REPLACING DIETARY ANTIBIOTICS WITH L-GLUTAMINE FOLLOWING WEANING AND TRANSPORT IN SWINE

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To my wife, Lisa, and my parents for all your support

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ABSTRACT

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In recent years, U.S. swine producers have received pressure from consumers to reduce antibiotic usage. With the increased consumer pressure, pork producers have sought out other technologies, including feed additives, to reduce antibiotic usage in commercial pork production. Therefore, the objective of Chapter 2 was to determine whether supplementing L-glutamine at cost-effective levels can replace dietary antibiotics to improve pig welfare and productivity following weaning and transport. Based on previous research, we hypothesized that withholding dietary antibiotics would negatively affect pigs while diet supplementation with 0.20% L-glutamine (**GLN**) would have similar effects on pig performance and health as antibiotics. Mixed sex pigs (N = 480; 5.62 ± 0.06 kg BW) were weaned (18.4 ± 0.2 d of age) and transported for 12 h in central Indiana, for two replicates, during the summer of 2016 and the spring of 2017. Pigs were blocked by BW and allotted to 1 of 3 dietary treatments [n = 10 pens/dietary treatment/replicate (8 pigs/pen)]; antibiotics [**A**; chlortetracycline (441 ppm) + tiamulin (38.6 ppm)], no antibiotics (**NA**), or GLN fed for 14 d. On d 15 to 34, pigs were provided common antibiotic free diets in two phases. Data were analyzed using PROC MIXED in SAS 9.4. Day 14 BW and d 0 to 14 ADG were greater (P = 0.01) for A (5.6% and 18.5%, respectively) and GLN pigs (3.8% and 11.4%, respectively) compared to NA pigs, with no differences between A and GLN pigs. Day 0 to 14 ADFI increased for A (P < 0.04; 9.3%) compared to NA pigs; however, no differences were detected comparing GLN to A and NA pigs. Once dietary treatments ceased, no

differences (P > 0.05) in growth performance among dietary treatments were detected. On d 13, plasma tumor necrosis factor alpha (**TNF-α**) was reduced (P = 0.02) in A (36.7 ± 6.9 pg/ml) and GLN pigs (40.9 ± 6.9 pg/ml) versus NA pigs (63.2 ± 6.9 pg/ml). Aggressive behavior tended to be reduced overall (P = 0.09; 26.4%) in GLN compared to A pigs, but no differences were observed between A and GLN versus NA pigs. Huddling, active, and eating/drinking behaviors were increased overall (P < 0.02; 179, 37, and 29%, respectively) in the spring replicate compared to the summer replicate. A subset of pigs from Chapter 2 were utilized, in Chapter 3, to evaluate the dietary treatment effects on intestinal morphology and gene expression. On d 33, mast cells/mm^2 were increased (*P* = 0.05) in GLN and NA pigs vs. A pigs (22.2% and 19.7%, respectively). On d 33, villus height:crypt depth tended to be increased (*P* = 0.07; 7.0%) in GLN and A pigs vs. NA pigs. On d 33, glucagon-like peptide 2 (**GLP-2**) mRNA abundance was decreased (*P* = 0.01; 50.3%) in GLN and NA pigs vs. A pigs. Crypt depth was increased (*P* = 0.01; 16.2%) and villus height:crypt depth ratio was reduced (*P* = 0.01; 9.6%) during the spring replicate compared to the summer replicate on d 33. On d 13, TNF-α and occludin mRNA abundance were increased (*P* ≤ 0.04; 45.9% and 106.5%, respectively) and zonula occludens-1 (**ZO-1**) mRNA abundance tended to be increased (*P* = 0.10; 19.2%) in the spring replicate compared to the summer replicate. Previous research and the results of Chapter 2 indicates that supplementing nursery diets with 0.20% GLN provides similar growth and health benefits as dietary antibiotics, but it is unknown whether greater inclusion levels will provide additional benefits. Therefore, the objective of Chapter 4 was to evaluate the impact of replacing dietary antibiotics with increasing levels of GLN on growth performance, health status, and production costs in pigs following weaning and transport. We hypothesized that diet supplementation with 0.20% to 1.00% GLN would incrementally improve pig health and productivity compared to dietary antibiotics. Mixed sex pigs (N = 308; 5.64 ± 0.06

kg BW) were weaned (19.1 ± 0.2 d of age) and transported in central Indiana during the autumn of 2017. Pigs were blocked by BW and allotted to 1 of 7 dietary treatments (n = 8 pens/dietary treatment); A [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)], NA, 0.20% GLN, 0.40% GLN, 0.60% GLN, 0.80% GLN, or 1.00% GLN fed for 14 d. On d 15 to 35, pigs were provided NA diets in two phases. Data were analyzed using PROC MIXED in SAS 9.4. Overall, ADG (P = 0.04; 6.4%) and ADFI (P = 0.04; 6.9%) were reduced in NA pigs vs. 0.40% GLN or A pigs. Increasing GLN in the diet tended to increase (linear; P = 0.10) ADG. Overall, increasing GLN in the diet tended to increase (linear; P = 0.08) d 35 BW. Day 35 BW was greater (P = 0.01) in 0.80% GLN and A pigs compared to NA, 0.20% GLN, and 0.60% GLN pigs, but no BW differences were detected between 0.80% GLN and A and 0.40% GLN and 1.00% GLN pigs. In addition, d 35 BW was greater (P = 0.01) for 0.40% GLN and 1.00% GLN compared to 0.20% GLN. Overall income over feed and therapeutic injectable antibiotics cost for enteric and unthrifty challenges was greater (P = 0.02) in 0.80% GLN pigs compared to NA, 0.20% GLN, and 0.60% GLN pigs, but no income over feed and therapeutic injectable antibiotics cost for enteric and unthrifty challenges differences were detected between 0.80% GLN pigs and 0.40% GLN, 1.00% GLN, and A pigs. Health challenges in swine herds negatively impact swine growth rate and performance. Therefore, utilizing the pigs from Chapter 2, the study objective for Chapter 5 was to quantify the impact of differences in rearing conditions through post hoc analysis on growth performance, tissue accretion rates, and production economics in pigs during different replicates (summer or spring). We hypothesized that pigs reared under health challenged conditions would have decreased growth performance and tissue accretion rates resulting in increased production costs compared to pigs reared with less health challenges. Data were analyzed using PROC MIXED and PROC NLMIXED in SAS 9.4. Therapeutic injectable antibiotics cost was reduced (P = 0.01; 246.7%) in

the spring replicate compared to the summer replicate. Income over feed and therapeutic injectable antibiotics cost was greater ($P = 0.01$; 23.1%; $16.62/pig) in the spring replicate compared to the summer replicate. Predicted ADG was greater ($P \leq 0.05$) in spring replicate barrows compared to the summer replicate barrows during the ranges of 22 to 38 and 119 to 177 days of age, respectively. Spring replicate gilts had greater ADG ($P \leq 0.05$) compared to summer replicate gilts during the ranges of 22 to 47 and 112 to 177 days of age, respectively. The maximum predicted empty body protein accretion rate for the summer replicate gilts and the spring replicate gilts is 145 and 156 g/d, respectively. In conclusion, GLN supplementation improved pig performance and health after weaning and transport similarly to A across studies and GLN shows promise as an antibiotic alternative with 0.40% GLN appearing to be the optimal level. Health challenges in pigs can have profound negative impacts on tissue accretion rates and key economic drivers for pork producers such as poorer feed efficiency and reduced hot carcass weight. The adverse health effects resulting in reduced growth performance, increased production costs ($16.62/pig), and negatively impact producer profitability.

CHAPTER 1. REPLACING DIETARY ANTIBIOTICS WITH L-GLUTAMINE FOLLOWING WEANING AND TRANSPORT IN SWINE: A LITERATURE REVIEW

1.1 Gut Barrier Function

The intestinal epithelium is a single layer of cells lining the gut lumen and is a very important barrier that separates the external environment from the internal environment of the body (Wang et al., 2015a). The epithelium has two critical functions: barrier to prevent the passage of harmful intraluminal items including foreign antigens, microorganisms and their toxins, and serve as a selective filter allowing the translocation and absorption of essential nutrients, electrolytes, and water (Groschwitz and Hogan, 2009; Moeser et al., 2017). Transcellular and paracellular pathways are the two major pathways that regulate selective intestinal permeability (Groschwitz and Hogan, 2009). Transcellular pathways aid in the transport of nutrients through enterocytes and are regulated by selective transporters for electrolytes, amino acids, short-chain fatty acids, and sugars (Groschwitz and Hogan, 2009). Paracellular pathways aid in the transport of nutrients between epithelial cells and is regulated by intercellular protein complexes such as tight junction proteins (Groschwitz and Hogan, 2009).

Dysfunction of the intestinal epithelium as protective barrier can lead to increased intestinal permeability and the translocation of potentially harmful pathogens. When an intestinal insult or breech of barrier function is detected, the immune cells within the gut recognize the violation and, through adaptive and innate immune responses, work to contain the threat and stop the spread of infection (Moeser et al., 2017; Pluske et al., 2018).

1.1.1 Tight Junction Proteins

Tight junction proteins, in addition to desmosomes and adherens junction proteins, provide cell to cell adhesion for epithelial cells (Hartsock and Nelson, 2008; Groschwitz and Hogan, 2009). Tight junction proteins are located in many regions of the body including: intestinal tissue, lung tissue, and blood-brain barrier (Lee, 2015). Tight junction proteins have a variety of roles and perform vital functions including: hold cells together, provide barrier function, and regulate nutrient transport through passive diffusion between the cells (Lee, 2015). Currently, there are about 50 known different tight junction proteins in the human digestive track (Lee, 2105). With respect to nutrient transport, tight junction protein complexes form semipermeable selective paracellular barriers (Hartsock and Nelson, 2008). Tight junction proteins assist in the passage of ions and solutes in between cells while limiting the translocation of unwanted or harmful substances such as: food antigens, bile, certain enzymes, endotoxins, and microorganisms (Wang et al., 2015a).

1.2 Weaning and Transport Stress

The weaning stress complex is a combination of many stressors that are initiated when the young pig is removed from the sow with this process alone being a large stressor (Campbell et al., 2013). In addition to weaning stress, other stressors that pigs can be challenged with are: abrupt diet change from all liquid to solid diet, feed and water withdrawal, social stress from mixing, transport, thermal stress, and pathogenic insults (Pluske et al., 1997, Lewis and Berry, 2006; Garcia et al, 2015). During transport pigs can be exposed to additional unique stressors that include: loading and unloading, restricted space, vibrations, high wind and temperature fluctuations, and shifting of posture during acceleration and deacceleration (Piñeiro et al., 2007; Garcia et al, 2015). The negative effects of weaning can be numerous including poor feed intake, reduced growth

performance, and altered intestinal morphology which can lead to malabsorption of nutrients and increased post-weaning diarrhea (Pluske et al., 1997). Albeit stressful, weaning stress and associated stressors is very difficult to completely ameliorate due to the necessity to remove the pigs from the mothers, mix with new cohorts, and transport pigs to different barns/production sites to be provided the potential benefits of reduction in infectious disease from multi-site production (Harris, 2000).

1.2.1 Weaning Stress

Weaning and transitioning from an all-liquid diet to solid feed form diets can negatively affect gut morphology as previous studies have reported weaned pigs to have reduced villous height three to seven days postweaning compared to non-weaned control pigs (Cera et al., 1988; Pluske et al., 1997). Additional studies determined that the metabolizable energy (**ME**) requirement for maintenance of the weaned pig is not being met until 5 d postweaning and ME intake does not equal pre-wean ME intake levels until nearly two weeks postweaning (as reviewed by Le Dividich and Herpin, 1994). The reduced amounts or lack of feed intake and subsequent reduced presence of feedstuffs in the intestinal lumen may be an underlying mechanism for reduced gut morphology postweaning as the presence of food is an important stimulus for functional maintenance and intestinal proliferation (Kelly et al., 1992; Pluske et al., 1997).

It is well established that mixing unfamiliar pigs often leads to fighting and increased cortisol levels (Bradshaw et al., 1996). This appears to be true in weaned pigs as well, as the mixing of weaned pigs resulted in elevated cortisol levels; however, cortisol levels returned to baseline levels 24 h post-mixing (Merlot et al., 2004). After mixing occurred, a previous study determined that mixed pigs alter their behavior to avoid conflicting encounters by reducing synchronization of activities and that mixed pigs have increased resting behavior compared to un-mixed control

pigs (Merlot et al., 2004). In addition, the authors recognize that the stress of mixing is difficult to discern from the physiological changes that occur during weaning and diet change (Merlot et al., 2004).

1.2.2 Transport Stress

1.2.2.1 Duration of Transport

In the United States swine production, pigs are typically transported at least once in their lives, but often times, pigs are transported multiple times. Transportation typically occurs to separate pigs by age groups as a biosecurity management tool to reduce the opportunity for pathogen reinfection (Harris, 2000). However, the stress associated from transport can have negative consequences on pigs (Chambers and Grandin, 2001). Duration of travel has been studied to determine the effect of time on stress response in pigs. Previous studies have determined that pigs transported less than 1 h undergo greater stress response compared to pigs transported longer durations (3 hours; Pérez et al., 2002; Pilcher et al., 2011). Pigs transported for less than 1 h have greater cortisol levels compared to pigs transported longer durations (Pérez et al., 2002). The authors hypothesize that pigs transported for shorter durations are not allowed sufficient time to recover from the stress of loading before they are unloaded, whereas pigs transported for longer durations have the opportunity to recover from the loading process while the transport is occurring before the added stress of unloading (Pérez et al., 2002; Pilcher et al., 2011).

1.2.2.2 Thermal Stress during Transport

Transport stress can include thermal stress as the ambient temperature can vary widely throughout the day causing fluctuation in temperatures within the trailers (Xiong et al., 2015) causing dehydration and weight loss (Wamnes et al., 2008). Furthermore, when pigs are exposed to elevated temperatures and thermal stress intestinal morphology and integrity is degraded

(Kpodo et al., 2018). Fluctuations in temperature can be dependent on pig location within the trailer, as the rear of the trailer most frequently resulted in maximum skin temperatures and pigs in the middle zone of the trailer most frequently had the minimum skin temperatures compared to the front of the trailer (Xiong et al., 2015). When trailers loaded with pigs are stopped, ambient temperatures within the trailer can increase quickly (+3-4 °C within 5 min; Xiong et al., 2015). Due to opportunity for temperature increases when trailers loaded with pigs are stopped, pork producers should take precautions to limit or eliminate additional stops when pigs are loaded on a trailer such as weaning pigs at multiple farms with a single trailer as pig welfare can be compromised due to increased ambient temperatures and thermal stress experienced by pigs already on the trailer.

1.2.2.3 Feed and Water Withdrawal during Transport

Transportation of pigs is typically performed in the absence of feed and water. A previous study determined that weaned pigs transported 16 h without feed and water had increased plasma protein levels compared to non-transported pigs or transported pigs provided feed and water (Garcia et al, 2015). The increase in plasma protein may be due to increased skeletal muscle proteolysis to provide amino acids to other tissues or systems such as the immune system (Souba et al., 1985a; Rhoads et al, 2007). After 24 h of transport, pigs transported without feed and water had greater BW loss percentage compared to pigs provided feed and water (Garcia et al., 2015). The authors conclude that pigs transported for 24 h or more should be supplemented with feed and water during transport as much of the weight loss is likely due to dehydration (Garcia et al., 2015). Water and feed withdrawal for 24 h postweaning negatively impacts growth performance and intestinal health markers (Horn et al., 2014; Horn et al., 2016; Horn et al., 2017).

1.2.3 Stress Modulates Gut Barrier Function

1.2.3.1 Weaning Stress Modulates Gut Barrier Function

Weaning is a stressful event as weaned pigs have increased circulating corticotrophin-releasing factor (**CRF**) and cortisol levels due to the stress-induced activation of the hypothalamic pituitary adrenal (**HPA**) axis compared to unweaned pigs (Moeser et al., 2017). In addition, weaned pigs (19 d of age) had increased intestinal permeability measured by reduced transepithelial electrical resistance (**TER**) and increased jejunal CRF receptor 1 protein levels compared to unweaned pigs (Moeser et al., 2007). However, when weaned pigs were treated with a CRF receptor antagonist, α-helical CRF(9–41), 30 min prior to weaning, intestinal permeability was not altered (Moeser et al, 2007). These results suggest that weaning stress induces intestinal permeability mediated by jejunal CRF receptors (Moeser et al., 2007).

1.2.3.2 Weaning Age Modulates Gut Barrier Function

Early weaning stress has also been associated with increased intestinal permeability. Early weaned pigs (15 to 21 d weaning age) had reduced TER indicative of increased intestinal permeability compared to late weaned pigs (23-28 d weaning age; Smith et al., 2010). In addition, early weaned pigs had increased serum CRF levels, jejunal CRF levels, and mast cells compared to late weaned pigs (Smith et al., 2010). The authors report that early weaning stress appears to elevate intestinal CRF concentration that is capable of inducing mast cell activation and intestinal permeability however they do not fully understand the signaling pathway involved (Smith et al., 2010).

