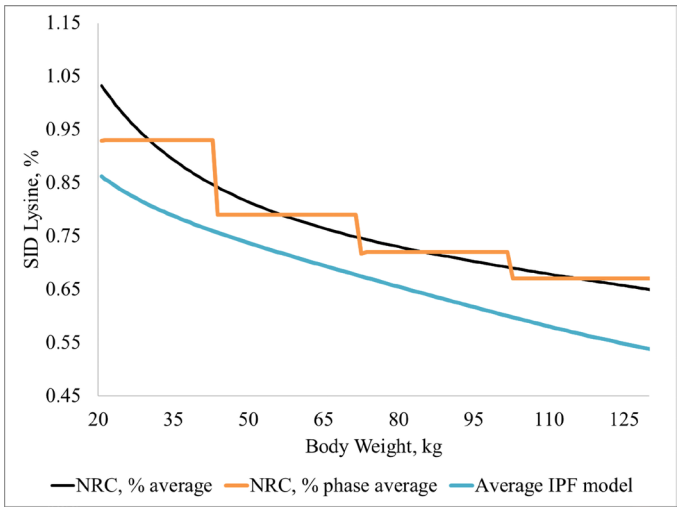

Figure 6-b.

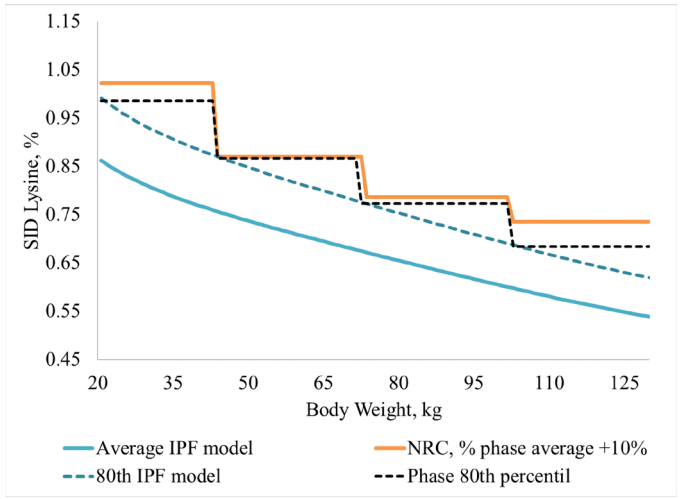

Figure 6-c.

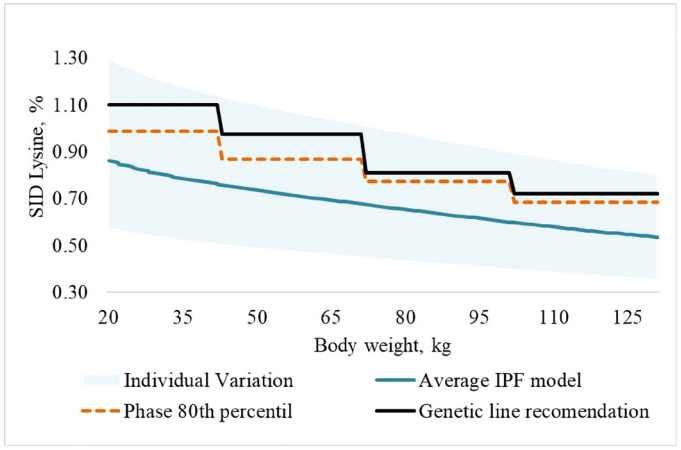

Figure 6-d.

Figure 6. Estimated standardized ileal digestible (SID) lysine (top graph) as a function of body weight of an average daily gain (DG) and daily feed intake (DFI) in a Canadian commercial farm. The average daily SID lysine requirements were estimated using the NRC (2012) and the IPF (Hauschild et al., 2012) models. The phase program was established using the average NRC estimate for 28 days.

nutrients during the growing phase (Figure 6-a). For a farm-tailored phase-feeding system, the use of 80th percentile pig estimated average requirements over the first three days of the phase (Hauschild et al., 2010; Remus et al., 2020b) is recommended to maximize growth (Figure 6-c). As estimating the 80th percentile pig might be difficult without having individual data, it is suggested to add 15% to the average SID Lys requirement estimated by the IPF model or to use the average NRC (2012) estimation at the beginning of the phase (Brossard et al., 2009; Hauschild et al., 2010; Remus et al., 2020b).

The second step in understanding animal variation and using this to our advantage in implementing group precision feeding (GPF), adapting the diet to the herd's daily requirements within each farm. Furthermore, it is in this step that caution must be advised: models used to estimate requirements within precision feeding systems must have been developed for this purpose (Remus et al., 2020b; Jacobs et al., 2022; Menendez et al., 2022). The traditional factorial methods estimates (e.g. NRC, 2012) cannot be directly applied to GPF or IPF because they have been calibrated to estimate population requirements for phase feeding programs, and likely no gain will be observed moving from phase feeding to GPF in this case (Figure 6-a). Traditional factorial models overestimate the average pig requirement by 15% (Hauschild et al., 2010; Remus et al., 2020; Figure 6-b); therefore, this information should be known when formulating diets to avoid excessive nutritional security margins.

The final step is the implementation of IPF. In this feeding program, each pig will receive daily tailored diets to account for the large among animal variation in nutritional requirements (Figure 6-d). A common pitfall is comparing different experiments using IPF and phase-feeding designed to maximize growth based on real-time data to conventional phase-feeding programs established based on reference populations. Each herd will have different requirements

depending on their growth performance, which makes the comparison difficult. However, the reduced proportions (%) of crude protein, Lys, phosphorus and calcium are repeatable among experiments (Andretta et al., 2014; Andretta et al., 2016b; Santos et al., 2018; Remus et al., 2019). Therefore, correctly conveying the concepts and training people to apply IPF is fundamental for this system's adoption and successful implementation.

To further develop IPF, we must improve our understanding of several animal metabolic processes. Precision feeding is still based on mathematical models and nutritional concepts developed for average population responses. When feeding individual pigs with daily tailored diets, these traditional nutritional concepts are not accurate and even sometimes incorrect (Remus et al., 2019; Remus et al., 2021). Therefore, it is necessary to distinguish the nutritional requirements of a population from those of an individual. For example, individual pigs can modulate growth and growth composition according to the level of available amino acids (Remus et al., 2019). Also, pigs can respond differently to the same amount of ingested amino acid due to differences in the efficiency of amino acid utilization (Remus et al., 2020a). These aspects are not considered in current nutritional models, which assume that the efficiency of animals using the available amino acids is constant. Similarly, the amino acid composition of whole body protein is also assumed to be constant, whereas it has been shown that it can vary. Similar results have been found for the efficiency of calcium and phosphorus utilization (Gonzalo et al., 2018). Understanding the metabolic processes responsible for the observed variation between individual animals in their ability to use dietary nutrients is challenging for nutritionists and modellers. However, it is required to improve the efficiency of livestock production further. Advances in IPF rely on developing sound nutritional concepts, and comprehensive biological models developed to estimate individual real-time nutrient requirements more precisely. The new understanding of individual metabolism and nutrition will allow animal science to move forward, opening up new opportunities for individual nutrition. Continuous and automatic monitoring of animals and farm resources will support production decisions at the farm level, the early detection of diseases and thus, decrease the use of antibiotics and avoid disease spread. This will ultimately enhance farm profitability, efficiency, and sustainability of the overall production system (Jacobs et al., 2022; Menendez et al., 2022).

Future Perspectives

Precision feeding is a major breakthrough in pig nutrition and one of the most promising avenues to promote high-quality and safe pork (optimal fatness) with the lowest environmental impact (60% less nutrient excretion) and high animal welfare standards. Fewer pollutants would mean improved population wellness and health, given the resulting reduction in odours, harmful waste, and the risks of water, air (e.g., ammonia and greenhouse gas emissions), and soil pollution. In addition, managing feeds, and animals through advanced computerized technologies make it possible to identify diseases early and apply individual treatments precisely, thus improving herd performance, dramatically reducing antibiotic use and thus contributing to improving public safety.

Combining knowledge- and data-driven models will further enhance our ability to use real-time farm data, opening up new opportunities that will enhance farm profitability, nutrient efficiency, and the sustainability of the overall animal production system. With the development of advanced computer and communication technologies and high-speed data-collection sensors, it is possible today to obtain numerous measurements at the animal, feed, building, and other farm levels. Besides the availability of these new technologies and data gathering, knowledge remains the most limiting factor to precisely providing each animal or a group of animals with the number of nutrients it needs to produce at the desired level. Understanding the metabolic processes responsible for the observed variation between individual animals in their ability to use dietary nutrients is challenging for nutritionists and modellers. However, it is required to improve the efficiency of livestock production systems further.

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Normal Vitamin Status in Pigs at Different Life Stages

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Summary

Recent evaluations of serum and tissue vitamin and mineral levels in swine have differed from previously published values from the late 1900's. While this could be due to newer laboratory analytics, the fact that swine housing, genetics, and nutrition have changed could be altering the previously published values. In particular, the values such as vitamin D and A have changed from previous years. This proceeding is set to review previous values, discuss potential changes, and introduce current values found in the literature.

Introduction

Vitamins and minerals are essential building blocks of life. As swine production has migrated into indoor housing, swine have less access to minerals found in the soil and less sunlight that can contribute to vitamin D production (Arnold et al., 2015). In order to address the needs of the pigs, nutritionists add vitamins and minerals to rations at various levels depending upon the phase of production. Flohr et al. (2016a) documented that United States nutritionists feed a margin of safety above the NRC (2012) recommendations to account for any vitamin degradation or manufacturing/ration handling challenges that may occur as the vitamin and mineral premixes are stored prior to use. The practice of supplementation above the NRC requirements is further supported by a recent study conducted by Hinson et al. (2022), which demonstrated that sows have reduced feed intake and their progeny have reduced vitamin levels and performance when they are fed NRC requirements compared to typical industry levels. Little information has been compiled over the last 15 years to document current vitamin and mineral tissue concentrations in healthy, modern genetics swine raised in the United States using modern swine production management practices.

