# REDUCING CRUDE PROTEIN LEVELS IN DIETS FED TO YOUNG PIGS TO OPTIMIZE GROWTH PERFORMANCE AND INTESTINAL HEALTH

BY

JOSEPH RAYMOND LIMBACH

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Animal Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2020

Urbana, Illinois

Master's Committee:

Professor Hans H. Stein, Chair
Professor Michael Ellis
Assistant Professor Maria Cattai de Godoy

# ABSTRACT

Two experiments were conducted to evaluate the effects of reducing the dietary crude protein (**CP**) concentration in diets fed to weanling pigs on growth performance, post-weaning diarrhea (**PWD**), blood characteristics, gut morphology, and products of protein fermentation. Both experiments used the same formulation of 3 corn-soybean diets to generate 3 experimental treatments: 3 diets contained 22% and 19% CP that provided amino acids (**AA**) at the requirement, and another diet contained 16% CP that did not provide AA to meet the requirement. A two-phase feeding program was used in both experiments. In the first experiment, thirty-six weaned pigs (7.87 ± 0.42 kg) were randomly allotted to the 3 dietary treatments with 12 replicate pigs per treatment to test the hypothesis that a reduction in dietary CP may improve blood characteristics associated with protein utilization, and improve serum vitamin levels, with a minimal negative effect on growth performance. Pigs were placed in individual metabolism crates, which acted as an additional stressor post-weaning. Results demonstrated that pig growth performance during the initial two weeks post-weaning was not affected ($P > 0.10$) by the reduction of dietary CP, however, overall body weight (**BW**), average daily gain (**ADG**), and the gain to feed ratio (**G:F**) were linearly ($P < 0.05$) decreased for the 28-d experiment. Blood urea nitrogen (**BUN**) concentration was the greatest ($P < 0.05$) on d 14, but the reduction of CP in the diet decreased ($P < 0.05$) BUN concentration. Both albumin and vitamin A in blood serum were the lowest ($P < 0.05$) on d 14. Serum vitamin E, however, decreased ($P < 0.05$) over the duration of the experiment. The hypothesis of the second experiment was that reducing CP in diets for weanling pigs, while still providing AA that meet the requirement, will reduce PWD and improve indicators of intestinal function, such as impacting gastrointestinal pH, improving intestinal morphology, and influencing the expression

of inflammatory and gut-protective genes, while having no negative effect on pig growth performance. One hundred-eighty weaned pigs (5.53 ± 0.88 kg) were randomly allotted to the 3 treatments with 12 replicate pens per treatment. Results demonstrated that overall BW, ADG, G:F, and diarrhea scores were linearly ($P < 0.05$) reduced during the 28-d experiment. Serum concentrations of total protein, albumin, peptide YY, and vitamins A and E were the lowest ($P < 0.05$) on d 13. Pigs fed the 16% CP diet had reduced ($P < 0.05$) serum albumin concentrations and tended ($P < 0.10$) to have reduced vitamin E concentrations compared with pigs fed the 22 or 19% CP diets. Serum BUN, haptoglobin, interleukin-1β, and interleukin-6 concentrations were the greatest ($P < 0.05$) on d 13, whereas serum concentrations of tumor necrosis factor-α and interleukin-10 were the greatest ($P < 0.05$) on d 6. Villus height and the villus height:crypt depth ratio in the jejunum, and crypt depth in the ileum were improved ($P < 0.05$) when pigs were fed the diet with 19% CP. In the stomach, pH tended ($P < 0.10$) to increase with the reduction of dietary CP, whereas in the ileum, pH was reduced ($P < 0.05$) with the reduction of dietary CP. Expression of interferon-γ, chemokine ligand 9, chemokine ligand 10, occludin, zonula occludens protein-1, trefoil factor-2, trefoil factor-3, mucin-2, GLUT2, and GLUT5 were all decreased ($P < 0.05$) when pigs were fed the 16% CP diet, whereas expression of transforming growth factor-β was increased when the 16% CP diet was fed instead of the 22 or 19% diets. In conclusion, low CP diets for weanling pigs may be used for the initial post-weaning period to reduce piglet susceptibility to PWD without largely impacting growth performance. If this strategy is to be fed for longer than the initial post-weaning period, meeting the AA requirement becomes crucial to maximizing protein synthesis, which effects both growth performance and maintenance of the gut architecture.

**Key words:** crude protein, growth performance, diarrhea, blood characteristics, pH, intestinal morphology, gene expression, pigs

# ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor, Dr. Hans Stein, for providing me with the opportunity to join your world-renowned team. I will be forever grateful to you for providing me with incredible opportunities to learn and develop professionally under your guidance and supervision. Some of my projects were frustrating at times, but your patience and understanding was undeniable. It was certainly an honor to be a part of the Stein Monogastric Nutrition Laboratory.

Secondly, I would like to acknowledge DSM for both the financial support needed to conduct these experiments and the collaboration on some laboratory analysis.

Next, I would like to express my gratitude to the members of my committee: Dr. Michael Ellis and Dr. Maria Cattai de Godoy. I am very appreciative of the knowledge and advice you have shared with me in my time here at the University of Illinois.

An enormous thank you deserves to be given to all the members of our team, both past and present: graduate students, post-docs, visiting scholars, farm employees, and laboratory workers. All of your support and collaboration was an integral part of completing my research. I would like to send a special thank you to Kate Stewart, our research manager at SRC. Thank you for your guidance in my projects and for allowing me to help at the farm so often; but perhaps more importantly, thank you for your friendship and support. I would also like to thank Dr. Charmaine Espinosa for her advice and assistance with some of my laboratory analysis.

Finally, I would like to acknowledge all of my friends. Thank you for your support through this stressful process. Whether we are playing sports or simply socializing, your company and encouragement have helped push me through this journey. A special thank you

goes to my three Colombian amigos, because without them, this journey would have been much more difficult.

Last, but certainly not least, I am grateful for my family that has always supported me. To my parents, Dennis and Twyla, I am beyond thankful for your unconditional love and support. I will forever be grateful that you push me to be a better man, even if that means I have to be away from home. To my siblings, Reagan and Thomas, thank you for taking care of things at home while I am gone and for always having eventful stories to share with me. I would also like to express my gratitude to my Grandma Janice and Grandma Lucille, both of which pushed me to the best scholar possible and showered with me love and support. This journey of attaining my Master's degree is an experience I will never forget.

# TABLE OF CONTENTS

# CHAPTER 1: INTRODUCTION

Weaning of pigs is accompanied with nutritional, environmental, or social stresses that result in a post-weaning lag in growth (Ravindran and Kornegay, 1993). In addition, a disruption in the function and integrity of the intestinal barrier and intestinal inflammation (Peace et al., 2011), both of which can cause diarrhea, are often experienced. Post-weaning diarrhea causes heavy economic losses in pig herds (Vondruskova et al., 2010). For decades, feed-grade antibiotics have been used to reduce pathogen infection (Roselli et al., 2005) and increase nutrient utilization, resulting in an improvement in growth performance (Close, 2000). One of the largest concerns with feeding antibiotics is that bacteria become resistant (Langlois et al., 1984). This concern has led to restrictions on the use of sub-therapeutic antibiotics in both the European Union and the United States. Therefore, nutritional alternatives to antibiotic growth enhancers have been proposed: diet acidification, mineral supplementation at pharmacological levels, probiotics, enzymes, and the reduction of dietary crude protein (**CP**; Close, 2000).

Excess amounts of CP may increase the amount of nitrogen entering the large intestine where it is fermented, creating an ideal environment for proliferation of bacteria and subsequently increasing diarrhea (Stein and Kil, 2006). Results from multiple studies have demonstrated that reducing dietary CP decreases the prevalence of diarrhea (Heo et al., 2008; Yue and Qiao, 2008) and products of proteolytic fermentation (Nyachoti et al., 2006; Heo et al., 2008; Rist et al., 2013; Almeida et al., 2017), while having no effect or a positive effect on growth performance (Le Bellego and Noblet, 2002; Htoo et al., 2007; Fang et al., 2019). However, negative effects of reducing dietary CP on growth performance have been also observed (Hansen et al., 1993; Nyachoti et al., 2006).

Low CP diets results in reduced blood urea nitrogen or plasma urea nitrogen (Nyachoti et al., 2006; Fang et al., 2019), indicating an improvement in amino acid utilization efficiency (Kohn et al., 2005). Concentrations of vitamins A and E, both of which are substrates used in the antioxidant system, may also influence health of the weaned pig (Buchet et al., 2017).

Previous work has demonstrated that reducing dietary CP can reduce the amount of fermentable substrate that may be used for bacteria proliferation and improve subsequent health of weaned pigs (Nyachoti et al., 2006). With this in mind, the objectives of this work were:

1. To determine if a reduction in dietary CP may improve blood characteristics associated with protein utilization, and improve serum vitamin levels, with minimal negative effects on growth performance.
2. To determine if a reduction in dietary CP, while providing amino acids to meet dietary requirement, may improve indicators of intestinal function, such as impacting gastrointestinal pH, decreasing concentrations of ammonia and volatile fatty acids in contents of the large intestine and feces, and decreasing the expression of genes related with inflammation and increasing the expression of genes associated with gut-function, while having minimal negative effects on pig growth performance.

## LITERATURE CITED

Almeida, V. V., A. J. C. Nuñez, A. P. Schinckel, P. V. A. Alvarenga, F. R. Castelini, Y. V. Silva-Guillen, and M. C. Thomaz. 2017. Interactive effect of dietary protein and dried citrus pulp levels on growth performance, small intestinal morphology, and hindgut fermentation of weanling pigs. J. Anim. Sci. 95:257-269. doi:10.2527/jas.2016.0498

Buchet, A., C. Belloc, M. Leblanc-Maridor, and E. Merlot. 2017. Effects of age and weaning conditions on blood indicators of oxidative status in pigs. PLoS. ONE. 12:e0178487-e0178487. doi:10.1371/journal.pone.0178487

Close, W. H. 2000. Producing pigs without antibiotic growth promoters. Adv. Pork Prod. 11:47-56.

Fang, L. H., Y. H. Jin, S. H. Do, J. S. Hong, B. O. Kim, T. H. Han, and Y. Y. Kim. 2019. Effects of dietary energy and crude protein levels on growth performance, blood profiles, and nutrient digestibility in weaning pigs. Asian Austral. J. Anim. Sci. 32:556-563. doi:10.5713/ajas.18.0294

Hansen, J. A., D. A. Knabe, and K. G. Burgoon. 1993. Amino acid supplementation of low-protein sorghum-soybean meal diets for 20- to 50-kilogram swine. J. Anim. Sci. 71:442-451. doi:10.2527/1993.712442x

Heo, J.-M., J.-C. Kim, C. F. Hansen, B. P. Mullan, D. J. Hampson, and J. R. Pluske. 2008. Effects of feeding low protein diets to piglets on plasma urea nitrogen, faecal ammonia nitrogen, the incidence of diarrhoea and performance after weaning. Arch. Anim. Nutr. 62:343-358. doi:10.1080/17450390802327811

Htoo, J. K., B. A. Araiza, W. C. Sauer, M. Rademacher, Y. Zhang, M. Cervantes, and R. T. Zijlstra. 2007. Effect of dietary protein content on ileal amino acid digestibility, growth performance, and formation of microbial metabolites in ileal and cecal digesta of early-weaned pigs. J. Anim. Sci. 85:3303-3312. doi:10.2527/jas.2007-0105

Kohn, R. A., M. M. Dinneen, and E. Russek-Cohen. 2005. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. J. Anim. Sci. 83:879-889. doi:10.2527/2005.834879x

Langlois, B. E., K. A. Dawson, T. S. Stahly, and G. L. Cromwell. 1984. Antibiotic resistance of fecal coliforms from swine fed subtherapeutic and therapeutic levels of chlortetracycline. J. Anim. Sci. 58:666-674. doi:10.2527/jas1984.583666x

Le Bellego, L., and J. Noblet. 2002. Performance and utilization of dietary energy and amino acids in piglets fed low protein diets. Livest. Prod. Sci. 76:45-58. doi:10.1016/S0301-6226(02)00008-8

Nyachoti, C. M., F. O. Omogbenigun, M. Rademacher, and G. Blank. 2006. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. J. Anim. Sci. 84:125-134. doi:10.2527/2006.841125x

Peace, R. M., J. Campbell, J. Polo, J. Crenshaw, L. Russell, and A. Moeser. 2011. Spray-dried porcine plasma influences intestinal barrier function, inflammation, and diarrhea in weaned pigs. J. Nutr. 141:1312-1317. doi:10.3945/jn.110.136796

Ravindran, V., and E. T. Kornegay. 1993. Acidification of weaner pig diets: A review. J. Sci. Food Agric. 62:313-322. doi:10.1002/jsfa.2740620402

Rist, V. T. S., E. Weiss, M. Eklund, and R. Mosenthin. 2013. Impact of dietary protein on microbiota composition and activity in the gastrointestinal tract of piglets in relation to gut health: a review. Animal. 7:1067-1078. doi:10.1017/S1751731113000062

Roselli, M., A. Finamore, M. S. Britti, P. Bosi, I. Oswald, and E. Mengheri. 2005. Alternatives to in-feed antibiotics in pigs: Evaluation of probiotics, zinc or organic acids as protective agents for the intestinal mucosa. A comparison of in vitro and in vivo results. Anim. Res. 54:203-218.

Stein, H. H., and D. Y. Kil. 2006. Reduced use of antibiotic growth promoters in diets fed to weanling pigs: Dietary tools, part 2. Anim. Biotech. 17:217-231. doi:10.1080/10495390600957191

Vondruskova, H., R. Slamova, M. Trckova, Z. Zraly, and I. Pavlik. 2010. Alternatives to antibiotic growth promoters in prevention of diarrhoea in weaned piglets: a review. Vet. Med. 55:199-224.

Yue, L. Y., and S. Y. Qiao. 2008. Effects of low-protein diets supplemented with crystalline amino acids on performance and intestinal development in piglets over the first 2 weeks after weaning. Livest. Sci. 115:144-152. doi:10.1016/j.livsci.2007.06.018

# CHAPTER 2: REVIEW OF THE LITERATURE – LOW CRUDE PROTEIN DIETS FED TO NURSERY PIGS

## INTRODUCTION

Weaning is one of the most stressful times in a pig's life. Maternal separation, co-mingling with pigs from other litters and/or nursery facilities, and the change of feed source leads to intestinal and immunological challenges that can disrupt the performance of a pig (Campbell et al., 2013). Any combination of these stressors can lead to a weakened immune system and gastrointestinal dysfunction, which increases the opportunity for pathogens to proliferate in the intestinal tract. This proliferation can potentially cause the loss of water and electrolytes through diarrhea, which then decreases pig growth performance. Post-weaning diarrhea (**PWD**) is one of the biggest challenges that pig producers face (Rhouma et al., 2017). Producers have used antimicrobial agents, or antibiotics, since the 1950s to control and prevent disease, as well as promote growth (Langlois et al., 1978; Cromwell, 2002; Phillips et al., 2004). Antibiotics may also be used to reduce morbidity and mortality in weanling pigs (Cromwell, 2002). However, as consumers become more interested in the food they are eating, they have become more concerned with the use of antibiotics in animal production and subsequent bacterial resistance, causing legislation on the use of antibiotics (Duttlinger et al., 2019). As of January 2017, the Food and Drug Administration's Veterinary Feed Directive (**VFD**) rule went into effect. The objective of the VFD is to eradicate the sub-therapeutic use of antibiotics for production purposes like growth promotion and feed efficiency. With the supervision of a licensed veterinarian, antibiotics are supposed to be used effectively and only when needed (FDA, 2015).

The increase in legislation has tasked nutritionists and producers with implementing alternative feed strategies to aid in the pig's transition after weaning and to combat the stresses that come along with weaning. The goal of these alternative nutritional strategies is to allow the pigs to recover from stressful events with the same efficiency as if antibiotics were used (Duttlinger et al., 2019). A review published by Stein and Kil (2006) discussed a variety of feeding schemes, such as feeding alternative cereal grains, decreasing the amount of dietary crude protein (**CP**), probiotic supplementation in the diet, plus other strategies that can be used to feed weanling pigs in the absence of feed-grade antibiotics. The concept behind feeding diverse cereal grains differs between the grains. In work by Pluske et al. (1996), inclusion of cooked rice, which has a low content of non-starch polysaccharides and a high content of easily fermentable carbohydrates, decreased carbohydrate fermentation in the large intestine and subsequently reduced the prevalence of intestinal infections. Probiotics, or direct-fed microbials (**DFM**), are live organisms that confer health benefits to the host and can be used to increase beneficial microbial populations in the gut (Liao and Nyachoti, 2017). Results from using probiotics are variable because health status plays a significant role in the improvement that is observed (Heo et al., 2013). Dietary supplementation of DFM is not as effective when provided to healthy animals or under sanitary environment conditions. Another option that can be used in the absence of antibiotics are acidifiers, which are molecules that can be used to acidify feed (Close, 2000). The immature development of the stomach and inability to produce enough hydrochloric acid (**HCl**) make it difficult for weaned pigs to maintain the optimum pH of 3.5 to efficiently digest protein and other nutrients (Doyle, 2001).

Another alternative strategy that can be used to improve intestinal function and growth performance of weanling pigs is to decrease the amount of CP in the diet (Stein and Kil, 2006).

By reducing dietary CP, the risk of gut disorders will be reduced because of reduced amounts of nitrogen reaching the large intestine (Wang et al., 2018). It is, therefore, hypothesized that reducing CP in the diet will decrease the amount of nitrogen reaching, and thus being fermented, in the large intestine. This reduction of nitrogen fermentation will create a less desirable environment for pathogenic bacteria to colonize and proliferate, resulting in an improvement in gut health and growth performance.

## PROTEIN DIGESTION

Crude protein consists of amino acid (**AA**) proteins and non-protein nitrogen. Amino acid protein is AA joined together by peptide bonds. After consumption of dietary protein, pepsinogen is secreted by chief cells in the fundic region (Bouhours et al., 1981). That pepsinogen is then activated to pepsin in the stomach by hydrogen ions in hydrochloric acid, which is secreted by parietal cells (Pond et al., 1995). Pepsin hydrolyzes between 15% and 50% of all peptide bonds in protein. If pH in the stomach is decreased to near 2, pepsin becomes more efficient, likely linked to the increase in HCl and its hydrogen ions that are needed to activate more pepsinogen (Pond et al., 1995). In total, the large protein molecules in feed are hydrolyzed to smaller polypeptide chains and passed into the small intestine (Wu, 2013). There, pancreatic enzymes such as trypsin, chymotrypsin, elastase, and carboxypeptidase act on these oligopeptides to reduce them to tri-peptides, di-peptides, or free AA (Wu, 2013). If the protein is not broken down into tri-peptides, di-peptides, or free AA, or if AA are present in excess amounts, they will not be absorbed by enterocytes in the small intestine and will continue to the large intestine where they will be fermented (NRC, 2012).

## PROTEIN FERMENTATION

High protein (21% - 24% CP) starter diets may increase microbial colonization and fermentation because of the undigested nutrients that pass through the stomach to the gastrointestinal tract (Ball and Aherne, 1987). These excess nutrients may provide an energy source for pathogens to proliferate. An increase in the end products of microbial fermentation, such as volatile fatty acids (**VFA**) and ammonia ($\mathbf{NH_3}$), are the result. Branched-chain fatty acids (**BCFA**), sulfur-containing metabolites, polyamines, and aromatic compounds such as phenolic and indolic compounds are also produced from protein fermentation (Wang et al., 2018). By lowering dietary CP, which ultimately means less excess nutrients, the occurrence and consistency of feces can be improved (Yue and Qiao, 2008). There are multiple ways to try to achieve the goal of decreasing CP. Bikker et al. (2006) discussed reducing CP in the diet and increasing fermentable carbohydrates in an effort to reduce nitrogen fermentation in weaned pigs. Hedemann et al. (2006) referenced Gibson and Wang (1994), showing that the increase in fermentable carbohydrates increases VFA production and lowers pH, which then negatively impacts the growth of pathogens. Increasing the inclusion of dietary fiber can also be used to dilute the amount of CP in the diet, thus decreasing protein fermentation (Rist et al., 2013; Jha and Berrocoso, 2016). This may be a result of increased carbohydrate fermentation increasing the need for nitrogen to be incorporated into microbial protein, and therefore, reducing the amount of protein available for fermentation in the hindgut.

Blood urea nitrogen (**BUN**) may be used as an indicator of AA utilization efficiency (Coma et al., 1995). Reduction of dietary CP results in a decrease in the amount of consumed nitrogen by the animal, and provided that the protein is balanced in AA, a greater proportion of dietary AA are used for protein synthesis and not as an energy-yielding material. Decreasing CP

in the diet decreases the amount of urea in the blood, measured as either plasma urea nitrogen (**PUN**) or BUN (Nyachoti et al., 2006; Heo et al., 2008; Yue and Qiao, 2008; Heo et al., 2009; Fang et al., 2019). Not only is BUN reduced with the reduction of dietary CP (Cho et al., 2008), but so are VFA levels in the small intestine (Nyachoti et al., 2006); this indicates that AA are being provided closer to the requirement and that less are being used for protein fermentation in the large intestine (de Lange et al., 2010). Protein is more likely to be fermented in the large intestine than carbohydrates because proteins in digesta are present along the entire gastrointestinal tract (Williams et al., 2005), whereas the carbohydrate fraction of digesta decreases as digesta moves distally. In the cecum, $NH_3$ and putrescine, which are synthesized after AA breakdown, were reduced with a decrease in dietary CP (Htoo et al., 2007). Similarly, acetic, isobutyric, and isovaleric acids, as well as total VFA concentrations were also decreased with a reduction in dietary CP (Htoo et al., 2007). This decrease in VFA in the intestine as CP content of the diet is reduced has been demonstrated several times (Nyachoti et al., 2006; Heo et al., 2008; 2009; Wang et al., 2011; Almeida et al., 2017). Reducing dietary CP from 21 to 17% reduces the cecum luminal ammonia nitrogen concentration, which was correlated with a lower fecal score and improvement in diarrhea (Opapeju et al., 2008).

However, results are not always consistent. Bikker et al. (2006) reported an increased $NH_3$ concentration in the ileum as CP was increased in the diet, but there were no differences in total VFA production. Alternatively, in the colon, there was a tendency for an increased $NH_3$ concentration and a greater total VFA concentration as dietary CP was increased. The microbiome and its diversity is a large factor in the nutrition and pathology of young pigs, especially directly post-weaning (Heo et al., 2013). The complexity surrounding the knowledge

and understanding of microbial activity is what creates the uncertainty about truly understanding these variable results.

## POST-WEANING DIARRHEA

de Lange et al. (2010) discussed that multiple authors have linked VFA and $NH_3$ production in the pathogenesis of diarrhea in pigs post-weaning. Microbial metabolites such as $NH_3$, amines, and VFA, which are influenced by dietary CP, may be associated with diarrhea in weaned pigs (Htoo et al., 2007), because microbial fermentation of excess protein and AA equates to an increase in the end products of fermentation. One of the most consistent results that is observed with feeding low CP, AA-supplemented diets is a reduction of diarrhea prevalence as CP is reduced (Wellock et al., 2006; Opapeju et al., 2008; Yue and Qiao, 2008); however, Le Bellego and Noblet (2002) and Htoo et al. (2007) reported no difference in fecal score with reduced CP in the diet. It is possible that these different responses are due to the variable health status of pigs.

## IMMUNE SYSTEM AND INFLAMMATION

Adaptive immunity is the body's response to an infection by a pathogen, resulting in antibody production to combat the antigen (Murphy and Weaver, 2016). Most immune cells originate from and mature in the bone marrow. After maturation, T cells and B cells, which are lymphocytes of adaptive immunity, are distributed to peripheral tissues or circulated through the body via the blood system (Murphy and Weaver, 2016). Once they encounter an antigen, B cells differentiate into plasma cells that secrete antibodies and T cells differentiate into effector T

cells, which activate cellular immune responses (Murphy and Weaver, 2016). In short, sensor cells such as macrophages and neutrophils, which are components of the innate immune system, detect inflammatory inducers that cause immune cells to generate mediators such as cytokines (Burger and Dayer, 2002). Cytokines are proteins associated with the processes of immune physiological homeostasis that act on cells to upregulate an immune response or act directly in termination of a pathogen (Burger and Dayer, 2002). A specialized subset of these secreted proteins are called chemokines, which are associated with the recruitment of chemokine receptors such as neutrophils to areas of infection or inflammation (Murphy and Weaver, 2016). Inflammation is the process where activated macrophages release cytokines and chemokines to recruit immune cells from the blood to aid in the destruction of a pathogen (Murphy and Weaver, 2016). Immune system stimulation, including inflammatory responses, plays a vital role as a defense mechanism for the animal (Suffredini et al., 1999; Opapeju et al., 2010). Immune challenge causes nutrients to be diverted away from growth to support the immune system, making immune function and minimizing inflammation key components to overcoming a stress like weaning in animals (Spurlock, 1997). Infectious diseases result in inflammation that can negatively impact the growth performance of pigs (van Heugten et al., 1994). Because nutrients are being directed towards supporting the immune system instead of growth of the animal, immune system stimulation and subsequent cytokine production influence growth factors and metabolism and decrease growth performance (Spurlock, 1997; Duan et al., 2013). Cytokines are sometimes difficult to classify due to their ability to have both pro- and anti-inflammatory actions. As for this review, cytokines are grouped as their actions were considered, either as pro- or anti-inflammatory.

A pro-inflammatory response is the body's recognition of threats to homeostasis, which results in the release of immune cells that cause the clinical signs of inflammation, such as increased regional blood flow and increased white blood cells flowing to the site of infection (Elsasser et al., 2008). Expression of tumor necrosis factor-α (**TNF-α**), interleukin-6 (**IL-6**), and interferon-γ (**IFN-γ**) were up-regulated due to stress and inflammation (Hu et al., 2013). Tumor necrosis factor-α is a pro-inflammatory cytokine that plays a role in tissue damage repair due to inflammation and may increase the ability of macrophages and epithelial cells to secrete interleukin-8 (**IL-8**; Bosi et al., 2004; Murphy and Weaver, 2016), which is produced by mononuclear cells and may influence cell barrier function (Nusrat et al., 2000). After expression in enterocytes and macrophages, the goal of IL-8 is to recruit other immune cells to sites of inflammation, as well as being associated with neutrophil movement to those same sites (Bosi et al., 2004). This function of IL-8 makes it a pro-inflammatory cytokine. Stimulation of porcine macrophages with an LPS-like extract resulted in up-regulation of mRNA expression of IL-8 (Sacco et al., 1996). The infection induced an immune response, which resulted in a greater secretion of IL-8 to activate more immune cells. Interleukin-1β (**IL-1β**), which is produced by macrophages and epithelial cells, is associated with T cell and macrophage activation (Murphy and Weaver, 2016). Macrophage activation indicates immune stimulation to protect the host from a pathogen or stressor, demonstrating that IL-1β is a pro-inflammatory cytokine. Subjecting porcine macrophages to an LPS-like extract also resulted in an increase in mRNA expression of IL-1β (Sacco et al., 1996). Produced by T cells, B cells, macrophages, and endothelial cells, IL-6 is part of T and B cell growth and differentiation, as well as acute phase protein production (Murphy and Weaver, 2016). It has been stated that IL-6 has both pro-inflammatory properties and influences anti-inflammatory properties (Opal and DePalo, 2000; Holm et al., 2009).