1.2.3.3 Other Stressors Modulate Gut Barrier Function

Other stressors have been shown to upregulate the CRF system in pigs and subsequently induce intestinal dysfunction. Pigs that were infected with *Salmonella typhimurium* were observed

to have increased ileal permeability and intestinal CRF protein expression (Boyer et al., 2015). When pigs were under chronic stress, due to chronic mixing and crowding stress, had increased intestinal permeability, decreased nutrient transport, and increased CRF gene expression compared to control pigs (Li et al., 2017). These results and the aforementioned studies demonstrate that weaning stress and other stressors can lead to intestinal dysfunction.

1.3 Glutamine

1.3.1 Biological Function

Glutamine (**GLN**) is a neutral amino acid with a polar side chain and has a chemical formula of $C_5H_{10}N_2O_3$ (Wu et al., 2011). Historically, GLN was thought to be a nonessential amino acid in the diet due to endogenous synthesis (Lacey and Wilmore, 1990). Furthermore, GLN analysis in feedstuffs and animal proteins is difficult because GLN is completely converted to glutamate under acid hydrolysis and as a result little research was conducted on GLN and little information was historically taught on the subject of GLN due to the difficulty in analysis and preconceived thoughts on ample endogenous GLN production (Wu et al., 2011). Evidence today supports that GLN is a conditionally essential amino acid where GLN is nutritionally nonessential for normal subjects; however, physiologically indispensable for subjects under certain conditions where endogenous synthesis is not sufficient (Lacey and Wilmore, 1990; Wang et al., 2015a).

Glutamine has been shown to have a role in many important biological functions throughout the body. Glutamine serves as a nitrogen carrier between tissues, regulator of acid-base balance through urinary ammonia production, substrate for metabolic pathways, regulator of cell signaling pathways, and precursor to nucleotides, nucleic acids, and protein (Lacey and Wilmore, 1990; Wu et al., 2011). With respect to the intestine, GLN serves additional specific biological and metabolic functions important for proper intestinal health, function, and mucosal barrier.

Glutamine is an important energy source for enterocytes; serves in the regulation of gene expression and proteins; involved in cellular signaling with respect to cell proliferation, differentiation, and apoptosis; and aids in antioxidation and immune responses (Souba et al., 1985a; Wu et al., 1996; Wang et al., 2015a).

1.3.2 Glutamine as Energy Source for Enterocytes

Glutamine is a primary energy source for rapidly dividing cells such as enterocytes (Souba et al., 1990b; Wu et al., 1996; Margaritis et a., 2005) with the intestine being the focal location of GLN utilization (Souba et al., 1985a; Souba et al., 1990a; Wu et al., 1996;). One hypothesis for this in carnivores and omnivores is the consumption of meat and raw muscle tissue being rich in GLN and due to an evolutionary adaptation where the intestine uses GLN and glucose as energy sources from enteral nutrition and the body switches to endogenous GLN, released from skeletal muscle which is also high in GLN, as the preferred energy source for enterocytes during times of fasting to spare glucose (Souba and Wilmore, 1985; Souba et al., 1990b). A previous study has shown that as animals, under catabolic conditions (e.g. stress, injury, or fasting), release amino acids (e.g. glutamine) into circulation through the accelerated proteolysis of skeletal muscle (Souba et al., 1985a), It has been determined that GLN comprises approximately 50% of total of amino acids being released (Cuthbertson, 1980). Conversely in the fed state, after a meal high in protein, portal blood GLN concentrations are elevated suggesting that the enterocytes' GLN needs are met and excess GLN can be used for other metabolic functions including replenishment of skeletal muscle reserves (Elwyn, 1968).

When animals have undergone glucocorticoid administration, the gastrointestinal (GI) tract significantly increases the uptake of GLN via the mesenteric artery (Souba et al., 1985b). Despite accelerated muscle proteolysis circulating GLN levels decrease due to the increase in uptake of

GLN by the GI tract and other tissues (Souba et al., 1985a;b). Upon uptake by the enterocyte, GLN can become the preferential energy source to conserve glucose for other functions when an animal is under stress. However, under homeostatic conditions the GI tract has been shown to consume glucose and following the administration of dexamethasone the GI tract releases glucose (Souba and Wilmore, 1985).

1.3.3 Glutamine Metabolism

Once GLN enters the cell either from circulation or from the lumen via a specific carrier, GLN can be used for energy metabolism. Glutamine can be converted to glutamate and ammonia via the enzyme glutaminase (Souba et al., 1985a; Souba et al., 1990a). Glutamate can then be converted to α-ketoglutarate and α-ketoglutarate can be completely oxidized in the Kreb's cycle (Souba et al., 1985a). The transformation of GLN to α-ketoglutarate and ultimately adenosine triphosphate (**ATP**) is quite efficient as 1 mol of GLN yields 30 mol of ATP while sparing glucose for other metabolic functions in other cells (Souba et al., 1985a).

1.3.4 Enzymology of Glutamine

There are two main enzymes that regulate glutamine metabolism: glutaminase and glutamine synthetase. Glutaminase catalyzes the hydrolysis of GLN to glutamate and ammonia (Souba et al., 1985a). Glutamine synthetase catalyzes the synthesis of GLN from glutamate and ammonia (Souba et al., 1985a). In most tissues, one enzyme, either glutaminase and glutamine synthetase, is higher in concentration within a cell to prevent futile cycling (Souba et al., 1985a). In the case of skeletal muscle, glutamine synthetase is more active for the synthesis and storage of GLN (Souba et al., 1985a). In the case of enterocytes, there is greater glutaminase activity for the conversion of GLN to glutamate to α-ketoglutarate for energy generation (Souba et al., 1990a).

With enterocyte having reduced glutamine synthetase activity compared to glutaminase, this may be one of the reasons that some trials fail to see a growth response to dietary glutamate. Glutamate can be converted to α-ketoglutarate for energy generation by the Kreb's cycle; however, there is limited glutaminase activity in the enterocyte to synthesize GLN from glutamate (Souba et al., 1990a). Potentially, there may not be sufficient GLN to meet the demands of weaned pigs under acute stress.

1.3.5 Glutamine Impacts on Intestinal Health

1.3.5.1 Morphology

Glutamine supplementation has been shown to reduce intestinal epithelial damage through improved intestinal morphology (Wu et al., 1996; Yi et al., 2005; Johnson and Lay, 2017). Improvements in villus height for pigs supplemented with GLN may be indicative of increased digestive and absorptive capacity allowing for greater nutrient uptake and improved intestinal health (Nabuurs et al., 1994; Wu et al., 1996; van Beers-Schreurs et al., 1998; Johnson and Lay, 2017). Glutamine can serve as an energy source for enterocytes supporting cellular metabolism and subsequently the structure and function of the small intestine (Wu et al., 1996; Pluske et al., 1997). Previous studies have worked to uncover possible underlying mechanisms that may support dietary GLN improving intestinal morphology, function, and health during a stress event such as weaning (Baskerville et al., 1980; Wu et al., 1996; He et al., 2019). When endogenous GLN levels are depleted to critically low levels and GLN is limited to enterocytes, which can occur during stress events like weaning, mucosal ulcerations, villous atrophy, and intestinal necrosis result in several species (Baskerville et al., 1980). On d 29 of lactation, GLN is the most abundant amino acid in sow's milk and is thought to play a critical role in the intestinal growth and development of nursing pigs (Wang et al., 2015a). In addition, GLN supplementation prevents jejunal atrophy

during the first week postweaning and potentially GLN fortified diets more closely match sow's milk and reduces the negative effects of weaning on intestinal morphology (Wu et al., 1996; Wang et al., 2015a). In addition, a previous study has shown GLN reduced apoptosis in the small intestine due to decreased activation of the unfolded protein response (He et al., 2019) thus reducing enterocyte turnover and potentially altering villus height:crypt depth. Based on these results, exogenous GLN supplementation may provide enterocytes increased levels of GLN to improve intestinal morphology and support proper cellular function.

1.3.5.2 Stress Hormones, Tight Junction Proteins, and Gut Barrier Function

Weaning is a stressful event to young pigs and can increase intestinal permeability and dysfunction (Moeser et al., 2017). Intestinal epithelial cells are held together by tight junction proteins to allow for proper intestinal barrier function. However, the erosion of tight junction proteins is associated with the pathology of many intestinal diseases such as inflammatory bowel disease and irritable bowel syndrome (Wang et al., 2015b). Glutamine supplementation has been shown to positively influence tight junction protein expression. When GLN was unavailable to Caco-2 cells, tight junction protein (Claudin-1 and occludin) content was reduced and TER was decreased demonstrating increased permeability; however, tight junction protein expression and permeability of the cultured cells returned to baseline levels with the addition of GLN to the culture media (Wang et al., 2015a).

Another potential pathway in which GLN provides improved intestinal health and ultimately benefit to the animal is through the possible reduction of stress hormone levels as it has been previously understood that CRF mediates intestinal permeability (Moeser et al., 2007; Smith et al., 2010). When pigs were supplemented with 1% GLN, jejunal CRF gene expression and protein levels were reduced compare to pigs not provided GLN (Wang et al., 2015b). In addition,

GLN supplemented pigs had increased claudin-1, occludin, ZO-2, and ZO-3 protein abundances and improved intestinal permeability through a lactulose/mannitol assay compared to control pigs (Wang et al., 2015b). Furthermore, in vitro work by Overman et al., (2012) demonstrated that when tissue from the ileum was exposed to CRF, TNF-α levels increased from mast cells and subsequently intestinal permeability increased. A previous study showed that plasma TNF-α levels were reduced when pigs were provided GLN compared to pigs not provided GLN (Duttlinger et al., 2019). These studies suggest that potentially GLN supplementation results in lower CRF levels which does not stimulate TNF-α to increase and as a result intestinal integrity is maintained.

In a previous study, pigs were either supplemented with 2% GLN diets or control diets containing no GLN for 4 d prior to castration (Hsu et al., 2012). On d 7 post-castration, GLN fed pigs had reduced plasma adrenocorticotropic hormone (ACTH) and cortisol levels than the control group (Hsu et al., 2012). The authors conclude that GLN was able to reduce the stress response associated with castration (Hsu et al., 2012).

In another study by Wang et al. (2015b) tissue was collected at 7 d post-weaning to determine the effects of GLN on tight junction proteins, CRF, and intestinal morphology. Seven days postweaning may be an appropriate duration to allow for the dietary effects of GLN to improve intestinal health while the control pigs are still exhibiting signs of catabolic weaning stress. Future work needs to investigate the proper duration, through serial harvests, to collect tissue sample postweaning where the potential maximum benefits of dietary GLN can be detected.

1.3.6 L-Glutamine and Improvements in Growth Performance

Glutamine is considered a conditionally essential amino acid where in most instances sufficient levels are maintained in the body through diet and endogenous synthesis (Wang et al., 2015a). However, in times of stress, GLN can be oxidized by the Krebs cycle to produce ATP for

rapidly dividing cells such as enterocytes (Wang et al., 2015a). As a result of the stress and subsequent GLN expenditure, GLN may be a nutritionally essential amino acid for neonates (Wang et al., 2015a).

Previous work has determined GLN to be effective in improving growth performance when fed at 1% (Wu et al., 1996; Wang et al., 2015b) in nursery diets. Despite the improvement in feed efficiency when GLN was fed at 1%, Wu et al., (1996) did not detect any improvements in growth performance when lower levels of GLN were fed. Recent work by Johnson and Lay, (2017) investigated the potential of GLN to be an antibiotic alternative when included in nursery diets at 0.20% for the first 14 d post-weaning. These trials resulted in improved growth performance compared to pigs not supplemented with dietary antibiotics and improved growth performance compared to pigs provided dietary antibiotics (Johnson and Lay, 2017). One possible reason why the aforementioned study detected growth performance improvements to feeding low levels (0.20%) of GLN may be due to the pigs having experienced the added stress of transport with weaning, further depleting endogenous GLN levels and creating the conditions for a greater GLN requirement.

1.3.7 L-Glutamine and Challenge Studies

L-glutamine supplementation has been determined to improve growth performance in weaned pigs. A study evaluated the effects of GLN following the administration of a health challenge (Yi et al., 2005). In a previous study, pigs were fed 2% added GLN for 12 d postweaning before being orally challenged with *E. coli* $K88^+$ (Yi et al., 2005). At 48 h after the challenge, the ADG of GLN pigs was equal to non-challenged pigs and challenged pigs not provided GLN had reduced growth performance compared to non-challenged pigs (Yi et al., 2005). In addition, GLN pigs had improved feed efficiency and intestinal morphology compared to challenged pigs not

provided GLN (Yi et al., 2005). These results indicate that GLN can maintain growth performance through the conservation of intestinal morphology similar to nonchallenge pigs during an enteric health challenge such as *E. coli* $K88^+$ infection (Yi et al., 2005).

1.4 Antibiotics in Swine Diets

The negative consequences associated with the stress of weaning and transport are commonly combated with antibiotics as antibiotics reduce pathogen load through microorganism suppression (as reviewed by Cromwell, 2006). Historically, swine producers could feed dietary antibiotics to improve growth performance as some dietary antibiotics could be fed at two different approved levels: subtherapeutic levels for growth promotion or therapeutic levels for the treatment or prevention of disease (as reviewed by Cromwell, 2006). More specifically, chlorotetracycline and tiamulin alters the commensal flora of the gut though inhibiting protein synthesis of the pathogens (Poulsen et al., 2001; Cornick, 2010). Tiamulin inhibits protein synthesis through the binding of the drug to the 50S ribosomal subunit of bacteria and inhibits peptidyl transferase activity (Poulsen et al., 2001). As a result of the reduction in pathogen concentration in the gastrointestinal tract, pig growth performance is reported to improve (Skinner et al., 2014). However, livestock producers have been urged to reduce dietary antibiotic usage and look for alternatives to antibiotics. Due to the Veterinary Feed Directive final rule that eliminated the use of medically important antibiotics for growth promotion, the use of medically important antibiotics can only be used under the guidance of a veterinarian through a veterinarian script (Schulz and Rademacher, 2017). Alternatives like GLN may help fill the new disease and stress management void.

1.5 Health Status Impact on Growth

1.5.1 Modeled Growth Differences

There is an overabundance of evidence that supports growth performance is reduced following a challenge exposure. Several previous studies have measured decreased growth rate when pigs were exposed to various health challenges or environmental stressors with the intent to stimulate the immune system in a controlled experimental setting (Schinckel et al., 1995; Williams et al., 1997a, b, c; Holck et al. 1998; Rakhshandeh and de Lange, 2012; Curry et al., 2017; Kvidera et al., 2017; Schweer et al., 2016; Schweer et al., 2017). The stressors or challenges administered or applied to the treatment pigs in the previous studies were: vaccination and lipopolysaccharide (**LPS**) injection (Schinckel et al., 1995), lack of sanitation, dietary antibiotics, and vaccination (Williams et al., 1997a,b,c), lack of sanitation and decreased space allowance in a commercial finisher (Holck et al. 1998); LPS injections (Rakhshandeh and de Lange, 2012; Kvidera et al., 2017), porcine epidemic diarrhea virus (**PEDV**) inoculation (Curry et al., 2017), porcine reproductive and respiratory syndrome (**PRRS**) and PEDV infection and coinfection (Schweer et al., 2016; 2017).

Interestingly, a previous study reported no differences in growth performance when pigs were infected with *Mycoplasma hyopneumoniae* (**M. hyo**) despite evidence of infection through increased cytokine levels, coughing, and pulmonary lesions (Escobar et al. 2002). The authors hypothesize that the surprising results may be due to the absence of other pathogens in a research setting that often exist under commercial conditions (Escobar et al., 2002). The pigs that were utilized in the study originated from a PRRS free herd, were early weaned to reduced pathogen transmission from the dam, and housed in disease containment chambers that had been thoroughly washed and sanitized. This hypothesis is supported from previous studies that reported pigs housed under commercial conditions often experience additional health challeges following viral

respiratory disease infection as secondary bacterial infections often follow (Brockmeier et al., 2002). The economic impact of single virus infection or virus coinfection has been studied and M. hyo infection was estimated to be $0.63/pig in lost productivity and economic impact and swine influenza virus **(SIV**) infection was estimated to be $3.23/pig in lost productivity and economic impact (Haden et al., 2012). However, when pigs were coinfected with M. hyo and SIV lost productivity and economic impact was estimated to be $10.12/pig as the production losses due to coinfection of the viruses were more than additive (Haden et al., 2012). There would be value in future research to investigate the growth, physiological, and nutrient requirement alterations to pigs that are coinfected with pathogens for pork producers to better understand the impact of disease coinfection and how to better manage flows of pigs that may suffer from such disease states.

Swine growth models can be useful tools to identify the impact of a treatment, detect alternate strategies to improve swine production efficiencies, and to estimate nutrient requirements for pigs of different ages and BW (Schinckel and de Lange, 1996). Despite the usefulness of swine growth models, fewer studies have modeled the growth curve response to health challenges or environmental stressors (Holck et al., 1998; Schinckel et al. 2002; Hamilton et al., 2003) relative to the aforementioned studies that measured the impact of health challenges on growth performance. In a study where pigs that were placed in commercial continuous-flow finishing barn had reduced BW, lean, and fat deposition that was 70% of pigs reared under improved sanitation conditions where pens where washed daily (Holck et al., 1998). An additional study modeled the growth response of pigs reared in an all-in all-out commercial finisher compared to pigs reared in a continuous-flow commercial finisher with barn and pigs with improved biosecurity management had improved growth rate due to the potential of reduced reinfection of pathogens from pigs of

different age groups compared to pigs reared in the all-in all-out setting (Schinckel et al., 2002). Hamilton et al. (2003) determined that pigs with reduced pen space allowance as an environmental stressor had subsequently reduced growth performance compared to pigs offered more floor space. Therefore, these results provide support that health challenges and environmental stressors do negatively impact growth rate of pigs and modeling helps illustrate the impact by visually displaying changes in slope and inflections points where growth rates are modified by the challenge exposure.