Discussion

Previous research studies have documented that vitamin and mineral concentrations differ across production phases and sample types. Two widely used publications for mineral and vitamin reference values were published in 1994 (Puls, 1994a; Puls, 1994b). While the publications provide specific values, the data is limited as it does not divide the information into both phases of production and tissue type. For example, in recent work conducted by Elefson et al. (unpublished), liver vitamin A levels are 4.7 ppm pre-suckle and 25 ppm after the piglet has suckled.

In order to assess vitamin and mineral status, samples are typically collected from muscle, liver, or serum. Serum sampling is the most common procedure used for assessment due to the ease of sample collection and the ability to collect the sample without euthanasia. However, not all vitamins and minerals are similarly stored in the three different collection sites which can lead to a misrepresentation of results. Knowing the appropriate location for sampling to best understand vitamin and mineral status is imperative. For example, vitamins A and D and minerals such as iron, copper, manganese, selenium and zinc are stored in the liver. Vitamin E is predomintantly stored within adipose tissue and cell membranes, and macrominerals are tightly regulated within serum and best measured in bone. Vitamin A concentration is tightly regulated within the blood through the use of retinol binding protein, and therefore, may not fully define vitamin status of the animal (Thorbjarnarson and Drummond, 1938; Quadro et al., 2000; Tanumihardjo, 2011). Hinson et al. (2022) demonstrated that serum vitamin A levels (0.280 vs 0.210 ppm) increase when additional vitamin A is added to the ration; however, the magnitude of the response is greater in the liver (19.55 vs 6.53 ppm) in response to increasing vitamin A levels in both the sow and nursery rations.

Assessment of vitamin and mineral status within a herd not only requires the determination of the appropriate sampling location, but also the health status of the animal and the process in which the sample is collected. Variation of findings can occur with different rations, dietary ingredients, and immune status of the animal. For example, during an infection, minerals such as iron and zinc may be sequestered in the liver (Klasing, 1994). Elevated zinc levels may be associated with feeding higher levels of zinc in the nursery to aid in controlling pathogenic organisms. Furthermore, the degree of hemolysis may result in elevated

Table 1. Previously published reference values for vitamins and minerals in the serum of swine[1].

	Phase Production					
	No Specified Age	Fetus	Weanling/ Nursery	Growing	Adult	Lactating Sow
Vitamin A, ppm[2, 3]	.	0.100-0.200[2]	0.400-0.500[2]	0.400-0.500[2]	0.400-0.500[2]	0.250-0.400[2]
			0.080-0.268[3]			0.128-0.393[3]
Vitamin D3, ng/ml3,4	.	.	5.0-23[1]	.	.	50-95[1]
	.	.	4.0-16[3]	.	.	25-111[3]
Calcium, ppm	75.1-134.7	.	.	.	.	.
Selenium, ppm	0.14-0.30	.	.	.	.	.
Zinc, ppm	0.7-1.5	.	.	.	.	.

[1]Vitamin values fom Puls, R. Vitamin levels in animal health. Sherpa International, 1994; Mineral values from Puls R. Mineral levels in animal health: diagnostic data, 2nd ed. Sherpa International, Clearbrook. In: British Columbia. ed. Puls R. 2nd ed. British Columbia, Canada: Sherpa International, 1994.
[2]Values are from dry weights
[3] Flohr et al., 2014.

Table 2. Vitamin and mineral concentrations in the serum of suckling (1-21 days of age), nursery (22-64 days of age), finisher pigs (65-165 days of age) and lactating sows.[1]

Nutrient, unit	Suckling Pig Range	Nursery Pig Range	Finishing Pig Range	Sow Range
Vitamin A, ppm[2, 3]	0.02-0.280	0.01-0.39	0.10-0.21	0.03-0.32
Vitamin D2, ng/ml3	nd	nd	nd	nd
Vitamin D3, ng/ml3,4	0.75-8.60	9.20-27.50	18.40-115.80	9.50-53.00
Calcium, ppm	75.1-134.7	50.1-120.4	83.5-100.9	75.9-133.4
Selenium, ppm	0.088-0.160	0.084-0.190	0.200-0.278	0.133-0.355
Zinc, ppm	0.3-10.4	0.5-1.2	0.5-2.0	0.6-4.4

[1]Suckling piglet n=17; Nursery n=13; Finisher n=11; Lactating Sow n=7
[2]Represented as retinol
[3] nd=non-determined. If the element of analysis was below the detectable limit, the lower limit threshold (1.5 ng/ml) was divided by two to provide a value which occurred in 2 muscle samples for vitamin A and all samples for vitamin D_2. Cohen and Ryan, 1989.
[4]Represented as 25(OH)D3

Table 3. Vitamin and mineral concentrations in the liver of suckling (1-21 days of age), nursery (22-64 days of age), finisher pigs (65-165 days of age) and lactating sows. Values represented as per unit of wet tissue weight.[1]

Nutrient, unit	Suckling Pig Range	Nursery Pig Range	Finishing Pig Range	Sow Range
Vitamin A, ppm[2, 3]	18-63	0.5-25.0	47-90	80-530
Calcium, ppm	59-145	63-128	67-118	60-121
Selenium, ppm	0.45-0.80	0.56-0.87	0.75-1.19	0.67-1.72
Zinc, ppm	27-120	42-562	51-313	38-91

[1]Suckling piglet n=17; Nursery n=13; Finisher n=11; Lactating Sow n=7
[2]Represented as retinol
[3]If the element of analysis was below the detectable limit (<1ppm), the lower limit threshold was divided by two to provide a value for 1 nursery pig. Cohen and Ryan, 1989.

concentrations of iron and potassium (Frank et al., 1978; Ji and Meng, 2011) but decreased vitamin E concentrations as a result of degradation (Hooser et al., 2000).

In a recent study by Greiner et al. (2022), some vitamin and mineral concentration ranges (presented in Table 2) differ from the values presented in Puls (1994a, 1994b; Table 1). For example, serum vitamin A (0.01-0.39 ppm in suckling/nursery pigs and 0.03-0.32 ppm in sows) and selenium levels (0.084-0.190 ppm in suckling/nursery pigs and 0.133-0.355 ppm for the sow) are lower than previously published values (vitamin A - 0.40-0.50 ppm in suckling and nursery pigs and 0.25-0.40 ppm in sows and selenium – 0.14-0.30 ppm with no specific age; Table 1). In addition, vitamin D_3 concentrations in the current study are lower in the suckling and nursery pig compared to the published values of 8-23 and 25-30 ng/ml, respectively (Flohr et al. 2016b). In the recent work conducted by Greiner et al. (2022), the vitamin D_3 levels were slightly lower than those documented by Flohr et al. (2016b). Other vitamins and mineral levels in the livers of swine are presented in Table 3.

In conclusion, recent studies have demonstrated that while some vitamin and mineral concentrations in modern day swine are not different from previously published ranges, other vitamins (A and D) and minerals (calcium and zinc) are different. The differing values may be related to ration differences, vitamin and mineral supplementation changes, or alterations in genetics or management practices. Recent publications indicate a need for additional studies focused on the analysis of multiple biological samples from healthy porcine to best determine the appropriate vitamin and mineral ranges for the modern pig to provide baseline values for future deficiency and requirement research.

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Vitamin D and Vitamin D Metabolites Impact on Calcium and Phosphorus Balance in Gestating Sows

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Summary

One-hydroxycholecalciferol (1-OH-D_3) and 25-hydroxycholecalciferol (25-OH-D_3) are vitamin D metabolites that may be added to diets for pigs. Because 1-OH-D3 is already hydroxylated at the 1-position, only the first hydroxylation in the liver at the 25-position is needed to convert the metabolite to calcitriol, which is the active form of vitamin D_3. Likewise, because the 25-OH-D_3 is already hydroxylated at the 25-position, only the second hydroxylation in the kidney at the 1-position is needed if this metabolite is used. It is possible that supplementation of diets with 25-OH-D_3 or 1-OH-D_3 increases absorption and retention of Ca and P by increasing the conversion efficiency to calcitriol compared with the conversion of cholecalciferol to calcitriol. Effects of supplementation of 1-OH-D_3 and dietary Ca and P in diets fed to gestating sows have been determined, and the effects of supplementation of 25-OH-D_3 and 1-OH-D_3 in diets without or with phytase have also been reported. Results of these experiments have indicated that Ca and P balance and concentrations of digestible energy and metabolizable energy in diets fed to late-gestating sows were not affected by Ca and P levels, but were increased by dietary supplementation with 1-OH-D_3. There was no interaction between dietary Ca and P and supplementation with 1-OH-D_3. Supplementation of 25-OH-D_3, 1-OH-D_3, or microbial phytase increased digestibility and retention of Ca and P. Supplementation of phytase did not affect digestibility of energy, but supplementation of 1-OH-D_3 increased digestibility of energy and concentration of metabolizable energy in diets containing no microbial phytase.

Introduction

Concentrations of blood Ca are tightly regulated by calcitonin, parathyroid hormone, and calcitriol (i.e., 1,25-dihydroxycholecalciferol), which is the active form of vitamin D_3 (Crenshaw, 2001). The regulation may change absorption of Ca and P from the intestinal tract, reabsorption of Ca and P from the kidneys, and formation or resorption of bone tissues (Renkema et al., 2008). In most diets for pigs, vitamin D_3 (i.e., cholecalciferol) is provided in vitamin premixes (Quisirumbay-Gaibor, 2019), and dietary vitamin D_3 needs to be converted to the active form before it can be utilized by animals. Cholecalciferol is transformed to calcitriol by two steps of hydroxylation (Henry, 2011). The first step, which takes place in the liver, involves hydroxylation of cholecalciferol at the 25-position to yield 25-hydroxycholecalciferol (**25-OH-D_3**). The second step, which takes place in the kidney, hydroxylates 25-OH-D_3 at the 1-position to yield 1,25-dihydroxycholecalciferol (i.e., calcitriol).