Although IL-6 is heavily involved in the production of acute phase proteins, it also down-regulates the production of IL-1β and TNF-α (Opal and DePalo, 2000). Interferon-γ is a glycoprotein released by activated T cells (Nusrat et al., 2000). Interferon-γ is also considered a pro-inflammatory cytokine. Interferon-γ is produced by macrophages in response to inflammation and is a factor for the activation of a cell-mediated response (Bosi et al., 2004). Pigs supplemented with a yeast product had reduced expression of IFN-γ in the jejunum, indicating a decrease in inflammation (de Laguna Ortega et al., 2019). mRNA expression of IFN-γ also positively correlated with intestinal concentrations of *Streptococcus*, total amines, cadaverine, and phenols, whereas IFN-γ was negatively correlated with intestinal concentrations of *Lactobacillus*, total SCFA, and butyrate (Yu et al., 2019b). Interleukin-21 (**IL-21**) is a pro-inflammatory cytokine that is produced by T cells and plays a key role in the differentiation of B and T cells (Murphy and Weaver, 2016). Chemokine (C-X-C motif) ligand 9 (**CXCL9**) and chemokine (C-X-C motif) ligand 10 (**CXCL10**) are both ligands for chemokine receptor 3 (**CKCR3**). They are produced by T cells and are involved with the migration of immune cells to the area of inflammation (Murphy and Weaver, 2016). Both CXCL9 and CXCL10 are pro-inflammatory cytokines. Chemokine (C-C motif) ligand 2 (**CCL2**) has a similar and complementary function as chemokine (C-X-C motif) ligand 8 (**CXCL8**). Recruitment of monocytes is the main function of CCL2 and CXCL8 is associated with attracting neutrophils, but both CCL2 and CXCL8 function to entice immune cells to decrease inflammation (Murphy and Weaver, 2016). This mode of action demonstrates that CCL2 is pro-inflammatory.

Anti-inflammatory cytokines inhibit the synthesis of IL-1β and cytokines that are involved with the suppression of inflammation (Opal and DePalo, 2000). Interleukin-4 (**IL-4**) is produced by T cells and mast cells, and is associated with B cell activation and differentiation of

$T_H2$ cells (Murphy and Weaver, 2016). $T_H2$ cells are helper T cells that produce IL-4, interleukin-5 (**IL-5**), interleukin-10 (**IL-10**), and interleukin-13 (**IL-13**), all of which antagonize macrophage function (Romagnani, 1999). This antagonistic effect indicates that IL-4 is anti-inflammatory. Interleukin-10, also known as cytokine synthesis inhibitory factor, is produced by macrophages, T cells, B cells, and dendritic cells (Murphy and Weaver, 2016). The fact that IL-10 causes a decrease in macrophage function makes it an anti-inflammatory cytokine (Murphy and Weaver, 2016). Interleukin-10 inhibits the production of cytokines like TNF-α and IFN-γ, so an increase in IL-10 improves the virus' chance of survival (Pestka et al., 2004). The mRNA expression of IL-10 has been shown to be positively correlated with intestinal concentrations of *Lactobacillus*, *Peptococcus*, and butyrate, and negatively correlated with intestinal concentrations of *Streptococcus*, total amines, and phenol (Yu et al., 2019b). Stromal fibroblasts produce interleukin-11 (**IL-11**), which has a similar mode of action as IL-4 in hematopoiesis, which is the generation of helper T cells in the blood (Murphy and Weaver, 2016). Interleukin-4 has similar properties with IL-6 because of their usage of the same receptor complex. Aside from its hematopoietic growth factor potential, IL-11 is also associated with the down-regulation of IL-1β, TNF-α, and IFN-γ synthesis (Opal and DePalo, 2000). Interleukin-13, an anti-inflammatory cytokine, is produced by T cells and is associated with B cell growth and differentiation, and it inhibits inflammatory cytokine synthesis by macrophages (Murphy and Weaver, 2016). Interleukin-13 shares a receptor with IL-4. The production of IL-13 is also associated with down-regulation of pro-inflammatory cytokines (Opal and DePalo, 2000). Produced by monocytes and T cells, transforming growth factor-β (**TGF-β**) is associated with helper cell generation and reduction of inflammation (Murphy and Weaver, 2016). Transforming growth factor-β also suppresses the synthesis of TNF-α and IFN-γ (Opal and DePalo, 2000).

Tumor necrosis factor-α, IL-1β, and IL-6 are pro-inflammatory cytokines that play an important role in the mediation of an inflammatory response, which includes production of acute phase proteins like haptoglobin (**Hp**; Chen et al., 2003; Opapeju et al., 2010). An acute phase protein is a plasma protein that originates in the liver, and is increased rapidly in response to an event such as weaning that disturbs homeostasis within the body of the pig (Chen et al., 2003). As production of pro-inflammatory cytokines increases, so do serum levels of Hp. Opapeju et al. (2010) determined that serum Hp followed the same trend as IL-1β, where feeding a high CP diet resulted in increased serum concentrations of both IL-1β and Hp. Haptoglobin can also be elevated in pigs with diarrhea (Chen et al., 2003) and in pigs with diseases that cause stress (Tóthová et al., 2013). Feeding lower CP diets, which may result in less frequency of diarrhea, may also result in decreased circulatory Hp in blood serum.

Immune activation can also be estimated by determining concentrations of immunoglobulin G (**IgG**; Ilsley et al., 2005; Lu et al., 2013). Because the placenta creates a barrier that immunoglobulins cannot cross, most of the IgG is passed from the blood to the colostrum in sows and is consumed within the initial 36 hours of life, which is vital for piglet survival (Salmon et al., 2009). At weaning, plasma IgG concentration can be linked to birth weight and rank, colostrum intake, and most importantly IgG concentration in the consumed colostrum (Devillers et al., 2011; Kielland et al., 2015). As described by Lu et al. (2013), IgG can bind to pathogens to neutralize their toxins as part of the immune response. Yin et al. (2008) referenced Gomez et al. (1998) and their discussion surrounding plasma IgG and its ability to maintain optimal gut function by preventing bacterial damage to the gut epithelial cells. Immunoglobulins G, A, and M are important serum immunoglobulins that play a role in the

protection of the extravascular compartments against pathogens. Increasing immunoglobulin synthesis indicates an enhanced immune response in pigs (Deng et al., 2007).

## FAT-SOLUBLE VITAMINS

Vitamins, like fat-soluble vitamins A and E, are often used as cofactors in the metabolism of nutrients (NRC, 2012). Vitamins A and E are also associated with the antioxidant system (Sharma and Buettner, 1993; Buchet et al., 2017). The goal of the antioxidant defense mechanism is to use scavenging antioxidants to minimize reactive oxygen species, which are substances that have one or more unpaired electrons, ultimately averting the production of free radical chain reactions (Zaken et al., 2001). These reactive oxygen species cause oxidative stress, which is involved with multiple diseases such as arteriosclerotic cardiovascular disease, metabolic disorders, and diabetes (Zaken et al., 2001). Free radicals can oxidize lipids and proteins into hydroperoxides, and if produced in excess, can cause cell and tissue injury (Buchet et al., 2017). Failure of the cell's defense mechanism to counter this oxidative damage leads to oxidative stress (Esrefoglu, 2012). Weaning induces oxidative stress, and it is possible that increased concentrations of antioxidants such as vitamins A and E may decrease oxidative stress in pigs, thus improving health and growth performance in weaning pigs (Wei et al., 2016). In a model shown by Beck (2007), decreasing antioxidant nutrients resulted in increased oxidative stress, which can lead to either decreased immune response or increased viral mutations, either of which result in an increase in viral pathogenicity.

Vitamin E, or α-tocopherol, status can be used to impact immunological functions by improving lymphocyte function (Jensen et al., 1988; Finch and Turner, 1996; Sivertsen et al., 2007). Vitamin E also reduces oxidative stress prompted by inflammation, increasing the ability

to mount an immune response (Esrefoglu, 2012). Usage of vitamin E for pro-oxidant neutralization, combined with a decrease in vitamin E consumption because of reduced feed intake after weaning, may be the reason for the drop in plasma concentration of vitamin E that is usually observed in pigs after weaning (Buchet et al., 2017). The reduction in plasma concentrations of vitamin E after weaning has been observed in multiple experiments (Håkansson et al., 2001; Lauridsen et al., 2011; Kim et al., 2016). Pigs with a reduced average daily gain (**ADG**) after weaning often have the lowest plasma vitamin E concentrations (Buchet et al., 2017).

Vitamin A, or retinol, also has a stimulatory effect on the immune response and prevents immunosuppression induced by steroids (Cohen and Cohen, 1973). Vitamin A is considered a key antioxidant for generating a cell-mediated immune response (Esrefoglu, 2012). Unlike vitamin E, vitamin A can be stored in the body, the liver specifically (NRC, 2012). It has been demonstrated that supplementing immunized 6-week-old chicks with vitamin A improved their protection against *E. coli* (Tengerdy and Nockels, 1975). Typically, plasma concentrations of vitamin A are not decreased as much as vitamin E in the post-weaning period (Buchet et al., 2017).

## GUT-PROTECTIVE PROTEINS

Dietary CP and the AA profile are important to maximize animal production and support animal health (Barekatain et al., 2019). The first line of defense against bacteria and pathogens is the intestinal mucosa barrier (Li et al., 2012). In the gut, epithelial cells are joined on their luminal side by complexes called tight junctions (Ballard et al., 1995). Tight junctions are composed of transmembrane proteins claudin and occludin, plus the peripheral protein zonula

occludens protein-1 (**ZO-1**; Clayburgh et al., 2004). The claudin family of proteins has adhesive properties and the ability to block the intracellular space (Chen et al., 2018). Occludin has 4 transmembrane-spanning domains and the extracellular domains likely interact with similar domains on adjacent cells (Ballard et al., 1995). The mRNA expression level of occludin is positively correlated with the intestinal concentration of total VFA, but is negatively correlated with intestinal concentrations of *Streptococcus*, total amines, cadaverine, and skatole (Yu et al., 2019b). The function of ZO-1 is to link the actin cytoskeletons to a membrane spanning tight junction protein like occludin (Ballard et al., 1995). Increased mRNA expression of ZO-1 is positively correlated with concentrations of total VFA and butyrate in the intestines, and negatively correlated with intestinal concentrations of *Streptococcus*, total amines, cadaverine, skatole, and phenol (Yu et al., 2019b).

Integrity of tight junctions is crucial to intestinal barrier function. The goal of the intestinal barrier is to prevent the translocation of bacteria and pathogens in the lumen of the intestine from reaching extra-intestinal sites (Li et al., 2012). The tight junctions also manage the paracellular pathway of permeability. Because tight junction proteins are the rate-limiting step in the paracellular pathway, an intact and undamaged epithelial barrier is crucial for protecting pigs from gut-derived pathogens (Groschwitz and Hogan, 2009; Li et al., 2012). Pigs that have diarrhea have increased intestinal permeability, which increases the potential of toxins to enter between epithelial cells (Wijtten et al., 2011a; Espinosa et al., 2020).

Reducing CP in diets for weaning pigs may decrease the fermentation of nitrogen, and thereby, reduce the synthesis of potentially harmful metabolites that may negatively impact the durability and function of tight junction proteins. Chen et al. (2018) demonstrated that when dietary CP was reduced by from 18 to 12% CP, claudin-3 and claudin-7 expression was also

decreased in finishing pigs. In contrast, Fan et al. (2017) reported that reducing CP in diets for finishing pigs increased expression of occludin and claudin.

Pro-inflammatory cytokines increase epithelial permeability and decrease expression of tight junction proteins, whereas anti-inflammatory cytokines maintain intestinal barrier function with increased expression of tight junction proteins (Hu et al., 2013). Supplementing diets for weaned pigs with a yeast cell wall product (0.05% inclusion rate) improved growth performance and increased the expression of tight junction proteins (Kyoung et al., 2019). Expression of TNF-α in the ileum and circulatory levels of TNF-α, IL-1β, and IL-6 were also reduced (Kyoung et al., 2019).

Mucins are glycoproteins that compose a major part of the mucus layer that functions to protect the intestinal epithelium (Kim et al., 2009). Produced by goblet cells in the small intestine and colon, mucin-2 (**MUC-2**) is the major intestinal mucin (Lillehoj et al., 2013). Dietary fiber increases mucin secretion (Ferrandis Vila et al., 2018). Interleukin-4 also stimulates an increase in MUC-2 expression, indicating that cytokine expression may influence intestinal changes like mucin synthesis (Ferrandis Vila et al., 2018). Reduction in mucin synthesis, and therefore a reduction in the mucosal layer, can cause intestinal inflammation and decreased gut barrier function, resulting in the potential for bacteria translocation (Puiman et al., 2011).

The trefoil factor family (**TFF**)-peptides, produced by mucin-producing epithelial cells, are also considered gut-protective proteins due to their role in upholding the surface integrity of epithelial cells (Hoffmann et al., 2001). This family includes trefoil factor-1 (**TFF-1**), trefoil factor-2 (**TFF-2**), and trefoil factor-3 (**TFF-3**). The stomach is a major producer of TFF-1 in mammals, but TFF-2 is primarily synthesized in the liver (Hoffmann et al., 2001). Unlike TFF-1 and TFF-2, TFF-3 is not located solely in the gut. It is also present in salivary glands and the

respiratory tract (Hoffmann et al., 2001). Expression of TFF-peptides is increased in humans with chronic inflammatory diseases, as well as in rats with gastric mucosal damage (Hoffmann et al., 2001). An increase in the expression of this family of peptides indicates an increase in protection of the gut. It is, however, likely that the mucus-secreting cells will not produce TFF-peptides unless metabolically induced to do so via infection or inflammation.

## NUTRIENT ABSORPTION

The plasma membrane GLUT transporters are some of the most important cellular nutrient transporters (Zhang et al., 2016). Facilitated diffusion using GLUT2 is responsible for transport of glucose, fructose, and galactose from the enterocytes to the intestinal space. GLUT2 can also translocate to the luminal membrane when concentrations of glucose in the lumen are in excess. Facilitated diffusion via GLUT5 is the method of transport across the luminal membrane for fructose and xylose. The most important glucose transporter from the lumen to the enterocytes is sodium-glucose linked transporter-1 (**SGLT-1**), which is an active sodium-dependent transporter that uses adenosine triphosphate (**ATP**) as the source of energy. An increase in the expression of these monosaccharide transporters indicates an improvement in the pig's ability to absorb glucose (Vigors et al., 2014; Zhang et al., 2016). Feeding a diet with greater CP content increased expression of GLUT2, which may result in an improvement in the pig's growth performance (Vigors et al., 2014). The fact that diets for pigs are usually low in fructose results in GLUT5 being less important in weaning pigs. Reduced CP diets may result in a decrease in the expression of GLUT5 in the duodenum, jejunum, and the ileum, which may be a measure of energy conservation (Zhang et al., 2016).

During the initial period post-weaning, a reduced villus height and crypt depth is usually observed (Zhao et al., 2007). A decrease in villus height indicates less surface area for absorption of nutrients and a shallower crypt depth implies a less rapid cell turnover for villus renewal (Yin et al., 2020). This impairment may result in a decrease in surface area, and a reduction in nutrient absorption. By reducing the nutrients absorbed, more are fermented in the large intestine, which can result in diarrhea (Wellock et al., 2008). The following observation was described in pigs post-weaning: shorter villus, but deeper crypts, were observed during the first week post-weaning, but by d 14, the morphology values had recovered; however, intestinal barrier function, measured as tight junction protein expression, had not recovered (Hu et al., 2013). Feeding pigs low CP diets may affect intestinal morphology and function (Yin et al., 2020), but data are inconclusive (Yu et al., 2019a). Reducing dietary CP increased crypt depth in the duodenum and jejunum on d 7 post-weaning and decreased the villus height:crypt depth ratio, but no differences in ileal morphology were observed (Almeida et al., 2017). On d 28 post-weaning, pigs had recovered and no differences were observed between pigs consuming a high or low CP diet. In contrast, reducing dietary CP decreased villus height and increased crypt depth in the duodenum and jejunum (Opapeju et al., 2008; Yue and Qiao, 2008), and Nyachoti et al. (2006) reported that CP content in the diet had no effect on intestinal morphology in the duodenum, jejunum, or ileum.

Villus height and crypt depth were negatively impacted when finishing pigs were fed diets where CP was reduced from 18 or 16% to 12 or 10%, respectively (Fan et al., 2017; Chen et al., 2018). These data indicate that a moderate reduction in dietary CP is acceptable, but negative effects can be observed if CP is decreased too much. The negative impact observed on

morphology decreases surface area for nutrient absorption, which may influence pig growth performance and nutrient utilization efficiency.

## GROWTH PERFORMANCE

The reduction in feed intake post-weaning contributes to the negative impact that weaning has on intestinal morphology (Zhao et al., 2007; Wijtten et al., 2011b), and cytokine synthesis may impact feed intake (Goodband et al., 2014). Peptide YY (**PYY**), a 36-AA peptide that is secreted by L-cells in the ileum and large intestine, is a gastrointestinal peptide that is important in the regulation of feed intake and energy homeostasis (Ueno et al., 2008). Plasma PYY concentrations increase within 15 minutes of eating a meal. Pigs fed full-fat rice bran had a reduced feed intake compared with pigs fed the control diet, and this was associated with reduced plasma concentrations of PYY (Casas and Stein, 2016). In humans, consuming a breakfast that was high in protein increased PYY levels (van der Klaauw et al., 2013).

As dietary CP is reduced, the largest concern is whether or not growth performance will be affected (Yu et al., 2019a). There are, however, many studies in which growth performance was unchanged or improved if dietary CP was reduced (Le Bellego and Noblet, 2002; Stein and Kil, 2006; Htoo et al., 2007; Heo et al., 2008; Opapeju et al., 2009; Toledo et al., 2014; Almeida et al., 2017; Fang et al., 2019). However, ADG and feed conversion ratio have been negatively impacted by the reduction of CP in the diet in other experiments (Hansen et al., 1993; Nyachoti et al., 2006; Wellock et al., 2006; Opapeju et al., 2008; Yue and Qiao, 2008). The cause of this poorer performance is not clear, but it may be due to an AA imbalance or deficiencies of other nutrients (Nyachoti et al., 2006). Similar results have been observed in poultry nutrition, where the decrease in performance may also be associated to a decrease in protein synthesis. This may

be because there are less free AA present in the blood of birds fed diets supplemented with synthetic AA than birds fed intact protein, which may be because free AA can be metabolized within the enterocytes (Faria Filho et al., 2005). Absorption of free AA and peptides, and the rates at which this takes place, may result in a reduced availability of AA needed to maximize protein synthesis (Pinchasov et al., 1990).

An alternative feeding strategy is that pigs can be fed diets with reduced amounts of CP for the initial one or 2 weeks post-weaning, which is the time when pigs are most susceptible to diarrhea, before supplementing them with a higher CP diet during the following period in an attempt to minimize performance losses. Opapeju et al. (2008) observed no difference in ADG during the first week post-weaning when feeding a low CP diet, but reported that overall growth performance over 3 weeks was negatively affected. A similar result was observed by Cho et al. (2008), with the exception that no difference in ADG was observed during the initial 2 weeks post-weaning when pigs were fed a diet with low CP and AA supplementation. Most of these studies lasted only a few weeks, which means the short time of the studies potentially limited the opportunity to observe compensatory gain as a result of better gut health (Stein and Kil, 2006). Pigs fed low CP diets followed by diets fortified with higher amounts of CP have tended to compensate for the previously poorer performance (Libal and Wahlstrom, 1976). Pigs fed a low CP diet in the starter phase achieved compensatory gain through the grower and finisher phases (Zimmerman and Khajarern, 1973).

## CONCLUSION

Reducing dietary CP with AA supplementation may be implemented as an alternative nutritional strategy in replacement of feed-grade antibiotics in diets fed to pigs post-weaning. By

reducing CP, and consequently reducing protein fermentation and its potentially harmful end-products due to decreased nitrogen passage, improved gut health and a decrease in diarrhea may be observed. However, more research needs to be conducted to determine the ideal duration of time post-weaning a reduced CP diet should be fed. The extent to which CP may be reduced without negatively affecting pig growth performance beyond the level that may be compensated for in later periods also needs to be determined. Because low CP diets are inferred to improve intestinal function in pigs, more research is also needed to evaluate the effects of feeding low CP, AA-supplemented diets on the inflammatory system, and how that may potentially affect gut physiology, and subsequent growth performance in pigs post-weaning.

## LITERATURE CITED

Almeida, V. V., A. J. C. Nuñez, A. P. Schinckel, P. V. A. Alvarenga, F. R. Castelini, Y. V. Silva-Guillen, and M. C. Thomaz. 2017. Interactive effect of dietary protein and dried citrus pulp levels on growth performance, small intestinal morphology, and hindgut fermentation of weanling pigs. J. Anim. Sci. 95:257-269. doi:10.2527/jas.2016.0498

Ballard, S. T., J. H. Hunter, and A. E. Taylor. 1995. Regulation of tight-junction permeability during nutrient absorption across the intestinal epithelium. Annu. Rev. Nutr. 15:35-55. doi:10.1146/annurev.nu.15.070195.000343

Barekatain, R., P. V. Chrystal, G. S. Howarth, C. J. McLaughlan, S. Gilani, and G. S. Nattrass. 2019. Performance, intestinal permeability, and gene expression of selected tight junction proteins in broiler chickens fed reduced protein diets supplemented with arginine, glutamine, and glycine subjected to a leaky gut model. Poult. Sci. 98:6761-6771. doi:10.3382/ps/pez393

Beck, M. A. 2007. Selenium and vitamin E status: Impact on viral pathogenicity. J. Nutr. 137:1338-1340. doi:10.1093/jn/137.5.1338

Bikker, P., A. Dirkzwager, J. Fledderus, P. Trevisi, I. le Huërou-Luron, J. P. Lallès, and A. Awati. 2006. The effect of dietary protein and fermentable carbohydrates levels on growth performance and intestinal characteristics in newly weaned piglets. J. Anim. Sci. 84:3337-3345. doi:10.2527/jas.2006-076

Bosi, P., L. Casini, A. Finamore, C. Cremokolini, G. Merialdi, P. Trevisi, F. Nobili, and E. Mengheri. 2004. Spray-dried plasma improves growth performance and reduces

inflammatory status of weaned pigs challenged with enterotoxigenic *Escherichia coli* K88. J. Anim. Sci. 82:1764-1772. doi:10.2527/2004.8261764x

Bouhours, D., J.-F. Bouhours, and P.-A. Bryon. 1981. Association of glycoproteins and pepsinogen in the secretory granules of fundic epithelial cells isolated from guinea pig stomach. Biochim. Biophys. Acta Gen. Subj. 672:288-296. doi:10.1016/0304-4165(81)90295-6

Buchet, A., C. Belloc, M. Leblanc-Maridor, and E. Merlot. 2017. Effects of age and weaning conditions on blood indicators of oxidative status in pigs. PLoS. ONE. 12:e0178487-e0178487. doi:10.1371/journal.pone.0178487

Burger, D., and J.-M. Dayer. 2002. Cytokines, acute-phase proteins, and hormones. Ann. N. Y. Acad. Sci. 966:464-473. doi:10.1111/j.1749-6632.2002.tb04248.x

Campbell, J. M., J. D. Crenshaw, and J. Polo. 2013. The biological stress of early weaned piglets. J. Anim. Sci. Biotechnol. 4:19-19. doi:10.1186/2049-1891-4-19

Casas, G. A., and H. H. Stein. 2016. Effects of full fat or defatted rice bran on growth performance and blood characteristics of weanling pigs. J. Anim. Sci. 94:4179-4187. doi:10.2527/jas.2016-0565

Chen, H.-H., J.-H. Lin, H.-P. Fung, L.-L. Ho, P.-C. Yang, W.-C. Lee, Y.-P. Lee, and R.-M. Chu. 2003. Serum acute phase proteins and swine health status. Can. J. Vet. Res. 67:283-290.

Chen, X., P. Song , P. Fan, T. He, D. Jacobs, C. L. Levesque, L. J. Johnston, L. Ji, N. Ma, Y. Chen, J. Zhang, J. Zhao, and X. Ma. 2018. Moderate dietary protein restriction optimized

gut microbiota and mucosal barrier in growing pig model. Front. Cell. Infect. Microbiol. 8. doi:10.3389/fcimb.2018.00246

Cho, J. H., Y. J. Chen, B. J. Min, J. S. Yoo, Y. Wang, and I. H. Kim. 2008. Effects of reducing dietary crude protein on growth performance, odor gas emission from manure and blood urea nitrogen and IGF-1 concentrations of serum in nursery pigs. Anim. Sci. J. 79:453-459. doi:10.1111/j.1740-0929.2008.00549.x

Clayburgh, D. R., L. Shen, and J. R. Turner. 2004. A porous defense: the leaky epithelial barrier in intestinal disease. Lab. Invest. 84:282-291. doi:10.1038/labinvest.3700050

Close, W. H. 2000. Producing pigs without antibiotic growth promoters. Adv. Pork Prod. 11:47-56.

Cohen, B. E., and I. K. Cohen. 1973. Vitamin A: adjuvant and steroid antagonist in the immune response. J. Immunol. 111:1376-1380.

Coma, J., D. Carrion, and D. R. Zimmerman. 1995. Use of plasma urea nitrogen as a rapid response criterion to determine the lysine requirement of pigs. J. Anim. Sci. 73:472-481. doi:10.2527/1995.732472x

Cromwell, G. L. 2002. Why and how antibiotics are used in swine production. Anim. Biotech. 13:7-27. doi:10.1081/ABIO-120005767

de Laguna Ortega, F. B., B. Bertaud, I. H. Kim, and Y. M. Kim. 2019. PSIV-10 Effect of a yeast product supplementation on growth performance, nutrient digestibility and cytokines mRNA expression in weanling piglets reared under low sanitary environment. J. Anim. Sci. 97:180-180. doi:10.1093/jas/skz122.317

de Lange, C. F. M., J. Pluske, J. Gong, and C. M. Nyachoti. 2010. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. Livest. Sci. 134:124-134. doi:10.1016/j.livsci.2010.06.117

Deng, Z.-Y., J.-W. Zhang, G.-Y. Wu, Y. Yin, Z. Ruan, T.-J. Li, W.-Y. Chu, X.-F. Kong, Y.-M. Zhang, Y.-W. Fan, R. Liu, and R.-L. Huang. 2007. Dietary supplementation with polysaccharides from Semen cassiae enhances immunoglobulin production and interleukin gene expression in early-weaned piglets. J. Sci. Food Agric. 87:1868-1873. doi:10.1002/jsfa.2908

Devillers, N., J. Le Dividich, and A. Prunier. 2011. Influence of colostrum intake on piglet survival and immunity. Animal. 5:1605-1612. doi:10.1017/S175173111100067X

Doyle, M. 2001. Alternatives to antibiotic use for growth promotion in animal husbandry. Food Research Institute, Department Food Microbiology & Toxicology.