1.5.2 Health Status Impact on Lean Accretion

In addition to the modeled growth curves, the aforementioned studies also modeled tissue accretion rates. The curve for empty body protein and/or fat-free lean gain takes on a shape similar to the ADG growth curve where the response criterion increases and then decreases as body weight increases. In addition, pig exposed to environmental stressors had decreased empty body protein accretion rate and fat-free lean gain compared to control pigs (Holck et al., 1998; Schinckel et al. 2002; Hamilton et al., 2003) suggesting that the challenge is limiting pigs from maximizing protein deposition and muscle growth.

Recent studies have utilized dual-energy X-ray absorptiometry (**DXA**) to measure body composition and tissue accretion rates when pigs were exposed to PRRS (Schweer et al., 2017) or PEDV (Curry et al., 2017). In these studies, with a controlled virus challenge, the pigs that were infected with the virus of interest had reduced lean accretion compared to non-infected control pigs (Schweer et al., 2017; Curry et al., 2017). In the first 42 days following PRRS infection, protein accretion was reduced by 24% in infected pigs compared to control (Schweer et al., 2017). However, lean accretion was not different during d 43-80 postinfection, but overall lean accretion was reduced 10% in PRRS infected pigs compared to controls (Schweer et al., 2017). In addition

to lean gain alterations, fat accretion rates were altered with infected pigs having decreased fat accretion rates compared to control pigs (Schweer et al., 2017; Curry et al., 2017). From d 0 to 80 following PRRS infection, fat accretion was reduced 20% in PRRS infected pigs compared to control pigs (Schweer et al., 2017). The altered tissue accretion rates may be due to the immune system demanding more energy and amino acids at a time when ADFI was decreased requiring a repartitioning of nutrients (Curry et al., 2017). The aforementioned studies determined that immune challenge decreases lean protein synthesis and accretion. Protein degradation rate increases due to reduced feed intake and need for amino acids to synthesize products and proteins for the immune system (Rhoads et al., 2007; Curry et al., 2017). The net results of these metabolic changes are, generally, reduce BW gain, poorer feed efficiency, and potentially carcasses with more adipose tissue as was observed by Schinckel et al. (2002) where the health challenged pigs had greater lipid accretion rates.

1.5.3 Health Status Impact on Lysine Requirement

Mechanistically and determined through modeling, when pigs are exposed to a health challenge, they have decreased protein and lean muscle synthesis due to a shift in amino acid needs for other tissues and systems, namely the immune system (Rhoads et al., 2007). Conversely, healthy pigs have increased growth rate, improved feed efficiency, and yield carcasses with higher percent lean and reduced fat percentage in part because healthier animals have reduced maintenance requirements. Previous research has determined that healthier pigs, subsequently, have altered nutrient requirements when compared to control pigs (Williams et al., 1997c).

Pigs with higher health status have a greater lysine requirement (g Lys/d) due to increased muscle tissue growth and more protein accretion (Williams et al., 1997c). The shape of the lysine curve is very similar to the protein accretion curve where the curves increase and then decrease as

BW increases as lysine is the first limiting amino acid for protein synthesis. Once protein accretion rate is determined, the lysine requirement for a pig can be estimated based on the biological relationship that has been previously determined for dietary lysine needs to support lean tissue growth (van Heughten, 2010). Skeletal muscle protein contains approximately 7.0% lysine and the efficiency of lysine utilization for protein gain is estimated to be 58% (van Heughten, 2010). Therefore, to gain 1 gram of skeletal muscle protein, a pig would need to consume 0.12 grams of digestible lysine. Production systems need to understand how management, health, genetics, and environment can impact nutrient requirements to be able to optimize nutrition for their animals under their conditions.

1.5.4 Potential Mechanisms

1.5.4.1 Increased Energy Requirement

The basic mechanisms responsible for the differences in growth and protein accretion rates are likely multifaceted. Previous work by Williams et al. (1997c) found that that health challenged pigs had increased alpha-1-acyglycoprotein concentrations and greater T lymphocyte CD4+:CD8+ ratios indicating a greater immune response compared to control pigs (Williams et al., 1997c). It has been previously determined that pigs with greater immune system activation have a greater glucose requirement than the immune system of control pigs (Kvidera et al., 2017). This is due to greater energy needs required by the immune system to mount an immune response, therefore, energy that could be shunted towards growth, specifically protein and muscle synthesis, may be repartitioned to the immune system (Kvidera et al., 2017).

1.5.4.2 Alterations in Muscle Physiology

Health challenged pigs also have increased circulating amino acid concentrations compared to control pigs indicating that the body is shunting its amino acid pool to other systems

and tissues in need during an immune challenge and potentially away from skeletal muscle deposition, one of the major amino acid pools of the body (Rhoads et al., 2007). Furthermore, pigs infected with rotavirus likely had inhibited skeletal muscle synthesis due alterations of the mammalian target of rapamycin (**mTOR**) pathway, specifically downregulated skeletal muscle ribosomal protein S6 phosphorylation (Rhoads et al., 2007). The mTOR pathway has been found to be important in the regulation of protein synthesis (Rhoads et al., 2007; Curry et al., 2017).

Additional changes in muscle physiology due to a health challenge were illustrated in a previous study where PRRS infected pigs had increased muscle myostatin mRNA levels compared to noninfected control pigs (Escobar et al., 2004). Increased muscle myostatin levels can potentially suppress skeletal muscle synthesis (Escobar et al., 2004). Protein accretion was reduced in PRRS infected pigs making it plausible that myostatin did alter protein accretion and skeletal muscle synthesis (Escobar, et al., 2004).

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CHAPTER 2. REPLACING DIETARY ANTIBIOTICS WITH 0.20% L-GLUTAMINE IN SWINE NURSERY DIETS: IMPACT ON HEALTH AND PRODUCTIVITY OF PIGS FOLLOWING WEANING AND TRANSPORT

2.1 Abstract

Antibiotic use has been limited in United States swine production. Therefore, the objective was to determine whether supplementing L-glutamine at cost-effective levels can replace dietary antibiotics to improve piglet welfare and productivity following weaning and transport. Based on previous research, we hypothesized that withholding dietary antibiotics would negatively affect pigs while diet supplementation with 0.20% L-glutamine (**GLN**) would have similar effects on pig performance and health as antibiotics. Mixed sex piglets (N = 480; 5.62 ± 0.06 kg BW) were weaned (18.4 ± 0.2d of age) and transported for 12 h in central Indiana, for two replicates, during the summer of 2016 and the spring of 2017. Pigs were blocked by BW and allotted to 1 of 3 dietary treatments [n = 10 pens/dietary treatment/replicate (8 pigs/pen)]; antibiotics [**A**; chlortetracycline (441 ppm) + tiamulin (38.6 ppm)], no antibiotics (**NA**), or GLN fed for 14 d. On d 15 to 34, pigs were provided common antibiotic free diets in two phases. Data were analyzed using PROC MIXED in SAS 9.4. Day 14 BW and d 0 to 14 ADG was greater ($P = 0.01$) for A (5.6 and 18.5%, respectively) and GLN pigs (3.8 and 11.4%, respectively) compared to NA pigs, with no differences between A and GLN pigs. Day 0 to 14 ADFI increased for A ($P < 0.04$; 9.3%) compared to NA pigs; however, no differences were detected when comparing GLN to A and NA pigs. Once dietary treatments ceased, no differences ($P > 0.05$) in productivity between dietary treatments were detected. On d 13, plasma tumor necrosis factor alpha (**TNF-α**) was reduced ($P = 0.02$) in A (36.7 ± 6.9 pg/ml) and GLN pigs (40.9 ± 6.9 pg/ml) versus NA pigs (63.2 ± 6.9 pg/ml). Aggressive behavior tended to be reduced overall ($P = 0.09$; 26.4%) in GLN compared to A pigs,

but no differences were observed between A and GLN versus NA pigs. Huddling, active, and eating/drinking behaviors were increased overall ($P < 0.02$; 179, 37, and 29%, respectively) in the spring replicate compared to the summer replicate. When hot carcass weight (**HCW**) was used as a covariate, loin depth and lean percentage was increased ($P = 0.01$; 4.0% and 1.1%, respectively) during the spring replicate compared to the summer replicate. In conclusion, GLN supplementation improved pig performance and health after weaning and transport similarly to A across replicates; however, the positive effects of A and GLN were diminished when dietary treatments ceased.

Keywords: antibiotics, L-glutamine, nursery, pigs, transport, weaning

2.2 Introduction

Weaning is a complex stressor associated with social, environmental and metabolic stress in pigs (Lallés et al., 2004). In newly weaned pigs, stress is induced by separation from the sow, relocation and mixing piglet groups, and a radical change in diet that often reduces or eliminates feed intake in the first 48 hours post-weaning (Brooks et al., 2001). As a result, piglets undergo a variety of physiological and metabolic changes that can negatively impact welfare. Changes may result from elevated blood cortisol levels (Moeser et al., 2007; Van der Meulen, et al., 2010), compromised feed intake (Maenz et al., 1994), altered intestinal morphology (Lallés et al., 2004), and dehydration due to the switch from an all liquid (milk) to a solid diet (Berry and Lewis, 2001). Unfortunately, in commercial production systems, weaning stress may be compounded by transport stress, which can induce significant weight loss with as little as 4 h of travel time (Hicks et al., 1998), and ambient temperature likely plays a critical role in determining total stress load incurred by piglets (Lambooy, 1988). Therefore, it is imperative that effective recovery strategies are developed to improve the welfare and productivity of pigs following these stressful events.

Historically, swine producers used dietary antibiotics to help newly weaned pigs overcome the stress of weaning and other associated stressors (Chiba, 2010). However, due to increased consumer concern regarding the use of antibiotics in animal production, and legislative action promoting antibiotic free diets, it has become increasingly important to develop alternatives that can help pigs recover from stressful events as effectively as dietary antibiotics. Previous research (Johnson and Lay, 2017) determined that inclusion of 0.20% L-glutamine (Ajinomoto North America, Inc., Raleigh, NC) in the diets of newly weaned and transported pigs could improve growth rate and well-being more effectively than dietary antibiotics ([chlortetracycline (Aureomycin, Zoetis, Parsippany, NJ) + tiamulin (Denagard, Elanco Animal Health, Greenfield, IN)]. However, this study was conducted under controlled conditions utilizing simulated transport and individual housing. Therefore, study objectives were to evaluate the impact of replacing dietary antibiotics with 0.20% L-glutamine on swine welfare, growth performance, health status, and carcass characteristics of pigs in a production environment following weaning and transport. We hypothesized that withholding dietary antibiotics would negatively impact the overall well-being of piglets, and that diet supplementation with 0.20% L-glutamine would have a similar effect on piglet health and productivity as dietary antibiotics in a production environment.

2.3 Materials and Methods

2.3.1 General

All procedures involving animal use were approved by the Purdue University Animal Care and Use Committee (protocol #1603001385), and animal care and use standards were based upon the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of Animal Science Societies, 2010). Mixed sex crossbred pigs [N = 480; 5.62 ± 0.06 kg initial BW; Duroc x (Landrace x Yorkshire)] were weaned and transported at 18.4 ± 0.2 d of age in central

Indiana and replicated during July of 2016 (summer replicate) and April of 2017 (spring replicate). The terms summer replicate and spring replicate refer only to the time of year in which the pigs were weaned and transported. One day prior to weaning and transport, all pigs were individually weighed, blocked by body weight, and randomly allotted to pens, and pens of pigs within BW blocks were allotted to 1 of 3 dietary treatments with 10 pens per dietary treatment per replicate. Each pen, initially, contained 8 pigs. Dietary treatments were antibiotics [**A**; chlortetracycline (441 ppm) + tiamulin (38.6 ppm)], no antibiotics (**NA**), or 0.20% L-glutamine (**GLN**).

2.3.2 Sentinel Pigs

On d 14.0 ± 1.9 d of age, calibrated thermochron temperature recorders (iButton model 1921H; accuracy ± 0.2ºC; Dallas Semiconductor, Maxim, Irving, TX) were implanted intra-abdominally into 12 selected mixed sex piglets (3 barrows and 3 gilts per replicate) to measure core body temperature (**T_C**) in 10 min intervals and then the hourly mean was calculated. For thermochron temperature recorder implantation, pigs were anesthetized (1 to 4% isoflurane), and then an incision (6 cm) was made on the abdomen, 3 cm lateral to the linea alba. Sterile thermochron temperature recorders were inserted in between the peritoneum and abdominal muscle and the incision site was closed. Immediately following surgery, all piglets were administered a broad-spectrum antibiotic (5 mg/kg IM every 3 d; Ceftiofur; Zoetis; Florham Park, NJ) to prevent infection at the surgical site, as well as analgesia (2.2 mg/kg IM; Flunixin meglumine; Merck Animal Health; Madison, NJ) immediately after surgery and 24 h post-surgery to control pain. Piglets were bandaged and then immediately returned to the sow after surgery where they remained until weaning.

2.3.3 Transportation

On the day of weaning and transport, selected pigs, including sentinel pigs, were removed from sows and herded up a ramp into a gooseneck livestock trailer (2.35 × 7.32 m; Wilson Trailer Company, Sioux City, IA) providing 0.07 m^2 per pig and within the range of 0.060 to 0.084 m^2 per pig required for 4.54 to 9.07 kg pigs, respectively (Federation of Animal Science Societies, 2010). The loading ramp to the trailer was 2.13 m in length providing an 11.0° incline, less than the recommended maximum of 20.0° (National Pork Board, 2015). Two data loggers (Hobo®; data logger temperature/RH; Onset®; Bourne, MA) were evenly spaced within the trailer to measure ambient temperature (**T_A**) and relative humidity (**RH**) in 5 min intervals. During transport, the trailer T_A and RH during the summer replicate was 29.4 ± 0.2°C and 64.3 ± 0.8%, respectively, and during the spring replicate was 11.0 ± 0.2°C and 63.1 ± 0.9%, respectively. Trailers were bedded with wood shavings and ventilation openings were adjusted based on the T_A (National Pork Board, 2015).

Piglets were transported as a group in the trailer for approximately 12 h and 819 km without feed or water. Total transport time was determined by adding loading time, time spent in the trailer, unloading time, and the time it took to be sorted into their respective pens in the nursery facility. The average time to wean and load the trailer was 55 min. The drivers were the same and followed the identical route for the summer replicate and spring replicate. Attention was given when developing the transport route such that approximately 50% two-lane roads and 50% four-lane roads were utilized for transport. The route was 273 km in length and was completed three times during the transport phase for each replicate. The route took, on average 3 h 16 min to complete. The driver was switched, and the truck was refueled after each time the 273 km route was completed. At the conclusion of the 12 h transport, piglets were unloaded from the trailer, individually weighed, and placed into pens. The average time to unload the trailer, weigh the pigs,

and place into pens was 1 h 10 min. All sentinel pigs were euthanized 24 h post-transport and body temperature recorders were removed.

2.3.4 Nursery Phase

Following transport, pigs were placed in their assigned pens and provided their respective dietary treatments for 14 d in 2 phases (d 0 to 14 post-weaning; Table 2.1). Following the dietary treatment period, all pigs were fed common antibiotic free diets from d 14 to the end of the nursery phase (d 34; Table 2.1). Diets were corn-soybean meal-based in meal form, fed in four phases, and were formulated to meet or exceed nutrient requirements (NRC, 2012) during the nursery period (Table 2.1). Pigs were weighed individually and feeders were weighed every 7 d during the nursery period to determine the response criteria of ADG, ADFI, and G:F.

Therapeutic antibiotic administration was recorded for the duration of the trial (weaning to market). The researchers and research farm staff were trained to identify pigs needing therapeutic injectable antibiotic treatment and were blinded to the study treatments. Pigs were treated when exhibiting clinical signs of illness. Treatment dose, product given, date given, pig and pen identification, and reason administered were recorded. Reason for therapeutic administration was then categorized for post-hoc analysis. Categories were: enteric challenge (e.g. scours or loose watery stool), respiratory challenge (e.g. coughing, thumping, or labored breathing), lameness (e.g. carrying a limb or difficulty walking or swollen joints), un-thriftiness (e.g. BW loss, poor gain, loss of body condition, or rough hair coat), and all other treatments (e.g. side paddling associated with *Streptococcus suis* infection, skin infection, and abscess).

The nursery facility where the initial 34 d of the trial was conducted contained pens (1.22 m × 1.37 m) that provided initially approximately 0.21 m^2 per pig. All pens contained 1, 5-hole dry self-feeder and a cup waterer to allow for *ad libitum* access to feed and water. The nursery

barn has a shallow pit for manure storage and completely slatted plastic floors. The nursery room operated on mechanical ventilation using a 4-stage digital controller (Airstream TC5-2V25A, Automated Production Systems, Assumption, IL, USA). During d 0 to 14 post-weaning, the nursery room average daily T_A during the summer replicate was 31.48 ± 1.82°C and during the spring replicate was 30.57 ± 0.68°C. From d 14 to 34 the nursery T_A was 28.70 ± 1.14°C and 25.99 ± 0.84°C for the summer and spring replicates, respectively.