One-hydroxycholecalciferol (**1-OH-D_3**) and 25-OH-D_3 are vitamin D metabolites that may be added to diets for pigs. Because 1-OH-D_3 is already hydroxylated at the 1-position, only the hydroxylation in the liver at the 25-position is needed to convert the metabolite to calcitriol. Likewise, because the 25-OH-D_3 is already hydroxylated at the 25-position, only the hydroxylation in the kidney at the 1-position is needed if this metabolite is used. Supplementation of diets with 25-OH-D_3 or 1-OH-D_3 may increase absorption and retention of Ca and P by increasing the conversion efficiency to calcitriol compared with the conversion of cholecalciferol to calcitriol. It is possible that effects on Ca and P balance differ between 25-OH-D_3 and 1-OH-D_3, but research to test this hypothesis has not been reported. It is also possible that effects of supplemental vitamin D metabolites (i.e., 1-OH-D_3 or 25-OH-D_3) are affected by the use of microbial phytase because both feed additives can change Ca and P metabolism in broilers (Han et al., 2009), but pig data to demonstrate this have not been reported. Therefore, the objective of this contribution is to summarize current knowledge and experiments about the effects of vitamin D metabolites on Ca and P balance in sows.

Table 1. Calcium and P balance, the apparent total tract digestibility (ATTD) of gross energy, and concentrations of digestible energy and metabolizable energy in diets containing different levels of Ca and P without 1-hydroxycholecalciferol (1-OH-D_3) or with 1-OH-D_3[1,2,3]

Item	Ca and P levels[b]	Normal		Low		SEM	*P*-value		
	1-OH-D_3, mg/kg diet	0	12.5	0	12.5		Ca and P Levels	1-OH-D_3	Interaction
Feed intake, kg/d		2.98	2.93	2.99	2.92	0.05	0.997	0.223	0.901
Fecal excretion, kg/d		0.40	0.33	0.36	0.31	0.06	0.096	0.001	0.441
Urine excretion, kg/d		10.51	11.26	12.10	13.91	3.40	0.327	0.554	0.804
ATTD of dry matter, %		85.04	87.72	86.80	88.31	2.17	0.076	0.003	0.365
Ca balance									
ATTD of Ca, %		11.31	30.25	18.21	30.49	12.17	0.407	0.001	0.439
Ca retention, % of intake		9.80	27.48	15.41	24.07	11.97	0.796	0.005	0.292
P balance									
ATTD of P, %		15.57	34.70	23.38	29.63	11.16	0.731	0.003	0.114
P retention, % of intake		12.44	28.03	21.93	28.51	11.52	0.223	0.010	0.269
Energy concentrations									
ATTD of gross energy, %		84.6	87.3	85.6	87.5	2.3	0.291	0.001	0.654
Digestible energy, kcal/kg		3,240	3,361	3,275	3,388	90	0.227	< 0.001	0.871
Metabolizable energy, kcal/kg		3,163	3,268	3,197	3,291	98	0.357	0.003	0.861

[1]Data from Lee and Stein (2022).
[2]Each least squares mean for each treatment represents 9 observations, respectively, except for the 2 diets containing normal or low Ca and P levels with no supplemental 1-OH-D_3 (n = 8).
[3]Normal level of Ca and P = 100% of the requirement for late gestation sows (0.72% Ca and 0.55% P); low level of Ca and P = 75% of the requirement for late gestation sows (0.54% Ca and 0.41% P; NRC, 2012).

Effects of Dietary Supplementation of Vitamin D Metabolites in Gestating Sows

Effects of dietary Ca and P and 1-OH-D_3 on digestibility and retention of Ca and P in sows

This experiment was conducted to test the hypothesis that supplementation of 1-OH-D_3 to diets for gestating sows containing Ca and P at or below the requirement increases apparent total tract digestibility (**ATTD**) and retention of Ca and P as well as the ATTD of gross energy (**GE**; Lee and Stein, 2022). The second hypothesis was that there is an interaction between dietary Ca and P concentrations and supplementation with 1-OH-D_3 in diets fed to gestating sows.

Diets were formulated using a 2 × 2 factorial arrangement with 2 levels of Ca and P (i.e., 100% or 75% of the requirement; NRC, 2012) without or with supplemental 1-OH-D_3 (Savint, Savint, Iluma Alliance, Durham, NC). Calcium to total P ratio in all diets was 1.3:1.0. Analyzed 1-OH-D_3 in the 2 diets containing the premix were 4.96 and 3.46 µg/kg, respectively, which are close to the commercially recommended dose of 5 µg/kg. The calculated level of vitamin D_3 in all diets was 1,660 IU/kg. All vitamins and minerals except Ca and P were included in all diets to meet or exceed nutrient requirements (NRC, 2012). The 4 diets were fed to 36 multiparous sows from d 91 to 105 of gestation. Sows were housed individually in metabolism crates during the experimental period. Daily feed allotments were provided in one daily meal that was fed at 0700 h throughout the experiment. The daily feed allowance was calculated as 1.5 × the maintenance energy requirement for late gestating sows based on the initial (day 90) body weight of sows (i.e., 100 kcal ME/kg body weight$^{0.75}$; NRC, 2012). Water was available at all times. The initial 5 days of each period were considered the adaptation period and urine were collected during the following 4 days. A color marker was included in the meal fed on day 6 and again in the meal fed on day 10. Fecal collections started when the first marker appeared in the feces and concluded when the second marker appeared. At the conclusion of the experiment, fecal samples were dried at 50 °C in a forced air oven and dried samples were ground; a sub-sample was collected for analysis. Diets, urine and fecal samples were analyzed for Ca, P, and GE and diet and fecal samples were also analyzed for dry matter.

No interactions between dietary Ca and P levels and supplemental 1-OH-D_3 were observed for Ca and P balance, the ATTD of dry matter (**DM**) and GE, or concentrations of DE and ME (Table 1). Dietary Ca and P did not affect Ca and P balance, the ATTD of DM and GE, or concentrations of DE and ME. However, although feed intake was not different among treatments, fecal excretion was less (P = 0.001) from sows fed diets supplemented with 1-OH-D_3 compared with sows fed diets with no 1-OH-D_3, which resulted in greater (P = 0.003) ATTD of DM in sows fed diets supplemented

with 1-OH-D_3 compared with sow fed no supplemental 1-OH-D_3. The ATTD of Ca and P and retention of Ca and P were greater ($P < 0.05$) if sows were fed diets supplemented with 1-OH-D_3 compared with sows fed no supplemental 1-OH-D_3. The ATTD of GE and concentrations of DE and ME increased ($P < 0.01$) by supplementing 1-OH-D_3 to the diets.

Effects of 25-OH-D_3 and 1-OH-D_3 on digestibility and retention of Ca and P in sows

A follow-up experiment was conducted to test the hypothesis that supplementation of diets for gestating sows with 25-OH-D_3 or 1-OH-D_3 increases Ca and P balance, the ATTD of GE and concentrations of ME in diets without or with microbial phytase (Lee et al., 2022). Diets were formulated using a 3 × 2 factorial with 3 inclusions of supplemental vitamin D metabolite (no metabolite, 25-OH-D_3, or 1-OH-D_3) and 2 inclusion levels of microbial phytase (0 or 1,000 units; Quantum Blue; AB Vista, Marlborough, UK). Diets were fed to 60 multiparous sows. All diets contained 90% of the requirement for Ca and P (NRC, 2012) and contained 1,660 IU/kg of vitamin D_3 as cholecalciferol from the vitamin-mineral premix. The daily feed allowance was 1.5 times the maintenance energy requirement for gestating sows based on the initial body weight of sows (i.e., 100 kcal ME/kg body weight$^{0.75}$; NRC, 2012). Water was available at all times. Total feces and urine samples during the collection period were collected and prepared for further analyses of Ca, P, and GE.

Results indicated that there was no difference in the ATTD of DM and GE among the 3 diets containing microbial phytase, but among diets without phytase, the ATTD of DM and GE was greater ($P < 0.05$) in diets containing 1-OH-D_3 compared with the diet without a vitamin D metabolite (interaction; $P < 0.05$; Table 2). If no phytase was added to diets, the DE was greater in the diet containing 1-OH-D_3 compared with the diet without a vitamin D metabolite, but if phytase was added to the diets, no difference among diets was observed (interaction; $P < 0.05$). In diets without microbial phytase, the ME was greater in diets containing either one of the 2 vitamin D metabolites than in the diet without one of the metabolites, but among diets with microbial phytase, the ME in the diet containing the 1-OH-D_3 metabolite was less than in the diet with 25-OH-D_3 (interaction; $P < 0.05$). No effects of microbial phytase on ATTD of DM or GE, or on concentrations of DE and ME were observed. Because the interactions between vitamin D metabolite and phytase in Ca and P balance were not significant, only main effects were included in the final model to analyze these parameters. Regardless of metabolite supplementation, use of microbial phytase increased ($P < 0.05$) the ATTD of Ca and P and Ca and P retention (Table 3). Regardless of dietary phytase, the ATTD of Ca and P was greater ($P < 0.05$) for sows fed a diet containing one of the vitamin D metabolites compared with sows fed a diet without a vitamin D metabolite. Calcium and P retentions were greater ($P < 0.05$) for sows fed a diet containing one of the 2 vitamin D metabolites compared with sows fed a diet without one of the metabolites.

Discussion

In both experiments, it was observed that supplementation of vitamin D metabolites increased the ATTD and retention of Ca and P in pigs, which was in agreement with previous data (Regassa et al., 2015; Zhang and Piao, 2021). This indicates that both of the vitamin D metabolites were effective in increasing digestibility of Ca and P and further implies that there is a beneficial effect of providing metabolites in which the first or the second hydroxylation has taken place, even if sows are provided diets that contain vitamin D_3 well above the requirement.

The increase in Ca and P balance by supplemental 1-OH-D_3 and 25-OH-D_3 in the second experiment may be a result of sows being fed below the requirements for Ca

Table 2. Apparent total tract digestibility (ATTD) of gross energy, and concentrations of digestible energy and metabolizable energy in diets fed to sows in late gestation[1]

Item	Microbial phytase:	0 unit/kg diet			1,000 unit/kg diet			SEM	*P*-value		
	Vitamin D3 metabolite:	-	25-OH-D_3	1-OH-D_3	-	25-OH-D_3	1-OH-D_3		Phytase	Vit D_3	Phytase × Vit D_3
ATTD of dry matter, %		85.45^{b}	87.57^{ab}	88.32^{a}	87.41^{ab}	88.48^{a}	86.77^{ab}	0.71	0.345	0.021	0.009
ATTD of gross energy, %		84.91^{b}	87.02^{ab}	87.65^{a}	86.35^{ab}	87.73^{a}	85.78^{ab}	0.77	0.852	0.023	0.021
Digestible energy, kcal/kg		3,303^{b}	3,385^{ab}	3,410^{a}	3,359^{ab}	3,413^{a}	3,337^{ab}	30	0.852	0.023	0.021
Metabolizable energy, kcal/kg		3,156^{c}	3,244^{ab}	3,249^{a}	3,219^{abc}	3,255^{a}	3,166^{bc}	32	0.889	0.088	0.029

$^{a\text{-}c}$Within a row, means without a common superscript differ ($P < 0.05$).