Duan, Y., F. Li, L. Li, J. Fan, X. Sun, and Y. Yin. 2013. n-6:n-3 PUFA ratio is involved in regulating lipid metabolism and inflammation in pigs. Br. J. Nutr. 111:445-451. doi:10.1017/S0007114513002584

Duttlinger, A. W., K. R. Kpodo, D. C. Lay, B. T. Richert, and J. S. Johnson. 2019. Replacing dietary antibiotics with 0.20% l-glutamine in swine nursery diets: Impact on health and productivity of pigs following weaning and transport. J. Anim. Sci. 97:2035-2052. doi:10.1093/jas/skz098

Elsasser, T. H., T. J. Caperna, C.-J. Li, S. Kahl, and J. L. Sartin. 2008. Critical control points in the impact of the proinflammatory immune response on growth and metabolism. J. Anim. Sci. 86:E105-E125. doi:10.2527/jas.2007-0634

Espinosa, C. D., R. S. Fry, M. E. Kocher, and H. H. Stein. 2020. Effects of copper hydroxychloride and dietary fiber on intestinal permeability, growth performance, and blood characteristics of nursery pigs. Anim. Feed Sci. Technol. 263:114447. doi:10.1016/j.anifeedsci.2020.114447

Esrefoglu, M. 2012. Oxidative stress and benefits of antioxidant agents in acute and chronic hepatitis. Hepat. Mon. 12:160-167. doi:10.5812/hepatmon.837

Fan, P., P. Liu, P. Song, X. Chen, and X. Ma. 2017. Moderate dietary protein restriction alters the composition of gut microbiota and improves ileal barrier function in adult pig model. Sci. Rep. 7:43412. doi:10.1038/srep43412

Fang, L. H., Y. H. Jin, S. H. Do, J. S. Hong, B. O. Kim, T. H. Han, and Y. Y. Kim. 2019. Effects of dietary energy and crude protein levels on growth performance, blood profiles, and nutrient digestibility in weaning pigs. Asian Austral. J. Anim. Sci. 32:556-563. doi:10.5713/ajas.18.0294

Faria Filho, D., P. Rosa, B. Vieira, M. Macari, and R. Furlan. 2005. Protein levels and environmental temperature effects on carcass characteristics, performance, and nitrogen excretion of broiler chickens from 7 to 21 days of age. Braz. J. Poult. Sci. 7:247-253.

FDA. 2015. Fact sheet: Veterinary feed directive final rule and next steps.

Ferrandis Vila, M., M. P. Trudeau, Y.-T. Hung, Z. Zeng, P. E. Urriola, G. C. Shurson, and M. Saqui-Salces. 2018. Dietary fiber sources and non-starch polysaccharide-degrading enzymes modify mucin expression and the immune profile of the swine ileum. PLoS. ONE. 13:e0207196-e0207196. doi:10.1371/journal.pone.0207196

Finch, J. M., and R. J. Turner. 1996. Effects of selenium and vitamin E on the immune responses of domestic animals. Res. Vet. Sci. 60:97-106. doi:10.1016/S0034-5288(96)90001-6

Gibson, G. R., and X. Wang. 1994. Regulatory effects of bifidobacteria on the growth of other colonic bacteria. J. Appl. Bacteriol. 77:412-420.

Gomez, G. G., O. Phillips, and R. A. Goforth. 1998. Effect of immunoglobulin source on survival, growth, and hematological and immunological variables in pigs. J. Anim. Sci. 76:1-7. doi:10.2527/1998.7611

Goodband, B., M. Tokach, S. Dritz, J. DeRouchey, and J. Woodworth. 2014. Practical starter pig amino acid requirements in relation to immunity, gut health and growth performance. J. Anim. Sci. Biotechnol. 5:12. doi:10.1186/2049-1891-5-12

Groschwitz, K. R., and S. P. Hogan. 2009. Intestinal barrier function: Molecular regulation and disease pathogenesis. J. Allergy Clin. Immunol. 124:3-20. doi:10.1016/j.jaci.2009.05.038

Håkansson, J., J. Hakkarainen, and N. Lundeheim. 2001. Variation in vitamin E, glutathione peroxidase and retinol concentrations in blood plasma of primiparous sows and their piglets, and in vitamin E, selenium and retinol contents in sows' milk. Acta Agric. Scand. Sect. A Anim. Sci. 51:224-234. doi:10.1080/09064700152717209

Hansen, J. A., D. A. Knabe, and K. G. Burgoon. 1993. Amino acid supplementation of low-protein sorghum-soybean meal diets for 20- to 50-kilogram swine. J. Anim. Sci. 71:442-451. doi:10.2527/1993.712442x

Hedemann, M. S., M. Eskildsen, H. N. Lærke, C. Pedersen, J. E. Lindberg, P. Laurinen, and K. E. B. Knudsen. 2006. Intestinal morphology and enzymatic activity in newly weaned pigs

fed contrasting fiber concentrations and fiber properties. J. Anim. Sci. 84:1375-1386. doi:10.2527/2006.8461375x

Heo, J.-M., J.-C. Kim, C. F. Hansen, B. P. Mullan, D. J. Hampson, and J. R. Pluske. 2008. Effects of feeding low protein diets to piglets on plasma urea nitrogen, faecal ammonia nitrogen, the incidence of diarrhoea and performance after weaning. Arch. Anim. Nutr. 62:343-358. doi:10.1080/17450390802327811

Heo, J. M., J. C. Kim, C. F. Hansen, B. P. Mullan, D. J. Hampson, and J. R. Pluske. 2009. Feeding a diet with decreased protein content reduces indices of protein fermentation and the incidence of postweaning diarrhea in weaned pigs challenged with an enterotoxigenic strain of *Escherichia coli*. J. Anim. Sci. 87:2833-2843. doi:10.2527/jas.2008-1274

Heo, J. M., F. O. Opapeju, J. R. Pluske, J. C. Kim, D. J. Hampson, and C. M. Nyachoti. 2013. Gastrointestinal health and function in weaned pigs: a review of feeding strategies to control post-weaning diarrhoea without using in-feed antimicrobial compounds. J. Anim. Physiol. Anim. Nutr. 97:207-237. doi:10.1111/j.1439-0396.2012.01284.x

Hoffmann, W., W. Jagla, and A. Wiede. 2001. Molecular medicine of TFF-peptides: from gut to brain. Histol. Histopathol. 16:319-334.

Holm, S., Z. Mackiewicz, A. K. Holm, Y. T. Konttinen, V.-P. Kouri, A. Indahl, and J. Salo. 2009. Pro-inflammatory, pleiotropic, and anti-inflammatory TNF-α, IL-6, and IL-10 in experimental porcine intervertebral disk degeneration. Vet. Pathol. 46:1292-1300. doi:10.1354/vp.07-VP-0179-K-FL

Htoo, J. K., B. A. Araiza, W. C. Sauer, M. Rademacher, Y. Zhang, M. Cervantes, and R. T. Zijlstra. 2007. Effect of dietary protein content on ileal amino acid digestibility, growth

performance, and formation of microbial metabolites in ileal and cecal digesta of early-weaned pigs. J. Anim. Sci. 85:3303-3312. doi:10.2527/jas.2007-0105

Hu, C. H., K. Xiao, Z. S. Luan, and J. Song. 2013. Early weaning increases intestinal permeability, alters expression of cytokine and tight junction proteins, and activates mitogen-activated protein kinases in pigs. J. Anim. Sci. 91:1094-1101. doi:10.2527/jas.2012-5796

Ilsley, S. E., H. M. Miller, and C. Kamel. 2005. Effects of dietary quillaja saponin and curcumin on the performance and immune status of weaned piglets. J. Anim. Sci. 83:82-88. doi:10.2527/2005.83182x

Jensen, M., C. Fossum, M. Ederoth, and R. V. J. Hakkarainen. 1988. The effect of vitamin E on the cell-mediated immune response in pigs. J. Vet. Med. Ser. B. 35:549-555. doi:10.1111/j.1439-0450.1988.tb00528.x

Jha, R., and J. F. D. Berrocoso. 2016. Dietary fiber and protein fermentation in the intestine of swine and their interactive effects on gut health and on the environment: A review. Anim. Feed Sci. Technol. 212:18-26. doi:10.1016/j.anifeedsci.2015.12.002

Kielland, C., V. Rootwelt, O. Reksen, and T. Framstad. 2015. The association between immunoglobulin G in sow colostrum and piglet plasma. J. Anim. Sci. 93:4453-4462. doi:10.2527/jas.2014-8713

Kim, C. H., D. Kim, Y. Ha, K. D. Cho, B. H. Lee, I. W. Seo, S. H. Kim, and C. Chae. 2009. Expression of mucins and trefoil factor family protein-1 in the colon of pigs naturally infected with *Salmonella typhimurium*. J. Comp. Pathol. 140:38-42. doi:10.1016/j.jcpa.2008.10.002

Kim, J. C., B. P. Mullan, J. L. Black, R. J. E. Hewitt, R. J. van Barneveld, and J. R. Pluske. 2016. Acetylsalicylic acid supplementation improves protein utilization efficiency while vitamin E supplementation reduces markers of the inflammatory response in weaned pigs challenged with enterotoxigenic *E. coli*. J. Anim. Sci. Biotechnol. 7:58. doi:10.1186/s40104-016-0118-4

Kyoung, H., M. Cho, H. Lee, S. Park, J. Kang, D. Mun, S. Kim, D. Seo, J. Lee, J. Choe, and M. Song. 2019. PSXIV-42 Late-Breaking: Effects of yeast cell wall product on growth performance, immune responses, and gene expression of tight junction proteins of weaned pigs. J. Anim. Sci. 97:333-334. doi:10.1093/jas/skz258.666

Langlois, B. E., G. L. Cromwell, and V. W. Hays. 1978. Influence of type of antibiotic and length of antibiotic feeding period on performance and persistence of antibiotic resistant enteric bacteria in growing-finishing swine. J. Anim. Sci. 46:1383-1396. doi:10.2527/jas1978.4651383x

Lauridsen, C., E.-M. Vestergaard, S. Højsgaard, S. K. Jensen, and M. T. Sørensen. 2011. Inoculation of weaned pigs with *E. coli* reduces depots of vitamin E. Livest. Sci. 137:161-167. doi:10.1016/j.livsci.2010.10.015

Le Bellego, L., and J. Noblet. 2002. Performance and utilization of dietary energy and amino acids in piglets fed low protein diets. Livest. Prod. Sci. 76:45-58. doi:10.1016/S0301-6226(02)00008-8

Li, X., S. Akhtar, and M. A. Choudhry. 2012. Alteration in intestine tight junction protein phosphorylation and apoptosis is associated with increase in IL-18 levels following

alcohol intoxication and burn injury. Biochim. Biophys. Acta. 1822:196-203. doi:10.1016/j.bbadis.2011.09.019

Liao, S. F., and M. Nyachoti. 2017. Using probiotics to improve swine gut health and nutrient utilization. Anim. Nutr. 3:331-343. doi:10.1016/j.aninu.2017.06.007

Libal, G. W., and R. C. Wahlstrom. 1976. Compensatory growth of swine following protein insufficiency. J. Anim. Sci. 3:455.

Lillehoj, E. P., K. Kato, W. Lu, and K. C. Kim. 2013. Cellular and molecular biology of airway mucins. In: K. W. Jeon, editor, Int. Rev. Cell Mol. Biol. No. 303. Academic Press. p. 139-202.

Lu, X., J. Liu, W. Fu, J. Zhou, Y. Luo, X. Ding, Y. Liu, and Q. Zhang. 2013. Genome-wide association study for cytokines and immunoglobulin G in swine. PLoS. ONE. 8:e74846-e74846. doi:10.1371/journal.pone.0074846

Murphy, K., and C. Weaver. 2016. Janeway's immunobiology. 9th edition ed. Garland science.

NRC. 2012. Nutrient requirements of swine. 11th edition ed. National Academies Press, Washington, D.C.

Nusrat, A., J. Turner, and J. Madara. 2000. Molecular physiology and pathophysiology of tight junctions. IV. Regulation of tight junctions by extracellular stimuli: Nutrients, cytokines, and immune cells. Am. J. Physiol. 279:G851-G857.

Nyachoti, C. M., F. O. Omogbenigun, M. Rademacher, and G. Blank. 2006. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. J. Anim. Sci. 84:125-134. doi:10.2527/2006.841125x

Opal, S. M., and V. A. DePalo. 2000. Anti-inflammatory cytokines. Chest. 117:1162-1172. doi:10.1378/chest.117.4.1162

Opapeju, F. O., D. O. Krause, R. L. Payne, M. Rademacher, and C. M. Nyachoti. 2009. Effect of dietary protein level on growth performance, indicators of enteric health, and gastrointestinal microbial ecology of weaned pigs induced with postweaning colibacillosis. J. Anim. Sci. 87:2635-2643. doi:10.2527/jas.2008-1310

Opapeju, F. O., M. Rademacher, G. Blank, and C. M. Nyachoti. 2008. Effect of low-protein amino acid-supplemented diets on the growth performance, gut morphology, organ weights and digesta characteristics of weaned pigs. Animal. 2:1457-1464. doi:10.1017/S175173110800270X

Opapeju, F. O., M. Rademacher, R. L. Payne, D. O. Krause, and C. M. Nyachoti. 2010. Inflammation-associated responses in piglets induced with post-weaning colibacillosis are influenced by dietary protein level. Livest. Sci. 131:58-64. doi:10.1016/j.livsci.2010.02.026

Pestka, S., C. D. Krause, D. Sarkar, M. R. Walter, Y. Shi, and P. B. Fisher. 2004. Interleukin-10 and related cytokines and receptors. Annu. Rev. Immunol. 22:929-979. doi:10.1146/annurev.immunol.22.012703.104622

Phillips, I., M. Casewell, T. Cox, B. De Groot, C. Friis, R. Jones, C. Nightingale, R. Preston, and J. Waddell. 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. J. Antimicrob. Chemother. 53:28-52. doi:10.1093/jac/dkg483

Pinchasov, Y., C. X. Mendonca, and L. S. Jensen. 1990. Broiler chick response to low protein diets supplemented with synthetic amino acids. Poult. Sci. 69:1950-1955. doi:10.3382/ps.0691950

Pluske, J. R., P. M. Siba, D. W. Pethick, Z. Durmic, B. P. Mullan, and D. J. Hampson. 1996. The incidence of swine dysentery in pigs can be reduced by feeding diets that limit the amount of fermentable substrate entering the large intestine. J. Nutr. 126:2920-2933. doi:10.1093/jn/126.11.2920

Pond, W. G., D. C. Church, and K. R. Pond. 1995. Basic animal nutrition and feeding. 4th Edition ed. John Wilet & Sons, Inc., United States of America.

Puiman, P. J., M. Jensen, B. Stoll, I. B. Renes, A. C. J. M. de Bruijn, K. Dorst, H. Schierbeek, M. Schmidt, G. Boehm, D. G. Burrin, P. T. Sangild, and J. B. van Goudoever. 2011. Intestinal threonine utilization for protein and mucin synthesis is decreased in formula-fed preterm pigs. J. Nutr. 141:1306-1311. doi:10.3945/jn.110.135145

Rhouma, M., J. M. Fairbrother, F. Beaudry, and A. Letellier. 2017. Post weaning diarrhea in pigs: risk factors and non-colistin-based control strategies. Acta Vet. Scand. 59:31. doi:10.1186/s13028-017-0299-7

Rist, V. T. S., E. Weiss, M. Eklund, and R. Mosenthin. 2013. Impact of dietary protein on microbiota composition and activity in the gastrointestinal tract of piglets in relation to gut health: a review. Animal. 7:1067-1078. doi:10.1017/S1751731113000062

Romagnani, S. 1999. Th1/Th2 cells. Inflamm. Bowel Dis. 5:285-294. doi:10.1097/00054725-199911000-00009

Sacco, R. E., S. K. Nibbelink, M. J. Baarsch, M. P. Murtaugh, and M. J. Wannemuehler. 1996. Induction of interleukin (IL)-1beta and IL-8 mRNA expression in porcine macrophages by lipopolysaccharide from *Serpulina hyodysenteriae*. Infect. Immun. 64:4369-4372.

Salmon, H., M. Berri, V. Gerdts, and F. Meurens. 2009. Humoral and cellular factors of maternal immunity in swine. Dev. Comp. Immunol. 33:384-393. doi:10.1016/j.dci.2008.07.007

Sharma, M. K., and G. R. Buettner. 1993. Interaction of vitamin C and vitamin E during free radical stress in plasma: An ESR study. Free Radical. Biol. Med. 14:649-653. doi:10.1016/0891-5849(93)90146-L

Sivertsen, T., E. Vie, A. Bernhoft, and B. Baustad. 2007. Vitamin E and selenium plasma concentrations in weanling pigs under field conditions in Norwegian pig herds. Acta Vet. Scand. 49:1-1. doi:10.1186/1751-0147-49-1

Spurlock, M. E. 1997. Regulation of metabolism and growth during immune challenge: an overview of cytokine function. J. Anim. Sci. 75:1773-1783. doi:10.2527/1997.7571773x

Stein, H. H., and D. Y. Kil. 2006. Reduced use of antibiotic growth promoters in diets fed to weanling pigs: Dietary tools, part 2. Anim. Biotech. 17:217-231. doi:10.1080/10495390600957191

Suffredini, A. F., G. Fantuzzi, R. Badolato, J. J. Oppenheim, and N. P. O'Grady. 1999. New insights into the biology of the acute phase response. J. Clin. Immunol. 19:203-214. doi:10.1023/A:1020563913045

Tengerdy, R. P., and C. F. Nockels. 1975. Vitamin E or vitamin A protects chickens against *E. coli* infection. Poult. Sci. 54:1292-1296. doi:10.3382/ps.0541292

Toledo, J. B., A. C. Furlan, P. C. Pozza, L. M. Piano, P. L. O. Carvalho, L. M. Peñuela-Sierra, and L. M. D. Huepa. 2014. Effect of the reduction of the crude protein content of diets supplemented with essential amino acids on the performance of piglets weighing 6–15kg. Livest. Sci. 168:94-101. doi:10.1016/j.livsci.2014.07.006

Tóthová, C., O. Nagy, G. Kovac, and S. Janciauskiene. 2013. The use of acute phase proteins as biomarkers of diseases in cattle and swine. Acute Phase Proteins. 103:138.

Ueno, H., H. Yamaguchi, M. Mizuta, and M. Nakazato. 2008. The role of PYY in feeding regulation. Regul. Pept. 145:12-16. doi:10.1016/j.regpep.2007.09.011

van der Klaauw, A. A., J. M. Keogh, E. Henning, V. M. Trowse, W. S. Dhillo, M. A. Ghatei, and I. S. Farooqi. 2013. High protein intake stimulates postprandial GLP1 and PYY release. Obesity. 21:1602-1607. doi:10.1002/oby.20154

van Heugten, E., J. W. Spears, and M. T. Coffey. 1994. The effect of dietary protein on performance and immune response in weanling pigs subjected to an inflammatory challenge. J. Anim. Sci. 72:2661-2669. doi:10.2527/1994.72102661x

Vigors, S., T. Sweeney, C. J. O'Shea, J. A. Browne, and J. V. O'Doherty. 2014. Improvements in growth performance, bone mineral status and nutrient digestibility in pigs following the dietary inclusion of phytase are accompanied by modifications in intestinal nutrient transporter gene expression. Br. J. Nutr. 112:688-697. doi:10.1017/S0007114514001494

Wang, D., X. S. Piao, Z. K. Zeng, T. Lu, Q. Zhang, P. F. Li, L. F. Xue, and S. W. Kim. 2011. Effects of keratinase on performance, nutrient utilization, intestinal morphology, intestinal ecology and inflammatory response of weaned piglets fed diets with different

levels of crude protein. Asian Austral. J. Anim. Sci. 24:1718-1728. doi:10.5713/ajas.2011.11132

Wang, Y., J. Zhou, G. Wang, S. Cai, X. Zeng, and S. Qiao. 2018. Advances in low-protein diets for swine. J. Anim. Sci. Biotechnol. 9:60. doi:10.1186/s40104-018-0276-7

Wei, H. K., H. X. Xue, Z. X. Zhou, and J. Peng. 2016. A carvacrol–thymol blend decreased intestinal oxidative stress and influenced selected microbes without changing the messenger RNA levels of tight junction proteins in jejunal mucosa of weaning piglets. Animal. 11:193-201. doi:10.1017/S1751731116001397

Wellock, I., P. Fortomaris, J. Houdijk, and I. Kyriazakis. 2006. The effect of dietary protein supply on the performance and risk of post-weaning enteric disorders in newly weaned pigs. Anim. Sci. 82:327-335. doi:10.1079/ASC200643

Wellock, I. J., P. D. Fortomaris, J. G. M. Houdijk, and I. Kyriazakis. 2008. Effects of dietary protein supply, weaning age and experimental enterotoxigenic *Escherichia coli* infection on newly weaned pigs: health. Animal. 2:834-842. doi:10.1017/S1751731108002048

Wijtten, P. J. A., J. v. d. Meulen, and M. W. A. Verstegen. 2011a. Intestinal barrier function and absorption in pigs after weaning: a review. Br. J. Nutr. 105:967-981. doi:10.1017/S0007114510005660

Wijtten, P. J. A., J. J. Verstijnen, T. A. T. G. van Kempen, H. B. Perdok, G. Gort, and M. W. A. Verstegen. 2011b. Lactulose as a marker of intestinal barrier function in pigs after weaning. J. Anim. Sci. 89:1347-1357. doi:10.2527/jas.2010-3571

Williams, B., M. Bosch, A. Awati, S. Konstantinov, H. Smidt, A. Akkermans, M. Verstegen, and S. Tamminga. 2005. In vitro assessment of gastrointestinal tract (GIT) fermentation: Fermentable substrates and microbial activity. Anim. Res. 54. doi:10.1051/animres:2005011

Wu, G. 2013. Amino acids: biochemistry and nutrition. CRC Press, Boca Raton, FL.

Yin, L., J. Li, H. Wang, Z. Yi, L. Wang, S. Zhang, X. Li, Q. Wang, J. Li, H. Yang, and Y. Yin. 2020. Effects of vitamin B6 on the growth performance, intestinal morphology, and gene expression in weaned piglets that are fed a low-protein diet. J. Anim. Sci. 98. doi:10.1093/jas/skaa022

Yin, Y., Z. Tang, Z. Sun, Z. Liu, T. Li, R. Huang, Z. Ruan, Z. Deng, B. Gao, L. Chen, G. Wu, and S. Kim. 2008. Effect of galacto-mannan-oligosaccharides or chitosan supplementation on cytoimmunity and humoral immunity in early-weaned piglets. Asian Austral. J. Anim. Sci. 21. doi:10.5713/ajas.2008.70408

Yu, D., W. Zhu, and S. Hang. 2019a. Effects of low-protein diet on the intestinal morphology, digestive enzyme activity, blood urea nitrogen, and gut microbiota and metabolites in weaned pigs. Arch. Anim. Nutr. 73:287-305. doi:10.1080/1745039X.2019.1614849

Yu, M., Z. Li, W. Chen, T. Rong, G. Wang, and X. Ma. 2019b. *Hermetia illucens* larvae as a potential dietary protein source altered the microbiota and modulated mucosal immune status in the colon of finishing pigs. J. Anim. Sci. Biotechnol. 10:50. doi:10.1186/s40104-019-0358-1

Yue, L. Y., and S. Y. Qiao. 2008. Effects of low-protein diets supplemented with crystalline amino acids on performance and intestinal development in piglets over the first 2 weeks after weaning. Livest. Sci. 115:144-152. doi:10.1016/j.livsci.2007.06.018

Zaken, V., R. Kohen, and A. Ornoy. 2001. Vitamins C and E improve rat embryonic antioxidant defense mechanism in diabetic culture medium. Teratology. 64:33-44. doi:10.1002/tera.1045

Zhang, S., Q. Yang, M. Ren, S. Qiao, P. He, D. Li, and X. Zeng. 2016. Effects of isoleucine on glucose uptake through the enhancement of muscular membrane concentrations of GLUT1 and GLUT4 and intestinal membrane concentrations of Na+/glucose co-transporter 1 (SGLT-1) and GLUT2. Br. J. Nutr. 116:593-602. doi:10.1017/S0007114516002439

Zhao, J., A. F. Harper, M. J. Estienne, K. E. Webb, Jr., A. P. McElroy, and D. M. Denbow. 2007. Growth performance and intestinal morphology responses in early weaned pigs to supplementation of antibiotic-free diets with an organic copper complex and spray-dried plasma protein in sanitary and nonsanitary environments. J. Anim. Sci. 85:1302-1310. doi:10.2527/jas.2006-434

Zimmerman, D. R., and S. Khajarern. 1973. Starter protein nutrition and compensatory responses in swine. J. Anim. Sci. 36:189-194. doi:10.2527/jas1973.361189x

# CHAPTER 3: EFFECT OF DIETARY CRUDE PROTEIN CONCENTRATION ON THE BLOOD PROFILE OF WEANLING PIGS DURING TIMES OF STRESS


## ABSTRACT

This experiment was conducted to test the hypothesis that a reduction in dietary crude protein (**CP**) may improve blood characteristics associated with protein utilization and improve serum vitamin levels, with a minimal negative effect on growth performance. Thirty-six weaned pigs (7.87 ± 0.42 kg) were randomly allotted to 1 of 3 dietary treatments with 12 replicate pigs per treatment. Three corn-soybean meal diets were used: 2 diets contained 22% or 19% CP, but had all indispensable amino acids (**AA**) included to meet the requirement. The last diet contained 16% CP and some of the indispensable AA in this diet were provided below the requirement. Pigs were housed individually in metabolism crates. Pig weights and daily feed allotments were recorded weekly. Blood serum samples were collected on d 1, 7, 14, 21, and 28. Growth performance data were analyzed using contrast statements and blood analytes were analyzed using repeated measures. Results demonstrated that the reduction of dietary CP had no effect ($P > 0.10$) on pig performance for the first 2 weeks post-weaning, but overall for the 28-d experiment, body weight, average daily gain, and the gain to feed ratio were linearly ($P < 0.05$) decreased as diet CP level was reduced. Blood urea nitrogen (**BUN**) concentrations were highest ($P < 0.05$) on d 14, but reducing dietary CP level reduced ($P < 0.05$) BUN. Albumin and vitamin A were the lowest ($P < 0.05$) on d 14 and vitamin E was decreased ($P < 0.05$) throughout the experiment. In conclusion, formulating weanling pig diets with AA below the requirement reduced overall pig growth performance and concentrations of BUN in blood serum.