2.3.5 Grow-Finish Phase

On day 34, all pigs were moved to the grow-finish facility for the remainder of the trial and pen integrity was maintained. Common antibiotic free diets were corn-soybean meal-DDGS-based diets provided in meal form to meet or exceed nutrient requirements (NRC, 2012) in six phases during the grow-finish period (Table 2.2). Pigs and feeders were weighed every 21 d during the grow-finish period to determine the response criteria of ADG, ADFI, and G:F.

The grow-finish facility contained pens (1.68 m × 4.27 m) that provided approximately 1.19 m^2 per pig. All pens contained one 2-hole dry self-feeder and a nipple waterer to allow for *ad libitum* access to feed and water. The grow-finish barn had a shallow pit for manure storage and completely slatted concrete floors. The barn was mechanically ventilated. During d 0 to 62 of the grow-finish phase, the room average daily T_A during the summer replicate was 22.35 ± 1.14°C and during the spring replicate was 25.47 ± 2.64°C. From d 62 to 125 the T_A was 19.87 ± 0.83°C and 25.74 ± 2.48°C for the summer and spring replicates, respectively.

2.3.6 Blood Parameters

Blood samples were collected (BD® vacutainers; Franklin Lakes, NJ; plasma; 5 mL) via jugular venipuncture immediately prior to transport, immediately post-transport, and 24 h post-transport from the sentinel animals. Blood samples were obtained at 0630 h on d 13 and 33 of the

nursery phase from one randomly selected pig per pen. Sex of the selected pig was balanced across treatments within day and balanced within pen across collection days. Plasma was collected by centrifugation at 4ºC and 1900 x g for 15 min, aliquoted and stored at -80°C. Plasma cortisol concentrations were analyzed using a commercially available radioimmunoassay (**RIA**) kit (minimum detectable level: 0.9 ng/mL; Cortisol RIA, Tecan Trading AG, Mannedorf, Switzerland) according to manufacturer's instructions. Plasma TNF-α concentrations were analyzed using a commercially available enzyme-linked immunosorbent assay (**ELISA)** kit (Swine TNF-α ELISA Kit; InvitrogenTM; Thermo Fisher Scientific; Waltham, MA) according to manufacturer's instructions. The intra-assay coefficients of variation were 9.0% and 8.6%, for cortisol and TNF-α, respectively. The inter-assay coefficient of variation for TNF-α was 12.4%.

2.3.7 Animal Behavior

Piglets were video-recorded for 14 d immediately following weaning and transport using ceiling mounted cameras (Panasonic WV-CP254H, Matsushita Electric Industrial Co. Ltd., Osaka, Japan) attached to a digital video recorder system (GeoVision VMS Software; GeoVision Inc., Tapei, Taiwan). Video was recorded both during the light and the dark periods (12 h : 12 h). Video files were later analyzed using Observer XT 11.5 behavioral analysis software (Noldus Information Technology B.V., Wageningen, The Netherlands) by four trained individuals that were blind to the treatments and maintained an agreement of 90% or greater. Individual behaviors were determined using an instantaneous scan sampling technique in 10 min intervals on d 2, 4, 8 and 12 post-weaning for 3 periods each day (0800 to 1000 h, 1100 to 1300 h, and 1400 to 1600 h) for sickness and other behaviors. Sickness behavior include huddling and other behaviors included active, resting, aggressive, eating/drinking, and non-visible. The percentage of pigs in each pen performing the specific behaviors was calculated for each timepoint. A definition for each behavior

is defined in an ethogram (Table 2.3). The absolute temperature range measured on each day of behavior analysis was as follows: d 2 for summer and spring replicates (30.30 to 32.70°C and 27.56 to 32.83°C, respectively), d 4 for summer and spring replicates (29.97 to 36.32°C and 30.60 to 33.61°C, respectively), d 8 for summer and spring replicates (29.42 to 35.43°C and 27.31 to 32.36°C, respectively, and d 12 for summer and spring replicates (28.97 to 36.86°C and 26.12 to 31.36°C, respectively).

2.3.8 Marketing

At the end of the 159-d experiment, pigs from each pen were individually tattooed with pen number and shipped approximately 48 km to Indiana Packers Corporation (Delphi, IN). Pigs were slaughtered under commercial conditions with carbon dioxide stunning. Standard carcass criteria of loin and backfat depth, hot carcass weight (**HCW**), fat-free lean index, and yield were collected. Fat depth and loin depth were measured with an optical probe (Fat-O-Meater, SFK Technology A/S, Herlev, Denmark) inserted between the third and fourth rib from the last rib (counting from the posterior of the carcass) and 7 cm from the dorsal midline of the hot carcass. Lean percentage was calculated according to the Indiana Packers Corporation (2015) formula and the fat-free lean percentage was calculated according to Schinckel et al. (2010) procedures.

2.3.9 Statistics

Data were analyzed as a randomized complete block design using the PROC MIXED procedure in SAS 9.4 (SAS Institute INC., Cary, NC), with pen as the experimental unit. The assumptions of normality of error, homogeneity of variance, and linearity were confirmed post-hoc. All injectable antibiotic administration and behavioral data were log-transformed to meet assumptions of normality; however, all log-transformed data are presented as arithmetic means for ease of interpretation. All non-transformed data are presented as LS means. For repeated analyses

for growth performance, each pen's respective parameter was analyzed using repeated measures and covariance structure was selected based on goodness of fit criteria with week as the repeated effect. Statistical significance was defined as $P \leq 0.05$ and a tendency was defined as $0.05 < P \leq 0.10$.

2.4 Results

2.4.1 Sentinel Data

Due to the trailer being considered one experimental unit, all sentinel data are for descriptive purposes only. Core body temperature was 40.1 ± 0.2 ºC and 38.9 ± 0.1 ºC during the summer replicate and spring replicate transport, respectively (Figure 2.1). Plasma cortisol and TNF-α concentrations during pre-transport, post-transport, and 24 h post-transport are presented in Table 2.4.

2.4.2 Blood Parameters

On d 13, plasma TNF-α was reduced ($P = 0.02$; 38.6%) in A and GLN pigs versus NA pigs, but no differences were detected between A and GLN pigs (Table 2.4). Tumor necrosis factor alpha was increased ($P = 0.01$; 70.6%) during the spring replicate compared to the summer replicate on d 33 (Table 2.4). No other plasma TNF-α differences were observed ($P > 0.13$) with any comparison (Table 2.4). No plasma cortisol differences were observed ($P > 0.14$) with any comparison (Table 2.4).

2.4.3 Growth Performance

2.4.3.1 Nursery Phase

When comparing the dietary treatments, ADG was greater overall ($P = 0.01$; 14.9%) from d 0 to 14 of the nursery period in A and GLN pigs compared to NA pigs, but no ADG differences

were detected between A and GLN pigs (Table 2.5). Overall, from d 0 to 34 of the nursery period, ADG was increased (P = 0.01; 7.9%) in A compared to NA pigs, but no differences were detected between A and NA versus GLN pigs (Table 2.5). An increase in ADFI was detected (P = 0.04) from d 0 to 14 of the nursery phase for A compared to NA pigs, but no differences were observed between A and NA versus GLN pigs (Table 2.5). Average daily feed intake tended to be greater (P = 0.09) from d 0 to 34 of the nursery period in A compared to NA pigs, but no differences were observed between A and NA versus GLN pigs (Table 2.5). Feed efficiency (G:F) was greater overall (P = 0.01; 7.7%) from d 0 to 14 of the nursery phase for A compared to NA and GLN pigs, but no differences were observed between NA and GLN pigs (Table 2.5). From d 0 to 34 of the nursery phase, G:F was increased (P = 0.01; 4.3%) in A compared to NA pigs, but no differences were observed for A and NA pigs compared to GLN pigs (Table 2.5). Day 14 BW was greater (P = 0.01) for A (5.6%) and GLN (3.8%) pigs compared to NA pigs; however, no differences were detected between A and GLN pigs (Table 2.5). Final BW was increased (P = 0.04; 4.8%) for A compared to NA pigs, but no differences were detected between A and NA versus GLN pigs (Table 2.5). No other dietary treatment growth performance differences (P > 0.05) were detected during the nursery phase.

Average daily feed intake tended to be reduced (P = 0.08; 5.1%) during the spring replicate compared to the summer replicate from d 0 to 14 of the nursery phase (Table 2.5). From d 14 to 34 of the nursery phase, ADG tended to be reduced (P = 0.09) and G:F was reduced (P = 0.01) during the summer replicate compared to the spring replicate (3.7 and 7.4%, respectively; Table 2.5). Overall, from d 0 to 34 of the nursery period, G:F was reduced (P = 0.04; 4.1%) during the summer replicate compared to the spring replicate (Table 2.5). No other replicate effects were observed during the nursery period (P > 0.05).

A diet x replicate interaction was detected (P = 0.04) from d 14 to 34 of the nursery phase where G:F was greater in the spring replicate in NA (0.69 ± 0.01) and GLN (0.68 ± 0.01) pigs compared to NA pigs (0.61 ± 0.01) during the summer replicate (data not presented). However, no differences were observed between A pigs (0.66 ± 0.01) during the spring replicate and A (0.64 ± 0.01) and GLN (0.63 ± 0.01) pigs during the summer replicate (data not presented). No other diet x replicate interactions were detected (P > 0.05; Table 2.5).

2.4.3.2 Grow-Finish Phase

No dietary treatment differences were observed (P > 0.17) during the grow-finish period (Table 2.5). From d 0 to 62 of the grow-finish phase, G:F was reduced (P = 0.01; 4.3%) during the summer replicate compared to the spring replicate (Table 2.5). Average daily gain, ADFI, and G:F were reduced (P = 0.01; 14.6, 4.4, and 12.1%, respectively) in the summer replicate compared to the spring replicate from d 62 to 125 of the grow-finish phase (Table 2.5). Overall, from d 0 to 125 of the grow-finish period, ADG and G:F were reduced (P = 0.01; 9.2 and 5.1%, respectively) in the summer replicate compared to the spring replicate (Table 2.5). Final BW at the end of the grow-finish period was reduced (P = 0.01; 9.82 kg decrease) in the summer replicate compared to the spring replicate (Table 2.5). No other growth performance differences were observed (P > 0.05) during the grow-finish period with any comparison (Table 2.5).

2.4.4 Treatment rate

2.4.4.1 Nursery Phase

A diet x replicate effect was detected (P = 0.04) where pigs treated for lameness from d 14 to 34 was greater in the spring replicate for GLN pigs (2.12 ± 1.00%) compared to all other treatments (data not presented). However, no differences were observed between A (0.56 ± 1.00%) and NA (0.00 ± 1.00%) pigs during the spring replicate, and A (0.48 ± 1.00%), GLN (0.00 ±

1.00%), and NA (0.00 ± 1.00%) pigs during the summer replicate (data not presented). There were no dietary treatment differences observed ($P > 0.05$) from d 0 to 14 (Table 2.6).

Pigs treated for Other reasons were greater ($P \leq 0.02$) from d 0 to 14 during the spring replicate compared to the summer replicate, regardless of dietary treatment (Table 2.6). No other replicate differences were observed ($P > 0.05$) for treatment rate (Table 2.6).

From d 0 to 14, GLN pigs tended ($P = 0.07$) to be treated for enteric challenges more often in the spring replicate (8.19 ± 2.31%) compared to A pigs (3.13 ± 2.31%), and A (3.13 ± 2.31%) and GLN (3.75 ± 2.31%) pigs during the summer replicate (data not presented). No other diet x replicate differences were detected ($P < 0.05$) during the nursery phase (Table 2.6).

2.4.4.2 Grow-Finish Phase

From d 62 to 125, treatment for unthriftiness was reduced ($P = 0.01$) in GLN (0.00 ± 0.37%) and NA pigs (0.31 ± 0.37%) compared to A pigs (1.00 ± 0.37%), but no differences were observed between GLN and NA pigs (Table 2.6). During d 62 to 125, enteric disease treatments tended ($P < 0.08$) to be reduced by A (0.00 ± 0.93%) pigs and greatest for the GLN (1.17 ± 0.93%) pigs with NA (0.34 ± 0.93%) pigs being intermediate (Table 2.6). No other treatment rate differences for the main effect of dietary treatment were observed ($P > 0.05$) with any comparison (Table 2.6).

Pigs treated for lameness were greater ($P < 0.02$) from d 0 to 62 and d 62 to 125 during the summer replicate compared to the spring replicate, regardless of dietary treatment (Table 2.6). Treatment for respiratory challenges were greater ($P < 0.01$) from d 0 to 62 during the summer replicate compared to the spring replicate (Table 2.6). Pigs treated for other challenges were greater ($P < 0.02$) during the summer replicate compared to the spring replicate from d 0 to 62 (Table 2.6). No other replicate differences were observed ($P > 0.05$) for treatment rate (Table 2.6).

2.4.5 Behavior

Aggressive behavior tended to be reduced overall ($P = 0.09$; 26.4%) in GLN compared to A pigs, but no differences were observed between A and GLN versus NA pigs (Table 2.7). No other diet differences were observed for behavior ($P > 0.05$) with any comparison (Table 2.7).

Huddling, active, and eating/drinking behaviors were increased overall ($P < 0.02$; 179, 37, and 29%, respectively) in the spring replicate compared to the summer replicate (Table 2.7; Figure 2.2). Non-visible behavior was greater ($P < 0.04$; 121%) in the summer replicate compared to the spring replicate (Table 2.7; Figure 2.2F). No other replicate differences were observed for behavior ($P > 0.05$) with any comparison (Table 2.7; Figure 2.2).

Huddling behavior was greater overall ($P < 0.01$) on d 2 and 4 compared to d 8 and 12 (Figure 2.2A). Active behavior was greater overall ($P < 0.01$) on d 2 compared to d 4, 8, and 12 (Figure 2.2B). In addition, active behavior was greater overall ($P < 0.01$) on d 8 and 12 compared to d 4 (Figure 2.2B). Resting behavior was greater overall ($P < 0.01$) on d 4, 8, and 12 compared to d 2 (Figure 2.2C). Aggressive behavior was greater overall ($P < 0.01$) on d 2 compared to d 4, 8, and 12 (Figure 2.2D). In addition, aggressive behavior was greater overall ($P < 0.01$) on d 4 compared to d 12 but no differences were observed on d 4 and 12 versus d 8 (Figure 2.2D). Eating/Drinking behavior was greater overall ($P = 0.01$) on d 8 and 12 compared to d 2 and 4 (Figure 2.2E). No other day differences were observed for behavior ($P > 0.05$) with any comparison (Table 2.7; Figure 2.2).

Active behavior was greater ($P < 0.01$) on d 2, 4, and 8 during the spring replicate compared to the summer replicate but was not different on d 12 (Figure 2.2B). Resting behavior was increased ($P < 0.01$) on d 2 during the summer replicate compared to the spring replicate; however, on d 12, resting behavior was greater during the spring replicate compared to the summer replicate (Figure 2.2C). Aggressive behavior tended to be greater ($P = 0.07$) on d 8 during the spring

replicate compared to the summer replicate (Figure 2.2D). Eating/drinking behavior was greater ($P < 0.01$) on d 2 and d 4 during the spring replicate compared to the summer replicate, but no differences were detected on d 8 and 12 (Figure 2.2E). No other behavioral differences were detected ($P < 0.05$) with any comparison (Table 2.7; Figure 2.2).

2.4.6 Carcass Characteristics

No dietary treatment effects were observed ($P > 0.60$) on carcass characteristics (Table 2.8). Hot carcass weight and loin depth were increased ($P < 0.01$; 5.4% and 5.5%, respectively) and carcass yield was reduced ($P < 0.01$; 2.0%) for pigs weaned in the spring replicate compared to the summer replicate when HCW was not used as a covariate in the statistical model (Table 2.8). When HCW was used as a covariate in the statistical analysis, loin depth and lean percentage were increased ($P = 0.01$; 4.0% and 1.1%, respectively) and carcass yield was reduced ($P = 0.01$; 2.3%) for pigs weaned in the spring replicate compared to the summer replicate (Table 2.8). Fat-free lean percentage during the spring replicate tended to be greater ($P = 0.07$; 1.3%) compared to the summer replicate when HCW was included as a covariate (Table 2.8). No other carcass characteristic differences were observed ($P > 0.05$) with any comparison (Table 2.8).