[1]Each least squares mean represents 10 observations except that there were only 9 observations for the 2 diets containing 25-OH-D_3.

Table 3. Apparent total tract digestibility (ATTD) of Ca and P and retention of Ca and P in diets fed to sows in late-gestation[1]

Item	Microbial phytase, unit/kg diet		SEM	*P*-value	Vitamin D_3 metabolite			SEM	*P*-value
	0	1,000			-	25-OH-D_3	1-OH-D_3		
ATTD of Ca, %	24.50	32.30	3.87	0.036	17.73[b]	30.38[a]	37.10[a]	4.27	< 0.001
Ca retention, % of intake	18.43	26.53	4.24	0.026	13.56[b]	25.47[a]	28.41[a]	4.59	0.003
ATTD of P, %	40.28	51.02	2.25	< 0.001	35.56[b]	48.51[a]	52.88[a]	2.51	< 0.001
P retention, % of intake	34.30	39.40	2.59	0.036	29.65[b]	41.94[a]	38.97[a]	2.85	< 0.001

[a-b]Within a row, means without a common superscript differ (P < 0.05).

[1]Each least squares mean represents 10 observations except that there were only 9 observations for the 2 diets containing 25-OH-D_3.

and P. The ATTD of P in lactating sows was not affected by adding 25-OH-D_3 to diets containing 100% of the Ca and P required by lactating sows (Zhang et al., 2019), but no interaction was observed between dietary Ca and P levels and supplementation of 1-OH-D_3 in the first experiment. It therefore appears that supplementation of late-gestation diets with 1-OH-D_3 positively affects Ca and P balance regardless of dietary Ca and P.

Effects of using both phytase and 1-OH-D_3 in P-deficient diets fed to broilers were additive (Snow et al., 2004), but this was not the case in diets for pigs (Biehl and Baker, 1996). Results from the second experiment demonstrated that effects of addition of phytase and vitamin D metabolites were not additive in diets for sows and the results, therefore, are in agreement with the data by Biehl and Baker (1996). Although there were no interactions between phytase and vitamin D metabolites for Ca and P balance, interactions were observed for the ATTD of DM and GE and concentration of DE and ME in diets, which to our knowledge has not been previously demonstrated. The mechanisms for these interactions are, however, not clear and additional research is needed to elucidate the reasons for this observation.

The level of vitamin D_3 in all diets was well above the presumed requirement for sows in gestation (i.e. 20 µg/kg; NRC, 2012), but the fact that the ATTD of Ca and P and retention of Ca and P increased by adding one of the vitamin D metabolites to the diets indicates that sows are not able to convert sufficient quantities of vitamin D_3 to calcitriol to maximize Ca and P balance. It is not clear why the vitamin D metabolites are so effective in sows fed diets containing vitamin D_3 in excess of the requirement. It appears that sows have difficulty hydroxylating vitamin D_3 to 1,25-dihydroxycholecalciferol, whereas use of one of the vitamin D metabolites results in increased synthesis of 1,25-dihydroxycholecalciferol. Conversion of 25-OH-D_3 to 1,25-dihydroxycholecalciferol in women in wk 12 of pregnancy is two-fold greater than in non-pregnant women (Hollis and Wagner, 2017), and it is, therefore, possible that pregnancy increases the need for calcitriol, but because we did not include non-pregnant females in this experiment we cannot confirm this hypothesis. It is also possible that the increase in 1,25-dihydroxycholecalciferol synthesis in sows fed a vitamin D metabolite is beneficial to their progeny because supplementation of diets for sows with 25-OH-D_3 resulted in increases in blood vitamin D, growth performance, and bone mineralization of their offspring (Witschi et al., 2011; Flohr et al., 2016). However, more research is needed to confirm this hypothesis.

To be converted to the calcitriol, 25-OH-D_3 skips the hydroxylation step in the liver and 1-OH-D_3 skips the hydroxylation step in the kidneys. The observation that the 2 vitamin D metabolites are equally effective in increasing ATTD of DM, GE, Ca, and P indicates that it is the double hydroxylation that is problematic for sows, whereas it appears to be less important which hydroxylation step needs to be completed if a vitamin D metabolite is provided.

The increases in DE and ME in diets containing 1-OH-D_3 were a result of increased ATTD of DM. This was observed in both experiments (Lee and Stein, 2022; Lee et al., 2022) and confirms that 1-OH-D_3 increases energy concentrations in diets by increasing the ATTD of DM when no phytase was used.

Conclusions

In conclusion, there were no interactions between levels of dietary Ca and P and supplemental 1-OH-D_3 and between use of vitamin D metabolites and microbial phytase on Ca and P balance in sows in late gestation. The ATTD and retention of Ca and P and concentrations of DE and ME in diets fed to sows in late gestation were not affected by dietary Ca and P, but supplementation of 25-OH-D_3 and 1-OH-D_3 increased the ATTD and retention of Ca and P. Supplementation of 1-OH-D_3 increased the ATTD of DM and GE and concentrations of DE and ME in diets containing no microbial phytase. No effect of phytase was observed for the ATTD of GE and concentrations of DE and ME in diets.

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Injecting Different Amounts of Iron – Effects on Blood Parameters During Late Nursery-Early Grower Phase

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Summary

Overall, iron status of young pigs has remained a hot topic within the swine industry ever since the industry-wide adoption of confinement production. Without iron supplementation shortly after birth, piglets become iron deficient quickly and remain in an iron-deficient state for several weeks until obtaining iron supplementation in the feed. Administering an iron injection at birth does improve overall growth and complete blood count (CBC) profile of piglets. However, the dosage of the iron injection administered impacts the magnitude and timing of peak hematological responses. Furthermore, following a standard iron injection (100 – 200 mg Fe) at birth there are still pigs that exhibit decreasing CBC profiles immediately before and after weaning. In an attempt to optimize hematological status at weaning, an experiment at UK that administered an additional iron injection several days prior to weaning resulted in an improved (i.e., increasing) CBC profiles at weaning compared to decreasing CBC profiles of pigs only receiving an iron injection at birth. Furthermore, pigs receiving the additional iron injection had a 1 kg increase in body weight at 28 days postweaning. Thus, the historical issue of iron deficiency anemia in young pigs remains a current reality worthy of continued research and its understanding has potential for significant improvement in health and performance.

Introduction

It is common practice in modern swine production to provide newborn piglets with supplemental iron to prevent iron deficiency, usually through an intramuscular (IM) injection of an iron complex, because piglets are born with very low iron stores (~ 50 mg of iron) and only receive ~ 1 mg of iron each per day through the sow milk (Venn et al., 1947). Moreover, it has been suggested that the iron requirement of piglets during lactation is mainly dependent on the growth rate of the piglet during the lactation period (Venn et al., 1947; Kamphues et al., 1992; Egeli and Framstad., 1998; Van Gorp et al., 2012). Modern swine production has undergone improvements in genetics which have resulted in increased litter sizes and growth rates of nursing piglets. Given these advancements, recent work has shown that the industry standard iron injection (100-200 mg Fe) administered to piglets shortly after birth is not sufficient to maintain the iron status of the pig throughout the lactation period (Bhattarai and Nielsen, 2015a; Perri et al., 2016). Unsurprisingly, faster-growing pigs in a litter are most susceptible to becoming iron deficient at weaning creating potential postweaning problems (Jolliff and Mahan, 2011).

Recently, Chevalier et al. (2021) reported that pigs administered 200 mg iron at birth had peak hemoglobin (Hb) concentration on d 17 that subsequently declined to weaning at d 22. Furthermore, pigs that only received 100 mg iron at birth had peak Hb at d 11 with a decline in Hb thereafter. These results agree with previous literature which suggests a possibility of pigs having sub-optimal hemoglobin concentrations (generally < 11 g/dL as defined by Thorn, 2010; Bhattarai and Nielsen, 2015b; Perri et al., 2016) at weaning. Interestingly, it has been demonstrated that pigs with optimal hemoglobin concentrations at weaning have greater growth performance in the subsequent postweaning periods (Bhattarai and Nielsen, 2015b; Fredericks et al., 2018). Furthermore, Olsen (2020) demonstrated that an additional iron injection given to piglets 4 to 6 days after the initial iron injection resulted in a much larger percentage (89 % vs. 20 %) of pigs having optimal Hb concentration (≥ 11 g/dL) at weaning and a 454 g increase in body weight (BW) 42 d postweaning. Thus, there is a continued need to reevaluate our iron supplementation practices as there may be a potential benefit to administration of an additional iron injection before weaning.

Experimental Procedures

Experiment 1

The objective of this study was to evaluate the course of the blood profile, growth performance, and tissue mineral concentration of pigs pre and postweaning after receiving various iron injection dosages at birth. This study has been published in the Journal of Swine Health and Production (Chevalier et al., 2021) and can be read in its entirety there; portions of the complete study are presented herein.

Crossbred pigs (n = 70) from 7 litters were used. At birth piglets were weighed and randomly allotted within litters to 5 different iron dextran injection treatments that consisted of 0, 50, 100, 200, and 300 mg iron through an intramuscular (IM) injection of an iron dextran product (100 mg/mL, Henry Schein Animal Health, Dublin, OH). Injections were administered after an initial blood sample was collected on day 0. Fifty pigs were used for blood sampling for hematological measures. Blood samples (3 mL) and BW were taken on day 0, 1, 2, 3, 4, 6, 8, 11, 13, 17, 22 (weaning), 23, 24, 25, 29, 38, and 52. Blood samples were then analyzed for a complete blood count (CBC) at the University of Kentucky Veterinary Diagnostic Laboratory. The CBC consisted of hemoglobin (Hb), hematocrit (HCT), red blood cell (RBC), white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

On day 22 (weaning), 38, and 52, a total of 15 pigs (3 pigs/treatment) for each time point were sacrificed for tissue samples (liver, heart, and spleen). The pigs sacrificed on day 22 were from the pigs not used for blood sampling, those sacrificed on day 38 included 5 pigs not used for blood sampling and 10 pigs used for blood sampling, while those sacrificed on day 52 were all from those included in the blood samplings. All tissues were ground and mixed to a homogenous mixture and a subsample digested and analyzed for trace mineral content (Fe, Zn, Cu, and Mn).