**Key words:** crude protein, stress, growth performance, blood characteristics

## INTRODUCTION

One of the contributing causes of decreased growth performance and increased mortality in weanling pigs is post-weaning diarrhea (Pluske et al., 1997). This can be caused by the abrupt change in the environment or by diets that affect the immune response and intestinal function of pigs (McCracken et al., 1999). Transition from sow milk that is highly digestible to a solid diet that is less palatable can lead to a reduction in feed intake, and subsequently malnourishment, which leads to a weakened immune system and a more vulnerable weaned pig (Campbell et al., 2013). Weanling pigs are susceptible to infections, diseases, and villous atrophy in the gut, which may indicate that the intestinal barrier function is disturbed after weaning (Wijtten et al., 2011). Intestinal permeability increases upon diarrhea occurrence, and this may allow the entry of toxins and pathogenic microorganisms through the epithelial cells (Zhang and Guo, 2009). High concentrations of dietary crude protein (**CP**) has a negative impact on post-weaning diarrhea (Wellock et al., 2008a; 2008b; Heo et al., 2009) and reductions in dietary CP may reduce diarrhea. By reducing dietary CP concentrations in the initial diets provided post-weaning, pig growth performance may be reduced, but this reduction may be compensated by improved growth rate during the following period (Stein and Kil, 2006). However, there are at this point, a lack of data to demonstrate the effect of dietary CP level on serum vitamin levels, especially during times of stress. Therefore, an experiment was conducted to test the hypothesis that a reduction in dietary CP may improve blood characteristics associated with protein utilization, and improve serum vitamin levels, with a minimal negative effect on growth performance.

## MATERIALS AND METHODS

The protocol for the experiment was approved by the Institutional Animal Care and Use Committee at the University of Illinois prior to initiation of the experiment. Pigs that were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN) were used.

### *Diets, Animals, and Experimental Design*

All diets were based on corn, soybean meal, and fish meal (Table 3.1). The three dietary treatments included: 1) high protein (22% CP), adequate amino acid (**AA**) content; 2) low protein (19% CP), adequate AA content; and 3) low protein (16% CP), AA content below the requirement (Tables 3.2 and 3.3). These diets were fed in a two-phase system, where the phase 1 diet was fed for 6 d and the phase 2 diet was fed during the following 22 d.

By the use of a randomized complete block design, thirty-six weanling pigs (20 ± 2 d old; 7.87 ± 0.42 kg) were blocked by pig body weight (**BW**) and placed in metabolism crates on the day of weaning, with 12 replicate pigs per treatment. Placement into metabolism crates was to add additional stress on the pigs. The average barn temperature over the experiment's duration was 81.9 ± 2.2°F. Pig BW was recorded at the beginning of the experiment and on d 6, d 13, d 20, and d 27. Feed allotments were recorded daily as well. A blood sample was collected from the jugular vein of each pig on d 1, d 7, d 14, d 21, and on d 28. The blood was collected in vacutainers for serum and was centrifuged at 2,000 × g at 4˚C for 15 min to recover the serum.

### *Sample Analyses*

Diets and ingredients were analyzed for DM (Method 930.15; AOAC, 2007), ash (Method 942.05; AOAC, 2007), CP (Method 990.03; AOAC, 2007), and GE using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL). Acid hydrolyzed ether extract

(**AEE**) was analyzed by acid hydrolysis using 3 *N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY) and followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY). Diets and ingredients were also analyzed for AA [Method 982.30 E (a, b, c); AOAC, 2007].

Serum samples were frozen at -20ºC before being analyzed for blood urea nitrogen (**BUN**), total protein, and albumin using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA, USA). Vitamins A and E in blood samples were analyzed using a high-performance liquid chromatography (**HPLC**) system coupled with fluorescence detection (Aebischer et al., 1999).

***Statistical Analysis***

Data were analyzed using the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with pig as the experimental unit. For growth performance, results for all treatment groups were analyzed using the PROC MIXED procedure (Littell et al., 1998; Stewart et al., 2010). Orthogonal contrast statements (linear and quadratic) were used to demonstrate effects of CP level. Fixed effects included day and treatment, whereas replicate was considered a random effect. For analysis of the blood profile, results for all treatment groups were analyzed as repeated measures using the PROC MIXED procedure (Littell et al., 1998; Stewart et al., 2010). Fixed effects included day and treatment, and the interaction between day and treatment. An REML estimation was conducted and treatment means were calculated using LSMeans in SAS. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 < P < 0.10$, respectively.

## RESULTS

### *Growth Performance*

There were no differences in average daily gain (**ADG**) and average daily feed intake (**ADFI**) among dietary treatments for d 1 to 6 and d 6 to 13 (Table 3.4). In the third week (d 13 to 20), the reduction of dietary CP from 22% to 16% linearly ($P < 0.05$) decreased ADG and the gain to feed ratio (**G:F**). Final BW of pigs on d 20 also tended to linearly ($P = 0.061$) decrease when CP was reduced from 22% to 16%. Likewise, during the final week (d 20 to 27), reduction of CP in the diet linearly ($P < 0.05$) decreased ADG and G:F and reducing dietary CP from 22% to 16% tended to linearly ($P = 0.059$) decrease ADFI. A linear ($P < 0.05$) decrease in pig final BW was also observed on d 27. From d 1 to 27, the decrease in dietary CP linearly ($P < 0.05$) reduced ADG and G:F.

### *Blood Characteristics*

There were no interactions between dietary treatments and day post-weaning for any of the measured blood characteristics (Table 3.5). Blood urea nitrogen increased ($P < 0.05$) during the first 2 weeks (d 1 to 14), but was reduced during the final 2 weeks of the experiment (d 14 to 28) as dietary CP was reduced. Blood urea nitrogen was also lower ($P < 0.05$) for pigs fed diets containing 19% or 16% CP compared with pigs fed the diet with 22% CP. There was no effect of dietary treatment on total serum protein at any point during the experiment, but albumin concentrations were reduced ($P < 0.05$) on d 14 and 21 compared with d 28. Likewise, serum vitamin A concentrations were greater ($P < 0.05$) on d 21 and 28 compared with d 7 or 14. In contrast, serum vitamin E concentrations continued to be reduced ($P < 0.05$) from d 1 to the end of the experiment, regardless of dietary treatment.

## DISCUSSION

Diets formulated with 22% or 19% CP both met AA requirements (NRC, 2012) because of the supplementation with crystalline AA. However, SID AA for the 16% CP diet did not meet requirements. The 22% CP diet contained a CP level reflective of what is present in typical starter diets. The 19% CP diet reflected a moderate reduction of dietary CP while still providing AA to meet the requirements (NRC, 2012). Due to an extremely low AA concentration in the originally formulated 16% CP diet, not all AA requirements could be met, even with supplementation of crystalline indispensable AA. The phase 1 diet with 16% CP had SID AA that were formulated to 70.73% of the requirement, due to the limitation of Phe in the diet and the phase 2 diet with 16% CP contained SID AA that were formulated to 80.56% of the NRC requirement, due to the limitation of His. The NRC (2012) outlines the "ideal protein," as the optimum balance of AA needed for maintenance and growth. As expected, supplementation below the requirement reduced pig growth performance. These data agree with results of multiple other studies demonstrating that feeding SID AA below the requirement results in suppressed growth (Nyachoti et al., 2006; Wellock et al., 2006; Opapeju et al., 2008; Wellock et al., 2008a; Yue and Qiao, 2008).

The transition from a highly digestible milk-based diet to a corn-soybean meal based diet may cause some of the stress that is generally associated with the low feed intake and poor weight gain that happens immediately after weaning (Opapeju et al., 2008). Placement of newly weaned pigs into metabolism crates, as was done in this experiment, is another likely cause of the low growth rate during the initial 2 weeks post-weaning. By the third experimental week, pigs appeared to have adjusted to the crates, and began eating and gaining weight better. Overall, the pigs consuming diets formulated with 22% CP and 19% CP had growth performance results

that were not different and pigs on these diets appeared to better handle the stress involved with diet transition and placement in metabolism crates compared with pigs fed the 16% CP diet.

Blood urea nitrogen is generally associated with utilization efficiency of AA and nitrogen excretion in pigs (Kohn et al., 2005). Blood urea nitrogen concentrations were the greatest on d 13, which may be associated with pigs catabolizing body protein and using those AA as a source of energy due to their low feed intake post-weaning. In total, the increase in BUN that was observed for pigs fed the 22% CP diet compared with the 19% CP diet indicates that there were excess AA in the 22% CP diet. However, as CP level was reduced, the balance of AA in the diet likely resulted in all AA being fed closer to the requirement and a greater proportion of AA were therefore utilized for protein synthesis, resulting in less AA being deaminated. The greater BUN of pigs fed the 16% CP diet than pigs fed the 19% CP diet indicates that pigs fed the 16% CP diet deaminated more AA, which may have been related to the lower ADFI for these pigs. They, therefore, used some of the absorbed AA for energy.

Diet change can cause physiological responses in the pig that tend to reduce feed intake and nutrient absorption (Campbell et al., 2013), which may be the reason for the low ADFI that was observed during the second week of the experiment. Likewise, the increase in BUN on d 14 that was observed for all diets may also have been a result of diet transition. The greater ADG during the last 2 weeks of the experiment likely resulted in greater protein synthesis, which required more AA for protein synthesis, and therefore, fewer AA were deaminated with reduced BUN as a consequence. The reduction in excessive amounts of CP may reduce the proliferation of pathogenic bacteria and decrease post-weaning diarrhea (Rist et al., 2013). The subsequent improvement in gut health due to fewer pathogens may result in an increase in the absorption of nutrients and AA, with a subsequent reduced need for nutrients from body reserves to be used,

which may have also contributed to the reduced BUN concentration. This effect is not observed for albumin and total protein because they are not measures of deaminated AA, as BUN is. Pigs were transitioned to the phase 2 diet at d 7. Perhaps adjustment/transition to the phase 2 diet is the reason albumin usually tends to decrease by d 14. The stress associated with weaning, as well as changing diets, may lead to a decrease in nutrients and AA being absorbed. Therefore, the decrease in protein synthesis results in a decrease in nutrients that need to be transported via albumin because the main function of albumin is binding and transporting nutrients including AA, fatty acids, and metabolites (Quinlan et al., 2005; Francis, 2010).

Vitamin A and vitamin E are both fat soluble vitamins (NRC, 2012), but their concentration in serum changes differently post-weaning. The reduction in serum concentration of vitamin E that was observed without a similar reduction in vitamin A is in agreement with previous data (Anderson et al., 1995; Kim et al., 2016; Buchet et al., 2017). Pigs are utilizing more vitamin E during times of stress (i.e., weaning), which may be the reason for the reduced serum concentrations after weaning. Vitamin E is needed to support the immune system (Nockels, 1979; Peplowski et al., 1980) and the reduced serum concentrations of vitamin E after weaning may be a result of the pig needing to activate the active immune system during this time (Kim et al., 2016). However, there are limited data documenting blood profiles of vitamins in pigs during the post-weaning period and it is, therefore, not known if reduced serum concentration of vitamin E, but not of vitamin A, is something that is usually observed during this period.

Low CP diets reduce diarrhea (Heo et al., 2009), and the current data indicate that a low CP diet, even with AA provided below the requirement, can be fed for 2 weeks post-weaning, without reducing pig growth performance. After that time, however, AA levels need to meet

requirements to optimize growth performance. However, because most piglet diarrhea occurs during the initial 2 weeks post-weaning, it may be possible to reduce diarrhea without reducing growth performance by feeding low CP diets during the initial 2 weeks post-weaning, and then placing pigs on a diet that meets the requirements for all AA. This conclusion is in agreement with data by Stein and Kil (2006).

## CONCLUSION

Results of the experiment demonstrated that pigs fed a diet with lower levels of CP have growth performance that is not different from that of pigs fed diets with higher concentrations of CP during the initial 2 weeks after weaning. However, after 2 weeks post-weaning, feeding a low protein diet reduces both ADG and G:F ratio. Blood urea nitrogen was the greatest at d 14 after weaning, but BUN was reduced as CP in the diet was decreased from 22 to 19%. Serum albumin concentrations were the lowest around 2 weeks post-weaning and a similar effect was observed for serum vitamin A and E concentrations. In conclusion, low CP diets can be utilized as a way to reduce BUN without affecting pig growth performance during the initial 2 weeks post-weaning.

## TABLES

**Table 3.1.** Nutrient composition of corn, soybean meal, and fish meal

| Item, % | Corn | Soybean meal | Fish meal |
|---|---|---|---|
| DM | 84.75 | 88.17 | 91.74 |
| GE, kcal/kg | 3,760 | 4,148 | 4,285 |
| CP | 6.54 | 45.80 | 64.10 |
| Acid-hydrolyzed ether extract | 3.45 | 1.68 | 7.07 |
| Ash | 0.90 | 6.77 | 20.88 |
| Indispensable AA | | | |
| Arg | 0.31 | 3.35 | 3.57 |
| His | 0.20 | 1.20 | 1.12 |
| Ile | 0.23 | 2.18 | 2.37 |
| Leu | 0.72 | 3.61 | 4.06 |
| Lys | 0.25 | 2.96 | 4.34 |
| Met | 0.14 | 0.64 | 1.60 |
| Phe | 0.32 | 2.46 | 2.35 |
| Thr | 0.25 | 1.86 | 2.42 |
| Trp | 0.04 | 0.60 | 0.52 |
| Val | 0.30 | 2.19 | 2.64 |
| Dispensable AA | | | |
| Ala | 0.46 | 2.02 | 3.86 |

**Table 3.1. (cont.)**

| | | | |
|---|---|---|---|
| Asp | 0.47 | 5.30 | 5.30 |
| Cys | 0.18 | 0.66 | 0.48 |
| Glu | 1.10 | 8.42 | 7.69 |
| Gly | 0.27 | 1.96 | 4.76 |
| Pro | 0.59 | 2.56 | 3.14 |
| Ser | 0.30 | 2.13 | 2.09 |
| Tyr | 0.21 | 1.79 | 1.85 |

**Table 3.2.** Ingredient composition of phase 1 and 2 experimental diets

| | Phase 1 | | | Phase 2 | | |
|---|---|---|---|---|---|---|
| Item | 22% CP | 19% CP | 16% CP[1] | 22% CP | 19% CP | 16% CP[2] |
| Ingredient, % | | | | | | |
| Ground corn | 44.28 | 51.33 | 59.84 | 51.26 | 57.70 | 65.43 |
| Soybean meal, 48% CP | 21.00 | 13.00 | 5.50 | 28.50 | 21.50 | 14.00 |
| Fish meal | 6.00 | 6.00 | 6.00 | 5.00 | 5.00 | 5.00 |
| Blood plasma | 3.50 | 3.50 | 3.50 | - | - | - |
| Dried whey | 20.00 | 20.00 | 20.00 | 10.00 | 10.00 | 10.00 |
| Soybean oil | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Limestone | 1.05 | 1.12 | 1.20 | 1.15 | 1.20 | 1.28 |
| L-Lysine, HCl | 0.32 | 0.57 | 0.24 | 0.29 | 0.51 | 0.41 |
| DL-Methionine | 0.12 | 0.19 | 0.04 | 0.09 | 0.15 | 0.07 |
| L-Threonine | 0.08 | 0.19 | 0.03 | 0.06 | 0.16 | 0.11 |
| L-Tryptophan | - | 0.04 | - | - | 0.02 | 0.02 |
| L-Valine | - | 0.12 | - | - | 0.08 | 0.03 |

**Table 3.2. (cont.)**

| | | | | | | |
|---|---|---|---|---|---|---|
| L-Isoleucine | - | 0.08 | - | - | - | - |
| L-Phenylalanine | - | 0.13 | - | - | 0.01 | - |
| L-Histidine | - | 0.08 | - | - | 0.02 | - |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin-mineral premix[3] | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Calculated values | | | | | | |
| ME, kcal/kg | 3,482 | 3,458 | 3,500 | 3,449 | 3,437 | 3,452 |
| CP, % | 22.50 | 19.27 | 16.39 | 22.15 | 19.34 | 16.39 |
| Ca, % | 0.85 | 0.85 | 0.85 | 0.80 | 0.80 | 0.80 |
| P[4], % | 0.65 | 0.61 | 0.58 | 0.56 | 0.53 | 0.50 |
| Amino acids[5], % | | | | | | |
| Arg | 1.21 | 0.98 | 0.76 | 1.28 | 1.07 | 0.86 |
| His | 0.52 | 0.52 | 0.37 | 0.51 | 0.46 | 0.37 |
| Ile | 0.83 | 0.77 | 0.57 | 0.83 | 0.71 | 0.58 |
| Leu | 1.70 | 1.51 | 1.34 | 1.63 | 1.46 | 1.29 |

**Table 3.2. (cont.)**

| | | | | | | |
|---|---|---|---|---|---|---|
| Lys | 1.50 | 1.50 | 1.06 | 1.35 | 1.35 | 1.09 |
| Met | 0.46 | 0.49 | 0.31 | 0.43 | 0.45 | 0.34 |
| Met + Cys | 0.82 | 0.82 | 0.61 | 0.74 | 0.74 | 0.60 |
| Phe | 0.90 | 0.88 | 0.63 | 0.90 | 0.79 | 0.65 |
| Phe + Tyr | 1.53 | 1.41 | 1.06 | 1.50 | 1.30 | 1.07 |
| Thr | 0.88 | 0.88 | 0.62 | 0.79 | 0.79 | 0.64 |
| Trp | 0.26 | 0.25 | 0.17 | 0.24 | 0.22 | 0.18 |
| Val | 0.96 | 0.95 | 0.71 | 0.90 | 0.86 | 0.69 |

[1] Phenylalanine was the limiting amino acid of this diet at 70.73% of requirement.

[2] Histidine was the limiting amino acid of this diet at 80.56% of requirement.

[3] Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

[4] Standardized total tract digestible P.

[5] Amino acids are indicated as standardized ileal digestible AA.

**Table 3.3.** Analyzed composition of phase 1 and 2 experimental diets

| | Phase 1 | | | Phase 2 | | |
|---|---|---|---|---|---|---|
| Item, % | 22% CP | 19% CP | 16% CP | 22% CP | 19% CP | 16% CP |
| DM | 87.50 | 86.50 | 87.34 | 87.45 | 88.26 | 88.12 |
| GE, kcal/kg | 3,988 | 3,987 | 3,948 | 4,007 | 4,003 | 3,972 |
| CP | 21.10 | 18.18 | 15.29 | 20.88 | 19.58 | 15.50 |
| Acid-hydrolyzed ether extract | 5.86 | 5.63 | 6.20 | 5.81 | 5.85 | 6.20 |
| Ash | 5.84 | 4.88 | 4.97 | 6.04 | 6.13 | 5.69 |
| Indispensable AA | | | | | | |
| Arg | 1.25 | 1.06 | 0.75 | 1.29 | 1.14 | 0.89 |
| His | 0.53 | 0.55 | 0.35 | 0.52 | 0.49 | 0.38 |
| Ile | 0.94 | 0.91 | 0.60 | 0.94 | 0.84 | 0.68 |
| Leu | 1.85 | 1.65 | 1.34 | 1.74 | 1.58 | 1.37 |
| Lys | 1.67 | 1.76 | 1.10 | 1.52 | 1.57 | 1.35 |

**Table 3.3. (cont.)**

| | | | | | | |
|---|---|---|---|---|---|---|
| Met | 0.48 | 0.49 | 0.32 | 0.45 | 0.45 | 0.34 |
| Phe | 1.03 | 1.04 | 0.68 | 1.01 | 0.90 | 0.74 |
| Thr | 1.10 | 1.10 | 0.79 | 0.90 | 0.86 | 0.71 |
| Trp | 0.26 | 0.29 | 0.19 | 0.24 | 0.22 | 0.16 |
| Val | 1.09 | 1.10 | 0.74 | 1.01 | 0.96 | 0.76 |
| Dispensable AA | | | | | | |
| Ala | 1.11 | 1.02 | 0.84 | 1.08 | 0.98 | 0.86 |
| Asp | 2.17 | 1.85 | 1.34 | 2.10 | 1.85 | 1.44 |
| Cys | 0.38 | 0.37 | 0.29 | 0.32 | 0.30 | 0.25 |
| Glu | 3.54 | 3.03 | 2.24 | 3.56 | 3.17 | 2.61 |
| Gly | 0.94 | 0.85 | 0.68 | 0.96 | 0.85 | 0.72 |
| Pro | 1.33 | 1.17 | 0.95 | 1.22 | 1.13 | 0.99 |
| Ser | 0.95 | 0.83 | 0.65 | 0.87 | 0.77 | 0.64 |
| Tyr | 0.73 | 0.65 | 0.50 | 0.69 | 0.62 | 0.52 |

**Table 3.4.** Growth performance of pigs consuming diets containing high, medium, and low levels of crude protein[1]

| Item | Dietary crude protein level, % | | | SEM | Contrast *P*-values | |
|---|---|---|---|---|---|---|
| | 22 | 19 | 16 | | Linear | Quadratic |
| d 1 to 6 | | | | | | |
| Initial BW[2], kg | 7.86 | 7.87 | 7.88 | 0.13 | 0.220 | 0.758 |
| ADFI[3], kg | 0.30 | 0.31 | 0.27 | 0.02 | 0.262 | 0.216 |
| d 6 to 13 | | | | | | |
| ADFI, kg | 0.36 | 0.37 | 0.31 | 0.04 | 0.415 | 0.459 |
| Final BW, kg | 8.33 | 8.51 | 8.23 | 0.32 | 0.819 | 0.553 |
| d 13 to 20 | | | | | | |
| ADG[3], kg | 0.38 | 0.35 | 0.25 | 0.03 | 0.001 | 0.380 |
| ADFI, kg | 0.57 | 0.58 | 0.52 | 0.02 | 0.144 | 0.288 |
| G:F[3] | 0.67 | 0.61 | 0.48 | 0.04 | 0.003 | 0.535 |
| Final BW, kg | 11.00 | 11.13 | 9.99 | 0.39 | 0.061 | 0.181 |
| d 20 to 27 | | | | | | |
| ADG, kg | 0.46 | 0.45 | 0.34 | 0.02 | <0.001 | 0.060 |
| ADFI, kg | 0.95 | 0.96 | 0.89 | 0.02 | 0.059 | 0.192 |
| G:F | 0.49 | 0.47 | 0.38 | 0.02 | 0.001 | 0.128 |
| Final BW, kg | 14.25 | 14.31 | 12.36 | 0.50 | 0.008 | 0.098 |
| d 1 to 13 | | | | | | |
| ADG, kg | 0.04 | 0.05 | 0.03 | 0.02 | 0.772 | 0.533 |
| ADFI, kg | 0.33 | 0.34 | 0.29 | 0.03 | 0.330 | 0.338 |
| G:F | 0.09 | 0.05 | 0.02 | 0.10 | 0.604 | 0.938 |
| d 1 to 27 | | | | | | |
| ADG, kg | 0.24 | 0.24 | 0.17 | 0.02 | 0.004 | 0.091 |
| ADFI, kg | 0.54 | 0.56 | 0.50 | 0.02 | 0.129 | 0.114 |
| G:F | 0.42 | 0.41 | 0.31 | 0.02 | 0.003 | 0.141 |

[1] Data are means of 12 observations per treatment.

[2] BW = body weight.

[3] ADG = average daily gain; ADFI = average daily feed intake; G:F = gain:feed ratio.

**Table 3.5.** Blood serum analytes from pigs fed diets containing high, medium, and low levels of crude protein[1]

| Item | % CP Content | Experimental Day | | | | | SEM | *P*-values | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 7 | 14 | 21 | 28 | | CP content | Day | CP*D |
| Blood Urea | 22 | 5.50 | 12.75 | 17.00 | 8.50 | 7.08 | 0.84 | 0.001 | <0.001 | 0.190 |
| Nitrogen (mg/dL) | 19 | 4.42 | 9.25 | 12.00 | 4.34 | 3.71 | | | | |
| | 16 | 4.97 | 12.25 | 14.25 | 5.26 | 4.68 | | | | |
| Total Protein | 22 | 4.91 | 4.83 | 4.83 | 4.85 | 4.90 | 0.11 | 0.414 | 0.458 | 0.336 |
| (g/dL) | 19 | 4.63 | 4.83 | 4.80 | 4.94 | 4.82 | | | | |
| | 16 | 4.83 | 4.81 | 4.55 | 4.76 | 4.65 | | | | |
| Albumin (g/dL) | 22 | 3.13 | 3.18 | 3.02 | 2.96 | 3.13 | 0.09 | 0.740 | <0.001 | 0.231 |
| | 19 | 2.92 | 3.17 | 3.04 | 3.06 | 3.07 | | | | |
| | 16 | 3.18 | 3.21 | 2.89 | 2.88 | 2.91 | | | | |
| Vitamin A | 22 | 207.17 | 187.58 | 195.17 | 276.75 | 238.88 | 17.68 | 0.727 | 0.001 | 0.881 |
| (ng/mL) | 19 | 191.08 | 193.33 | 207.58 | 246.45 | 237.46 | | | | |
| | 16 | 197.67 | 180.38 | 180.67 | 250.33 | 229.50 | | | | |
| Vitamin E | 22 | 4,455.17 | 1,890.17 | 1,144.25 | 1,019.75 | 885.00 | 224.00 | 0.299 | <0.001 | 0.714 |
| (ng/mL) | 19 | 4,420.79 | 2,233.67 | 1,288.83 | 1,018.76 | 920.70 | | | | |
| | 16 | 4,941.70 | 2,794.01 | 1,263.63 | 932.63 | 940.17 | | | | |

[1] Data are means of 11-12 observations per treatment.

## LITERATURE CITED

Aebischer, C.-P., J. Schierle, and W. Schüep. 1999. Simultaneous determination of retinol, tocopherols, carotene, lycopene, and xanthophylls in plasma by means of reversed-phase high-performance liquid chromatography, Methods Enzymol. No. 299. Academic Press. p. 348-362.

Anderson, L. E., Sr, R. O. Myer, J. H. Brendemuhl, and L. R. McDowell. 1995. The effect of excessive dietary vitamin A on performance and vitamin E status in swine fed diets varying in dietary vitamin E. J. Anim. Sci. 73:1093-1098. doi:10.2527/1995.7341093x

AOAC. 2007. Official methods of analysis of AOAC International. AOAC International, Gaithersburg, MD.