2.5 Discussion

The need to wean and transport pigs is necessary to reduce the risk of infectious disease through multi-site production (Harris, 2000). However, the resultant stress response can reduce growth performance and welfare in newly weaned pigs (Chambers and Grandin, 2001; Campbell et al., 2013), especially in the absence of dietary antibiotics (Heo et al., 2013). Despite this, the use of in-feed antibiotics has been reduced in swine production due to consumer preference, legislative action, and concerns about antibiotic resistance (Smith et al., 2010), putting the welfare and

productivity of newly weaned and transported pigs at risk and necessitating the development of effective alternatives. Recent work has described improved welfare and productivity in piglets provided GLN compared to A and NA following weaning and simulated transport (Johnson and Lay, 2017). In accordance with the aforementioned study, piglets provided GLN after weaning and transport in the present study had improved growth performance compared to NA pigs during the 14-d dietary treatment period, regardless of replicate. However, no growth performance differences were detected between GLN and A pigs in the current study. Although reasons for this discrepancy are currently unknown, it may be due to differences in study design since the transport procedure was simulated and piglets were individually housed in the previous study (Johnson and Lay, 2017). While the mechanism(s) of action for improved growth performance has yet to be discerned, GLN can serve as energy source for enterocytes thus reducing jejunal atrophy and intestinal epithelial damage (Wu et al., 1996; Yi et al., 2005; Wang et al., 2015a,b). Therefore, it is possible that piglets provided supplemental GLN had improved intestinal barrier function leading to greater pathogen resistance, reduced translocation of bacteria (Peng, 2004; Wang et al., 2015a,b) and subsequently an improvement in growth performance (Jiang et al., 2009; Johnson and Lay, 2017). Nevertheless, the advantages observed in early nursery growth performance may suggest that GLN supplementation could serve as an alternative to dietary antibiotics in production systems.

Although growth performance was improved in GLN and A pigs during the dietary treatment period and the advantage was maintained for the overall nursery period, no differences were detected when compared to NA pigs from d 14 to market when all pigs were fed a common antibiotic free diet. However, these results were expected as previous studies have described a loss of growth performance differences once dietary antibiotic treatments (Skinner et al., 2014) or

dietary formulation treatments (Dritz et al., 1996) cease. This may be due to pen to pen variability differences that diminished the growth rate advantages as the studies progressed or the performance advantages of feeding dietary treatments are limited only to the period when fed. Therefore, it could be suggested that feeding GLN to pigs for a longer duration could have extended the growth benefits. However, further work would be needed to confirm this hypothesis and any increase in growth performance would need to be balanced with the cost of including GLN in diets for a longer period of time.

Tumor necrosis factor alpha is a proinflammatory cytokine and elevated levels of plasma TNF-α can be indicative of systemic inflammation and immune system activation (Kalliolias and Ivashkiv, 2016). An activated immune system is energetically expensive to the pig as the glucose requirement is increased (Kvidera et al., 2017). This increase in glucose requirement by the activated immune system consumes energy that could be used for growth. As a result, growth may be inhibited during an immune challenge. In the present study the reduced plasma TNF-α concentrations of A and GLN compared to NA fed pigs could be indicative of reduced whole-body inflammatory response, which would decrease the immune system energy requirement as previously described (Kvidera et al., 2017). As a result, more energy would likely be available for growth in the A and GLN fed pigs and may partially explain the improved performance compared to the NA fed pigs. While reasons for this reduction in TNF-α are currently unknown, it is possible that an improvement in intestinal health caused the reduction in TNF-α for A and GLN fed pigs since decreased intestinal barrier function is associated with an increase in bacterial translocation and systemic inflammation in pigs (Pearce et al., 2014). However, more research is needed to confirm this hypothesis.

Cortisol is often used by researchers as a physiological indicator of stress in pigs and is often increased during stress exposure (Marchant-Forde et al., 2012). One of the most stressful periods during a pig's life is weaning and transport (Campbell et al., 2013). However, previous studies in weaned pigs transported under TN conditions have shown that although cortisol levels will increase during transport, they return to baseline or reduced levels at unloading (Bradshaw et al., 1996, Averós et al., 2009). In contrast, when pigs are weaned and transported under HS conditions, cortisol levels remain elevated post-transport then are reduced to near baseline levels the next day (Johnson et al., 2018). In accordance with the aforementioned reports, although a 38% numerical reduction in post-transport cortisol levels were observed in spring replicate transported sentinel pigs, those transported during the summer replicate in the present study had a 457% numerical increase in circulating cortisol levels following transport. Despite the fact that the weaning and transport process appeared to be more stressful (as indicated by numerically elevated cortisol levels) during the summer replicate, no replicate or dietary differences were observed on d 13 and 33 post-transport. This is likely due to the fact that pigs had recovered from the acute stressor and cortisol levels had returned to near baseline as time progressed as previously described (Johnson et al., 2018).

Weaning and transport are stressful to piglets and may result in behavioral changes including increased aggression and activity that are indicative of distress (Lewis and Berry, 2006; Wamnes et al., 2008). As such, newly weaned and transported piglets in the present study exhibited behavioral signs of distress immediately following transport, which subsequently declined as time progressed following weaning and transport. These behaviors ranged from increased activity, which may be indicative of greater exploratory behavior and stress (Bøe, 1993), to greater huddling behavior that may have been due to greater sub-clinical illness (Hennessy et al., 2001), and an

increase in aggressive behavior likely due to fighting and establishing a social hierarchy (Meese and Ewbank, 1973; Blackshaw et al., 1987; Colson et al, 2012). However, despite the improved growth performance, dietary A and GLN supplementation treatments did not appear to alleviate this post-weaning and transport behavioral stress response relative to NA treated pigs. In addition, aggressive behavior tended to be greater in A compared to GLN pigs, which may be a sign of resource guarding (i.e., feed; Drake et al., 2008) in group-housed pigs. Therefore, potential mechanisms may have been that A pigs spent more time guarding feed as this was the only resource available in the pen or that they felt better and were therefore more capable of doing so. However, because GLN and A pigs had similar ADFI, but differ in levels of aggression, it is still unclear whether the increase in aggressive behavior was due to resource guarding and further research should be performed to determine the cause.

In addition to the impact of weaning and transport as well as dietary treatments on piglet behavior, replicate effects were also observed. Increased resting behavior was observed during the summer replicate on d 2 post-weaning and transport compared to the spring replicate and this may have been due to greater exhaustion and dehydration during the summer replicate as previously reported (Berry and Lewis, 2001). Furthermore, pigs weaned and transported in the spring replicate exhibited greater huddling behavior compared to those weaned and transported in the summer replicate. Although a specific reason has yet to be elucidated, this response may have been related to T_A and pigs' need for supplemental heat (Hay et al., 2001). This is because the nursery T_A during the summer replicate was at the upper end of the recommended thermoneutral zone and the spring replicate nursery T_A was at the lower end of the recommended thermoneutral zone for nursery pigs (Federation of Animal Science Societies, 2010). Therefore, the increase in summer replicate nursery T_A may have diminished the need for huddling (Hay et al., 2001). Furthermore, this nursery

T_A difference may have been responsible for a reduction in eating/drinking and active behavior during the summer replicate in an effort to reduce heat increment from feed consumption during the time of day when behavior was analyzed (Coffey et al., 1982; Nienaber et al., 1999).

Therapeutic injectable antibiotics are one of many options currently available to aid in the control of pathogens and disease in addition to good biosecurity practices, vaccinations, and dietary antibiotics (Maes, 2008). An increase in treatment rate with therapeutic antibiotics can be an indicator of illness in swine herds. In the present study, A pigs had fewer therapeutic antibiotic treatments for enteric challenges compared to GLN pigs during the spring replicate from d 0 to 14 post-weaning, but no differences were detected during the summer replicate. While this may indicate that dietary antibiotic treatments were more effective at reducing pathogen load compared to GLN, the lack of overall dietary treatment differences may suggest that the timing of weaning and transport throughout the year influences the impact of GLN on therapeutic treatments. Regardless, the increase in therapeutic treatments did not appear to coincide with a depression in growth performance and this may be due to differences in the mode of action between A and GLN treatments whereby dietary antibiotics reduce pathogen colonization (Pluske et al., 2002) while GLN improves gut barrier function in pigs (Wang et al., 2015a,b). Further work is needed to explore the combined feeding of multiple nutraceuticals that have shown performance benefits independently to determine if the effect of combining them is additive.

In the present study, no dietary treatment effects were observed for carcass trait differences, confirming previous reports that providing dietary additives (i.e., antibiotics) for a limited period in the nursery phase would have no impact on carcass composition (Skinner et al., 2014). While the effects of providing GLN on carcass characteristics in pigs are unknown, previous reports in broilers reported that GLN supplementation during heat stress improves meat yield (Dai et al.,

2011). However, because broilers were provided GLN until harvest in the aforementioned study and pigs in the present study were only provided GLN for the first 14 d post-weaning, it is likely that the lack of carcass trait differences are related to the timing of dietary inclusion. Nevertheless, a lack of dietary treatment differences confirms that GLN would not have negative effects on carcass traits compared to A and NA diets.

Despite the lack of dietary treatment differences on carcass characteristics, pigs weaned in the spring replicate had greater HCW and loin depth and increased lean percentage and fat-free lean percentage when HCW was used as a covariate compared to summer replicate weaned pigs. While the mechanism(s) for the improvement in carcass characteristics are unknown, we speculate that health status may have impacted the carcass differences observed in the current study due to the differences in therapeutic antibiotic treatment rate between replicates. This response appears to be consistent with previous work by Holck et al. (1998) and Williams et al. (1997) who reported improved carcass characteristics when pigs were reared under higher health status. This suggests that poorer health status may have decreased growth rate and subsequently reduced lean tissue accretion rate. This potential advantage in health status during the spring replicate weaned pigs may have allowed the pigs to grow and deposit lean tissue at a rate closer to their genetic potential because previous studies determined that when pigs were exposed to chronic immune system activation in a health compromised environment, cytokine concentration was elevated (Williams et al., 1997) thereby suppressing lean growth. This is further explained by Zamir et al. (1994) where rats administered with an IL-1 receptor antagonist had reduced skeletal muscle catabolism when IL-1 was administered. Thus, based on these relationships, less environmental pathogens as indicated by reduced therapeutic antibiotic use could have decreased immune system and cytokine

activation thus allowing the potential for increased muscle accretion rate due to less skeletal muscle catabolism.

2.6 Conclusion

Weaning and transport is stressful to pigs and antibiotics have been routinely used to help young pigs overcome these challenges. Despite the advantages in growth performance and productivity found from the use of dietary antibiotics, alternatives to antibiotics are needed. It was proposed that L-glutamine supplementation could serve as an antibiotic alternative following weaning and transport and allow pigs to perform similarly to those given dietary antibiotics. We determined that L-glutamine supplemented at 0.20% improved pig health and productivity after weaning and transport similarly to antibiotics during the nursery phase; however, the positive effects of dietary antibiotics and L-glutamine were diminished during the grow-finish phase. However, pigs not provided dietary antibiotics had decreased growth rate during the nursery phase. Future work should address the mechanism(s) by which L-glutamine supplementation improves pig growth performance following weaning and transport.

2.7 References

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Table 2.1 Composition of nursery diets

	Phase 1[1]			**Phase 2[2]**			**Phase 3[3]**	**Phase 4[4]**
Item	A[5]	GLN[6]	NA[7]	A	GLN	NA		
Ingredient, % as fed								
Corn	30.81	31.18	31.38	37.52	37.89	38.09	51.63	57.38
SBM, 48% CP	13.95	13.95	13.95	18.00	18.00	18.00	25.65	30.70
Dried Distillers Grain with Solubles	---	---	---	---	---	---	---	5.00
Soybean Oil	5.00	5.00	5.00	5.00	5.00	5.00	3.00	---
Choice White Grease	---	---	---	---	---	---	---	3.00
Limestone	0.79	0.79	0.79	0.74	0.74	0.74	0.86	1.33
Monocalcium Phosphate	0.40	0.40	0.40	0.49	0.49	0.49	0.49	0.74
Vitamin Premix[8]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace Mineral Premix[9]	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Selenium Premix[10]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Phytase[11]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.30	0.35
Plasma Protein	6.50	6.50	6.50	2.50	2.50	2.50	---	---
Spray Dried Blood Meal	1.50	1.50	1.50	1.50	1.50	1.50	---	---
Soy Concentrate	4.00	4.00	4.00	3.00	3.00	3.00	2.50	---
Select Menhaden Fish Meal	5.00	5.00	5.00	4.00	4.00	4.00	4.00	---
Dried Whey	25.00	25.00	25.00	25.00	25.00	25.00	10.00	---
Lactose	5.00	5.00	5.00	---	---	---	---	---
Lysine-HCL	0.07	0.07	0.07	0.20	0.20	0.20	0.28	0.40
DL-Methionine	0.22	0.22	0.22	0.23	0.23	0.23	0.18	0.17
L-Threonine	0.04	0.04	0.04	0.09	0.09	0.09	0.12	0.14
L-Tryptophan	---	---	---	0.01	0.01	0.01	0.01	0.00
Zinc Oxide	0.375	0.375	0.375	0.375	0.375	0.375	0.375	---
Copper Sulphate	---	---	---	---	---	---	---	0.10
Aureomycin 50[12]	0.40	---	---	0.40	---	---	---	---
Denagard 10[13]	0.18	---	---	0.18	---	---	---	---
L-Glutamine[14]	---	0.20	---	---	0.20	---	---	---
Banminth 48[15]	---	---	---	---	---	---	---	0.10
Clarifly, 0.67%[16]	---	---	---	---	---	---	0.08	0.07
Calculated chemical composition								
ME, kcal/kg	3536	3536	3536	3510	3510	3510	3418	3396
Fat, %	7.27	7.27	7.27	7.36	7.36	7.36	5.73	5.86
CP, %	24.62	24.62	24.62	22.87	22.87	22.87	22.29	21.28
SID Lys, %	1.55	1.55	1.55	1.45	1.45	1.45	1.35	1.25
Ca, %	0.90	0.90	0.90	0.85	0.85	0.85	0.80	0.75
Total P, %	0.75	0.75	0.75	0.71	0.71	0.71	0.64	0.57

Table 2.1 continued

Avail. P, %	0.60	0.60	0.60	0.55	0.55	0.55	0.45	0.36
Analyzed chemical composition								
Summer replicate								
GE, kcal/kg	4217	4251	4173	4172	4146	4184	---	---
CP, %	24.42	25.62	23.85	22.30	22.38	22.46	22.07	22.00
Total Lys, %	1.30	1.35	1.26	1.13	1.18	1.11	---	---
Total Glu, %[17]	3.15	3.43	3.11	2.78	2.88	2.68	---	---
Chlortetracycline, ppm[18]	467	0	0	468	0	0	---	---
Spring replicate								
GE, kcal/kg	4266	4199	4079	4174	4193	4129	---	---
CP, %	25.36	26.37	22.51	22.78	23.02	24.68	22.37	21.14
Total Lys, %	1.58	1.75	1.40	1.54	1.51	1.51	---	---
Total Glu, %	3.70	4.23	3.17	3.68	3.81	3.62	---	---
Chlortetracycline, ppm	436	0	0	436	0	0	---	---

[1]Fed d 0 to 7 post-weaning and transport
[2]Fed d 7 to 14 post-weaning and transport
[3]Fed d 14 to 21 post-weaning and transport
[4]Fed d 21 to 34 post-weaning and transport
[5]Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)]
[6]Pigs provided 0.20% L-glutamine
[7]Pigs provided no dietary antibiotics
[8]Provided per kilogram of the diet: vitamin A, 6,615 IU; vitamin D_3, 662 IU; vitamin E, 44 IU; vitamin K, 2.2 mg; riboflavin, 8.8 mg; pantothenic acid, 22 mg; niacin, 33 mg; B_{12}, 38.6 mg
[9]Provided available minerals per kilogram of the diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15 mg; copper, 11.3 mg; iodine, 0.46 mg
[10]Provided 0.3 ppm Se
[11]Provided 600 FTU per kg of the diet
[12]Aureomycin (Zoetis, Parsippany, NJ) provided 441 ppm chlortetracycline in the diet
[13]Denagard (Elanco Animal Health, Greenfield, IN) provided 38.6 ppm tiamulin in the diet
[14]Ajinomoto North America, Inc., Raleigh, NC
[15]Banminth (Phibro Animal Health Corporation, Teaneck, NJ) provided 106 ppm pyrantel tartrate in the diet
[16]Clarifly (Central Life Sciences, Schaumburg, IL) provided 5.4 ppm (Phase 3) and 4.7 ppm (Phase 4) diflubenzuron in the diet
[17]Samples submitted to Ajinomoto for glutamic acid analysis
[18]Samples submitted to Zoetis, Parsippany, NJ for chlortetracycline analysis