The sow lactation diet was a corn-soybean meal based diet that was formulated to meet or exceed NRC (2012) nutrient requirement estimates for gestating and lactating sows. Furthermore, the lactation diet was formulated to supply an added 100 mg/kg iron as ferrous sulfate and was provided to all sows ad libitum during lactation. A common nursery diet was provided to all pigs after weaning; it was formulated to meet or exceed the nutrient requirement estimates (NRC, 2012) of 7 to 25 kg pigs and was supplemented with 100 mg/kg iron as ferrous sulfate.

Experiment 2

Because of the peak Hb occurring about d 17 in the previous experiment, the objective of the present experiment was to evaluate the effects of administering an additional iron injection 4 days before weaning on growth performance, hematological status, and tissue mineral concentration both pre and postweaning.

A total of 136 crossbred pigs [82 barrows and 54 gilts; (Yorkshire x Landrace) x Large White] from 20 litters with an initial BW of 5.48 ± 1.08 kg were assigned to either a control (CON) or an added-injection(+Fe) group at around 14-20 days of age (depending on the scheduled weaning date) through a pairing scheme. At birth all pigs were subjected to normal farm processing procedures (tail docking, ear notching, and clipping needle teeth). At this same time, all piglets received a 150 mg iron intramuscular (IM) injection of iron dextran (Henry Schein Animal Health, Dublin, OH) in the right side of the neck. The pairing process selected pigs based on the following basis: two same-sex pigs from the same litter with a BW difference of < 0.9 lbs. Within a given pair, one pig was assigned to the +Fe group and the other pig to the CON group. Pigs assigned to the +Fe group received an additional 150 mg iron IM injection 4 days before weaning (14-20 days). In contrast, the pigs allotted to the CON group received no additional injection at this time.

Pigs were weaned to a nursery site 4 days following the additional iron injection of the treatment pigs (18-24 days of age for the pigs). In the nursery, pigs were allotted to pens based on BW, treatment, and sex. Pens consisted of 3 to 5 pigs per pen and were equalized between treatments. A nursery diet was formulated to meet or exceed the NRC (2012) nutrient requirement estimates for 7 to 25 kg pigs and provided ad libitum access to both the CON and +Fe groups. The nursery diet was formulated to supply an added 100 mg/kg iron as ferrous sulfate. The experiment continued for 28 days in the nursery.

A total of 8 pigs (4 pigs per treatment) were selected for sacrifice on days -4 (pre-wean), 0 (weaning), 14, and 28. All pigs used for tissue mineral determination in this experiment were barrows. Liver and spleen samples were collected from the sacrificed pigs. All data (growth, CBC, and tissue mineral content) was subjected to ANOVA by using the GLM procedure in SAS (Statistical Analysis system, Cary, NC) with the individual pig as the experimental unit.

Results and Discussion

Experiment 1

Pigs that did not receive an iron injection at birth had the lowest numerical BW by d 8 that continued through d 52 (Table 1). By the end of Wk 2 (d 8-14) there was a quadratic increase in ADG as iron injection dosage increased. Following Wk 3 (d 14-22), the last week preweaning, the differences in ADG between treatments were even more noticeable. Average daily gain continued to be improved in a linear and quadratic fashion through Wk 4 and 5 (d 22-29 and 29-38; the first two weeks postweaning). There were no differences in weekly ADG thereafter; however, the linear

Table 1. Effects of iron injection dosage on individual BW and ADG during pre and postweaning (Experiment 1)*

	Iron injection, mg iron						P-value	
Variable	0	50	100	200	300	SEM	L	Q
BW (kg)								
d 0	1.45	1.45	1.44	1.46	1.49	0.05	.51	.73
d 1	1.60	1.58	1.56	1.63	1.64	0.06	.42	.63
d 2	1.76	1.74	1.71	1.77	1.86	0.07	.20	.26
d 3	1.96	1.93	1.93	1.98	2.06	0.07	.22	.42
d 4	2.13	2.10	2.11	2.15	2.25	0.08	.15	.40
d 6	2.55	2.53	2.59	2.59	2.70	0.08	.14	.63
d 8	2.97	3.01	3.08	3.05	3.19	0.10	.10	.92
d 11	3.57	3.76	3.84	3.73	3.89	0.12	.12	.60
d 14	4.20	4.55	4.59	4.51	4.64	0.15	.11	.31
d 17	4.75	5.33	5.35	5.32	5.34	0.18	.08	.09
d 22	5.48	6.69	6.62	6.67	6.63	0.25	.01	.01
d 23	5.27	6.44	6.44	6.51	6.39	0.24	.01	.01
d 24	5.44	6.93	6.87	6.93	6.77	0.26	.01	< .01
d 25	5.60	7.28	7.24	7.34	7.09	0.27	< .01	< .001
d 29	6.87	8.81	8.88	9.12	8.72	0.32	< .01	< .001
d 38	10.87	13.91	14.02	14.15	14.12	0.49	< .001	< .001
d 44	14.90	17.53	18.03	18.37	18.20	0.65	< .01	.01
d 52	20.14	22.77	24.02	23.48	23.51	0.77	.01	.01
ADG (g)								
d 0-8	189.7	195.5	205.0	199.2	212.3	7.92	.06	.89
d 8-14	204.7	257.3	250.8	241.9	241.3	12.39	.26	.04
d 14-22	160.1	266.7	253.4	271.2	247.1	18.96	.01	< .01
d 22-29	199.2	303.8	323.3	349.0	299.4	19.49	< .01	< .0001
d 29-38	445.0	566.4	571.8	559.0	599.4	24.94	< .001	.05
d 38-44	616.6	641.6	665.2	692.2	683.9	39.05	.14	.44
d 44-52	748.2	747.9	855.1	730.0	758.2	40.13	.76	.34
d 22-52	504.8	558.1	597.6	580.5	576.9	21.93	.04	.02
d 0-52	366.6	418.5	442.8	430.9	432.4	14.72	.01	.01

* Means represent 10 pigs/treatment that were assigned to 1 of 5 iron injection dosages administered on day 0. All pigs were weaned on day 22. On d 44 and d 52 means represent 8 pigs/treatment.

BW = body weight; ADG = average daily gain; d = day; SEM = standard error of the mean; L = linear; Q = quadratic.

and quadratic increase (P = .01) remained for overall ADG from birth (d 0-52). The improved ADG associated with increasing iron injection dosage resulted in heavier BW seen first statistically (P = .01) at weaning on d 22. The BW response to increasing iron dosage remained linear and quadratic (P ≤ 0.01) from d 22 to d 52.

The days leading up to weaning (day 17 to 21) have been shown to be important in regard to hematological measures declining below optimal levels (hemoglobin > 11g/dL) after receiving a standard iron injection administered early in life (Jolliff and Mahan, 2011; Bhattarai and Nielsen, 2015a; Perri et al., 2016). Optimizing the iron status at weaning has shown beneficial effects on subsequent growth performance (Fredricks et al., 2018) which may be a function of the improved oxygen transport, immune function, vitality, and metabolism (Von der Recke and Heisel, 2014). In agreement with the current study, Williams et al. (2020) reported linear and quadratic improvements in ADG from day 3 to 21 after pigs were administered increasing doses of iron (0, 50, 100, 150, and 200 mg iron) at processing.

In addition to poor growth performance, pigs that received no iron injection had the lowest Hb concentration at all sampling times with the exception of day 52 by which time it recovered (Figure 1). For Hb concentrations, a treatment effect was observed as well as effects of day and treatment × day interaction (P < .001, Figure 1). Both the 50 and 100 mg iron injection treatments had absolute hemoglobin concentrations that peaked at day 6 whereas the Hb concentration for the 200 and 300 mg iron treatments peaked at day 17 and then began to decline. This is in agreement with the theoretical model proposed by Van Gorp et al. (2012), suggesting that the iron supply from the initial iron injection will only last approximately 17 to 18 days. Additionally, as was observed with Hb values, the HCT, RBC, WBC, MCV, MCH, and MCHC were all impacted by the iron injection dosage as there was a treatment effect, day effect, and treatment × day interaction (P < .01, Figures 2-7).