Buchet, A., C. Belloc, M. Leblanc-Maridor, and E. Merlot. 2017. Effects of age and weaning conditions on blood indicators of oxidative status in pigs. PLoS. ONE. 12:e0178487-e0178487. doi:10.1371/journal.pone.0178487

Campbell, J. M., J. D. Crenshaw, and J. Polo. 2013. The biological stress of early weaned piglets. J. Anim. Sci. Biotechnol. 4:19-19. doi:10.1186/2049-1891-4-19

Francis, G. L. 2010. Albumin and mammalian cell culture: implications for biotechnology applications. Cytotechnology. 62:1-16. doi:10.1007/s10616-010-9263-3

Heo, J. M., J. C. Kim, C. F. Hansen, B. P. Mullan, D. J. Hampson, and J. R. Pluske. 2009. Feeding a diet with decreased protein content reduces indices of protein fermentation and the incidence of postweaning diarrhea in weaned pigs challenged with an enterotoxigenic strain of *Escherichia coli*. J. Anim. Sci. 87:2833-2843. doi:10.2527/jas.2008-1274

Kim, J. C., B. P. Mullan, J. L. Black, R. J. E. Hewitt, R. J. van Barneveld, and J. R. Pluske. 2016. Acetylsalicylic acid supplementation improves protein utilization efficiency while vitamin E supplementation reduces markers of the inflammatory response in weaned pigs challenged with enterotoxigenic *E. coli*. J. Anim. Sci. Biotechnol. 7:58. doi:10.1186/s40104-016-0118-4

Kohn, R. A., M. M. Dinneen, and E. Russek-Cohen. 2005. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. J. Anim. Sci. 83:879-889. doi:10.2527/2005.834879x

Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76:1216-1231. doi:10.2527/1998.7641216x

McCracken, B. A., M. E. Spurlock, M. A. Roos, F. A. Zuckermann, and H. R. Gaskins. 1999. Weaning anorexia may contribute to local inflammation in the piglet small intestine. J. Nutr. 129:613-619. doi:10.1093/jn/129.3.613

Nockels, C. F. 1979. Protective effects of supplemental vitamin E against infection. In: Fed. Proc. p. 2134-2138.

NRC. 2012. Nutrient requirements of swine. 11th edition ed. National Academies Press, Washington, D.C.

Nyachoti, C. M., F. O. Omogbenigun, M. Rademacher, and G. Blank. 2006. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. J. Anim. Sci. 84:125-134. doi:10.2527/2006.841125x

Opapeju, F. O., M. Rademacher, G. Blank, and C. M. Nyachoti. 2008. Effect of low-protein amino acid-supplemented diets on the growth performance, gut morphology, organ weights and digesta characteristics of weaned pigs. Animal. 2:1457-1464. doi:10.1017/S175173110800270X

Peplowski, M. A., D. C. Mahan, F. A. Murray, A. L. Moxon, A. H. Cantor, and K. E. Ekstrom. 1980. Effect of dietary and injectable vitamin E and selenium in weanling swine antigenically challenged with sheep red blood cells. J. Anim. Sci. 51:344-351. doi:10.2527/jas1980.512344x

Pluske, J. R., D. J. Hampson, and I. H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livest. Prod. Sci. 51:215-236. doi:10.1016/S0301-6226(97)00057-2

Quinlan, G. J., G. S. Martin, and T. W. Evans. 2005. Albumin: Biochemical properties and therapeutic potential. Hepatology. 41:1211-1219. doi:10.1002/hep.20720

Rist, V. T. S., E. Weiss, M. Eklund, and R. Mosenthin. 2013. Impact of dietary protein on microbiota composition and activity in the gastrointestinal tract of piglets in relation to gut health: a review. Animal. 7:1067-1078. doi:10.1017/S1751731113000062

Stein, H. H., and D. Y. Kil. 2006. Reduced use of antibiotic growth promoters in diets fed to weanling pigs: Dietary tools, part 2. Anim. Biotech. 17:217-231. doi:10.1080/10495390600957191

Stewart, L. L., B. G. Kim, B. R. Gramm, R. D. Nimmo, and H. H. Stein. 2010. Effect of virginiamycin on the apparent ileal digestibility of amino acids by growing pigs. J. Anim. Sci. 88:1718-1724. doi:10.2527/jas.2009-2063

Wellock, I., P. Fortomaris, J. Houdijk, and I. Kyriazakis. 2006. The effect of dietary protein supply on the performance and risk of post-weaning enteric disorders in newly weaned pigs. Anim. Sci. 82:327-335. doi:10.1079/ASC200643

Wellock, I. J., P. D. Fortomaris, J. G. M. Houdijk, and I. Kyriazakis. 2008a. Effects of dietary protein supply, weaning age and experimental enterotoxigenic *Escherichia coli* infection on newly weaned pigs: health. Animal. 2:834-842. doi:10.1017/S1751731108002048

Wellock, I. J., P. D. Fortomaris, J. G. M. Houdijk, and I. Kyriazakis. 2008b. Effects of dietary protein supply, weaning age and experimental enterotoxigenic *Escherichia coli* infection on newly weaned pigs: performance. Animal. 2:825-833. doi:10.1017/S1751731108001559

Wijtten, P. J. A., J. v. d. Meulen, and M. W. A. Verstegen. 2011. Intestinal barrier function and absorption in pigs after weaning: a review. Br. J. Nutr. 105:967-981. doi:10.1017/S0007114510005660

Yue, L. Y., and S. Y. Qiao. 2008. Effects of low-protein diets supplemented with crystalline amino acids on performance and intestinal development in piglets over the first 2 weeks after weaning. Livest. Sci. 115:144-152. doi:10.1016/j.livsci.2007.06.018

Zhang, B., and Y. Guo. 2009. Supplemental zinc reduced intestinal permeability by enhancing occludin and zonula occludens protein-1 (ZO-1) expression in weaning piglets. Br. J. Nutr. 102:687-693. doi:10.1017/S0007114509289033

# CHAPTER 4: EFFECTS OF DIETARY CRUDE PROTEIN LEVEL ON GROWTH PERFORMANCE, BLOOD CHARACTERISTICS, AND INDICATORS OF GUT FUNCTIONALITY IN WEANLING PIGS


## ABSTRACT

The hypothesis was that reducing crude protein (**CP**) in diets for weanling pigs, while providing amino acids (**AA**) to meet the dietary requirement, reduces post-weaning diarrhea and will improve indicators of intestinal function, while having no negative effect on pig growth performance. These indicators were decreasing gastrointestinal pH, decreasing concentrations of ammonia and volatile fatty acids in contents of the large intestine and feces, and decreasing expression of genes related with inflammation and increasing the expression of genes associated gut-function. One hundred-eighty weaned pigs (5.53 ± 0.88 kg) were randomly allotted to 3 treatments with 12 replicate pens per treatment. Treatments included 3 corn-soybean meal diets containing 22, 19, or 16% CP. Diets with 22 or 19% CP were adequate in amino acids, but the 16% CP diet provided AA below the requirement. Diets were fed for 28 d. Daily feed provisions and pig weights were recorded at the start of the experiment and weekly thereafter. Fecal scores were assessed every other day. Blood samples were collected from one pig per pen on d 1, 6, 13, 20, and 27 and 1 pig per pen was euthanized on d 12. Data for growth performance, fecal scores, morphology, intestinal pH, VFA concentrations, and relative gene abundance were analyzed by contrast statements and data for blood characteristics were analyzed using repeated measures. Results indicated that reducing dietary CP linearly ($P < 0.05$) reduced overall average daily gain (**ADG**), the gain to feed ratio (**G:F**), body weight (**BW**), and fecal scores of pigs. Total protein, albumin, peptide YY, and vitamins A and E in plasma were lowest ($P < 0.05$) on d 13, but pigs fed the 16% CP diet had reduced ($P < 0.05$) albumin and tended ($P < 0.10$) to have reduced

vitamin E compared with pigs fed other diets. Blood urea nitrogen, haptoglobin, interleukin-1β, and interleukin-6 serum concentrations were the greatest ($P < 0.05$) on d 13, while tumor necrosis factor-α and interleukin-10 concentrations were the greatest ($P < 0.05$) on d 6. Villus height in the jejunum and crypt depth in the ileum were improved ($P < 0.05$), whereas ammonia concentration in the feces on d 27 were the greatest ($P < 0.05$) when the 19% CP diet was fed. The pH in the stomach tended ($P < 0.10$) to be increased, whereas pH in the ileum was reduced ($P < 0.05$) as dietary CP decreased. Expression of interferon-γ, chemokine ligand 9, chemokine ligand 10, occludin, zonula occludens protein-1, trefoil factor-2, trefoil factor-3, mucin-2, GLUT2, and GLUT5 were all increased ($P < 0.05$) when pigs were fed the 22% CP diet, whereas expression of transforming growth factor-β was decreased when the 22% CP diet was fed instead of the 19 or 16% CP diets. In conclusion, reducing CP in diets for weanling pigs reduces fecal score and expression of genes associated with inflammation, but this does not improve growth performance. The strategy of feeding low CP diets balanced with AA may be used to mitigate diarrhea during the initial post-weaning period.

**Key words:** crude protein, growth performance, blood characteristics, diarrhea, intestinal morphology, pH, volatile fatty acids, ammonia, gene expression

## INTRODUCTION

Concerns with antibiotic resistance, plus the increased focus on restriction and legislation around the usage of feed-grade antibiotics as growth promoters, makes identifying alternative strategies to managing gut health important. Post-weaning diarrhea (**PWD**) is one of the most serious threats the swine industry faces and is one of the contributing causes to reduced growth performance and increased mortality in pigs after weaning (Rhouma et al., 2017; Pluske et al.,

1997). In a review published by Stein and Kil (2006), several dietary strategies were suggested to control post-weaning diarrhea and mortality without using antibiotic growth promoters, and one of these strategies was to reduce dietary crude protein (**CP**).

High protein (21% - 24% CP) starter diets may increase microbial colonization and fermentation because of the undigested nutrients that pass through the stomach to the gastrointestinal tract (Ball and Aherne, 1987). High pH and increased concentrations of metabolism byproducts such as ammonia (**$NH_3$**) and volatile fatty acids (**VFA**) may be used as indicators for stomach and intestinal health (Nyachoti et al., 2006). High CP diets increase buffering in the stomach, which leads to an increased intestinal pH and ultimately a more ideal environment for the propagation of pathogens. Opapeju et al. (2008) demonstrated that reduced levels of CP in the diet reduces the cecum luminal ammonia nitrogen concentration, which was correlated with a lower fecal score.

Weanling pigs are susceptible to infections, diseases, and villous atrophy in the gut, which may indicate that the intestinal barrier function is disturbed after weaning (Wijtten et al., 2011). Diarrhea occurrence increases intestinal permeability, which may allow the entry of toxins and pathogenic microorganism through the epithelial cells (Zhang and Guo, 2009). Tight junctions are made up of integral membrane proteins, mainly occludin, claudin, zonula occludens protein-1 (**ZO-1**). The integrity of the tight junctions is one of the important components of the intestinal mucosal barrier function (Ballard et al., 1995).

The concern associated with the low CP approach is the loss of pig growth performance (Nyachoti et al., 2006; Opapeju et al., 2008; Wellock et al., 2006; Wellock et al., 2008; Yue and Qiao, 2008). By reducing dietary CP concentrations in the initial diets provided post-weaning,

pig growth performance may be reduced, but this reduction may be compensated by an improved growth rate during the following period (Stein and Kil, 2006).

We demonstrated in previous experiments, that reducing levels of CP in the diet reduced blood urea nitrogen (**BUN**) concentrations; however, the same effect was not observed for total protein or albumin concentrations. The objective of this experiment was to test the hypothesis that a reduction in dietary CP, while still meeting the amino acid (**AA**) requirement, will improve indicators of intestinal function while having no negative effect on pig growth performance. These indicators are decreasing gastrointestinal pH, decreasing concentrations of ammonia and volatile fatty acids in contents of the large intestine and feces, and decreasing expression of genes related with inflammation and increasing the expression of those associated gut-function.

## MATERIALS AND METHODS

The protocol for the experiment was approved by the Institutional Animal Care and Use Committee at the University of Illinois prior to initiation of the experiment. Pigs that were the offspring of Line 359 boars and Camborough females (Pig Improvement Company, Hendersonville, TN) were used.

### *Diets, Animals, and Experimental Design*

The three dietary treatments were based on corn, soybean meal, and fish meal (Table 4.1). Treatments were as follows: 1) high protein (22% CP), adequate AA content; 2) low protein (19% CP) with adequate AA content; and 3) low protein (16% CP), AA content below the requirement. These diets were fed using a two-phase feeding system, where the phase 1 diet was fed for 7 d and the phase 2 diet being fed for the following 21 d (Table 4.2).

A total of 180 weanling pigs (20 ± 2 d old; 5.53 ± 0.88 kg) were allotted to the 3 dietary treatments using a randomized complete block design, with pigs blocked by body weight (**BW**). There were 5 pigs per pen and 12 pen replicates per treatment. The average barn temperature over the experiment's duration was 83.5 ± 8.0°F. Pig body weight was recorded at the start of the experiment and on d 7, 14, 21, and 28. Daily feed allotments were recorded and the weight of feed left in each feeder was recorded on d 7, 14, 21, and 28.

During the experiment, fecal scores of pigs were assessed visually within pen as a whole, and recorded every other day. One person assigned scores ranging from 1 to 5 (1 = normal feces; 2 = moist feces; 3 = mild diarrhea; 4 = severe diarrhea; and 5 = watery diarrhea). The frequency of diarrhea in each pen was calculated by counting pigs with a diarrhea score of 3 or greater.

A blood sample was collected from the jugular vein of one pig per pen in the mornings of d 1, 6, 13, 20, and 27. The same pig was used for blood collection throughout the experiment. The blood was collected in vacutainers for collection and serum was recovered after centrifugation at 2,000 × g at 4˚C for 15 min. Fecal samples from that same pig were collected on d 13, 20, and 27 (after the blood collection) for analysis of fecal $NH_3$ and VFA. For analysis of VFA and $NH_3$, approximately 5 g of feces were collected. After collection, fecal samples were placed in 15 mL tubes and samples were stabilized in 2*N* HCl and stored at -20°C until analyzed for concentrations of VFA. An additional 10 g of feces were collected and stored at -20°C for analysis of fecal dry matter.

One pig per pen (a different pig than the pig that blood and feces were collected from) was euthanized via captive bolt stunning on d 12. After killing, samples from the cecum and colon were collected to determine concentrations of $NH_3$ and VFA. The same sampling,

stabilizing, and storage techniques was used for the contents of the cecum and colon as was used for the fecal samples.

In the euthanized pigs, pH of the stomach, ileum, and colon was determined. The ileal mucosa, 30 cm anterior to the ileocecal valve, was scraped gently, snap frozen in liquid $N_2$, and stored at -80°C until used for mRNA and protein determination to quantify regulatory genes. Jejunum and ileum samples between 2 and 3 cm long were collected approximately 60 cm from the pylorus, for analysis of morphology.

***Sample Analyses***

Diets and ingredients were analyzed for DM (Method 930.15; AOAC, 2007), ash (Method 942.05; AOAC, 2007), CP (Method 990.03; AOAC, 2007), and GE using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL). Acid hydrolyzed ether extract (**AEE**) was analyzed by acid hydrolysis using 3 *N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY) followed by fat extraction (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY). Diets and ingredients were also analyzed for AA [Method 982.30 E (a, b, c); AOAC, 2007].

Serum samples were frozen at -20ºC for the analysis for BUN, total protein, and albumin using a Beckman Coulter Clinical Chemistry AU analyzer (Beckman Coulter, Inc., Brea, CA, USA). One blood sample was used to analyze for vitamins A and E. Another sample was analyzed for interleukin 1-beta (**IL-1ß**), interleukin 6 (**IL-6**), interleukin 10 (**IL-10**), and tumor necrosis factor-α (**TNF-α**) using individual ELISA kits (R&D Systems, Inc., Minneapolis, MN, USA). A serum sample was analyzed for immunoglobulin G (**IgG**), peptide YY (**PYY**), and haptoglobin (**Hp**; Bethyl Laboratories Inc., Montgomery, TX, Phoenix Pharmaceuticals Inc., Burlingame, CA, GenWay Biotech Inc., San Diego, CA, respectively). Vitamins A and E

concentrations in blood samples were analyzed using a high-performance liquid chromatography (**HPLC**) unit coupled with fluorescence detection (Aebischer et al., 1999).

Concentrations of VFA (acetate, propionate, butyrate, valerate, isovalerate, and isobutyrate) and $NH_3$ were analyzed according to Erwin et al. (1961) using a Hewlett-Packard (Hewlett Packard, Avondale, PA) Model 5890A gas chromatograph equipped with a flame ionization detector on a column (1.8 m × 4 mm i.d.) packed with GP 10% SP-1200/1% H3P04 on 80/100 chromosorb W/AW (Chromosorb® W/AW-DMCS, Supelco, Bellefonte, PA). Nitrogen was the carrier gas used with a flow rate of 45 mL/min. The oven, injection port, and detector port temperatures were 125, 175, and 180°C, respectively.

The pH in intestinal contents was obtained via the *in situ* method described by Morgan et al. (2014). Immediately postmortem, a pH electrode was inserted into the digesta in the lumen of the stomach, ileum, and colon while ensuring that the pH electrode did not touch the wall of the organs. This measurement was conducted in duplicate.

Total RNA from the ileal mucosa was extracted using a Qiagen RNeasy Mini Kit (Qiagen Inc., Germantown, MD) as per manufacturer's instructions and RNA was treated with DNase (Qiagen RNase-free DNase set; Qiagen Inc., Germantown, MD). The primers used to quantify the relative abundance of genes are located in Table 4.4. Gene expression for tight junction proteins (occludin, claudin-1, ZO-1), mucus synthesis (**MUC-2**), and energy metabolism (**GLUT2**, **GLUT5**) were analyzed. The following pro-inflammatory genes were measured: TNF-α, IL-1β, IL-6, interleukin-8 (**IL-8**), interleukin-21 (**IL-21**), interferon-γ (**IFN-γ**), chemokine ligand 2 (**CCL2**), chemokine ligand 9 (**CXCL9**), and chemokine ligand 10 (**CXCL10**). The following anti-inflammatory genes were also measured: interleukin-4 (**IL-4**), IL-10, interleukin 11 (**IL-11**), interleukin 13 (**IL-13**), and transforming growth factor-β (**TGF-β**). Encoding

intestinal peptide transporter 1 (**SLC15A1**), trefoil factor-1 (**TFF-1**), trefoil factor-2 (**TFF-2**), and trefoil factor-3 (**TFF-3**) were quantified as well.

The ileum and jejunum samples were cut at the mesenteric side and pinned with the serosa side down on a piece of cardboard (Nabuurs et al., 1993). Samples were fixed in 10% neutral buffered formalin until processing. After fixation, each sample was cut in 2-3 mm thick cross-sections and embedded in paraffin for slide preparation. From each sample, 3 to 4 transverse sections were selected and stained with hematoxylin and eosin. Slides were scanned using a 17-megapixel Canon Rebel 3 Ti camera mounted to a Meiji 5300 (Veterinary Diagnostic Pathology, LLC, Fort Valley, VA). Ten villi and the associated crypts were measured using ImageJ software (National Institute of Health). Villus height was measured from the villus tip to the crypt mouth and the crypts were measured from the crypt mouth to the top of the crypt valley.

***Statistical Analysis***

Data were analyzed using the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with pen as the experimental unit. For growth performance, fecal scores, ammonia, and VFA production, and gene expression, results for all treatment groups were analyzed using the PROC MIXED procedure (Littell et al., 1998; Stewart et al., 2010). Fixed effects included day and treatment, and replicate was considered a random effect. Orthogonal contrast statements (linear and quadratic) were used to demonstrate the effects of CP level. For the blood profile analysis, results for all treatment groups were analyzed as repeated measures using the PROC MIXED procedure (Littell et al., 1998; Stewart et al., 2010). Fixed effects included day and treatment, and the interaction between day and treatment. An REML estimation was conducted

and treatment means were calculated using LSMeans in SAS. Statistical significance and tendencies were considered at $P < 0.05$ and $0.05 < P < 0.10$, respectively.

## RESULTS

### *Growth Performance*

There were no differences among treatments in average daily gain (**ADG**), average daily feed intake (**ADFI**), the gain to feed ratio (**G:F**), or BW during the first week post-weaning (Table 4.5). For the second wk (d 7 to 14), there was a linear ($P < 0.05$) reduction in ADG and G:F as CP level decreased, which also led to a linear ($P < 0.05$) decrease in pig BW on d 14. In week 3 (d 14 to 21), reduction of CP in the diet linearly ($P < 0.05$) decreased ADG, ADFI, G:F, and final BW. The same results were observed for the final week of the experiment (d 21 to 28), where the reduction in dietary CP linearly ($P < 0.05$) reduced ADG, G:F, and final BW. In terms of cumulative performance, reducing CP in the diet resulted in a linear ($P < 0.05$) decrease in ADG and G:F for the first 2 weeks post-weaning (d 1 to 14) and for the 2 weeks after that (d 14 to 28). Over the entire experimental period (d 1 to 28), there was a linear ($P < 0.05$) decrease in ADG and G:F and a tendency for a reduced ($P = 0.084$) ADFI when CP in the diet was reduced.

### *Fecal Scores*

Reducing CP in the diet linearly ($P < 0.05$) reduced fecal scores in pigs for each of the first 2 weeks post-weaning (d 1 to 7 and d 7 to 14; Table 4.6), but there were no differences ($P > 0.10$) in fecal scores during the following 2 weeks. Overall, fecal scores were linearly ($P < 0.05$) reduced by decreasing the dietary CP level.

***Blood Characteristics***

Reduction in CP had a greater ($P < 0.05$) effect on reducing BUN on d 20 and 27 than on d 13 (d by BUN interaction, $P < 0.05$; Table 4.7). Blood urea nitrogen concentration was the lowest ($P < 0.05$) on d 1 and the greatest ($P < 0.05$) on d 13. A tendency for an interaction ($P = 0.080$) between CP and day was also observed for serum albumin concentrations because a greater increase in albumin concentrations were observed with increasing dietary CP on d 20 and 27 compared with d 13. Total protein concentration and serum albumin concentration were the lowest ($P < 0.05$) on d 13 compared with d 1, d 20, and d 27. Pigs consuming the 16% CP diet also had reduced ($P < 0.05$) serum albumin concentrations when compared with pigs consuming diets with greater levels of CP.

Serum IgG concentrations were the greatest ($P < 0.05$) on d 1, but no influence of dietary treatments were observed on d 6, 13, 20, or 27. Regardless of diet, serum PYY concentrations were reduced ($P < 0.05$) from d 1 to d 13; and on d 20, the serum PYY concentrations were the greatest ($P < 0.05$). An interaction ($P < 0.01$) between diet CP level and d after weaning was observed for serum Hp concentration with concentrations being reduced ($P < 0.05$) with level of CP on d 6, 13, and 20, but not on d 27. Serum vitamin A concentrations were reduced ($P < 0.05$) from d 6 to d 13 regardless of dietary treatment, but then ($P < 0.05$) increased on d 20 and 27. Likewise, serum vitamin E concentration was reduced ($P < 0.05$) from d 6 to d 13, but then increased ($P < 0.05$) on d 20 and 27. Reducing dietary CP also tended ($P = 0.068$) to reduce serum vitamin E concentrations.

Although there was no effect of dietary treatment on serum cytokine concentration, serum TNF-α and IL-10 were the greatest ($P < 0.05$) on d 6 whereas serum IL-1β and IL-6 were the greatest ($P < 0.05$) on d 13.

***Morphology of the Jejunum and Ileum***

In the jejunum, villus height and villus height:crypt depth ratio increased from pigs fed the fed the 19 or 16% CP diets compared with pigs fed the 22% CP diet (quadratic ($P < 0.05$); Table 4.8). Reducing dietary CP from 22% to 19 or 16% resulted in a reduction (quadratic, $P < 0.05$) in crypt depth in the ileum.

***Gastrointestinal pH, Volatile Fatty Acid, and Ammonium Concentrations***

Reduction in dietary CP tended ($P = 0.099$) to linearly increase the pH in the stomach of pigs on d 12 (Table 4.9). In the ileum, pH was linearly ($P < 0.05$) reduced as CP in the diet was reduced, but there was a quadratic ($P < 0.05$) effect in the colon, where pH was the lowest when pigs were fed the diet with 19% CP.

In the cecum, there was no effect ($P > 0.10$) of CP level on $NH_3$ concentration. As for VFA concentration, there tended ($P = 0.086$) to be a quadratic decrease in propionate when pigs consumed the diet with 19% CP. There also tended ($P = 0.085$) to be a linear increase in isovalerate concentration as CP in the diet was reduced. However, CP in the diet had no effect on total VFA concentration.

In the colon, dietary CP had no effect on $NH_3$ concentration. There tended ($P = 0.079$) to be a quadratic increase in butyrate concentration when pigs consumed the diet with 19% CP. Dietary CP level had no effect on total VFA concentration.

On d 13, there was no effect ($P > 0.10$) of dietary CP level on $NH_3$ or total VFA concentration in feces (Table 4.10), but a tendency ($P = 0.076$) for a quadratic increase in fecal $NH_3$ concentration on d 20 was observed as dietary CP was reduced. There was a linear ($P < 0.05$) reduction in butyrate concentration as CP decreased in the diet. There tended ($P = 0.084$) to

be a quadratic increase in isobutyrate concentration, and a quadratic ($P < 0.05$) increase in isovalerate concentration was observed when pigs were fed the 19% CP diet. On d 27, there was a quadratic ($P < 0.05$) increase in $NH_3$ concentration when pigs consumed the 19% CP diet, but dietary CP level had no effect on the concentration of individual VFA or on total VFA concentration.

***mRNA Abundance in the Ileal Mucosa***

In mucosa from the ileum, mRNA abundance of the pro-inflammatory genes IFN-γ and CXCL10 was linearly ($P < 0.05$) decreased as CP in the diet was reduced (Table 4.11). A quadratic ($P < 0.05$) decrease and a tendency ($P = 0.078$) for a quadratic decrease in mRNA abundance of IL-8 and CXCL9, respectively, were also observed as dietary CP decreased. As for anti-inflammatory mRNA abundance, there was a quadratic ($P < 0.05$) increase in IL-10 abundance and a tendency ($P = 0.070$) for a linear increase in TGF-β abundance as CP was reduced.

Abundance of occludin linearly ($P < 0.05$) decreased with the reduction of CP in the diet and a tendency ($P = 0.051$) for a quadratic decrease in ZO-1 abundance was observed as dietary CP decreased. Abundance of TFF-2 and TFF-3, 2 other gut-protective proteins, were linearly ($P < 0.05$) decreased as CP content was reduced in the diet. Abundance of MUC-2 was linearly ($P < 0.05$) reduced as dietary CP content was reduced, and the abundance of GLUT2 and GLUT5 also linearly ($P < 0.05$) decreased with reduced dietary CP.