Table 2.2 Composition of grow-finish diets

Item	**Phase 1[1]**	**Phase 2[2]**	**Phase 3[3]**	**Phase 4[4]**	**Phase 5[5]**	**Phase 6[6]**
Ingredient, % as fed						
Corn	61.47	64.65	66.40	71.10	82.38	68.67
SBM, 48% CP	23.20	16.15	9.75	5.25	4.25	15.10
Dried Distillers Grain with Solubles	10.00	15.00	20.00	20.00	10.00	10.00
Choice White Grease	2.00	1.00	1.00	1.00	1.00	3.00
Limestone	1.37	1.35	1.39	1.32	1.16	1.26
Monocalcium Phosphate	0.47	0.32	0.05	0.00	0.10	0.27
Vitamin Premix	0.150[7]	0.150[7]	0.125[8]	0.120[9]	0.100[10]	0.150[7]
Trace Mineral Premix	0.10[11]	0.09[12]	0.08[13]	0.07[14]	0.05[15]	0.10[11]
Selenium Premix	0.050[16]	0.050[16]	0.050[16]	0.050[16]	0.025[17]	0.050[16]
Phytase[18]	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.35	0.35	0.30	0.30	0.25	0.30
Lysine-HCL	0.42	0.46	0.48	0.46	0.37	0.42
DL-Methionine	0.11	0.08	0.05	0.01	0.00	0.10
L-Threonine	0.130	0.130	0.120	0.105	0.095	0.160
L-Tryptophan	0.010	0.030	0.035	0.040	0.030	0.030
Paylean 2.25[19]	---	---	---	---	---	0.15
Availa Zn 120[20]	---	---	---	---	---	0.042
Clarifly, 0.67%	0.07[21]	0.09[22]	0.07[21]	0.08[23]	0.09[22]	0.10[24]
Calculated chemical composition						
ME, kcal/kg	3373	3337	3351	3359	3371	3438
Fat, %	5.29	4.69	5.06	5.15	4.73	6.40
CP, %	19.34	17.59	15.99	14.18	11.90	16.01
SID Lys, %	1.10	0.98	0.85	0.73	0.60	0.90
Ca, %	0.70	0.65	0.60	0.55	0.50	0.60
Total P, %	0.50	0.47	0.41	0.38	0.35	0.42
Avail. P, %	0.32	0.30	0.26	0.24	0.20	0.26
Analyzed chemical composition						
Summer replicate						
CP, %	19.13	18.08	14.92	14.66	11.73	15.64
Spring replicate						
CP, %	19.25	17.33	16.73	15.59	12.29	16.85

[1]Fed d 0 to 21 of the grow-finish phase
[2]Fed d 21 to 42 of the grow-finish phase
[3]Fed d 42 to 62 of the grow-finish phase
[4]Fed d 62 to 83 of the grow-finish phase

Table 2.2 continued

[5]Fed d 83 to 104 of the grow-finish phase
[6]Fed d 104 to 125 of the grow-finish phase
[7]Provided per kilogram of the diet: vitamin A, 3,969 IU; vitamin D_3, 397 IU; vitamin E, 26 IU; vitamin K, 1.3 mg; riboflavin, 5.3 mg; pantothenic acid, 13 mg; niacin, 20 mg; B_{12}, 23.2 mg
[8]Provided per kilogram of the diet: vitamin A, 3,308 IU; vitamin D_3, 331 IU; vitamin E, 22 IU; vitamin K, 1.1 mg; riboflavin, 4.4 mg; pantothenic acid, 11 mg; niacin, 17 mg; B_{12}, 19.3 mg
[9]Provided per kilogram of the diet: vitamin A, 3,175 IU; vitamin D_3, 318 IU; vitamin E, 21 IU; vitamin K, 1.1 mg; riboflavin, 4.2 mg; pantothenic acid, 11 mg; niacin, 16 mg; B_{12}, 18.5 mg
[10]Provided per kilogram of the diet: vitamin A, 2,646 IU; vitamin D_3, 265 IU; vitamin E, 18 IU; vitamin K, 0.9 mg; riboflavin, 3.5 mg; pantothenic acid, 9 mg; niacin, 13 mg; B_{12}, 15.4 mg
[11]Provided per available minerals kilogram of the diet: iron, 97 mg; zinc, 97 mg; manganese, 12 mg; copper, 9 mg; iodine, 0.37 mg
[12]Provided per available minerals kilogram of the diet: iron, 87 mg; zinc, 87 mg; manganese, 11 mg; copper, 8 mg; iodine, 0.33 mg
[13]Provided per available minerals kilogram of the diet: iron, 78 mg; zinc, 78 mg; manganese, 10 mg; copper, 7.2 mg; iodine, 0.29 mg
[14]Provided per available minerals kilogram of the diet: iron, 68 mg; zinc, 68 mg; manganese, 8 mg; copper, 6.3 mg; iodine, 0.26 mg
[15]Provided per available minerals kilogram of the diet: iron, 48.5 mg; zinc, 48.5 mg; manganese, 6 mg; copper, 4.5 mg; iodine, 0.18 mg
[16]Provided 0.3 ppm Se
[17]Provided 0.15 ppm Se
[18]Provided 600 FTU per kg of the diet
[19]Paylean (Elanco Animal Health, Greenfield, IN) provided 7.5 ppm ractopamine HCl in the diet
[20]Zinpro Corporation, Eden Prairie, MN
[21]Clarifly (Central Life Sciences, Schaumburg, IL) provided 4.7 ppm diflubenzuron in the diet
[22]Clarifly (Central Life Sciences, Schaumburg, IL) provided 6.0 ppm diflubenzuron in the diet
[23]Clarifly (Central Life Sciences, Schaumburg, IL) provided 5.4 ppm diflubenzuron in the diet
[24]Clarifly (Central Life Sciences, Schaumburg, IL) provided 6.7 ppm diflubenzuron in the diet

Table 2.3 Ethogram used for behavioral observations

Category	Behavior	Definition
Sickness Behavior	Huddling	When 3 or more pigs are touching while lying down and 50% of a pig's body is touching another pig.
Other	Active	Piglets are walking about or interacting in a non-aggressive manner with each other or their environment.
	Resting	Piglets are lying, either ventral or lateral, either alone or loosely in groups, with gaps of spaces between them.
	Aggressive	Piglets are engaged in agonistic interactions.
	Eating/Drinking	The piglet has its nose in the feeder or its mouth on the waterer.
	Non-visible	When piglet moves out of view and cannot be observed.

Table 2.4 Effect of dietary treatment on blood plasma parameter concentrations

	Replicate		**Diet**				***P***		
Parameter	Summer[1]	Spring[2]	A[3]	GLN[4]	NA[5]	SE	D[6]	R[7]	D x R
Sentinel pigs[8]									
Pre-transport[9]									
TNF-α[10], pg/ml	19.11	27.11	---	---	---	10.21	---	---	---
Cortisol, µg/L	25.24	54.80	---	---	---	13.91	---	---	---
Post-transport[11]									
TNF-α, pg/ml	3.27	12.58	---	---	---	10.24	---	---	---
Cortisol, µg/L	140.64	34.19	---	---	---	19.60	---	---	---
24 h post-transport[12]									
TNF-α, pg/ml	32.53	34.41	---	---	---	11.38	---	---	---
Cortisol, µg/L	37.01	19.06	---	---	---	9.96	---	---	---
Experimental Data[13]									
Day 13									
TNF-α, pg/ml	47.88	46.02	36.73[a]	40.92[a]	63.19[b]	6.94	0.02	0.82	0.14
Cortisol, µg/L	28.1	25.18	26.80	26.39	26.72	2.25	0.99	0.25	0.95
Day 33									
TNF-α, pg/ml	45.33	77.32	62.03	54.78	67.16	5.84	0.31	0.01	0.92
Cortisol, µg/L	46.79	53.95	52.68	48.46	49.96	4.55	0.78	0.15	0.40

[1]Pigs weaned and transported for 12 h during July 2016
[2]Pigs weaned and transported for 12 h during April 2017
[3]Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)] for 14 d post-weaning and transport and then fed common antibiotic free diets
[4]Pigs provided 0.20% L-glutamine for 14 d post-weaning and transport and then fed common antibiotic free diets
[5]Pigs provided no dietary antibiotics for 14 d post-weaning and transport and then fed common antibiotic free diets
[6]Dietary treatment
[7]Replicate
[8]6 sentinel pigs per replicate was selected for blood parameter descriptive data
[9]Blood samples were collected immediately prior to transport
[10]Tumor necrosis factor alpha
[11]Blood samples were collected immediately post-transport
[12]Blood samples were collected 24 h post-transport
[13]A total of 10 pens were used per dietary treatment per replicate with 1 pig per pen closest to the pen mean BW was selected for plasma cortisol concentration analysis
[a,b]Letters indicate significant differences ($P \leq 0.05$) within a row and dietary treatment

Table 2.5 Effect of dietary treatment on nursery and grow-finish growth performance[1]

	Replicate		Diet				*P*		
Parameter	Summer[2]	Spring[3]	A[4]	GLN[5]	NA[6]	SE	D[7]	R[8]	D x R
Nursery Period									
Day 0 to 14									
Initial BW, kg	5.64	5.51	5.58	5.59	5.57	0.29	0.99	0.70	0.99
ADG, g	210	206	224^{a}	210^{a}	189^{b}	10.19	0.01	0.56	0.82
ADFI, g	274	260	277^{a}	272^{ab}	253^{b}	13.21	0.04	0.08	0.92
G:F	0.80	0.80	0.84^{a}	0.79^{b}	0.77^{b}	0.01	0.01	0.91	0.17
Day 14 BW, kg	8.44	8.46	8.65^{a}	8.50^{a}	8.19^{b}	0.52	0.01	0.83	0.97
Day 14 to 34									
ADG, g	439	455	458	447	436	12.05	0.21	0.09	0.43
ADFI, g	693	674	702	680	669	22.81	0.16	0.19	0.63
G:F	0.63	0.68	0.65	0.66	0.65	0.01	0.78	0.01	0.04
Day 0 to 34									
ADG, g	347	355	364^{a}	352^{ab}	337^{b}	10.18	0.01	0.23	0.58
ADFI, g	525	509	532^{x}	517^{xy}	503^{y}	17.43	0.09	0.12	0.77
G:F	0.70	0.73	0.73^{a}	0.71^{ab}	0.70^{b}	0.01	0.03	0.01	0.07
Day 34 BW, kg	17.20	17.62	17.78^{a}	17.49^{ab}	16.96^{b}	0.74	0.04	0.11	0.69
Grow-finish Period									
Day 0 to 62									
ADG, kg	0.76	0.77	0.78	0.76	0.76	0.01	0.32	0.37	0.62
ADFI, kg	1.79	1.75	1.80	1.76	1.75	0.03	0.40	0.14	0.88
G:F	0.44	0.46	0.45	0.46	0.45	0.01	0.80	0.01	0.36
Day 62 BW, kg	64.72	65.5	65.99	65.02	64.31	0.96	0.22	0.32	0.76
Day 62 to 125									
ADG, kg	0.82	0.96	0.88	0.89	0.90	0.02	0.41	0.01	0.36
ADFI, kg	2.83	2.96	2.87	2.91	2.90	0.05	0.72	0.01	0.42
G:F	0.29	0.33	0.30	0.31	0.31	0.01	0.17	0.01	0.62
Day 0 to 125									
ADG, kg	0.79	0.87	0.83	0.83	0.83	0.01	0.95	0.01	0.58
ADFI, kg	2.31	2.35	2.33	2.33	2.32	0.03	0.97	0.21	0.60
G:F	0.37	0.39	0.38	0.38	0.38	0.01	0.54	0.01	0.56
Final BW, kg	117.37	127.19	122.77	121.73	122.34	1.23	0.83	0.01	0.64

[1]A total of 10 pens were used per dietary treatment per replicate
[2]Pigs weaned and transported for 12 h during July 2016
[3]Pigs weaned and transported for 12 h during April 2017

Table 2.5 continued

[4]Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)] for 14 d post-weaning and transport and then fed common antibiotic free diets
[5]Pigs provided 0.20% L-glutamine for 14 d post-weaning and transport and then fed common antibiotic free diets
[6]Pigs provided no dietary antibiotics for 14 d post-weaning and transport and then fed common antibiotic free diets
[7]Dietary treatment
[8]Replicate
[a,b]Letters indicate significant differences ($P \leq 0.05$) within a row and dietary treatment
[x,y]Letters indicate tendencies ($0.05 < P \leq 0.10$) within a row and dietary treatment

Table 2.6 Effect of dietary treatment on therapeutic antibiotic treatment rate during the nursery period[1]

	Replicate		Diet				*P*		
Parameter	Summer[2]	Spring[3]	A[4]	GLN[5]	NA[6]	SE	D[7]	R[8]	D x R
Nursery Period									
Day 0 to 14									
Enteric[9]	4.59	5.29	3.13	5.97	5.72	2.31	0.31	0.38	0.07
Lame[10]	1.67	0.88	1.26	1.64	0.94	1.02	0.73	0.27	0.89
Unthrifty[11]	1.46	0.65	0.94	0.97	1.25	1.02	0.92	0.22	0.48
Respiratory[12]	---	---	---	---	---	---	---	---	---
Other[13]	0.00	1.06	0.63	0.35	0.63	0.86	0.86	0.02	0.86
Day 14 to 34									
Enteric	0.48	0.19	0.00	0.48	0.52	0.66	0.36	0.33	0.37
Lame	0.16	0.89	0.52^{b}	1.06^{a}	0.00^{b}	1.00	0.08	0.06	0.04
Unthrifty	0.34	1.07	0.52	0.58	1.03	0.80	0.64	0.14	0.57
Respiratory	0.16	0.18	0.00	0.27	0.24	0.53	0.58	0.94	0.20
Other	0.00	0.18	0.00	0.27	0.00	0.53	0.31	0.27	0.31
Grow-finish Period									
Day 0 to 62									
Enteric	0.19	0.63	0.56	0.34	0.34	0.67	0.81	0.31	0.77
Lame	1.00	0.00	0.28	0.28	0.95	1.06	0.41	0.02	0.41
Unthrifty	0.82	0.19	0.89	0.00	0.62	0.99	0.24	0.17	0.16
Respiratory	9.96	1.30	5.84	5.95	5.11	2.92	0.60	<0.01	0.77
Other	1.59	0.19	0.56	1.17	0.95	1.11	0.69	0.02	0.45
Day 62 to 125									
Enteric	0.22	0.78	0.00^{y}	1.17^{x}	0.34^{y}	0.93	0.08	0.19	0.58
Lame	1.19	0.00	1.11	0.34	0.34	1.05	0.21	0.01	0.21
Unthrifty	0.19	0.56	1.12	0.00	0.00	0.93	0.01	0.28	0.30
Respiratory	10.24	7.17	8.83	7.81	9.48	4.20	0.81	0.49	0.86
Other	0.19	0.00	0.28	0.00	0.00	0.56	0.37	0.32	0.37

[1]A total of 10 pens were used per dietary treatment per replicate
[2]Pigs weaned and transported for 12 h during July 2016
[3]Pigs weaned and transported for 12 h during April 2017
[4]Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)] for 14 d post-weaning and transport and then fed common antibiotic free diets
[5]Pigs provided 0.20% L-glutamine for 14 d post-weaning and transport and then fed common antibiotic free diets
[6]Pigs provided no dietary antibiotics for 14 d post-weaning and transport and then fed common antibiotic free diets
[7]Dietary treatment

Table 2.6 continued

[8]Replicate
[9]Percent of pigs within pen treated with therapeutic antibiotics for enteric challenge
[10]Percent of pigs within pen treated with therapeutic antibiotics for lameness
[11]Percent of pigs within pen treated with therapeutic antibiotics for un-thriftiness
[12]Percent of pigs within pen treated with therapeutic antibiotics for respiratory challenge
[13]Percent of pigs within pen treated with therapeutic antibiotics for all other conditions
[a,b]Letters indicate significant differences ($P \leq 0.05$) within a row and dietary treatment
[x,y]Letters indicate tendencies ($0.05 < P \leq 0.10$) within a row and dietary treatment

Table 2.7 Effect of dietary treatment on behavior (% of time) from day 2 to 12 post-weaning[1]

	Replicate		**Diet**				***P***		
Behavior	Summer[2]	Spring[3]	A[4]	GLN[5]	NA[6]	SE	D[7]	R[8]	D x R
Huddling[9], %	5.52	15.38	10.3	8.58	11.2	1.46	0.92	<0.01	0.84
Active[10], %	9.14	12.49	10.9	10.64	10.71	0.55	0.78	<0.01	0.14
Resting[11], %	77.55	73.07	73.6	77.13	74.94	1.33	0.12	0.34	0.33
Aggressive[12], %	1.39	1.57	1.74[x]	1.28[y]	1.41[xy]	0.19	0.09	0.14	0.7
Eat/Drink[13], %	8.7	11.26	10.7	9.96	9.14	0.51	0.17	<0.01	0.18
Non-visible[14], %	0.75	0.34	0.83	0.41	0.36	0.37	0.26	0.04	0.67

[1]A total of 10 pens were used per dietary treatment per replicate
[2]Pigs weaned and transported for 12 h during July 2016
[3]Pigs weaned and transported for 12 h during April 2017
[4]Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)] for 14 d post-weaning and transport and then fed common antibiotic free diets
[5]Pigs provided 0.20% L-glutamine for 14 d post-weaning and transport and then fed common antibiotic free diets
[6]Pigs provided no dietary antibiotics for 14 d post-weaning and transport and then fed common antibiotic free diets
[7]Dietary treatment
[8]Replicate
[9]When 3 or more pigs are touching while lying down and 50% of a pig's body is touching another pig; collected independent of other behaviors
[10]Piglets are walking about or interacting in a non-aggressive manner with each other or their environment
[11]Piglets are lying, either ventral or sternal, either alone or loosely in groups, with gaps of spaces between them
[12]Piglets are engaged in agonistic interactions
[13]The piglet has its nose in the feeder or its mouth on the waterer
[14]When piglet moves out of view and cannot be observed
[x,y]Letters indicate tendencies ($0.05 < P \leq 0.10$) within a row dietary treatment

Table 2.8 Effect of dietary treatment on carcass characteristics[1]