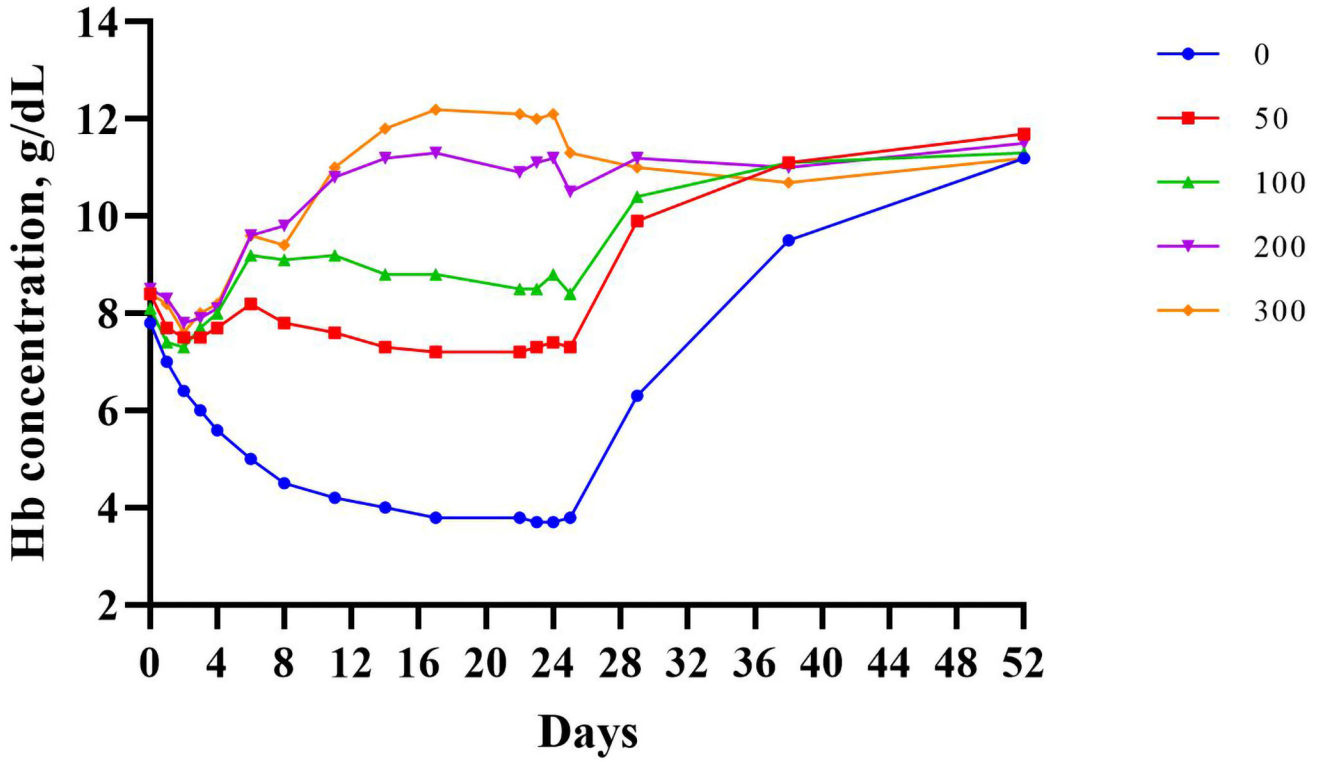

Figure 1. Effects of iron injection dosage on hemoglobin (Hb) concentration (Experiment 1; as presented in Chevalier et al., 2021).

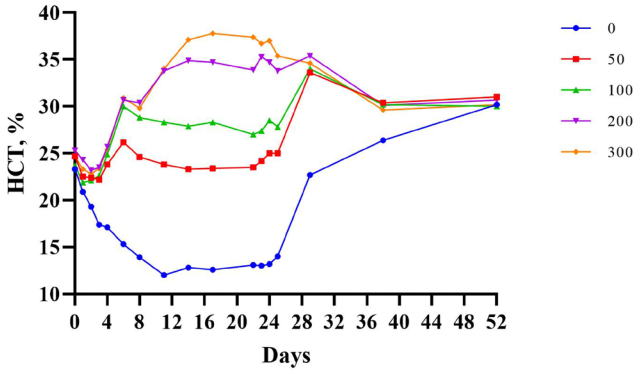

Figure 2. Effects of iron injection dosage on hematocrit (HCT) content (Experiment 1; as presented in Chevalier et al., 2021).

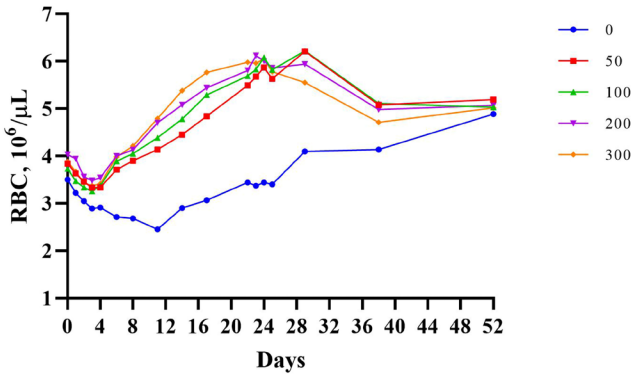

Figure 3. Effects of iron injection dosage on red blood cell (RBC) count (Experiment 1; as presented in Chevalier et al., 2021).

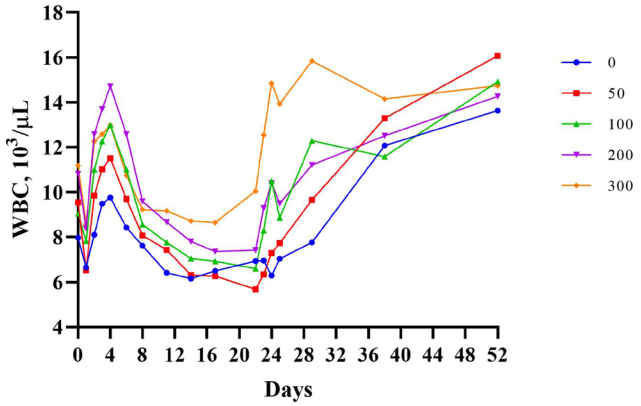

Figure 4. Effects of iron injection dosage on white blood cell (WBC) count (Experiment 1; as presented in Chevalier et al., 2021).

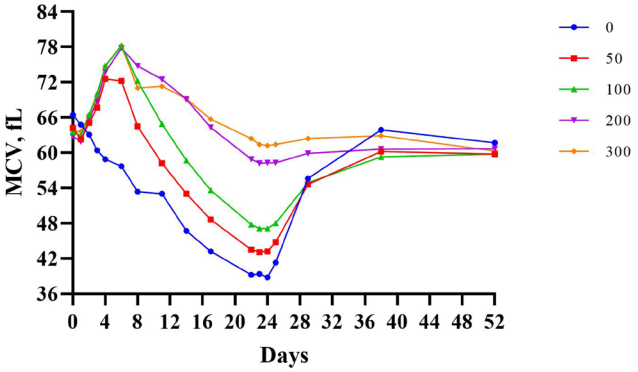

Figure 5. Effects of iron injection dosage on mean corpuscular volume (MCV) (Experiment 1; as presented in Chevalier et al., 2021).

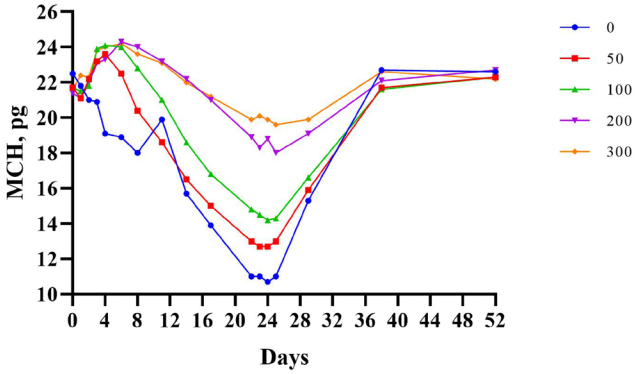

Figure 6. Effects of iron injection dosage on mean corpuscular hemoglobin (MCH) (Experiment 1; as presented in Chevalier et al., 2021).

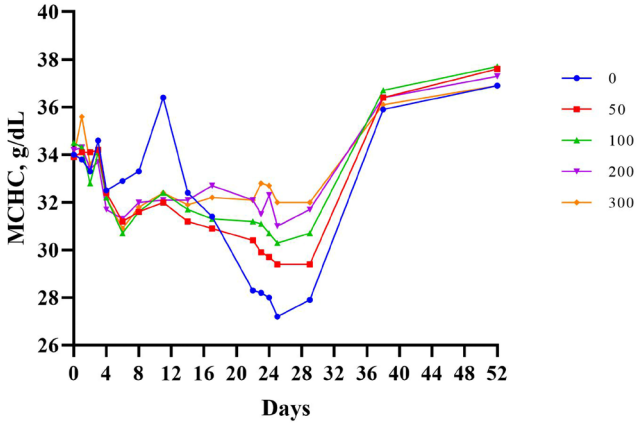

Figure 7. Effects of iron injection dosage on mean corpuscular hemoglobin concentration (MCHC) (Experiment 1; as presented in Chevalier et al., 2021).

Liver iron concentration (Table 2) was higher in response to increasing iron injection dosage at weaning (d 22) and d 38 ($P < 0.01$ and $P = 0.02$; respectively). While there was a significant linear response at both time points, a major observation was that the 300 mg iron treatment had tremendously higher liver iron concentrations at weaning that declined by 3 38 while all other treatments increased liver iron content from weaning to d 38. By d 52, there were no differences in liver iron content. The liver is a major site for ferritin and hemosiderin which are iron storage compounds that act as a reserve which is used for hemoglobin synthesis (Dallman, 1986). Iron transport through the body is dependent on the transport protein transferrin. Transferrin delivers iron at a rate dependent on the pace of RBC production which is dependent on the overall iron status of the individual (Huebers and Finch, 1984).

Experiment 2

At the time of administration of the additional iron injection to the treatment group, the CON and +Fe group

Table 2: Effects of iron injection dosage on liver mineral content (Experiment 1)*

	Iron injection, mg Fe						P-value	
Variable	**0**	**50**	**100**	**200**	**300**	**SEM**	**L**	**Q**
d 22								
BW (kg)	6.39	6.61	5.03	6.20	6.04	0.53	.71	.37
Liver wt (g)	193.03	223.70	159.17	213.15	214.97	18.46	.44	.48
Liver wt (% BW)	3.03	3.40	3.18	3.43	3.58	0.21	.13	.91
Fe (mg/kg DM)	95.8	143.0	204.5	402.9	1652.5	348.73	< 0.01	.15
Zn (mg/kg DM)	287.8	247.6	296.9	211.5	276.0	23.75	.47	.26
Cu (mg/kg DM)	413.6	415.6	482.8	448.1	413.7	56.34	.99	.43
Mn (mg/kg DM)	7.1	6.4	6.3	6.4	8.6	1.21	.36	.25
d 38								
BW, kg	9.73	14.94	14.30	13.84	14.51	1.16	.08	.09
Liver wt (g)	367.40	602.57	536.17	538.03	570.47	67.93	.20	.26
Liver wt (% BW)	3.72	3.99	3.74	3.89	3.91	0.25	.72	.94
Fe (mg/kg DM)	380.1	627.1	586.5	610.8	654.2	57.61	.02	.13
Zn (mg/kg DM)	146.0	137.8	117.2	100.8	107.4	17.26	.08	.35
Cu (mg/kg DM)	159.6	73.9	99.9	121.9	85.7	29.48	.38	.58
Mn (mg/kg DM)	5.8	6.1	7.3	5.7	6.6	0.35	.49	.38
d 52								
BW (kg)	21.19	22.27	24.49	22.53	22.61	2.38	.80	.52
Liver wt (g)	803.00	806.83	870.63	865.17	828.57	90.84	.78	.60
Liver wt (% BW)	3.80	3.63	3.56	3.84	3.66	0.11	.99	.69
Fe (mg/kg DM)	742.4	796.5	819.6	786.0	913.4	90.74	.27	.81
Zn (mg/kg DM)	114.5	129.5	132.6	182.1	180.2	17.78	.01	.58
Cu (mg/kg DM)	35.5	25.2	35.1	33.3	30.1	3.81	.80	.90
Mn (mg/kg DM)	4.2	5.5	3.3	2.8	3.1	0.67	.06	.54

* Pigs were administered 1 of 5 iron injection dosage treatments at day 0. All pigs were weaned on day 22. A total of 3 pigs/treatment were used for tissue analysis. All data are reported as least squares means.