## DISCUSSION

Via the use of crystalline AA, both the 22% and 19% CP diets provided AA to meet the requirement (NRC, 2012). In contrast, concentrations of SID AA did not meet the requirement in the 16% CP diet. The 22% CP diet represented CP levels typically present in starter diets. The 19% CP diet represented an intermediate reduction of dietary CP while still providing AA to meet the requirement (NRC, 2012). Because AA concentration was extremely low in the originally formulated 16% CP diet, not all AA requirements could be met, even with supplementation of crystalline indispensable AA. The concentration of SID Phe in the phase 1 diet with 16% CP was 71.73% of the NRC requirement and the concentration of SID His in the phase 2 diet with 16% CP was 80.56% of the NRC requirement. Consequently, the concentrations of other indispensable AA in the phase 1 and the phase 2 diets were limited to 71.73% and 80.56% of the requirement because it was assumed that Phe and His would be the first limiting AA in these diets. Pig growth performance was reduced when supplementing AA below the requirement, which is in agreement with other studies (Opapeju et al., 2008; Wellock et al., 2008; Yue and Qiao, 2008). However, although the reduction in overall growth performance was linear as dietary CP was reduced, it appears the 16% CP diet is the driver behind the statistical significance that was observed. Because both the 22% CP diet and the 19% CP diet contained AA in quantities that presumably met the requirement, it was expected that no difference in growth performance between these 2 diets would be observed. In previous experiments, it was observed that reducing CP and fortifying diets with crystalline AA had no negative effect, or even demonstrated a positive effect, on pig performance (Kerr et al., 1995; Htoo et al., 2007). It is, therefore, likely that although the 19% CP diet used in the current

experiment was supposed to meet all requirements for indispensable AA, this diet may have been marginal in 1 or more AA.

The weaning process is one of the most stressful times in a pig's life and a decrease in growth performance and an increase in mortality may be observed during this time (Campbell et al., 2013). This was also illustrated in this experiment, where pigs had a low ADG from d 1 to d 14 post-weaning. Although growth performance was low for all diets, there was not a large difference in ADG between dietary treatments from d 1 to d 14. Pig growth performance was improved by the third week after weaning. Overall ADG and G:F were reflective of the amount of CP in the diet, where increased CP level resulted in an improvement in growth performance parameters. Given that the 16% CP diet was limiting in most indispensable AA, protein synthesis was likely not maximized in pigs fed this diet, which resulted in the reduced overall ADG and final pig BW. However, the lack of significant differences among diets during the initial 2 weeks post-weaning indicates that low CP diets can be fed for the first few weeks post-weaning with only small differences in ADG and BW. This small decrease in growth performance may potentially allow for compensatory gain later in the nursery period if pigs are provided access to a diet with adequate concentrations of AA after the period with restricted intake of AA (Stein and Kil, 2006; Kil and Stein, 2010). Indeed, it has been demonstrated that pigs fed low CP diets during the initial post-weaning period can be fed a higher CP diet afterwards with no negative effect on overall growth performance (Heo et al., 2008). However, results of the current experiment indicate that if a diet that is limiting in indispensable AA is fed for more than 2 weeks post-weaning, final pig BW will be reduced compared with pigs fed diets that are adequate in indispensable AA.

In addition to the potential reduction in growth, weaning can result in increased susceptibility to diarrhea, infections, and gut disorders (Lallès et al., 2004). The observation that a reduction in CP resulted in a decrease in fecal consistency is in agreement with results of multiple other studies (Stein and Kil, 2006; Yue and Qiao, 2008).

The reason BUN was at the greatest concentration on d 13 post-weaning is likely that BUN is an indicator for AA utilization efficiency (Coma et al., 1995). The low ADG from d 1 to 14, in combination with the increase in BUN, indicates that pigs were not effectively utilizing AA for protein synthesis. The increase in BUN concentrations post-weaning may also be associated with the low feed intake that was observed, resulting in pigs catabolizing their body protein and using those AA as a source of energy. The reduction in BUN as dietary CP was reduced is also in agreement with previous data (Cho et al., 2008; Yue and Qiao, 2008). The greater BUN of pigs fed the 22% CP diet compared with pigs fed the 19 or 16% CP diets indicates that there were excess AA in this diet. The lower BUN of pigs consuming the 19 or 16% diets implies that AA were provided closer to the requirement, which is also in agreement with diet formulations. The lowest BUN in pigs is expected if diets have a balanced supply of SID AA close to or below the requirement, and the present data confirm this hypothesis. A decrease in protein synthesis was likely the reason for the reduced ADG in pigs fed the 19 or 16% CP diets when compared with pigs fed the 22% CP diet. Similarly, this is likely the reason albumin, a carrier protein, was reduced as well. This is also the reason that after d 14, when ADG increased, serum albumin concentration also increased due to the increase in protein synthesis, and therefore, an increased need for transport of AA.

The hormone PYY plays a critical role in the regulation of food intake and energy homeostasis (Ueno et al., 2008). Pigs fed full-fat rice bran had a decrease in PYY concentrations

associated with a decrease in ADFI (Casas and Stein, 2016). Although there was no effect of dietary CP concentration on PYY concentration, PYY decreased during the initial 2 weeks post-weaning when ADFI and ADG of pigs were low, but as ADG increased after d 13, the concentration of PYY also increased. However, the lack of an effect of dietary CP on serum concentrations of PYY indicates that PYY is not directly correlated with pig ADG. It is likely that PYY concentrations instead are correlated with the efficiency of utilization of energy after absorption, but data to confirm this hypothesis have not been reported.

Although the goal of the "ideal protein" concept (NRC, 2012) is to maximize growth, support of the immune system and health is not a consideration (Resink and van Kempen, 2019). With this in mind, IgG and and Hp were analyzed. Immunoglobulin G was used as an indicator of immune activation (Ilsley et al., 2005). Absorption of IgG from colostrum is generally completed 24 hours post-birth due to gut closure (Rooke et al., 2003). That is the reason the concentration of serum IgG in newborn pigs generally is high on d 1 of life, and then decreases during the following 30 days (Porter and Hill, 1970). A similar effect of day ($P < 0.05$) was observed in this experiment, where IgG was greater on the d of weaning than during the following 4 weeks. Values for serum IgG concentration that were analyzed in the current experiment were similar to those reported in previous experiments (Gomez et al., 1998; Yin et al., 2008). The decrease in serum IgG from d 1 to d 7 post-weaning was also observed previously (Rooke et al., 2003) and this may be because of protein catabolism during this time, or because of the increase in body size that occurs after weaning causing a dilution effect (Curtis and Bourne, 1971). The decrease in serum IgG concentration that was observed after weaning indicates that pigs had reduced immune ability, or had not restored their immune status to pre-

weaning levels (Cho et al., 2006). A lower IgG concentration in serum indicates a reduced ability of the animal to prevent bacterial damage to the intestinal gut surface (Deng et al., 2007).

Haptoglobin is an acute phase protein that increases during an inflammatory response (Dritz et al., 1995; Eckersall et al., 1996). The observation that serum Hp was reduced with a decrease in dietary CP content during the initial 3 weeks post-weaning indicates that pigs fed the diet with 16% CP had a greater immune response than pigs fed the diets with 22 or 19% CP. Therefore, it appears that although pigs fed the 16% CP diet had reduced ADG, these pigs had an improved immune response, which was also reflected in the improved fecal scores that were observed. However, pigs that are consuming the 16% CP diet also have an increased serum Hp concentration on d 27. This may be associated with these pigs experiencing dietary stress as a result of their diet being restricted in AA concentration.

Fat-soluble vitamins A and E, along with other vitamins, are coenzymes for nutrient metabolism (NRC, 2012). They are also involved in the antioxidant system, which can be overwhelmed by free radical production linked to immune activation (Buchet et al., 2017). The reduction of serum vitamin E without a reduction in vitamin A post-weaning that was observed in this experiment is in agreement with previous data (Buchet et al., 2017). Likewise, the observation that serum vitamin E concentration is reduced during the post-weaning period compared with the pre-weaning period is also in agreement with previous data (Sivertsen et al., 2007; Lauridsen et al., 2011; Kim et al., 2016), which may indicate that humoral and cell-mediated immune responses are reduced during this period (Bonnette et al., 1990). The current data indicate that serum concentrations of vitamin E are reduced after weaning, which support the hypothesis that pigs are utilizing vitamin E to support their active immune system. This also

agrees with the data for IgG, indicating that these pigs were not building up immunity during the experimental period.

The villus height was shorter in the jejunum and ileum of pigs used in this experiment compared with pigs from other experiments (Zhao et al., 2007; Hu et al., 2013; Yin et al., 2020). The reduction in villus height that was observed after weaning decreases the surface area for nutrient absorption, which may explain the poor growth performance during the initial 2 weeks post-weaning. The increase in villus height in the jejunum when CP was reduced from 22 to 19% CP may be associated with a reduction in undigested oligosaccharides passing through the small intestine. The content of CP in the diet was mainly manipulated via the reduction of soybean meal, so decreasing soybean meal inclusion likely reduced the amount of oligosaccharides consumed. Because young pigs are not fully able to ferment the oligosaccharides in soybean meal, feeding diets with more soybean meal decreases villus height and negatively impacts growth performance in pigs (Li et al., 1990; Li et al., 1991). However, villus height was reduced when dietary CP was decreased from 19 to 16%, which may be associated with a reduction in protein synthesis in these pigs due to limited AA supply that may be needed for cell proliferation (Núñez et al., 1996; Gu and Li, 2004). In the ileum, crypt depth was the greatest for pigs consuming the diet with 22% CP, which is in agreement with other data (Opapeju et al., 2008). Although no effect was observed in the ileum, Gu and Li (2004) also reported an increase in crypt depth in the jejunum when diets with greater dietary CP were fed. Crypt hypertrophy, or elongated crypts, is an alteration of gut morphology that may be associated with pathogen colonization or a feed antigen (Opapeju et al., 2008). The increase in crypt depth that was observed with the higher CP diets aligns with the greater concentration of soybean meal in the diet and the increase in fecal score of those pigs.

Although the data are inconsistent, it has been stated that excess protein in the diet may result in protein fermentation in the large intestine, and subsequently cause diarrhea (Rist et al., 2013). In the current experiment, pigs fed diets with an increased concentration of dietary CP had a lower pH in the stomach, but an increased pH in the ileum. With increased amounts of CP being consumed, pigs secrete more hydrochloric acid, as a measure of compensation to aid in the digestion of the increased amount of protein (Htoo et al., 2007). Diets with an increased concentration of CP have a high buffering capacity, meaning that intestinal pH will increase and a more favorable environment for the proliferation of bacteria will be created (Partanen and Mroz, 1999; Htoo et al., 2007). The increase in pH also is in agreement with the trend in fecal score, where increasing dietary CP resulted in an increased prevalence of looser feces. More CP in the diet results in increased pH in the ileum, which creates a more ideal environment for bacteria proliferation, resulting in more diarrhea. Another reason for the increase in pH that was observed in the ileum may be overcompensation of bicarbonate secretion from the pancreas. Due to the pH decrease in the stomach, the duct cells in the pancreas may secrete extra bicarbonate to buffer the digesta in the ileum (Argenzio and Southworth, 1975). Results of pH measurements from the colon are difficult to explain. The decrease of dietary CP was achieved via a reduction of soybean meal inclusion in the diet with corn used as a replacement. As CP in the diet was reduced, corn inclusion, and consequently carbohydrate inclusion, increased. Carbohydrate fermentation occurs mostly in the colon, so the decrease in pH in the colon when the diet was decreased from 22% to 19% CP may be a result of this increase in fermentable carbohydrates (Rist et al., 2013). However, in the diet with 16% CP, pH was increased compared with the 19% CP diet, which may be a result of less CP entering the hindgut, and therefore, less substrate for protein fermentation.

The pH in the stomach, ileum, and colon, and products of fermentation such as $NH_3$ and VFA are indicators of intestinal health (Htoo et al., 2007). However, in this experiment, data for $NH_3$ and VFA in the cecum and colon were erratic and did not appear to be influenced by dietary CP. The microbiota in the hindgut of pigs are unstable over the first week post-weaning. It takes 2 to 3 weeks post-weaning before fermentation capacity of the microbiota is developed (Jensen, 1998). It is, therefore, possible that the lack of differences in cecal and colonic $NH_3$ and VFA is a result of pigs struggling in the most post-weaning period and that the microbiota needed for fermentation were not developed.

The increase in $NH_3$ concentration in feces for pigs fed the 19% CP diet reflects the increased VFA concentration that was observed from pigs fed this diet. It is possible that the reason for this observation is that pigs fed the 22% CP diet had damaged microbes in the large intestine, and therefore, had a decreased capacity for fermentation and subsequent VFA and $NH_3$ synthesis. However, pigs fed the 19% CP diet had less excess CP, and therefore, less intestinal inflammation, which also resulted in reduced fecal scores that were observed for pigs fed this diet.

The balance of intestinal immunity plays a key role in an animal's health and growth performance (Liu et al., 2014). An important mediator of this balance is the intestinal mucosa. Aside from managing the exchange of ions, liquids, and nutrients across the intestinal epithelium, the intestinal mucosa also acts as a barrier against invading pathogens (Schenk and Mueller, 2008), who may induce an inflammatory response. A pathogenic bacteria challenge induces inflammation in animals and humans (Savkovic et al., 2003; Srinivasan and McSorley, 2006; Liu et al., 2014). The goal of a pro-inflammatory response is to release immune system hormones (i.e., cytokines) that cause a cascade of events that may be associate with

inflammation, such as the redirection of white blood cells to the needed location and alterations in nutrient utilization as an immune challenge becomes more difficult (Elsasser et al., 2008). The increase in inflammation results in an up-regulation of pro-inflammatory genes. The expression of IL-8, IFN-γ, CXCL9, and CXCL10 were all reduced by decreasing the amount of CP and AA in the diet, indicating less inflammation in the ileum. This activation of the immune system enables the recruitment of immune cells associated with inflammation (Zhou et al., 2003; Liu et al., 2014). The pro-inflammatory cytokine IFN-γ, as well as TNF-α and IL-1β, is also key in facilitating an immune response (Al-Sadi et al., 2009). Reducing CP in the diet results in less inflammation and a decrease in IFN-γ abundance. Because CXCL10 is also known as interferon γ-induced protein 10, it makes sense that it follows a similar trend as IFN-γ (Liu et al., 2011). As CP in the diet increased, expression of IFN-γ increased and the same was the case for CXCL10. It is also logical that CXCL9 decreased with CP in the diet, especially knowing that CXCL9 and CXCL10 both use the same CXCR3 receptor (Guo et al., 2018).

Transforming growth factor-β is an anti-inflammatory cytokine that is involved in the suppression of infiltrating cells (Chen et al., 2018b) and containment of inflammation (Al-Sadi et al., 2009). The increase in TGF-β observed in the mucosa of pigs fed lower CP diets indicates a reduction in inflammation. However, up-regulation of anti-inflammatory genes was not consistently observed in this study. It is possible that feeding the low CP diet aids in management of the immune system, and the immune system had no need to turn on transcription of the anti-inflammatory genes (Hu et al., 2013). This may also be the reason MUC-2 abundance was the lowest in the ileal mucosa of pigs consuming the 16% CP diet. Mucins are important in the local defense against pathogens and enteric bacteria (Betscher et al., 2010). A result of feeding the lower CP diet is a reduction in inflammation-related gene abundance, indicating less

mucus secretion is needed to protect the ileum. Alternatively, the decrease in mucus secretion in pigs consuming the 16% CP diet may be associated with a limitation in protein synthesis for cell turnover because the diet is limiting in AA.

Reduction in dietary CP had no effect on cytokine concentrations in the blood, indicating that the response to inflammation was localized in the ileum. However, the stress from weaning triggered an increase in circulating inflammatory cytokines, hence the increase of TNF-α, IL-1β, IL-6, and IL-10 on either d 6 or d 13 post-weaning (Kim et al., 2011).

The link between weaning stressors, feed intake post-weaning, and subsequent gut-function has been well documented (Pluske et al., 1997; Lallès et al., 2004; Horn et al., 2014). The intestinal barrier is composed of enterocyte membranes, secreted mucus, and tight junction proteins (Lambert, 2009). The tight junction complex is a multi-protein complex that is involved with epithelial permeability (Nusrat et al., 2000). The complex is composed of membrane-bound proteins, occludin and claudin, plus the adhesion molecule for the junction, ZO-1 (Li et al., 2012). Reduction in dietary CP reduced the abundance of occludin and ZO-1, indicating a decrease in permeability and "looser" junction. The TFF peptides (i.e., TFF-1, TFF-2, and TFF-3) are released by mucus-secreting cells and also have a protective effect on epithelial cells in the gastrointestinal tract (Hoffmann et al., 2001; Kim et al., 2009). If pigs are secreting less mucus when fed low CP diets, a reduced expression of the TFF proteins may be a consequence. In combination with the decreased expression of tight junction proteins, the decrease in abundance of these gut-protective proteins implies a decrease in gut function and a potential impairment in the intestinal barrier. When finishing pigs are consuming a diet with dietary CP reduced from 18 to 12% CP, claudin-3 and claudin-7 expression was decreased (Chen et al., 2018c). It was reported by Hu et al. (2013) that impairment in the intestinal barrier resulted in up-regulation of

pro-inflammatory cytokines and a decrease in the expression of tight junction proteins; however, that was not observed in this experiment. Increased pro-inflammatory cytokines may cause a disruption of the intestinal barrier, resulting in an increase in intestinal permeation of antigens in the lumen (Nusrat et al., 2000; Bruewer et al., 2006; Shen and Turner, 2006; Al-Sadi et al., 2009). Another explanation for the increase in gut-protective protein expression as dietary CP is increased is that these genes are needed in greater quantities as a control mechanism to mitigate the increased inflammation that is a consequence of greater dietary CP. Alternatively, perhaps a consequence of feeding the 16% CP diet that provided AA below the requirement is that pigs do not have enough AA to maintain the rapid cell turnover rates in the ileum, thus the decrease in gut-protective protein abundance. This would also align with results observed for villus height in the jejunum.

Some of the most important cellular nutrient transporters are the GLUT transporters, which play a key role in metabolism (Zhang et al., 2016). Glucose and galactose are transported via facilitated diffusion using GLUT2. Facilitated diffusion is also the method of transport for fructose and xylose using GLUT5. The increase in GLUT2 expression in the ileal mucosa indicates increased glucose availability for the pig (Vigors et al., 2014). Feeding a high CP diet increased GLUT2 expression (Vigors et al., 2014) and a similar response was observed in this experiment. This is decrease in glucose transporter expression may also be associated with the decrease in the expression of tight junction proteins, and so pigs are absorbing more glucose via the paracellular pathway.

## CONCLUSION

Results of this experiment indicate that pigs can be fed diets with reduced CP for the initial 2 weeks post-weaning without a large difference in growth performance, but the consistency of feces were improved as a result of reduced dietary CP. However, feeding the low CP diets for more than 2 weeks post-weaning negatively impacted ADG and G:F. Blood urea nitrogen and albumin were both decreased when pigs were fed reduced CP diets. Reducing dietary CP while upholding the "ideal protein" concept improved intestinal morphology in both the jejunum and ileum. The decrease in dietary CP content increased pH in the stomach and decreased pH in the ileum. Reducing dietary CP also reduced the expression of pro-inflammatory genes IL-8, IFN-γ, CXCL9, and CXCL10; additionally, expression of the anti-inflammatory gene TGF-β was greater in pigs fed lower levels of CP. However, the relative abundance of gut-protective proteins occludin, ZO-1, TTF-2, TFF-3, and MUC-2 were all decreased with the reduction of CP in the diet. Similarly, GLUT2 and GLUT5 were both less expressed in pigs consuming the 16% CP diet. In conclusion, feeding low CP diets that are balanced for AA may be an alternative feeding strategy for the initial period post-weaning to maximize the health of pigs and reduce feeding excess nitrogen without sacrificing growth performance or intestinal morphology. However, low CP diets should only be fed for the initial 2 weeks post-weaning.

## TABLES

**Table 4.1.** Nutrient composition of corn, soybean meal, and fish meal

| | Ingredient | | |
|---|---|---|---|
| Item, % | Corn | Soybean meal | Fish meal |
| DM | 85.89 | 87.54 | 91.39 |
| GE, kcal/kg | 3,868 | 4,196 | 4,384 |
| CP | 6.49 | 46.03 | 65.16 |
| Acid-hydrolyzed ether extract | 2.97 | 1.46 | 6.93 |
| Ash | 1.13 | 6.23 | 19.30 |
| Indispensable AA | | | |
| Arg | 0.33 | 3.37 | 3.57 |
| His | 0.20 | 1.22 | 1.34 |
| Ile | 0.25 | 2.26 | 2.60 |
| Leu | 0.75 | 3.59 | 4.28 |
| Lys | 0.25 | 2.95 | 4.32 |
| Met | 0.16 | 0.68 | 1.67 |
| Phe | 0.32 | 2.36 | 2.44 |
| Thr | 0.24 | 1.79 | 2.39 |
| Trp | 0.05 | 0.65 | 0.55 |
| Val | 0.33 | 2.30 | 2.98 |
| Dispensable AA | | | |

**Table 4.1. (cont.)**

| | | | |
|---|---|---|---|
| Ala | 0.48 | 2.01 | 3.93 |
| Asp | 0.47 | 5.29 | 5.40 |
| Cys | 0.16 | 0.65 | 0.47 |
| Glu | 1.17 | 8.46 | 7.94 |
| Gly | 0.28 | 1.97 | 4.54 |
| Pro | 0.58 | 2.28 | 2.79 |
| Ser | 0.30 | 2.07 | 1.99 |
| Tyr | 0.20 | 1.72 | 1.88 |

**Table 4.2.** Ingredient composition of phase 1 and 2 experimental diets

| | Phase 1 | | | Phase 2 | | |
|---|---|---|---|---|---|---|
| Item | 22% CP | 19% CP | 16% CP[1] | 22% CP | 19% CP | 16% CP[2] |
| Ingredient, % | | | | | | |
| Ground corn | 44.28 | 51.33 | 59.84 | 51.26 | 57.70 | 65.43 |
| Soybean meal, 48% CP | 21.00 | 13.00 | 5.50 | 28.50 | 21.50 | 14.00 |
| Fish meal | 6.00 | 6.00 | 6.00 | 5.00 | 5.00 | 5.00 |
| Blood plasma | 3.50 | 3.50 | 3.50 | - | - | - |
| Dried whey | 20.00 | 20.00 | 20.00 | 10.00 | 10.00 | 10.00 |
| Soybean oil | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Limestone | 1.05 | 1.12 | 1.20 | 1.15 | 1.20 | 1.28 |
| L-Lysine, HCl | 0.32 | 0.57 | 0.24 | 0.29 | 0.51 | 0.41 |
| DL-Methionine | 0.12 | 0.19 | 0.04 | 0.09 | 0.15 | 0.07 |
| L-Threonine | 0.08 | 0.19 | 0.03 | 0.06 | 0.16 | 0.11 |
| L-Tryptophan | - | 0.04 | - | - | 0.02 | 0.02 |
| L-Valine | - | 0.12 | - | - | 0.08 | 0.03 |

**Table 4.2. (cont.)**

| | | | | | | |
|---|---|---|---|---|---|---|
| L-Isoleucine | - | 0.08 | - | - | - | - |
| L-Phenylalanine | - | 0.13 | - | - | 0.01 | - |
| L-Histidine | - | 0.08 | - | - | 0.02 | - |
| Salt | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Vitamin-mineral premix[3] | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 | 0.15 |
| Calculated values | | | | | | |
| ME, kcal/kg | 3,482 | 3,458 | 3,500 | 3,449 | 3,437 | 3,452 |
| CP, % | 22.50 | 19.27 | 16.39 | 22.15 | 19.34 | 16.39 |
| Ca, % | 0.85 | 0.85 | 0.85 | 0.80 | 0.80 | 0.80 |
| P[4], % | 0.65 | 0.61 | 0.58 | 0.56 | 0.53 | 0.50 |
| Amino acids[5], % | | | | | | |
| Arg | 1.21 | 0.98 | 0.76 | 1.28 | 1.07 | 0.86 |
| His | 0.52 | 0.52 | 0.37 | 0.51 | 0.46 | 0.37 |
| Ile | 0.83 | 0.77 | 0.57 | 0.83 | 0.71 | 0.58 |
| Leu | 1.70 | 1.51 | 1.34 | 1.63 | 1.46 | 1.29 |

**Table 4.2. (cont.)**

| | | | | | | |
|---|---|---|---|---|---|---|
| Lys | 1.50 | 1.50 | 1.06 | 1.35 | 1.35 | 1.09 |
| Met | 0.46 | 0.49 | 0.31 | 0.43 | 0.45 | 0.34 |
| Met + Cys | 0.82 | 0.82 | 0.61 | 0.74 | 0.74 | 0.60 |
| Phe | 0.90 | 0.88 | 0.63 | 0.90 | 0.79 | 0.65 |
| Phe + Tyr | 1.53 | 1.41 | 1.06 | 1.50 | 1.30 | 1.07 |
| Thr | 0.88 | 0.88 | 0.62 | 0.79 | 0.79 | 0.64 |
| Trp | 0.26 | 0.25 | 0.17 | 0.24 | 0.22 | 0.18 |
| Val | 0.96 | 0.95 | 0.71 | 0.90 | 0.86 | 0.69 |

[1] Phenylalanine was be the limiting amino acid of this diet at 70.73% of requirement.

[2] Histidine was be the limiting amino acid of this diet at 80.56% of requirement.

[3] Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D3 as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B12, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

[4] Standardized total tract digestible P.

[5] Amino acids are indicated as standardized ileal digestible AA.