	Replicate		Diet				*P*		
Parameter	Summer[2]	Spring[3]	A[4]	GLN[5]	NA[6]	SE	D[7]	R[8]	D x R
No HCW[9] covariate									
HCW, kg	92.42	97.44	95.32	95.54	93.93	1.32	0.60	<0.01	0.70
Loin Depth, mm	63.95	67.46	65.79	65.85	65.48	0.72	0.93	<0.01	0.60
Backfat, mm	21.35	22.05	21.73	21.64	21.73	0.59	0.99	0.31	0.40
Yield, %	77.18	75.67	76.55	76.36	76.36	0.19	0.68	<0.01	0.46
Lean, %[10]	54.42	54.61	54.51	54.55	54.47	0.25	0.97	0.53	0.54
Fat-free lean, %[11]	48.69	48.79	48.74	48.79	48.69	0.30	0.97	0.76	0.50
HCW covariate									
Loin Depth, mm	64.43	66.99	65.72	65.74	65.68	0.69	0.99	0.01	0.66
Backfat, mm	22.02	21.41	21.64	21.49	22.00	0.49	0.75	0.33	0.57
Yield, %	77.33	75.52	76.52	76.33	76.42	0.17	0.69	0.01	0.63
Lean, %	54.20	54.82	54.54	54.60	54.38	0.23	0.78	0.04	0.71
Fat-free lean, %	48.41	49.06	48.77	48.85	48.58	0.27	0.77	0.07	0.68

[1]A total of 10 pens were used per dietary treatment per replicate
[2]Pigs weaned and transported for 12 h during July 2016
[3]Pigs weaned and transported for 12 h during April 2017
[4]Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)] for 14 d post-weaning and transport and then fed common antibiotic free diets
[5]Pigs provided 0.20% L-glutamine for 14 d post-weaning and transport and then fed common antibiotic free diets
[6]Pigs provided no dietary antibiotics for 14 d post-weaning and transport and then fed common antibiotic free diets
[7]Dietary treatment
[8]Replicate
[9]Hot carcass weight
[10]Equation used: 54.672154 - (0.412525 × backfat, mm) - (0.002982 × hot carcass weight, kg × 2.20462) + (0.1433242 × loin depth, mm) (Indiana Packers Corporation, 2015)
[11]Equation used: 51.2 - (0.510 × backfat, mm) + (0.131 × loin depth, mm) (Schinckel et al., 2010)

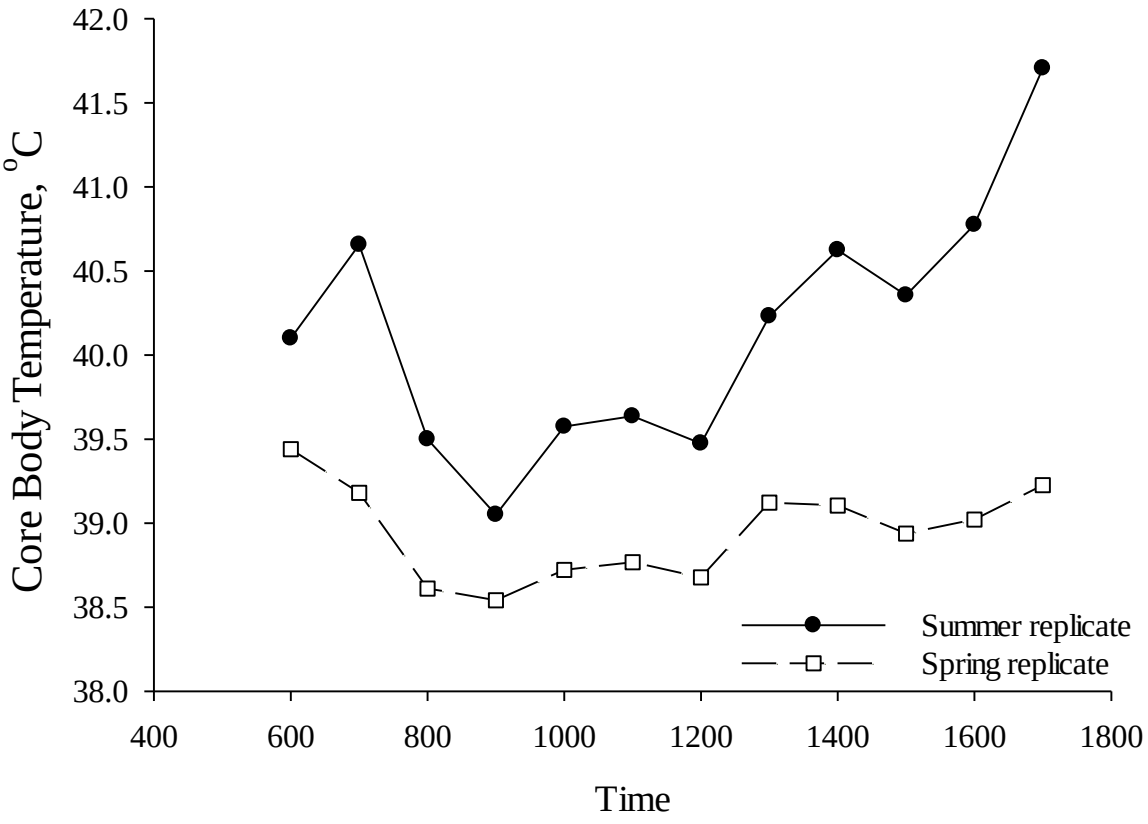

Figure 2.1 Descriptive data of core body temperature over time during weaning and transport in the summer of 2016 and the spring of 2017.

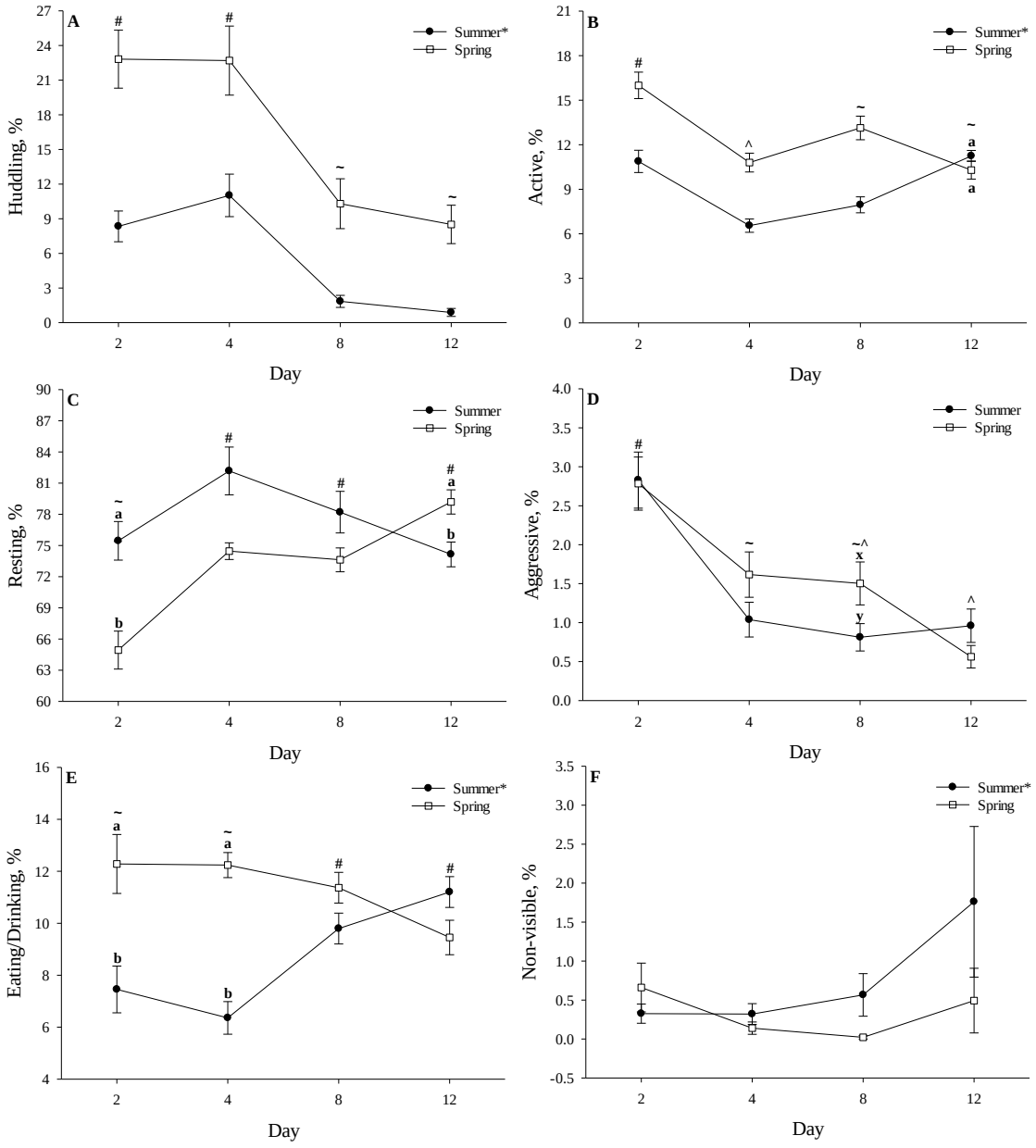

Figure 2.2 The effects of replicate (summer or spring) on (A) huddling, (B) active, (C) resting, (D) aggressive, (E) eating/drinking, and (F) non-visible behavior on d 2, 4, 8, and 12 post-weaning and transport. An asterisk (*) on the legend indicates overall replicate differences ($P < 0.05$), #,~,^ symbols indicate overall day differences ($P < 0.05$), ^{a,b}letters indicate replicate by day differences ($P \leq 0.05$), and ^{x,y}letters indicate replicate by day tendencies ($0.05 < P \leq 0.10$).

CHAPTER 3. REPLACING DIETARY ANTIBIOTICS WITH 0.20% L-GLUTAMINE IN SWINE NURSERY DIETS: IMPACT ON INTESTINAL PHYSIOLOGY FOLLOWING WEANING AND TRANSPORT

3.1 Abstract

Previous research by our lab demonstrates that supplementing 0.20% L-glutamine (**GLN**) in the diets of newly weaned and transported pigs improves growth rate to a similar extent as providing dietary antibiotics. However, the effects of replacing dietary antibiotics with GLN on intestinal physiology is currently unknown. Therefore, the study objective was to evaluate the impact of replacing dietary antibiotics with GLN on intestinal morphology and gene expression of pigs in a production environment following weaning and transport. Based on previous research, we hypothesized that removing dietary antibiotics would have a negative impact on intestinal physiology, whereas GLN supplementation would have similar effects on intestinal physiology as dietary antibiotics. Mixed sex pigs (N=480; 5.62 ± 0.06 kg BW) were weaned (18.4 ± 0.2d of age) and transported for 12 h in central Indiana, for two replicates, during the summer of 2016 and the spring of 2017. Pigs were blocked by BW and allotted to 1 of 3 dietary treatments [n=10 pens/dietary treatment/replicate (8 pigs/pen)]; antibiotics [**A**; chlortetracycline (441 ppm) + tiamulin (38.6 ppm)], no antibiotics (**NA**), or GLN fed for 14 d. From d 14 to 34, pigs were fed common antibiotic free diets in two phases. Data were analyzed using PROC MIXED in SAS 9.4. On d 33 (end of common diet period), mast cells/mm^2 were increased (P = 0.05) in GLN and NA pigs vs. A pigs (22.2% and 19.7%, respectively). On d 33, villus height:crypt depth tended to be increased (P = 0.07; 7.0%) in GLN and A pigs vs. NA pigs. On d 33, glucagon-like peptide 2 (**GLP-2**) mRNA abundance was decreased (P = 0.01; 50.3%) in GLN and NA pigs vs. A pigs and mast cells/mm^2 were increased (P = 0.05) in GLN and NA pigs vs. A pigs (22.2% and 19.7%,

respectively). Crypt depth was increased overall on d 33(P = 0.01; 16.2%) during the spring replicate compared to the summer replicate. Villus height:crypt depth was reduced (P = 0.01; 9.6%) during the spring replicate compared to the summer replicate on d 33. On d 13 (end of diet treatment period), TNF-α and occludin mRNA abundance was increased ($P \leq 0.04$; 45.9% and 106.5%, respectively) and ZO-1 mRNA abundance tended to be increased (P = 0.10; 19.2%) in the spring replicate compared to the summer replicate. In conclusion, GLN supplementation tended to improve villus height:crypt depth similarly to A pigs. In addition, A pigs had increased GLP-2 gene expression and reduced mast cell/mm^2 compared to GLN and NA pigs.

Keywords: antibiotics, L-glutamine, nursery, pigs, transport, weaning

3.2 Introduction

A young pig is exposed to many stressors during the weaning period with weaning, itself, being a large stressor (as reviewed by Campbell et al., 2013). Additional stressors that coincide with weaning can include: transport, mixing, thermal stress, water and food withdrawal, and pathogenic insults (Lewis and Berry, 2006). The consequences of these stressors can include lost animal performance, altered intestinal morphology (Lallés et al., 2004), higher incidence of disease and morbidity, and in severe cases increased mortality (as reviewed by Campbell et al., 2013). To mitigate the negative impacts of these stressors on pig health and well-being, swine producers have traditionally implemented the use of dietary antibiotics once pigs reached wean-to-finish production sites (Cromwell, 2005). Unfortunately, due to changing consumer preference, antibiotic resistance concerns (Smith et al., 2010) and legislative ban on antibiotics used for growth promotion producers use of dietary antibiotics has declined in recent years leading to an increased requirement for alternatives that are as effective as dietary antibiotics in promoting growth and reducing stress.

Recent work has described either improved (Johnson and Lay, 2017) or maintained (Duttlinger et al., 2019) growth performance in pigs provided diets supplemented with 0.20% L-glutamine (**GLN;** Ajinomoto North America, Inc., Raleigh, NC) compared to traditional dietary antibiotics [chlortetracycline (Aureomycin, Zoetis, Parsippany, NJ) + tiamulin (Denagard, Elanco Animal Health, Greenfield, IN)]. In addition, physiological and health advantages resulting from GLN supplementation have been described such as improved morphological indicators of intestinal health (Johnson and Lay, 2017) and a reduction in pro-inflammatory cytokines (Duttlinger et al., 2019). Although, the mechanism(s) involved in the aforementioned GLN advantages relative to dietary antibiotics are still unclear, they may be related to improvements in intestinal epithelial barrier function because L-glutamine improves tight junction protein abundance in pigs on d 7 postweaning (Wang et al., 2015b). Therefore, the study objective was to evaluate the impact of replacing dietary antibiotics with GLN on the intestinal morphology and tight junction protein gene expression of pigs in a production environment following weaning and transport. Based on previous research (Johnson and Lay, 2017; Duttlinger et al., 2019), we hypothesized that removing dietary antibiotics would negatively impact intestinal physiology while supplementing diets with 0.20% GLN diet would have similar effects on intestinal morphology and tight junction protein gene expression as dietary antibiotics in a production environment.

3.3 Materials and Methods

3.3.1 General

All procedures involving animal use were approved by the Purdue University Animal Care and Use Committee (protocol #1603001385), and animal care and use standards were based upon the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of

Animal Science Societies, 2010). All live animal methods have been previously described in great detail by Duttlinger et al. (2019). Briefly, crossbred pigs consisting of barrows and gilts [N = 480; 5.62 ± 0.06 kg initial BW; Duroc x (Landrace x Yorkshire)] were weaned and transported approximately 12 h at 18.4 ± 0.2 d of age in central Indiana. The trial was conducted in two replicates during July 2016 (summer replicate) and April 2017 (spring replicate). The terms summer replicate and spring replicate refer only to the time of year in which the pigs were weaned and transported. On the day prior to weaning and transport, pigs were weighed individually, blocked by BW, and randomly allotted to pens, and pens of pigs within BW blocks were allotted to 1 of 3 dietary treatments and 10 pens per dietary treatment per replicate (n = 20 pens/dietary treatment). Initially, each pen contained 8 pigs. Dietary treatments were antibiotics [**A**; chlortetracycline (441 ppm) + tiamulin (38.6 ppm)], no antibiotics (**NA**), or GLN from d 0 to 14 post-weaning and transport followed by NA diets fed to all pigs from d 14 to 34 post-weaning and transport.

3.3.2 Transportation

The transportation procedure was previously described by Duttlinger et al. (2019). In brief, selected pigs, including sentinel pigs, were removed from sows and loaded into a gooseneck livestock trailer (2.35 × 7.32 m; Wilson Trailer Company, Sioux City, IA) providing 0.07 m^2 per pig. During transport, the trailer ambient temperature (**T_A**) and relative humidity (**RH**) during the summer replicate was 29.4 ± 0.2°C and 64.3 ± 0.8%, respectively, and during the spring replicate was 11.0 ± 0.2°C and 63.1 ± 0.9%, respectively. Trailers were bedded with wood shavings and ventilation openings were adjusted based on the T_A (National Pork Board, 2015). Pigs were transported, without feed or water, as a group in the trailer for approximately 12 h and 819 km. At

the conclusion of the 12 h transport, pigs were unloaded from the trailer, individually weighed, and placed into pens.

3.3.3 Nursery Phase

Following transport, pigs were placed in their assigned pens and provided their respective dietary treatments for 14 d in 2 phases (d 0 to 14 post-weaning and transport; Table 3.1). Following the dietary treatment period, all pigs were fed common antibiotic free diets from d 14 to the end of the nursery phase (d 34; Table 3.1). Diets, fed in 4 phases, were corn-soybean meal-based in meal form and were formulated to meet or exceed nutrient requirements (NRC, 2012) during the nursery period (Table 3.1). The nursery facility where the 34 d of the trial was conducted is described by Duttlinger et al. (2019).