BW = body weight; d = day; SEM = standard error of the mean; L = linear; Q = quadratic.

Table 3: Effects of an additional Fe injection on growth performance (Experiment 2)[1].

	Treatment[2]			
Item	**CON**	**+Fe**	**SEM**	**P-value**
BW, kg				
d -4	5.43	5.46	0.02	0.21
d 0 (weaning)	6.49	6.51	0.03	0.73
d 7	8.22	8.38	0.05	0.03
d 14	11.50	11.96	0.09	<0.001
d 21	16.49	17.29	0.14	<0.001
d 28	21.65	22.61	0.19	<0.001
ADG, g				
d -4 to 0	266.1	261.2	5.74	0.55
d 0 to 7	246.5	268.4	6.86	0.03
d 7 to 14	469.1	510.9	7.67	<0.001
d 14 to 21	661.2	704.6	11.60	0.01
d 21 to 28	737.3	760.4	11.10	0.14
d -4 to 28	498.0	526.2	5.57	<0.001

[1] Treatment means represent 60 pigs/treatment; reduced to 56 pigs/treatment after d 14. Weaning is represented as d 0.
[2] CON = control group; +Fe = the added injection group.

Table 4. Effects of an additional Fe injection on CBC profile (Experiment 2)[1].

	Treatment[2]			
Item	**CON**	**+Fe**	**SEM**	**P-value**
d -4				
Hb, g/dL	10.83	10.75	0.15	0.74
HCT, %	32.94	32.78	0.44	0.80
RBC, M/µL	5.62	5.70	0.06	0.39
WBC, K/µL	7.89	8.06	0.26	0.64
MCV, fL	58.69	57.63	0.57	0.19
MCH, pg	19.33	18.92	0.20	0.15
MCHC g/dL	32.90	32.83	0.13	0.65
d 0 (weaning)				
Hb, g/dL	10.33	12.06	0.14	<.0001
HCT, %	31.31	36.65	0.43	<.0001
RBC, M/µL	5.83	6.26	0.07	<.0001
WBC, K/µL	7.71	9.32	0.29	0.0002
MCV, fL	53.77	58.77	0.57	<.0001
MCH, pg	17.74	19.35	0.20	<.0001
MCHC g/dL	32.98	32.93	0.15	0.82
d 14				
Hb, g/dL	11.55	11.90	0.10	0.02
HCT, %	35.16	35.63	0.32	0.30
RBC, M/µL	6.41	6.29	0.06	0.17
WBC, K/µL	14.42	14.69	0.41	0.64
MCV, fL	54.91	56.78	0.42	0.002
MCH, pg	18.05	18.97	0.15	<.0001
MCHC g/dL	32.85	33.39	0.12	0.001
d 28				
Hb, g/dL	12.83	12.79	0.11	0.82
HCT, %	38.13	38.10	0.35	0.94
RBC, M/µL	6.72	6.62	0.07	0.30
WBC, K/µL	13.23	12.78	0.51	0.53
MCV, fL	56.85	57.68	0.40	0.15
MCH, pg	19.13	19.39	0.15	0.21
MCHC g/dL	33.66	33.62	0.17	0.86

[1] Treatment means represent 60 pigs/treatment; reduced to 56 pigs/treatment after d 14. Weaning is represented as d 0.
[2] CON = control group; +Fe = the added injection group.

Table 5. Effects of an additional Fe injection on liver and spleen Fe concentration (DM basis, mg/kg; Experiment 2)[1].

	Treatment[2]			
Item	**CON**	**+Fe**	**SEM**	**P-value**
d -4				
Liver Fe		495.1		
Spleen Fe		151.1		
d 0 (weaning)				
Liver Fe	274.4	809.2	125.88	0.02
Spleen Fe	750.6	1024.2	100.01	0.10
d 14				
Liver Fe	476.5	536.4	36.26	0.29
Spleen Fe	802.0	885.0	82.92	0.51
d 28				
Liver Fe	598.9	600.4	41.29	0.98
Spleen Fe	711.1	847.1	117.15	0.44

[1] Means at d -4 represent 8 pigs; treatment means at d 0, 14 and 28 represent 4 pigs/treatment.
[2] CON = control group; +Fe = the added injection group.

had an initial BW difference of 0.03 kg (Table 3), which was essentially unchanged at weaning (0.02 kg). Once weaned, pigs from the +Fe group had greater ADG during weeks 1, 2, and 3 (all $P < 0.03$). Altogether the +Fe pigs had an increased ($P < 0.001$) ADG for the entire experimental period (d-4 to 28; $P < 0.001$) leading to a heavier final BW of approximately 1 kg. The current performance response agrees with previous work (Kamphues et al., 1982) which reported that pigs administered a second iron injection one week prior to a weaning at 28 days had an increase in daily BW gain (380 g vs. 362 g) through the following three weeks in the nursery. More recent work (Williams et al., 2020), however, found no advantage after administering an additional iron injection. Notably, Williams et al. (2020) administered the second iron injection on d 11 and weaned pigs on d 21 (thus, about 10 days preweaning) compared to the current experiment where the second injection was administered at 4 days preweaning.

Like the BW similarity at the time of the additional iron injection at d -4, pigs of both groups had similar CBC profiles. However, unlike the BW similarity at weaning, with regard to hematological effects (Table 4), the +Fe pigs had higher (all $P < 0.0002$) Hb, HCT, RBC, WBC, MCV, and MCH values. Interestingly, also at weaning the CON group had a numerical decrease in Hb, HCT, WBC, MCV, and MCH content compared to the previous sampling at d -4 while all of these measures increased from d -4 to weaning in the +Fe treatment group. Hemoglobin concentration continued to be higher ($P < 0.02$) in the +Fe group at d 14 sampling but not at d 28. Furthermore, at d 14, the MCV, MCH, and MCHC content was higher ($P \leq 0.002$) in pigs administered the additional iron injection. By the end of the experiment (d 28) there were no differences in the CBC profiles for both groups of pigs. The improvement in hematological measures at weaning after administering the additional iron injection 4 days previous was expected and agrees with other work in which an additional iron injection improved hematological measures (e.g., Haugegaard et al. [2008] administered a second injection at d 20 and weaned at d 34 and Williams et al. [2020] who was previously mentioned).

As with the hematological profile, the CON pigs had a numerical decrease in liver iron content (Table 5) compared to pigs sampled at d -4. The reduction in tissue iron concentration may be from the body pulling iron from tissue reserves to support normal erythropoiesis and hemoglobin synthesis. Dallman (1986) suggests that of all the iron sites (hemoglobin, serum iron, etc.), the storage sites are the last to be depleted in an iron deficient state. The +Fe group had a much higher ($P = 0.02$) liver iron content at weaning compared to the CON group. Furthermore, at weaning there was a tendency observed for a higher ($P = 0.10$) spleen iron content of the +Fe pigs.

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Effects and Functions of Copper on Nutrient Utilization in Growing Pigs

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Summary

Copper is involved in metabolic reactions and is important for oxidation-reduction reactions, transport of oxygen and electrons, and protection against oxidative stress. It is common to include high levels of Cu (i.e., 75 to 250 mg/kg) in nursery diets and, in some cases, grower-finisher diets may also include high levels of Cu to improve pig growth performance and health. However, the mechanism(s) by which dietary Cu exerts these positive effects on pig performance has never been fully elucidated. Results of recent experiments indicate that the consistently observed improvement in growth performance upon Cu supplementation is likely a result of the ability of dietary Cu to modulate intestinal microbial populations, stimulate secretion of neuropeptide Y and growth hormone, indirectly improve the immune response, and influence post-absorptive metabolism of lipids in pigs. However, the optimum amount and duration of feeding supplemental Cu in diets for pigs need to be further investigated. Future research also needs to focus on determining potential interactions of Cu with non-nutritive feed additives.

Introduction

Copper is involved in metabolic reactions including cellular respiration, tissue pigmentation, hemoglobin formation, and connective tissue development. Copper is an essential component of several metalloenzymes including ceruloplasmin, cytochrome C oxidase, lysyl oxidase, cytosolic Cu-Zn superoxide dismutase, extracellular Cu-Zn superoxide dismutase 3, monoamine oxidase, and tyrosinase (Manto, 2014). Copper, therefore, is important for oxidation-reduction reactions, transport of oxygen and electrons, and protection against oxidative stress. The requirement for Cu by pigs is influenced by dietary factors and age of the animal. Neonatal and growing pigs usually require 5 to 10 mg of Cu per kg of diet for normal metabolism and as pigs get older, the requirement for Cu decreases (NRC, 2012). The Cu that is included in pig diets usually originates from plant and animal based feed ingredients or from inorganic sources. Cereal grains, oilseed meals, and plant coproducts typically contain 4 to 30 mg/kg of Cu, but the amount of Cu present within each plant feed ingredient may vary depending on the variety, type of soil on which plants grow, maturity stage, and climatic conditions during growth (Underwood and Suttle, 1999). Supplemental Cu is provided by fortifying complete diets and premixes with inorganic Cu, which can be in the form of Cu sulfate (CuSO4), Cu chloride, chelated Cu, Cu amino acid complexes, monovalent Cu oxide, and Cu hydroxychloride.

Although the requirement for Cu is low, it is common practice to include high levels of Cu (i.e., 75 to 250 mg/kg) in nursery diets, and in some cases, grower-finisher diets may also include high levels of Cu. The objective of this paper is to present the current understanding of nutritional value of Cu and effects of pharmacological levels of Cu on growth performance, intestinal health, nutrient digestibility, gut microbiome, and lipid metabolism of pigs. Gaps that need to be addressed to maximize inclusion of Cu in diets to improve growth performance will also be discussed.