**Table 4.3.** Analyzed composition of phase 1 and 2 experimental diets

| | Phase 1 | | | Phase 2 | | |
|---|---|---|---|---|---|---|
| Item, % | 22% CP | 19% CP | 16% CP | 22% CP | 19% CP | 16% CP |
| DM | 88.82 | 88.28 | 88.20 | 87.92 | 87.88 | 87.86 |
| GE, kcal/kg | 4,076 | 4,032 | 3,989 | 4,053 | 4,046 | 4,022 |
| CP | 21.99 | 19.13 | 15.27 | 21.74 | 18.75 | 16.18 |
| Acid-hydrolyzed ether extract | 5.24 | 4.97 | 5.29 | 5.12 | 5.33 | 5.80 |
| Ash | 6.23 | 6.26 | 5.96 | 5.64 | 5.61 | 5.51 |
| Indispensable AA | | | | | | |
| Arg | 1.26 | 0.94 | 0.83 | 1.34 | 1.07 | 0.91 |
| His | 0.54 | 0.51 | 0.39 | 0.53 | 0.46 | 0.39 |
| Ile | 0.96 | 0.82 | 0.67 | 0.94 | 0.79 | 0.70 |
| Leu | 1.81 | 1.53 | 1.38 | 1.72 | 1.49 | 1.34 |
| Lys | 1.61 | 1.53 | 1.17 | 1.48 | 1.42 | 1.20 |

**Table 4.3. (cont.)**

| | | | | | | |
|---|---|---|---|---|---|---|
| Met | 0.53 | 0.51 | 0.32 | 0.46 | 0.45 | 0.39 |
| Phe | 1.02 | 0.92 | 0.72 | 1.01 | 0.85 | 0.73 |
| Thr | 1.03 | 0.94 | 0.74 | 0.87 | 0.83 | 0.71 |
| Trp | 0.26 | 0.26 | 0.18 | 0.23 | 0.22 | 0.18 |
| Val | 1.11 | 1.01 | 0.81 | 1.01 | 0.94 | 0.79 |
| Dispensable AA | | | | | | |
| Ala | 1.10 | 0.94 | 0.87 | 1.06 | 0.95 | 0.86 |
| Asp | 2.14 | 1.65 | 1.44 | 2.12 | 1.73 | 1.48 |
| Cys | 0.37 | 0.31 | 0.33 | 0.31 | 0.26 | 0.25 |
| Glu | 3.52 | 2.80 | 2.43 | 3.58 | 2.98 | 2.58 |
| Gly | 0.94 | 0.76 | 0.69 | 0.95 | 0.83 | 0.72 |
| Pro | 1.18 | 0.98 | 0.92 | 1.16 | 1.01 | 0.92 |
| Ser | 0.93 | 0.75 | 0.68 | 0.91 | 0.75 | 0.63 |
| Tyr | 0.72 | 0.59 | 0.53 | 0.71 | 0.61 | 0.52 |

**Table 4.4.** Forward and reverse primer sequences used for quantitative reverse transcription-PCR

| Item | Primer Sequences | | Citation |
|---|---|---|---|
| | Forward | Reverse | |
| Pro-Inflammatory | | | |
| TNF-α | AGCACTGAGAGCATGATCCG | GACATTGGCTACAACGTGGG | Ferrandis Vila et al., 2018 |
| IL-1β | CCAATTCAGGGACCCTACC | CATGGCTGCTTCAGAAACCT | Lapthorne et al., 2015 |
| IL-6 | TGAACTCCCTCTCCACAAGC | GGCAGTAGCCATCACCAGA | Lapthorne et al., 2015 |
| IL-8 | AAGCTTGTCAATGGAAAAGAG | TGATTCTCATCAAGCAGGTCTCC | Petrov et al., 2014 |
| IL-21 | GGCACAGTGGCCCATAAATC | GCAGCAATTCAGGGTCCAAG | Kiros et al., 2011 |
| IFN-γ | GCTTTTCAGCTTTGCGTGACT | TCACTCTCCTCTTTCCAATTCTTC | Ferrandis Vila et al., 2018 |
| CCL2 | CCGAAGCTTGAATCCTCATC | TAGCAGCAGGTGACTGGAGA | Ondrackova et al., 2013 |
| CXCL9 | AGCAGTGTTGCCTTGCTTTTGGGTATCATC | GCTGGTGTTGATGCAGGAACAACGTCC | Ondrackova et al., 2013 |

**Table 4.4. (cont.)**

| | | | |
|---|---|---|---|
| CXCL10 | CCCACATGTTGAGATCATTGC | CATCCTTATCAGTAGTGCCG | Ondrackova et al., 2013 |
| Anti-Inflammatory | | | |
| IL-4 | CCAACCCTGGTCTGCTTACTG | TTGTAAGGTGATGTCGCACTTGT | Sweeney et al., 2012 |
| IL-10 | CACTGCTCTATTGCCTGATCTTCC | AAACTCTTCACTGGGCCGAAG | Xun et al., 2015 |
| IL-11 | CAAATTCCCAGCTGACGGAGA | GTAGGAAAACAGGTCTGCTCG | Ferrandis Vila et al., 2018 |
| IL-13 | CTGACCACCAGCATGCAGTACT | GCTGCAGTCGGAGATGTTGA | Royaee et al., 2004 |
| TGF-β | CACCCCAGATCCTCCTACCT | GTCAGCACTAGCAGCCACAG | Chen et al., 2018a |
| Gut-Protective Proteins | | | |
| OCLN | TCCTGGGTGTGATGGTGTTC | CGTAGAGTCCAGTCACCGCA | Hu et al., 2013 |
| CLDN1 | AGAAGATGCGGATGGCTGTC | CCCAGAAGGCAGAGAGAAGC | Hu et al., 2013 |
| ZO1 | AAGCCCTAAGTTCAATCACAATCT | ATCAAACTCAGGAGGCGGC | Hu et al., 2013 |

**Table 4.4. (cont.)**

| | | | |
|---|---|---|---|
| TFF-1 | CCATGGAGCACAAGGTGA | AGGGTGGAAGCACCACGGGA | Scholven et al., 2009 |
| TFF-2 | CAAGAGTCTGAGGAGTGCGTCA | GACATGGGGAAGAAGCACC | Scholven et al., 2009 |
| TFF-3 | GGGAGTATGTGGGCCTGTC | AGGTGCATTCTGTTTCCTGC | Scholven et al., 2009 |
| MUC-2 | GGCTGCTCATTGAGAGGAGT | ATGTTCCCGAACTCCAAGG | Ferrandis Vila et al., 2018 |
| Miscellaneous | | | |
| GLUT2 | TTTTGGGTGTTCCGCTGGAT | GAGGCTAGCAGATGCCGTAG | Saqui-Salces et al., 2017 |
| GLUT5 | TGTGTGGCTCCTGGTAACAC | TCGGCCATGTTCGATTCCTT | Saqui-Salces et al., 2017 |
| SLC15A1 | CAGACTTCGACCACAACGGA | TTATCCCGCCAGTACCCAGA | Fiesel et al., 2014 |

**Table 4.5.** Growth performance of pigs consuming diets containing high, medium, and low levels of crude protein[1]

| Item | Dietary crude protein level, % | | | SEM | Contrast *P*-values | |
|---|---|---|---|---|---|---|
| | 22 | 19 | 16 | | Linear | Quadratic |
| d 1 to 7 | | | | | | |
| Initial BW[2], kg | 5.54 | 5.52 | 5.54 | 0.23 | 0.639 | 0.175 |
| ADG[3], kg | 0.08 | 0.07 | 0.08 | 0.01 | 0.894 | 0.439 |
| ADFI[3], kg | 0.13 | 0.13 | 0.14 | 0.01 | 0.563 | 0.447 |
| G:F[3] | 0.63 | 0.60 | 0.57 | 0.03 | 0.236 | 0.946 |
| Final BW, kg | 6.10 | 6.06 | 6.06 | 0.24 | 0.604 | 0.764 |
| d 7 to 14 | | | | | | |
| ADG, kg | 0.11 | 0.11 | 0.09 | 0.01 | 0.034 | 0.527 |
| ADFI, kg | 0.21 | 0.20 | 0.20 | 0.01 | 0.506 | 0.269 |
| G:F | 0.55 | 0.55 | 0.44 | 0.03 | 0.006 | 0.155 |
| Final BW, kg | 6.93 | 6.82 | 6.66 | 0.27 | 0.021 | 0.848 |
| d 14 to 21 | | | | | | |
| ADG, kg | 0.29 | 0.22 | 0.19 | 0.01 | <0.001 | 0.279 |
| ADFI, kg | 0.44 | 0.39 | 0.38 | 0.02 | 0.009 | 0.262 |
| G:F | 0.65 | 0.58 | 0.50 | 0.02 | <0.001 | 0.767 |
| Final BW, kg | 8.93 | 8.45 | 7.99 | 0.36 | <0.001 | 0.961 |
| d 21 to 28 | | | | | | |
| ADG, kg | 0.44 | 0.43 | 0.34 | 0.02 | <0.001 | 0.027 |
| ADFI, kg | 0.67 | 0.65 | 0.62 | 0.03 | 0.205 | 0.996 |
| G:F | 0.64 | 0.67 | 0.53 | 0.02 | <0.001 | 0.001 |
| Final BW, kg | 12.05 | 11.46 | 10.35 | 0.46 | <0.001 | 0.317 |
| d 1 to 14 | | | | | | |
| ADG, kg | 0.10 | 0.09 | 0.09 | 0.00 | 0.071 | 0.890 |
| ADFI, kg | 0.16 | 0.16 | 0.17 | 0.01 | 0.504 | 0.575 |
| G:F | 0.59 | 0.57 | 0.50 | 0.02 | 0.007 | 0.282 |
| d 14 to 28 | | | | | | |

**Table 4.5. (cont.)**

| | | | | | | |
|---|---|---|---|---|---|---|
| ADG, kg | 0.36 | 0.33 | 0.26 | 0.02 | <0.001 | 0.246 |
| ADFI, kg | 0.56 | 0.52 | 0.51 | 0.02 | 0.172 | 0.727 |
| G:F | 0.65 | 0.63 | 0.51 | 0.01 | <0.001 | 0.007 |
| d 1 to 28 | | | | | | |
| ADG, kg | 0.24 | 0.21 | 0.17 | 0.01 | <0.001 | 0.381 |
| ADFI, kg | 0.37 | 0.34 | 0.34 | 0.01 | 0.084 | 0.325 |
| G:F | 0.64 | 0.62 | 0.51 | 0.01 | <0.001 | 0.010 |

[1] Data are means of 10-12 observations per measurement.

[2] BW = body weight.

[3] ADG = average daily gain; ADFI = average daily feed intake; G:F = gain:feed ratio.

**Table 4.6.** Fecal scores of nursery pigs on diets with high, medium, and low levels of crude protein[1]

| Item | Dietary crude protein level, % | | | SEM | Contrast *P*-values | |
|---|---|---|---|---|---|---|
| | 22 | 19 | 16 | | Linear | Quadratic |
| Fecal Score | | | | | | |
| d 1 to 7 | 1.67 | 1.50 | 1.25 | 0.13 | 0.031 | 0.803 |
| d 8 to 14 | 1.67 | 1.42 | 1.21 | 0.12 | 0.009 | 0.886 |
| d 15 to 21 | 1.64 | 1.44 | 1.31 | 0.16 | 0.140 | 0.879 |
| d 22 to 28 | 1.17 | 1.15 | 1.04 | 0.06 | 0.137 | 0.561 |
| d 1 to 28 | 1.52 | 1.36 | 1.19 | 0.08 | 0.005 | 0.925 |

[1] Fecal scores were as follows: 1 - normal feces, 2 - moist feces, 3 - mild diarrhea, 4 - severe diarrhea, 5 - watery diarrhea.

**Table 4.7.** Blood serum analytes from pigs consuming diets containing high, medium, and low levels of crude protein[1]

| Item | % CP | Experimental Day | | | | | | *P*-values | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Content | 1 | 6 | 13 | 20 | 27 | SEM | CP content | Day | CP*D |
| Blood Urea | 22 | 5.83 | 9.33 | 11.50 | 8.33 | 9.33 | 0.80 | 0.008 | <0.001 | 0.011 |
| Nitrogen (mg/dL) | 19 | 5.42 | 8.50 | 12.28 | 7.25 | 5.25 | | | | |
| | 16 | 5.81 | 6.67 | 9.42 | 5.25 | 4.83 | | | | |
| Total Protein | 22 | 4.36 | 4.14 | 3.84 | 4.28 | 4.46 | 0.10 | 0.601 | <0.001 | 0.870 |
| (g/dL) | 19 | 4.37 | 4.24 | 3.89 | 4.34 | 4.41 | | | | |
| | 16 | 4.31 | 4.25 | 3.76 | 4.19 | 4.32 | | | | |
| Albumin (g/dL) | 22 | 2.55 | 2.48 | 2.19 | 2.21 | 2.56 | 0.08 | <0.001 | <0.001 | 0.080 |
| | 19 | 2.64 | 2.55 | 2.25 | 2.25 | 2.35 | | | | |
| | 16 | 2.45 | 2.41 | 2.00 | 2.03 | 2.21 | | | | |
| Peptide YY | 22 | 5.28 | 4.95 | 4.01 | 5.81 | 4.47 | 0.53 | 0.392 | <0.001 | 0.148 |
| (ng/mL) | 19 | 5.44 | 4.84 | 3.34 | 5.07 | 4.70 | | | | |
| | 16 | 5.03 | 6.52 | 3.58 | 6.58 | 5.38 | | | | |
| Immunoglobulin G | 22 | 13.34 | 6.58 | 5.99 | 4.84 | 4.41 | 0.91 | 0.984 | <0.001 | 0.597 |
| (mg/mL) | 19 | 12.18 | 7.61 | 5.73 | 5.84 | 4.65 | | | | |
| | 16 | 11.92 | 7.77 | 5.80 | 5.66 | 4.70 | | | | |
| Haptoglobin | 22 | -- | 0.54 | 1.42 | 0.71 | 0.70 | 0.11 | 0.426 | <0.001 | 0.008 |
| (mg/mL) | 19 | -- | 0.57 | 1.22 | 0.65 | 0.37 | | | | |
| | 16 | -- | 0.30 | 1.19 | 0.55 | 0.88 | | | | |

**Table 4.7. (cont.)**

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Tumor necrosis | 22 | 77.70 | 147.15 | 114.79 | 122.06 | 121.19 | 8.49 | 0.700 | <0.001 | 0.276 |
| factor-α (pg/mL) | 19 | 85.47 | 152.35 | 117.33 | 129.03 | 131.97 | | | | |
| | 16 | 86.51 | 140.92 | 121.70 | 141.43 | 107.60 | | | | |
| Interleukin 1-beta | 22 | 13.42 | 13.25 | 28.79 | 6.70 | 8.44 | 1.65 | 0.649 | <0.001 | 0.960 |
| (pg/mL) | 19 | 13.26 | 12.16 | 27.58 | 5.75 | 6.88 | | | | |
| | 16 | 13.98 | 11.78 | 26.68 | 5.29 | 6.52 | | | | |
| Interleukin-6 | 22 | 7.06 | 4.22 | 23.43 | 3.48 | 2.63 | 1.08 | 0.872 | <0.001 | 0.656 |
| (pg/mL) | 19 | 6.94 | 4.23 | 23.51 | 4.83 | 2.93 | | | | |
| | 16 | 6.81 | 5.26 | 24.39 | 3.79 | 3.47 | | | | |
| Interleukin-10 | 22 | 9.74 | 13.68 | 12.75 | 10.69 | 7.06 | 0.99 | 0.108 | <0.001 | 0.338 |
| (pg/mL) | 19 | 10.03 | 14.33 | 12.04 | 8.42 | 5.94 | | | | |
| | 16 | 8.85 | 11.18 | 9.64 | 7.75 | 5.99 | | | | |
| Vitamin A[2] | 22 | -- | 186.07 | 133.48 | 286.90 | 324.82 | 15.32 | 0.222 | <0.001 | 0.851 |
| (ng/mL) | 19 | -- | 167.53 | 124.26 | 250.96 | 303.08 | | | | |
| | 16 | -- | 172.33 | 108.24 | 249.50 | 288.06 | | | | |
| Vitamin E[2] | 22 | -- | 1,415.55 | 797.05 | 1,340.52 | 1,163.88 | 79.43 | 0.068 | <0.001 | 0.439 |
| (ng/mL) | 19 | -- | 1,396.78 | 730.64 | 1,037.64 | 1,010.28 | | | | |
| | 16 | -- | 1,183.94 | 717.83 | 1,145.56 | 1,042.69 | | | | |

[1] Data are means of 9-12 observations per treatment.

[2] Day 1 data were analyzed as a covariate due to imbalance of values across treatment. Original values (ng/mL) for vitamin A were: 22% CP, 148.29; 19% CP, 137.16; 16% CP, 175.46. Original values (ng/mL) for vitamin E were: 22% CP, 4,497.50; 19% CP, 3,503.73; 16% CP, 3,477.75.

**Table 4.8.** Effects of dietary crude protein level on the morphology of the jejunum and ileum of nursery pigs[1]

| Item | Dietary crude protein level, % | | | SEM | Contrast *P*-values | |
|---|---|---|---|---|---|---|
| | 22 | 19 | 16 | | Linear | Quadratic |
| Jejunum | | | | | | |
| Villus height, µm | 278.33 | 327.96 | 300.82 | 11.82 | 0.198 | 0.013 |
| Crypt depth, µm | 212.67 | 192.30 | 209.81 | 13.10 | 0.878 | 0.247 |
| Villus height:crypt depth ratio | 1.44 | 1.85 | 1.54 | 0.11 | 0.554 | 0.015 |
| Ileum | | | | | | |
| Villus height, µm | 242.14 | 207.39 | 248.14 | 20.52 | 0.834 | 0.132 |
| Crypt depth, µm | 200.03 | 161.00 | 171.71 | 7.89 | 0.009 | 0.008 |
| Villus height:crypt depth ratio | 1.22 | 1.30 | 1.37 | 0.12 | 0.391 | 0.980 |

[1] Data are means of 11-12 observations per treatment.

**Table 4.9.** pH and concentration of volatile fatty acids in the contents from cecum and colon of nursery pigs on diets with high, medium, and low levels of crude protein[1]

| Item | Dietary crude protein level, % | | | SEM | Contrast *P*-values | |
|---|---|---|---|---|---|---|
| | 22 | 19 | 16 | | Linear | Quadratic |
| pH | | | | | | |
| Stomach | 2.90 | 2.97 | 3.43 | 0.22 | 0.099 | 0.455 |
| Ileum | 7.07 | 6.99 | 6.68 | 0.11 | 0.016 | 0.382 |
| Colon | 6.70 | 6.52 | 6.69 | 0.07 | 0.967 | 0.023 |
| Cecum | | | | | | |
| Ammonia, mg/g | 0.20 | 0.17 | 0.19 | 0.02 | 0.892 | 0.338 |
| Volatile fatty acids | | | | | | |
| Acetate | 74.76 | 67.12 | 77.67 | 6.28 | 0.742 | 0.226 |
| Propionate | 35.25 | 30.97 | 39.39 | 2.93 | 0.335 | 0.086 |
| Butyrate | 16.39 | 15.91 | 14.88 | 1.85 | 0.573 | 0.904 |
| Isobutyrate | 0.27 | 0.29 | 0.41 | 0.07 | 0.153 | 0.471 |
| Isovalerate | 0.32 | 0.34 | 0.46 | 0.07 | 0.085 | 0.431 |
| Valerate | 2.92 | 3.11 | 3.50 | 0.51 | 0.444 | 0.866 |
| Total [VFA[2]], µmol/g | 130.64 | 118.06 | 137.17 | 10.51 | 0.667 | 0.221 |
| Colon | | | | | | |
| Ammonia, mg/g | 0.34 | 0.31 | 0.31 | 0.04 | 0.582 | 0.832 |
| Volatile fatty acids | | | | | | |
| Acetate | 78.48 | 73.22 | 73.11 | 5.71 | 0.445 | 0.665 |
| Propionate | 26.67 | 27.35 | 28.70 | 2.18 | 0.459 | 0.886 |
| Butyrate | 10.97 | 14.05 | 11.12 | 1.58 | 0.936 | 0.079 |
| Isobutyrate | 0.84 | 0.83 | 0.86 | 0.14 | 0.929 | 0.887 |
| Isovalerate | 1.05 | 1.13 | 1.14 | 0.21 | 0.778 | 0.885 |
| Valerate | 2.45 | 3.04 | 2.77 | 0.39 | 0.543 | 0.339 |
| Total [VFA], µmol/g | 120.42 | 119.61 | 117.69 | 9.27 | 0.810 | 0.954 |

[1] Data are means of 9-12 observations per measurement.

[2] VFA = volatile fatty acids.

**Table 4.10.** Concentration of volatile fatty acids in feces of nursery pigs fed diets with high, medium, and low levels of crude protein[1]

| Item | Dietary crude protein level, % | | | SEM | Contrast *P*-values | |
|---|---|---|---|---|---|---|
| | 22 | 19 | 16 | | Linear | Quadratic |
| Feces | | | | | | |
| d 13 | | | | | | |
| Ammonia, mg/g | 0.62 | 0.58 | 0.60 | 0.05 | 0.758 | 0.637 |
| Volatile fatty acids | | | | | | |
| Acetate | 73.53 | 70.39 | 70.75 | 6.05 | 0.747 | 0.815 |
| Propionate | 22.31 | 19.68 | 19.51 | 2.37 | 0.408 | 0.672 |
| Butyrate | 8.89 | 8.89 | 6.94 | 1.33 | 0.303 | 0.547 |
| Isobutyrate | 1.79 | 1.34 | 1.45 | 0.16 | 0.139 | 0.170 |
| Isovalerate | 2.42 | 1.91 | 2.11 | 0.23 | 0.319 | 0.208 |
| Valerate | 2.47 | 2.22 | 2.28 | 0.29 | 0.626 | 0.678 |
| Total [VFA[2]], µmol/g | 111.41 | 105.95 | 103.41 | 9.86 | 0.570 | 0.904 |
| d 20 | | | | | | |
| Ammonia, mg/g | 0.62 | 0.78 | 0.59 | 0.08 | 0.739 | 0.076 |
| Volatile fatty acids | | | | | | |
| Acetate | 108.93 | 112.12 | 104.50 | 4.47 | 0.403 | 0.242 |
| Propionate | 37.97 | 39.51 | 34.02 | 2.39 | 0.209 | 0.213 |
| Butyrate | 17.08 | 15.66 | 12.80 | 1.08 | 0.008 | 0.594 |
| Isobutyrate | 2.38 | 3.00 | 2.13 | 0.34 | 0.611 | 0.084 |
| Isovalerate | 3.31 | 4.47 | 3.04 | 0.51 | 0.715 | 0.047 |
| Valerate | 4.29 | 4.84 | 4.53 | 0.48 | 0.725 | 0.463 |
| Total [VFA], µmol/g | 174.70 | 179.17 | 162.15 | 8.29 | 0.236 | 0.241 |
| d 27 | | | | | | |
| Ammonia, mg/g | 0.66 | 0.85 | 0.67 | 0.07 | 0.940 | 0.030 |
| Volatile fatty acids | | | | | | |
| Acetate | 88.44 | 87.52 | 89.69 | 3.31 | 0.716 | 0.619 |
| Propionate | 28.24 | 28.98 | 31.61 | 2.29 | 0.239 | 0.700 |

**Table 4.10. (cont.)**

| | | | | | | |
|---|---|---|---|---|---|---|
| Butyrate | 12.69 | 12.46 | 12.25 | 0.68 | 0.592 | 0.987 |
| Isobutyrate | 1.84 | 2.28 | 2.02 | 0.23 | 0.548 | 0.165 |
| Isovalerate | 2.36 | 3.25 | 2.84 | 0.34 | 0.294 | 0.100 |
| Valerate | 3.87 | 4.10 | 4.22 | 0.36 | 0.416 | 0.894 |
| Total [VFA], µmol/g | 138.95 | 137.62 | 142.63 | 6.74 | 0.622 | 0.624 |

[1] Data are means of 9-12 observations per measurement.

[2] VFA = volatile fatty acids.

**Table 4.11.** Least squares means ($log_2$-backtransformed) for expression of genes in the ileal mucosa of pigs consuming diets containing high, medium, and low levels of crude protein[1]

| Item | Dietary crude protein level, % | | | SEM | Contrast *P*-values | |
|---|---|---|---|---|---|---|
| | 22 | 19 | 16 | | Linear | Quadratic |
| pro-inflammatory | | | | | | |
| TNF-α | 1.04 | 1.15 | 1.19 | 0.07 | 0.136 | 0.649 |
| IL-1β | 0.71 | 0.73 | 0.71 | 0.11 | 0.992 | 0.885 |
| IL-6 | 1.01 | 0.81 | 0.98 | 0.17 | 0.875 | 0.308 |
| IL-8 | 0.87 | 1.07 | 0.69 | 0.12 | 0.184 | 0.036 |
| IL-21 | 1.07 | 1.04 | 1.05 | 0.14 | 0.895 | 0.914 |
| IFN-γ | 1.46 | 1.04 | 0.69 | 0.19 | 0.009 | 0.890 |
| CCL2 | 0.85 | 0.99 | 0.75 | 0.11 | 0.410 | 0.140 |
| CXCL9 | 1.20 | 1.44 | 0.90 | 0.19 | 0.155 | 0.078 |
| CXCL10 | 1.41 | 1.07 | 0.67 | 0.16 | <0.001 | 0.476 |
| anti-inflammatory | | | | | | |
| IL-4 | 0.87 | 1.11 | 1.48 | 0.28 | 0.133 | 0.933 |
| IL-10 | 0.94 | 0.84 | 0.98 | 0.04 | 0.525 | 0.024 |
| IL-11 | 0.58 | 0.81 | 0.96 | 0.17 | 0.101 | 0.758 |
| IL-13 | 0.92 | 1.34 | 1.19 | 0.27 | 0.449 | 0.402 |
| TGF-β | 0.86 | 0.92 | 1.09 | 0.08 | 0.070 | 0.581 |
| gut-protective proteins | | | | | | |
| Occludin | 1.08 | 1.09 | 0.63 | 0.14 | 0.016 | 0.133 |
| Claudin-1 | 1.04 | 1.23 | 1.09 | 0.51 | 0.930 | 0.765 |
| ZO-1 | 0.96 | 1.01 | 0.88 | 0.04 | 0.165 | 0.051 |
| TFF-1 | 0.33 | 0.58 | 0.39 | 0.24 | 0.849 | 0.491 |
| TFF-2 | 1.47 | 1.11 | 0.55 | 0.17 | <0.001 | 0.275 |
| TFF-3 | 1.22 | 1.03 | 0.59 | 0.12 | <0.001 | 0.178 |
| MUC-2 | 1.23 | 1.27 | 0.73 | 0.15 | 0.011 | 0.079 |
| miscellaneous | | | | | | |
| GLUT2 | 1.25 | 1.06 | 0.60 | 0.21 | 0.024 | 0.449 |

**Table 4.11. (cont.)**

| | | | | | | |
|---|---|---|---|---|---|---|
| GLUT5 | 1.31 | 1.10 | 0.77 | 0.15 | 0.015 | 0.598 |
| SLC15A1 | 0.85 | 0.71 | 0.55 | 0.18 | 0.232 | 0.930 |

[1] Data are means of 10-12 observations per measurement.

## LITERATURE CITED

Aebischer, C.-P., J. Schierle, and W. Schüep. 1999. Simultaneous determination of retinol, tocopherols, carotene, lycopene, and xanthophylls in plasma by means of reversed-phase high-performance liquid chromatography, Methods Enzymol. No. 299. Academic Press. p. 348-362.

Al-Sadi, R., M. Boivin, and T. Ma. 2009. Mechanism of cytokine modulation of epithelial tight junction barrier. Front. Biosci. 14:2765-2778. doi:10.2741/3413

AOAC. 2007. Official methods of analysis of AOAC International. AOAC International, Gaithersburg, MD.

Argenzio, R., and M. Southworth. 1975. Sites of organic acid production and absorption in gastrointestinal tract of the pig. Am. J. Physiol. 228:454-460. doi:10.1152/ajplegacy.1975.228.2.454

Ball, R. O., and F. X. Aherne. 1987. Influence of dietary nutrient densitiy, level of feed intake and weaning age on young pigs. I. performance and body composition. Can. J. Anim. Sci. 67:1093-1103. doi:10.4141/cjas87-115

Ballard, S. T., J. H. Hunter, and A. E. Taylor. 1995. Regulation of tight-junction permeability during nutrient absorption across the intestinal epithelium. Annu. Rev. Nutr. 15:35-55. doi:10.1146/annurev.nu.15.070195.000343

Betscher, S., A. Beineke, L. Schönfeld, and J. Kamphues. 2010. Effects of diet's physical form (grinding intensity; meal/pellets) on morphological and histological parameters (e.g. ratio of neutral to acid mucins) of the gastrointestinal tract in weaned piglets. Livest. Sci. 134:149-151. doi:10.1016/j.livsci.2010.06.122

Bonnette, E. D., E. T. Kornegay, M. D. Lindemann, and C. Hammerberg. 1990. Humoral and cell-mediated immune response and performance of weaned pigs fed four supplemental vitamin E levels and housed at two nursery temperatures. J. Anim. Sci. 68:1337-1345. doi:10.2527/1990.6851337x

Bruewer, M., S. Samarin, and A. Nusrat. 2006. Inflammatory bowel disease and the apical junctional complex. Ann. N. Y. Acad. Sci. 1072:242-252. doi:10.1196/annals.1326.017

Buchet, A., C. Belloc, M. Leblanc-Maridor, and E. Merlot. 2017. Effects of age and weaning conditions on blood indicators of oxidative status in pigs. PLoS. ONE. 12:e0178487-e0178487. doi:10.1371/journal.pone.0178487

Campbell, J. M., J. D. Crenshaw, and J. Polo. 2013. The biological stress of early weaned piglets. J. Anim. Sci. Biotechnol. 4:19-19. doi:10.1186/2049-1891-4-19

Casas, G. A., and H. H. Stein. 2016. Effects of full fat or defatted rice bran on growth performance and blood characteristics of weanling pigs. J. Anim. Sci. 94:4179-4187. doi:10.2527/jas.2016-0565

Chen, C.-H., Y.-H. Lin, C.-H. Chen, Y.-H. Wang, M.-L. Yeh, T.-L. Cheng, and C.-Z. Wang. 2018a. Transforming growth factor beta 1 mediates the low-frequency vertical vibration enhanced production of tenomodulin and type I collagen in rat Achilles tendon. PLoS. ONE. 13:e0205258. doi:10.1371/journal.pone.0205258

Chen, L., T. Yang, D.-W. Lu, H. Zhao, Y.-L. Feng, H. Chen, D.-Q. Chen, N. D. Vaziri, and Y.-Y. Zhao. 2018b. Central role of dysregulation of TGF-β/Smad in CKD progression and potential targets of its treatment. Biomed. Pharmacother. 101:670-681. doi:10.1016/j.biopha.2018.02.090

Chen, X., P. Song , P. Fan, T. He, D. Jacobs, C. L. Levesque, L. J. Johnston, L. Ji, N. Ma, Y. Chen, J. Zhang, J. Zhao, and X. Ma. 2018c. Moderate dietary protein restriction optimized gut microbiota and mucosal barrier in growing pig model. Front. Cell. Infect. Microbiol. 8. doi:10.3389/fcimb.2018.00246

Cho, J. H., Y. J. Chen, B. J. Min, H. J. Kim, O. S. Kwon, K. S. Shon, I. H. Kim, S. J. Kim, and A. Asamer. 2006. Effects of essential oils supplementation on growth performance, IgG concentration and fecal noxious gas concentration of weaned pigs. Asian Austral. J. Anim. Sci. 19:80-85. doi:10.5713/ajas.2006.80

Cho, J. H., Y. J. Chen, B. J. Min, J. S. Yoo, Y. Wang, and I. H. Kim. 2008. Effects of reducing dietary crude protein on growth performance, odor gas emission from manure and blood urea nitrogen and IGF-1 concentrations of serum in nursery pigs. Anim. Sci. J. 79:453-459. doi:10.1111/j.1740-0929.2008.00549.x

Coma, J., D. Carrion, and D. R. Zimmerman. 1995. Use of plasma urea nitrogen as a rapid response criterion to determine the lysine requirement of pigs. J. Anim. Sci. 73:472-481. doi:10.2527/1995.732472x

Curtis, J., and F. J. Bourne. 1971. Immunoglobulin quantitation in sow serum, colostrum and milk and the serum of young pigs. Biochim. Biophys. Acta. 236:319-332. doi:10.1016/0005-2795(71)90181-4

Deng, Z.-Y., J.-W. Zhang, G.-Y. Wu, Y. Yin, Z. Ruan, T.-J. Li, W.-Y. Chu, X.-F. Kong, Y.-M. Zhang, Y.-W. Fan, R. Liu, and R.-L. Huang. 2007. Dietary supplementation with polysaccharides from Semen cassiae enhances immunoglobulin production and

interleukin gene expression in early-weaned piglets. J. Sci. Food Agric. 87:1868-1873. doi:10.1002/jsfa.2908

Dritz, S. S., J. Shi, T. L. Kielian, R. D. Goodband, J. L. Nelssen, M. D. Tokach, M. M. Chengappa, J. E. Smith, and F. Blecha. 1995. Influence of dietary β-glucan on growth performance, nonspecific immunity, and resistance to *Streptococcus suis* infection in weanling pigs. J. Anim. Sci. 73:3341-3350. doi:10.2527/1995.73113341x

Eckersall, P. D., P. K. Saini, and C. McComb. 1996. The acute phase response of acid soluble glycoprotein, α1-acid glycoprotein, ceruloplasmin, haptoglobin and C-reactive protein, in the pig. Vet. Immunol. Immunopathol. 51:377-385. doi:10.1016/0165-2427(95)05527-4

Elsasser, T. H., T. J. Caperna, C.-J. Li, S. Kahl, and J. L. Sartin. 2008. Critical control points in the impact of the proinflammatory immune response on growth and metabolism. J. Anim. Sci. 86:E105-E125. doi:10.2527/jas.2007-0634

Erwin, E. S., G. J. Marco, and E. M. Emery. 1961. Volatile fatty acid analyses of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44:1768-1771. doi:10.3168/jds.S0022-0302(61)89956-6

Ferrandis Vila, M., M. P. Trudeau, Y.-T. Hung, Z. Zeng, P. E. Urriola, G. C. Shurson, and M. Saqui-Salces. 2018. Dietary fiber sources and non-starch polysaccharide-degrading enzymes modify mucin expression and the immune profile of the swine ileum. PLoS. ONE. 13:e0207196-e0207196. doi:10.1371/journal.pone.0207196

Fiesel, A., D. K. Gessner, E. Most, and K. Eder. 2014. Effects of dietary polyphenol-rich plant products from grape or hop on pro-inflammatory gene expression in the intestine, nutrient

digestibility and faecal microbiota of weaned pigs. BMC Vet. Res. 10:196. doi:10.1186/s12917-014-0196-5

Gomez, G. G., O. Phillips, and R. A. Goforth. 1998. Effect of immunoglobulin source on survival, growth, and hematological and immunological variables in pigs. J. Anim. Sci. 76:1-7. doi:10.2527/1998.7611

Gu, X., and D. Li. 2004. Effect of dietary crude protein level on villous morphology, immune status and histochemistry parameters of digestive tract in weaning piglets. Anim. Feed Sci. Technol. 114:113-126. doi:10.1016/j.anifeedsci.2003.12.008

Guo, M., P. Chang, E. Hauke, B. M. Girard, K. Tooke, J. Ojala, S. M. Malley, H. Hsiang, and M. A. Vizzard. 2018. Expression and function of chemokines CXCL9-11 in micturition pathways in cyclophosphamide (CYP)-induced cystitis and somatic sensitivity in mice. Front. Syst. Neurosci. 12. doi:10.3389/fnsys.2018.00009

Heo, J.-M., J.-C. Kim, C. F. Hansen, B. P. Mullan, D. J. Hampson, and J. R. Pluske. 2008. Effects of feeding low protein diets to piglets on plasma urea nitrogen, faecal ammonia nitrogen, the incidence of diarrhoea and performance after weaning. Arch. Anim. Nutr. 62:343-358. doi:10.1080/17450390802327811

Hoffmann, W., W. Jagla, and A. Wiede. 2001. Molecular medicine of TFF-peptides: from gut to brain. Histol. Histopathol. 16:319-334.

Horn, N., F. Ruch, G. Miller, K. M. Ajuwon, and O. Adeola. 2014. Impact of acute water and feed deprivation events on growth performance, intestinal characteristics, and serum stress markers in weaned pigs. J. Anim. Sci. 92:4407-4416. doi:10.2527/jas.2014-7673

Htoo, J. K., B. A. Araiza, W. C. Sauer, M. Rademacher, Y. Zhang, M. Cervantes, and R. T. Zijlstra. 2007. Effect of dietary protein content on ileal amino acid digestibility, growth performance, and formation of microbial metabolites in ileal and cecal digesta of early-weaned pigs. J. Anim. Sci. 85:3303-3312. doi:10.2527/jas.2007-0105

Hu, C. H., K. Xiao, Z. S. Luan, and J. Song. 2013. Early weaning increases intestinal permeability, alters expression of cytokine and tight junction proteins, and activates mitogen-activated protein kinases in pigs. J. Anim. Sci. 91:1094-1101. doi:10.2527/jas.2012-5796

Ilsley, S. E., H. M. Miller, and C. Kamel. 2005. Effects of dietary quillaja saponin and curcumin on the performance and immune status of weaned piglets. J. Anim. Sci. 83:82-88. doi:10.2527/2005.83182x

Jensen, B. B. 1998. The impact of feed additives on the microbial ecology of the gut in young pigs. J. Anim. Feed Sci. 7:45-64 journal article. doi:10.22358/jafs/69955/1998

Kerr, B. J., F. K. McKeith, and R. A. Easter. 1995. Effect on performance and carcass characteristics of nursery to finisher pigs fed reduced crude protein, amino acid-supplemented diets. J. Anim. Sci. 73:433-440. doi:10.2527/1995.732433x

Kil, D. Y., and H. H. Stein. 2010. Board Invited Review: Management and feeding strategies to ameliorate the impact of removing antibiotic growth promoters from diets fed to weanling pigs. Can. J. Anim. Sci. 90:447-460. doi:10.4141/cjas10028

Kim, C. H., D. Kim, Y. Ha, K. D. Cho, B. H. Lee, I. W. Seo, S. H. Kim, and C. Chae. 2009. Expression of mucins and trefoil factor family protein-1 in the colon of pigs naturally

infected with *Salmonella typhimurium*. J. Comp. Pathol. 140:38-42. doi:10.1016/j.jcpa.2008.10.002

Kim, J. C., B. P. Mullan, J. L. Black, R. J. E. Hewitt, R. J. van Barneveld, and J. R. Pluske. 2016. Acetylsalicylic acid supplementation improves protein utilization efficiency while vitamin E supplementation reduces markers of the inflammatory response in weaned pigs challenged with enterotoxigenic *E. coli*. J. Anim. Sci. Biotechnol. 7:58. doi:10.1186/s40104-016-0118-4

Kim, M.-H., J.-Y. Yang, S. D. Upadhaya, H.-J. Lee, C.-H. Yun, and J. K. Ha. 2011. The stress of weaning influences serum levels of acute-phase proteins, iron-binding proteins, inflammatory cytokines, cortisol, and leukocyte subsets in Holstein calves. J. Vet. Sci. 12:151-157.

Kiros, T. G., J. van Kessel, L. A. Babiuk, and V. Gerdts. 2011. Induction, regulation and physiological role of IL-17 secreting helper T-cells isolated from PBMC, thymus, and lung lymphocytes of young pigs. Vet. Immunol. Immunopathol. 144:448-454. doi:10.1016/j.vetimm.2011.08.021

Lallès, J.-P., G. Boudry, C. Favier, N. Le Floc'h, I. Luron, L. Montagne, I. P. Oswald, S. Pié, C. Piel, and B. Sève. 2004. Gut function and dysfunction in young pigs: Physiology. Anim. Res. 53:301-316.

Lambert, G. P. 2009. Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects. J. Anim. Sci. 87:E101-E108. doi:10.2527/jas.2008-1339

Lapthorne, S., J. E. Bines, F. Fouhy, N. L. Dellios, G. Wilson, S. L. Thomas, M. Scurr, C. Stanton, P. D. Cotter, and P. M. Pereira-Fantini. 2015. Changes in the colon microbiota

and intestinal cytokine gene expression following minimal intestinal surgery. World J. Gastroenterol. 21:4150.

Lauridsen, C., E.-M. Vestergaard, S. Højsgaard, S. K. Jensen, and M. T. Sørensen. 2011. Inoculation of weaned pigs with *E. coli* reduces depots of vitamin E. Livest. Sci. 137:161-167. doi:10.1016/j.livsci.2010.10.015

Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, J. D. Hancock, G. L. Allee, R. D. Goodband, and R. D. Klemm. 1990. Transient hypersensitivity to soybean meal in the early-weaned pig. J. Anim. Sci. 68:1790-1799. doi:10.2527/1990.6861790x

Li, D. F., J. L. Nelssen, P. G. Reddy, F. Blecha, R. D. Klemm, D. W. Giesting, J. D. Hancock, G. L. Allee, and R. D. Goodband. 1991. Measuring suitability of soybean products for early-weaned pigs with immunological criteria. J. Anim. Sci. 69:3299-3307. doi:10.2527/1991.6983299x

Li, X., S. Akhtar, and M. A. Choudhry. 2012. Alteration in intestine tight junction protein phosphorylation and apoptosis is associated with increase in IL-18 levels following alcohol intoxication and burn injury. Biochim. Biophys. Acta. 1822:196-203. doi:10.1016/j.bbadis.2011.09.019

Liu, M., S. Guo, J. M. Hibbert, V. Jain, N. Singh, N. O. Wilson, and J. K. Stiles. 2011. CXCL10/IP-10 in infectious diseases pathogenesis and potential therapeutic implications. Cytokine Growth Factor Rev. 22:121-130. doi:10.1016/j.cytogfr.2011.06.001

Liu, Y., M. Song, T. M. Che, J. J. Lee, D. Bravo, C. W. Maddox, and J. E. Pettigrew. 2014. Dietary plant extracts modulate gene expression profiles in ileal mucosa of weaned pigs

after an *Escherichia coli* infection. J. Anim. Sci. 92:2050-2062. doi:10.2527/jas.2013-6422

Morgan, N. K., C. L. Walk, M. R. Bedford, and E. J. Burton. 2014. In vitro versus in situ evaluation of the effect of phytase supplementation on calcium and phosphorus solubility in soya bean and rapeseed meal broiler diets. Br. Poult. Sci. 55:238-245. doi:10.1080/00071668.2014.880876

Nabuurs, M. J., A. Hoogendoorn, E. J. van der Molen, and A. L. van Osta. 1993. Villus height and crypt depth in weaned and unweaned pigs, reared under various circumstances in the Netherlands. Res. Vet. Sci. 55:78-84. doi:10.1016/0034-5288(93)90038-h

NRC. 2012. Nutrient requirements of swine. 11th edition ed. National Academies Press, Washington, D.C.

Núñez, M. C., J. D. Bueno, M. V. Ayudarte, A. Almendros, A. Ríos, M. D. Suárez, and A. Gil. 1996. Dietary restriction induces biochemical and morphometric changes in the small intestine of nursing piglets. J. Nutr. 126:933-944. doi:10.1093/jn/126.4.933

Nusrat, A., J. Turner, and J. Madara. 2000. Molecular physiology and pathophysiology of tight junctions. IV. Regulation of tight junctions by extracellular stimuli: Nutrients, cytokines, and immune cells. Am. J. Physiol. 279:G851-G857.

Nyachoti, C. M., F. O. Omogbenigun, M. Rademacher, and G. Blank. 2006. Performance responses and indicators of gastrointestinal health in early-weaned pigs fed low-protein amino acid-supplemented diets. J. Anim. Sci. 84:125-134. doi:10.2527/2006.841125x

Ondrackova, P., L. Leva, Z. Kucerova, M. Vicenova, M. Mensikova, and M. Faldyna. 2013. Distribution of porcine monocytes in different lymphoid tissues and the lungs during experimental *Actinobacillus pleuropneumoniae* infection and the role of chemokines. Vet. Res. 44:98. doi:10.1186/1297-9716-44-98

Opapeju, F. O., M. Rademacher, G. Blank, and C. M. Nyachoti. 2008. Effect of low-protein amino acid-supplemented diets on the growth performance, gut morphology, organ weights and digesta characteristics of weaned pigs. Animal. 2:1457-1464. doi:10.1017/S175173110800270X

Partanen, K. H., and Z. Mroz. 1999. Organic acids for performance enhancement in pig diets. Nutr. Res. Rev. 12:117-145.

Petrov, A., M. Beer, and S. Blome. 2014. Development and validation of a harmonized TaqMan-based triplex real-time RT-PCR protocol for the quantitative detection of normalized gene expression profiles of seven porcine cytokines. PLoS. ONE. 9:e108910-e108910. doi:10.1371/journal.pone.0108910

Pluske, J. R., D. J. Hampson, and I. H. Williams. 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livest. Prod. Sci. 51:215-236. doi:10.1016/S0301-6226(97)00057-2

Porter, P., and I. R. Hill. 1970. Serological changes in immunoglobulins IgG, IgA and IgM and *Escherichia coli* antibodies in the young pig. Immunology. 18:565-573.

Resink, J. W., and T. A. T. G. van Kempen. 2019. Protective effects of amino acids after weaning. In: M. L. Chizzotti, editor, Energy and protein metabolism and nutrition No. 138. Wageningen Academic Publishers, Wageningen, The Netherlands. p. 339-340.

Rhouma, M., J. M. Fairbrother, F. Beaudry, and A. Letellier. 2017. Post weaning diarrhea in pigs: risk factors and non-colistin-based control strategies. Acta Vet. Scand. 59:31. doi:10.1186/s13028-017-0299-7

Rist, V. T. S., E. Weiss, M. Eklund, and R. Mosenthin. 2013. Impact of dietary protein on microbiota composition and activity in the gastrointestinal tract of piglets in relation to gut health: a review. Animal. 7:1067-1078. doi:10.1017/S1751731113000062

Rooke, J. A., C. Carranca, I. M. Bland, A. G. Sinclair, M. Ewen, V. C. Bland, and S. A. Edwards. 2003. Relationships between passive absorption of immunoglobulin G by the piglet and plasma concentrations of immunoglobulin G at weaning. Livest. Prod. Sci. 81:223-234. doi:10.1016/S0301-6226(02)00260-9

Royaee, A. R., R. J. Husmann, H. D. Dawson, G. Calzada-Nova, W. M. Schnitzlein, F. A. Zuckermann, and J. K. Lunney. 2004. Deciphering the involvement of innate immune factors in the development of the host response to PRRSV vaccination. Vet. Immunol. Immunopathol. 102:199-216. doi:10.1016/j.vetimm.2004.09.018

Saqui-Salces, M., Z. Huang, M. F. Vila, J. Li, J. A. Mielke, P. E. Urriola, and G. C. Shurson. 2017. Modulation of intestinal cell differentiation in growing pigs is dependent on the fiber source in the diet. J. Anim. Sci. 95:1179-1190. doi:10.2527/jas.2016.0947

Savkovic, S. D., A. Koutsouris, and G. Hecht. 2003. PKCζ participates in activation of inflammatory response induced by enteropathogenic *E. coli*. Am. J. Physiol. Cell Physiol. 285:C512-C521. doi:10.1152/ajpcell.00444.2002

Schenk, M., and C. Mueller. 2008. The mucosal immune system at the gastrointestinal barrier. Best Pract. Res. Clin. Gastroenterol. 22:391-409. doi:10.1016/j.bpg.2007.11.002

Scholven, J., D. Taras, S. Sharbati, J. Schön, C. Gabler, O. Huber, D. M. zum Büschenfelde, N. Blin, and R. Einspanier. 2009. Intestinal expression of TFF and related genes during postnatal development in a piglet probiotic trial. Cell. Physiol. Biochem. 23:143-156.

Shen, L., and J. R. Turner. 2006. Role of epithelial cells in initiation and propagation of intestinal inflammation. Eliminating the static: tight junction dynamics exposed. Am. J. Physiol. Gastrointest. Liver. Physiol. 290:G577-G582. doi:10.1152/ajpgi.00439.2005

Sivertsen, T., E. Vie, A. Bernhoft, and B. Baustad. 2007. Vitamin E and selenium plasma concentrations in weanling pigs under field conditions in Norwegian pig herds. Acta Vet. Scand. 49:1-1. doi:10.1186/1751-0147-49-1

Srinivasan, A., and S. J. McSorley. 2006. Activation of *Salmonella*-specific immune responses in the intestinal mucosa. Arch. Immunol. Ther. Exp. 54:25-31. doi:10.1007/s00005-006-0003-5

Stein, H. H., and D. Y. Kil. 2006. Reduced use of antibiotic growth promoters in diets fed to weanling pigs: Dietary tools, part 2. Anim. Biotech. 17:217-231. doi:10.1080/10495390600957191

Sweeney, T., C. B. Collins, P. Reilly, K. M. Pierce, M. Ryan, and J. V. O'Doherty. 2012. Effect of purified β-glucans derived from *Laminaria digitata*, *Laminaria hyperborea* and *Saccharomyces cerevisiae* on piglet performance, selected bacterial populations, volatile fatty acids and pro-inflammatory cytokines in the gastrointestinal tract of pigs. Br. J. Nutr. 108:1226-1234. doi:10.1017/S0007114511006751

Ueno, H., H. Yamaguchi, M. Mizuta, and M. Nakazato. 2008. The role of PYY in feeding regulation. Regul. Pept. 145:12-16. doi:10.1016/j.regpep.2007.09.011

Vigors, S., T. Sweeney, C. J. O'Shea, J. A. Browne, and J. V. O'Doherty. 2014. Improvements in growth performance, bone mineral status and nutrient digestibility in pigs following the dietary inclusion of phytase are accompanied by modifications in intestinal nutrient transporter gene expression. Br. J. Nutr. 112:688-697. doi:10.1017/S0007114514001494

Wellock, I., P. Fortomaris, J. Houdijk, and I. Kyriazakis. 2006. The effect of dietary protein supply on the performance and risk of post-weaning enteric disorders in newly weaned pigs. Anim. Sci. 82:327-335. doi:10.1079/ASC200643

Wellock, I. J., P. D. Fortomaris, J. G. M. Houdijk, and I. Kyriazakis. 2008. Effects of dietary protein supply, weaning age and experimental enterotoxigenic *Escherichia coli* infection on newly weaned pigs: health. Animal. 2:834-842. doi:10.1017/S1751731108002048

Wijtten, P. J. A., J. J. Verstijnen, T. A. T. G. van Kempen, H. B. Perdok, G. Gort, and M. W. A. Verstegen. 2011. Lactulose as a marker of intestinal barrier function in pigs after weaning. J. Anim. Sci. 89:1347-1357. doi:10.2527/jas.2010-3571

Xun, W., L. Shi, H. Zhou, G. Hou, T. Cao, and C. Zhao. 2015. Effects of curcumin on growth performance, jejunal mucosal membrane integrity, morphology and immune status in weaned piglets challenged with enterotoxigenic *Escherichia coli*. Int. Immunopharmacol. 27:46-52. doi:10.1016/j.intimp.2015.04.038

Yin, L., J. Li, H. Wang, Z. Yi, L. Wang, S. Zhang, X. Li, Q. Wang, J. Li, H. Yang, and Y. Yin. 2020. Effects of vitamin B6 on the growth performance, intestinal morphology, and gene expression in weaned piglets that are fed a low-protein diet. J. Anim. Sci. 98. doi:10.1093/jas/skaa022

Yin, Y., Z. Tang, Z. Sun, Z. Liu, T. Li, R. Huang, Z. Ruan, Z. Deng, B. Gao, L. Chen, G. Wu, and S. Kim. 2008. Effect of galacto-mannan-oligosaccharides or chitosan supplementation on cytoimmunity and humoral immunity in early-weaned piglets. Asian Austral. J. Anim. Sci. 21. doi:10.5713/ajas.2008.70408

Yue, L. Y., and S. Y. Qiao. 2008. Effects of low-protein diets supplemented with crystalline amino acids on performance and intestinal development in piglets over the first 2 weeks after weaning. Livest. Sci. 115:144-152. doi:10.1016/j.livsci.2007.06.018

Zhang, B., and Y. Guo. 2009. Supplemental zinc reduced intestinal permeability by enhancing occludin and zonula occludens protein-1 (ZO-1) expression in weaning piglets. Br. J. Nutr. 102:687-693. doi:10.1017/S0007114509289033

Zhang, S., Q. Yang, M. Ren, S. Qiao, P. He, D. Li, and X. Zeng. 2016. Effects of isoleucine on glucose uptake through the enhancement of muscular membrane concentrations of GLUT1 and GLUT4 and intestinal membrane concentrations of Na+/glucose co-transporter 1 (SGLT-1) and GLUT2. Br. J. Nutr. 116:593-602. doi:10.1017/S0007114516002439

Zhao, J., A. F. Harper, M. J. Estienne, K. E. Webb, Jr., A. P. McElroy, and D. M. Denbow. 2007. Growth performance and intestinal morphology responses in early weaned pigs to supplementation of antibiotic-free diets with an organic copper complex and spray-dried plasma protein in sanitary and nonsanitary environments. J. Anim. Sci. 85:1302-1310. doi:10.2527/jas.2006-434

Zhou, X., J. A. Girón, A. G. Torres, J. A. Crawford, E. Negrete, S. N. Vogel, and J. B. Kaper. 2003. Flagellin of enteropathogenic *Escherichia coli* stimulates interleukin-8 production in T84 cells. Infect. Immun. 71:2120-2129.

# CHAPTER 5: CONCLUSIONS

Results from this work indicate that reducing the crude protein (**CP**) content in diets for weanling pigs can be used during the initial 2 weeks post-weaning to reduce post-weaning diarrhea (**PWD**) without having a great impact on growth performance. However, if low CP diets are fed longer than the initial 2 weeks post-weaning, it is important to provide amino acids (**AA**) to meet the requirement in order to sustain average daily gain. Reducing CP while maintaining AA balance also maximized villus height and villus height:crypt depth in the jejunum and positively impacted crypt depth in the ileum.

Reducing dietary CP reduced blood urea nitrogen, indicating that AA are being fed closer to the requirement; however, serum albumin concentrations were also decreased when reducing CP in the diet. Reducing dietary CP resulted in increased pH in the stomach, but a lower pH in the ileum, which may be associated with the decrease in fecal consistency that was observed.

The reduction of dietary CP increased the expression of pro-inflammatory genes and decreased the expression of anti-inflammatory genes in the ileum, indicating less inflammation in pigs fed low CP diets. However, reducing dietary CP had no effect on cytokines in blood serum, indicating that the inflammation observed from feeding increased levels of CP was localized in the ileum.

Overall, low CP diets that provide AA to meet the animal's requirement can be utilized as an alternative nutritional strategy to using antibiotics in diets fed to weanling pigs to optimize the balance of maximizing growth performance, reducing PWD, and enhancing gut health in pigs post-weaning.