Growth Promoting Levels of Cu

One of the alternatives for antibiotic growth promoters is dietary pharmacological levels of Cu. Supplementing Cu to diets fed to weanling pigs at 75 to 250 mg/kg may reduce post weaning diarrhea and improve average daily gain (**ADG**) and average daily feed intake (Cromwell et al., 1998; Hill et al., 2000; Perez et al., 2011). Reduction in diarrhea frequency and increased feed efficiency were also observed when a high concentration of Cu was included in diets for pigs (Espinosa et al., 2017). Supplemental Cu also increases feed efficiency of nursery pigs exposed to a heat stress challenge (Espinosa et al., 2019b).

Addition of Cu at 250 mg/kg in diets for weanling pigs containing 5% animal fat improved growth performance, and it was speculated that this is due to the ability of Cu to improve animal fat utilization (Dove and Haydon, 1992; Dove, 1993). Therefore, a recent ex-

periment was conducted to determine the energetic value of dietary Cu in comparison with choice white grease (CWG; Espinosa et al., 2021). Pigs were fed diets with increasing concentrations of extracted fat by adding 2.0, 4.0, or 6.0% CWG to a diet based on corn, soybean meal, and distillers dried grains with solubles, which contained no CWG. Based on the improvement on feed efficiency that was observed upon supplementation of CWG to the diets, a prediction equation for the energetic value of each percent of CWG was generated. Two additional diets were formulated by adding 150 mg/kg of added Cu to the diet without added CWG and to the diet with 2% added CWG. Results indicated that supplementation of Cu to the diet without added CWG and to the diet containing 2% CWG improved feed efficiency of pigs. Based on the prediction equation, it was calculated that the improvement obtained by Cu supplementation was similar to the improvement in feed efficiency obtained by adding 2.8 to 3.8% CWG to the diets (Figure 1).

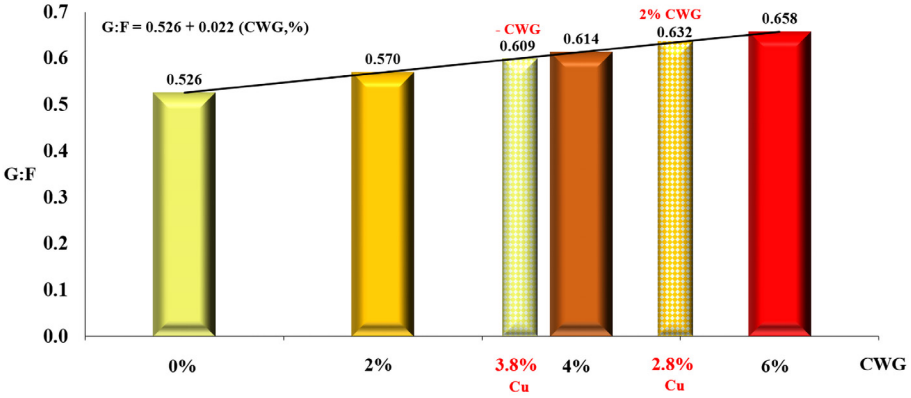

Figure 1. Choice white grease (CWG) equivalency of 150 mg/kg of Cu.

Effect of Cu on fat and energy digestibility

To understand how Cu increases feed efficiency in pigs, an experiment was conducted to test the hypothesis that supplemental Cu increases digestibility of fat and energy in pigs. In this experiment, 64 pigs were allotted to eight dietary treatments (Espinosa et al., 2021). A basal diet based on corn, soybean meal, and corn bran was formulated. Three additional diets were then formulated by adding 15, 30, and 45% distillers dried grains with solubles to the basal diet. The last four diets were formulated by adding 150 mg/kg of Cu to the first four diets. Results demonstrated that the apparent total tract digestibility (**ATTD**) of gross energy in diets containing supplemental Cu was not different from values for diets without supplemental Cu (Table 1). However, Cu improved the ATTD of fat by reducing the endogenous loss of fat (i.e., from 11.23 to 7.14 g/kg dry matter intake; Table 2). However, values for the true total tract digestibility of fat in diets without or with supplemental Cu were not different.

Effect of Cu on gut microbiome and health of pigs

One hypothesized mode of action for Cu is that Cu affects the bacteriostatic properties in the intestinal tract with a subsequent improvement in gastrointestinal health and immune function of pigs. Therefore, an experiment was designed to test the hypothesis that supplemental Cu changes concentration of microbial protein in the small intestine or

Table 1. Apparent total tract digestibility (ATTD) of dry matter (DM), gross energy (GE), and acid hydrolyzed ether extract (AEE) of pigs fed diets with increasing concentrations of distillers dried grains with solubles (DDGS) without or with 150 mg/kg Cu[1].

	No added Cu				150 mg/kg Cu					P-value		
Item	0% DDGS	15% DDGS	30% DDGS	45% DDGS	0% DDGS	15% DDGS	30% DDGS	45% DDGS	Pooled SEM	DDGS	Cu	DDGS × Cu
ATTD of DM	82.2	81.9	83.4	84.9	81.5	82.1	83.2	84.4	0.60	<0.001	0.399	0.882
ATTD of GE	82.3	81.7	82.8	84.4	81.6	81.7	82.4	83.4	0.69	0.002	0.204	0.819
ATTD of AEE	41.4	38.5	53.6	64.0	52.7	55.0	63.7	70.0	3.88	<0.001	<0.001	0.594

[1]Data are least squares means of 8 observations for all treatments.

Table 2. Regression coefficients of apparent total tract digested acid hydrolyzed ether extract (AEE; g/kg dry matter intake) on dietary AEE intake (g/kg dry matter) of pigs fed diets with increasing concentrations of distillers dried grains with solubles (DDGS) without or with 150 mg/kg Cu[1].

Item	Regression equation	Slope		Intercept		R^2	Estimated TTTD[2] of AEE	Estimated endogenous loss of fat[3]
		SE	P-value	SE	P-value			
DDGS	y = 0.8282x – 11.23	0.0660	<0.001	2.6737	<0.001	0.85	0.828	11.23[y]
DDGS + Cu	y = 0.8185x – 7.14	0.0404	<0.001	1.6305	<0.001	0.94	0.819	7.14[x]

[x,y]Means that do not have a common superscript tended to differ (P < 0.10).
[1]Regression analyses of apparent total tract digested AEE on dietary AEE intake was linear (P < 0.01).
[2]TTTD = true total tract digestibility.
[3]Gram per kilogram dry matter intake.

in the large intestine of pigs (Espinosa et al., 2019a). Results indicated that supplementation of Cu to diets reduced the concentrations of total volatile fatty acids and microbial protein in feces. Therefore, these observations indicate that the improved feed efficiency that was observed in pigs upon Cu supplementation is likely due to the effect of Cu in reducing selected microbial populations in the intestinal tract. This may have reduced the number of toxins and pathogenic microorganisms that could have negatively affected intestinal health, which may have reduced incidence of diarrhea and positively influenced immune response in pigs. Indeed, supplementation of Cu has been reported to positively influence cytokine concentrations and superoxide dismutase in blood serum of weanling pigs (Gonzales-Eguia, 2009; Espinosa et al., 2020a)

Effect of Cu on post-absorptive metabolism of lipids

In addition to the impact on intestinal health of pigs fed dietary Cu because of reduced microbial populations, the mode of action of Cu also was hypothesized to be related to systemic effects. Administration of high concentration of Cu via intravenous injection improved growth performance in previous experiments. This response was hypothesized to be attributed to the effect of Cu on increasing the mRNA expressions of growth hormone releasing hormone and neuropeptide Y in the hypothalamus of pigs (Li et al., 2008; Yang et al., 2011). In rabbits and fish, the effect of Cu on improving body mass gain is attributed to its role in increasing mRNA expression of fatty acid binding proteins and fatty acid transport proteins (Chen et al., 2016; Lei et al., 2017). To test the hypothesis that similar results can be obtained in pigs, another experiment was designed to investigate effects of Cu on feed efficiency and mRNA abundance of genes involved in lipid metabolism of pigs (Espinosa et al., 2020b). It was demonstrated that supplementation of Cu to diets increased mRNA abundance of lipoprotein lipase, fatty acid binding protein, and carnitine palmitoyl transferase 1B in the subcutaneous adipose tissue of pigs. Therefore, it was concluded that it is possible that the improved growth performance of pigs fed the Cu-supplemented diets is a result of improved lipid metabolism, which may have improved energy utilization.

Gaps

Supplementation of Cu to diets for weanling and young growing pigs has been a common practice due to its consistent improvement in growth performance. However, this may not be the case with finishing pigs as the response in growth performance is quite variable (Coble et al., 2018). Davis et al. (2002) demonstrated increased final body weight of finishing pigs fed diets supplemented with 175 mg/kg of Cu. However, Carpenter et al. (2017) and Forouzandeh et al. (2022) reported no positive effect of supplementing Cu at 130 to 250 mg/kg. In contrast, finishing pigs had increased ADG and feed efficiency when fed diets containing 20 mg/kg of supplemental Cu (Wen et al., 2022). Therefore, the optimum amount and duration of feeding supplemental Cu in diets fed to pigs also need to be further investigated.

Aside from Cu, other alternatives to antibiotic growth promoters are commonly used. These alternatives include probiotics, acidifiers, prebiotics, phytobiotics, and dietary pharmacological levels of Zn (Liu et al., 2018). Use of exogenous enzymes (e.g., xylanase and phytase) in pig diets is also rapidly increasing to enhance nutrient digestibility and pig performance. In some cases, commercial pig diets contain one or more of these feed ingredients; however, the mechanism of action and interactions among these products have not been fully elucidated. Therefore, further research needs to focus on determining potential interactions of Cu with non-nutritive feed additives.

Conclusion

Dietary inclusion of 75 to 250 mg/kg of Cu reduces diarrhea frequency and improves growth performance of

pigs. Results of several experiments demonstrated that the consistent improvement in growth performance of young growing pigs upon Cu supplementation to diets is likely a result of the ability of dietary Cu to modulate intestinal microbial populations, stimulate secretion of neuropeptide Y and growth hormone, indirectly improve the immune response, and influence post-absorptive metabolism of lipids in pigs. To maximize the use of Cu in pig diets, potential interactions of Cu with non-nutritive feed additives, as well as the optimum amount and duration of feeding supplemental Cu in diets fed to pigs need to be further investigated.

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