3.3.4 Intestinal Tissue Collection

Intestinal tissue samples and intestinal content samples were obtained on d 13 (end of diet treatment period) and d 33 (end of common diet period) of the nursery phase from one randomly selected pig per pen. Sex of the selected pig was balanced across treatments and BW blocks within day and balanced within pen across collection days. Pigs were euthanized by CO_2 gas asphyxiation followed by exsanguination (AVMA, 2013).

A 15 cm section of jejunum was collected 1.2 m and 1.8 m from the pyloric sphincter from pigs on day 13 and on day 33, respectively. From this 15 cm section, a 10 cm section of jejunal tissue was removed, rinsed with phosphate buffered saline to remove digesta, cut into small pieces, and placed into a Whirl-Pak® (Nasco, Fort Atkinson, WI) bag, snap frozen in liquid nitrogen, and stored at -80 °C until further processing for gene expression analyses. In addition, a 5 mm thick cross-section of the jejunum was obtained from the remaining 5 cm section of jejunal tissue, rinsed with phosphate buffered saline and placed into a cryomold (25 mm × 20 mm × 5 mm, Tissue-

Tek® Cryomold®, Sakura Fintek USA, Inc. Torrance, CA) and covered with OCT compound (Tissue-Plus® O.C.T. Compound, Fisher HealthCare, Houston, TX). The cryomold containing the intestinal tissue cross-section was frozen in liquid nitrogen cooled 2-methylbutane (Sigma-Aldrich, St. Louis, MO) and stored at -80 °C until further processing for histological analyses (Hermes et al., 2013).

3.3.5 Histology

Jejunum sections were processed by the Purdue Histology Research Laboratory using an 0.1% Aqueous Toluidine Blue stain and 0.1% Fast Green FCF Counterstain to allow mast cells and gross morphology to be visualized. Cross sections of preserved tissues were cut at 5 µm. Sections were hydrated in distilled water and placed on slides. Slides were stained in 0.1% Aqueous Toluidine Blue stain for 10 min then rinsed well with distilled water. Slides were then stained in 0.1% Fast Green FCF counterstain for 1 min. Slides were then quickly dehydrated in methanol. Tissues were cleared and mounted with synthetic resin.

Histological slides contained two cross-sections of intestinal tissue per pig. Three fields of view of each cross-section were imaged (MoticamBTW, Motic, Hong Kong, China) at 10X magnification for later analysis for a total of 6 fields of view per pig. ImageJ software (NIH, Bethesda, MD) was used to measure all villi height and crypt depth from the obtained images. Six fields of view per pig were also used for mast cell quantification in the submucosa. Mast cells were counted in the submucosa at 40X in 2 cross-sections and 3 fields of view per cross-section with results presented as mast cells per mm^2. The field of view contained gridlines forming 200 µm × 200 µm and the number of mast cells were counted in a grid measuring 3 squares × 3 squares. The criteria were set that at least 1 mast cell needed to be contained in the upper left square of the grid to ensure that the field of view being counted was positioned in the correct region of the submucosa

that contained mast cells. All histology measures were made by trained individuals who were blinded to dietary treatments. All villus and crypt depth measurements were conducted by one trained individual. For mast cell quantification, the trained observer who quantified the sample was included as a random effect in the statistical analysis to account for any observer differences.

3.3.6 Gene expression

Total RNA was isolated from jejunal tissue utilizing a commercially available kit (RNeasy® Mini Kit, Qiagen, Germantown, MD). Total RNA concentration was quantified using a spectrophotometer (Nanodrop Technologies, Wilmington, DE; model ND-1000) at 260 nm. The RNA purity was determined by examining the absorbance ratios at 260 and 280 nm, respectively. All RNA samples had 260/280 ratio above 2.0. The isolated RNA samples (1 μg of RNA) were reversed transcribed into complementary DNA (**cDNA**) with a commercially available kit (High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor, Applied Biosystems by Thermo Fisher Scientific, Waltham, MA).

Real-time quantitative polymerase chain reaction (**qPCR**) amplification was conducted with 5 μL of Fast SYBR^{TM} Green Master Mix (Applied Biosystems by Thermo Fisher Scientific, Waltham, MA), 0.25 μL of each forward and reverse primers (Table 3.2; manufactured by Integrated DNA Technologies, Inc., Coralville, IA), 1.5 μL of nuclease free water, and 3 μL of cDNA template. Glyceraldehyde-3-phosphate dehydrogenase (**GAPDH**) was utilized as the housekeeping gene. The mRNA abundance values were normalized, for each sample and target gene, to GAPDH according to the $2^{-\Delta\Delta CT}$ method (Thermo Fisher Scientific, 2016).

3.3.7 Statistics

Statistical analyses were performed as previously described (Duttlinger et al., 2019). Briefly, data were analyzed as a randomized complete block design using the PROC MIXED procedure in SAS 9.4 (SAS Institute INC., Cary, NC). The assumptions of normality of error, homogeneity of variance, and linearity were confirmed post-hoc. Pen was the experimental unit and fixed effects included diet, replicate, and all interactions with BW block included as a random effect. Plate and observer were included as a random effect for analyses of gene expression and mast cells, respectively. Statistical significance was defined as $P \leq 0.05$ and a tendency was defined as $0.05 < P \leq 0.10$.

3.4 Results

3.4.1 Jejunum Histology

On d 33, mast cells/mm^2 were increased ($P = 0.05$) in GLN and NA pigs vs. A pigs (22.2% and 19.7%, respectively; Table 3.3, Figure 3.1). On d 33, villus height:crypt depth tended to be increased ($P = 0.07$) in GLN and A pigs vs. NA pigs (7.0% and 7.0%, respectively; Table 3.3, Figure 3.2). No other jejunal histology differences due to diet were detected ($P > 0.21$) with any comparison (Table 3.3).

Crypt depth was increased ($P = 0.01$; 16.2%) during the spring replicate compared to the summer replicate on d 33 (Table 3.3). Villus height:crypt depth was reduced ($P = 0.01$; 9.6%) during the spring replicate compared to the summer replicate on d 33 (Table 3.3, Figure 3.2). No other jejunal histology differences due to replicate were detected ($P > 0.23$) with any comparison (Table 3.3).

3.4.2 Gene Expression

On d 13, a tendency for a diet by replicate interaction ($P = 0.06$) was observed for GLP-2 gene expression where the GLP-2 mRNA abundance in GLN pigs was increased ($P = 0.01$) during the spring replicate vs. summer replicate. However, the no GLP-2 mRNA abundance differences were detected ($P > 0.32$) for A or NA pigs during the spring replicate vs. the summer replicate (Table 3.4).

On d 33, GLP-2 mRNA abundance was decreased ($P = 0.01$) in GLN and NA pigs vs. A pigs (61.0% and 39.6%, respectively; Table 3.4). No other mRNA abundance differences due to diet were observed ($P > 0.12$) with any comparison (Table 3.4).

On d 13, TNF-α and occludin mRNA abundance was increased ($P < 0.05$; 45.9% and 106.5%, respectively) in the spring replicate compared to the summer replicate, regardless of dietary treatment (Table 3.4). On d 13, ZO-1 mRNA abundance tended to be increased overall ($P = 0.10$; 19.2%) in the spring replicate compared to the summer replicate (Table 3.4). No other mRNA abundance differences due to replicate were observed ($P > 0.12$) with any comparison (Table 3.4).

3.5 Discussion

Weaning and transport is necessary in U.S. swine production despite the well-described negative impact on the health, performance, and welfare of young pigs (Rioja-Lang et al., 2019). Dietary antibiotics have routinely been provided to reduce enteric challenges, decrease morbidity and mortality, and improve overall health in order to help pigs overcome the effects of weaning and transport stress (Cromwell, 2005). However, despite the advantages to pig health and welfare, U.S. swine producers are working to limit or reduce dietary antibiotics due to consumer pressure and recent legislation limiting the feeding of antibiotics for growth promotion. Unfortunately, this

can have the unintended effect of reducing pig health and research demonstrates that eliminating prophylactic dietary antibiotics increases the rate of therapeutic treatments for disease (DANMAP, 2001) and increases post-weaning mortality in pigs (Heo et al., 2013). Therefore, it is imperative that effective alternatives are developed to improve pig welfare outcomes.

Previous work has reported that pigs provided GLN have growth performance that surpassed (Johnson and Lay, 2017) or equaled that of dietary antibiotics (Duttlinger et al., 2019). While, the mechanism(s) involved that allow for this GLN advantage are currently unknown, it is hypothesized that improvements in intestinal health are partially responsible as Johnson and Lay (2017) observed significant improvements in morphological markers of intestinal health for GLN compared to A or NA pigs. In agreement with the aforementioned report (Johnson and Lay, 2017) GLN and A pigs had an increase in villus height:crypt depth when compared to NA pigs and this improvement was likely driven by a numerical reduction in crypt depth for NA compared to GLN and A pigs. Interestingly, the improved villus height:crypt depth for GLN pigs in the present study was not observed until d 33 post-weaning when all pigs were on common diets for approximately 2.5 weeks as opposed to a previous report in which morphological differences were detected while pigs were still on dietary treatments (d 14 post-weaning; Johnson and Lay, 2017). Few studies, if any, that we are aware of have investigated intestinal morphological differences after the experimental GLN diets have stopped being fed. However, it is possible that the the delayed morphological response in the present study may be due to refractory response to reduced cell turnover that likely occurred when the GLN dietary treatments were being fed. This is because GLN reduces apoptosis in the small intestine due to decreased activation of the unfolded protein response (He et al., 2019). As a result, this would cause increased cell lifespan. Therefore, it is

possible that GLN supplementation from d 0 to 14 postweaning reduced enterocyte turnover, which may have increased the villus height:crypt depth.

Although villus height:crypt depth was improved to a greater extent for GLN compared to A and NA pigs when measured at d 14 postweaning and transport in the previous study (Johnson and Lay, 2017), no differences were detected between GLN and A pigs in the present study. While reasons for these discrepancies are currently unclear, they may be due to trial design differences in which the Johnson and Lay (2017) study used simulated transport and individual housing compared to physical transport and group housing under production conditions in the present study. Furthermore, a previous study reported that morphological markers of intestinal health were improved on d 7 postweaning for pigs provided GLN-supplemented diets, but no differences were detected on d 14 postweaning (Wu et al., 1996). These data may suggest that morphological improvements in intestinal health may have been detected for GLN compared to A pigs in the present study if measures were taken earlier; however, this hypothesis would need to be tested in future studies.

In addition to morphological improvements, A pigs had greater GLP-2 gene expression compared to GLN and NA pigs. Antibiotics shift microbiota populations of the gastrointestinal tract (Rettedal et al., 2009; Gresse et al., 2017), and changes in microbial populations can increase the production of short chain fatty acids (SCFA; Sakata et al., 1999). Furthermore, previous work determined that SCFA increases GLP-2 plasma concentrations (Tappenden and Mcburney, 1998). Because GLP-2 is linked to intestinal growth via increasing enterocyte proliferation (as reviewed by Burrin et al., 2003; Petersen et al., 2001), it could be hypothesized that the A treatment shifted the microbiome to populations of bacteria that produce increased levels of SCFA. As a result of this shift, GLP-2 levels may have increased epithelial cell proliferation to support improved

intestinal growth, health, and barrier function and this may have contributed to the improved morphological biomarkers observed in A pigs in the present study. Surprisingly, despite the fact that morphological improvements were observed for GLN pigs, no differences in GLP-2 were detected in the present study. However, this may be due to GLN benefiting enterocytes directly through absorption (e.g. energy source, antioxidant, reducing apoptosis, etc.) as previously reported (Souba et al., 1985; He et al., 2019) rather than altering microbiota population thus not stimulating increased GLP-2 gene expression.

In addition to greater GLP-2 gene expression, A pigs had decreased mast cell counts compared to GLN and NA pigs. Greater intestinal integrity of A pigs may be the cause of reduced mast cells/mm^2. Mast cells are important for the localized immune response and are increased in number and activated when bacterial ligands are detected due to bacteria translocation resulting in a rapid release of proinflammatory cytokines (McLamb et al., 2013). Reasons why mast cells were reduced for A pigs but not reduced for GLN pigs despite similar improvements in intestinal morphology may be due to increased GLP-2 gene expression potentially improving intestinal barrier function as previous studies have determined that GLP-2 improves intestinal barrier function (Yu et al., 2014) and chlortetracycline reduces intestinal permeability (Song et al., 2013) resulting in a reduced immune response as indicated by decreased mast cells/mm^2. Despite a lack of intestinal markers indicating improved intestinal health via gene expression at the timepoints measured in the present study, a previous study reported that GLN and A pigs have similar growth performance during the nursery period (Duttlinger et al., 2019).

Despite mast cell differences in the present study, no dietary treatment differences were detected for tight junction protein (e.g. claudin 1, occludin, and ZO-1) gene expression. The role of tight junction proteins is to form multiprotein complexes that improve epithelial barrier function

and regulate paracellular permeability. However, when the protein complex is altered or degraded then increased paracellular permeability and bacterial translocation can occur (Shen et al., 2011). Previous work determined that jejunal tight junction protein abundance is increased in weaned pigs provided 1.0% GLN compared to control pigs (Wang et al., 2015). The differences detected by Wang et al. (2015) are interesting given that GLN appears to positively impact tight junction protein abundance, but there are many differences between the study conducted by Wang et al. (2015) and the present study. The concentration of supplemented GLN differed with 1.0% GLN Wang et al. (2015) and 0.20% GLN in the present study and may have been a contributing factor as to why no differences were detected in tight junction protein gene expression in the present study. In addition, the assay for tight junction protein and timing differed as the previous study by Wang et al., (2015) utilized Western blot analysis to measure protein abundance and was conducted at d 7 post-weaning compared to gene expression measured via PCR from tissue collected on d 13 and d 33 postweaning in the present study. These differences in GLN concentration, assay type, and timing of tissue collection may be contributing to the lack of differences in the present study and it is possible that differences in tight junction protein expression would have been detected if intestinal tissue had been collected earlier post-weaning and transport.

In addition to dietary treatment effects on intestinal histology, the spring replicate had increased crypt depth and decreased villus height:crypt depth on d 33 postweaning compared to the summer replicate. No previous studies, to our knowledge, have investigated the effect of seasonal replicate on intestinal morphological measures; however, increased crypt depth is indicative of greater crypt hyperplasia and may be associated with increased crypt-cell production to replace enterocytes damaged from intestinal insults (as reviewed by Pluske et al., 1997). As

previously reported, the spring replicate had a decrease in ADFI from d 0 to 14 postweaning (Duttlinger et al., 2019). Because decreased feed intake negatively impacts villus height:crypt depth (as reviewed by Pluske et al., 1997). Therefore, it is possible that the increased crypt depth and reduced villus height:crypt depth observed for the spring replicate was a refractory response to the decreased feed intake from d 0 to 14 postweaning.

In addition to the impact on intestinal morphology, an increase in gene expression for tight junction proteins (e.g., occludin, ZO-1) and pro-inflammatory cytokines (e.g., TNF-α) was observed in the spring compared to the summer replicate on d 13 postweaning. No previous studies that we are aware of have investigated the effect of seasonal replicate on intestinal gene expression. However, the increased gene expression of tight junction proteins may have been due to reduced health status of the spring replicate as indicated by increased therapeutic antibiotic treatment rate and decreased feed intake postweaning (Duttlinger et al., 2019) since decreased feed intake causes increased intestinal permeability (Spreeuwenberg et al., 2001, Pearce et al., 2013). While it seems counterintuitive that an increase in tight junction protein gene expression could indicate greater intestinal permeability, previous studies have observed increased tight junction protein abundance when measured 14 d postweaning compared to 7 d postweaning (Xiao et al., 2014) and we hypothesized that this is a response by the intestine to improve barrier function following weaning stress. Therefore, the increase in tight junction protein gene expression for the spring replicate on d 13 postweaning may have been in response to greater stress caused by reduced feed intake and health status for the spring replicate (Duttlinger et al., 2019). Furthermore, this increase may have been partially mediated by greater production of pro-inflammatory cytokines as indicated by increased TNF-α gene expression for spring replicate pigs because increased intestinal permeability is associated with increased intestinal inflammation (Pearce et al., 2014).

These data provide value to the swine industry in that A and GLN can improve intestinal morphology. Furthermore, these results provide continued evidence that withholding dietary antibiotics decreases intestinal health and may result in poor health outcomes for pigs and negative financial impacts on the swine industry. Possibilities as to why no differences in tight junction proteins were observed in the present study may be due to the use of PCR to quantify changes in gene expression as Wang et al. (2015) utilized Western blot assays and were conducted at 7 days post-weaning rather than 13 and 33 days post-weaning as was conducted in the present study. Future work is needed to conduct research through serial tissue collection to determine when additional morphological changes due to L-glutamine supplementation can be elucidated and if any changes in tight junction protein gene expression is associated with these time points.

3.6 Conclusion

Weaning and transport negatively affect pigs and dietary antibiotics have routinely been given to help overcome these stressors. However, U.S. swine producers have interest in reducing antibiotic usage and replacing antibiotics with other beneficial technologies. It was determined that diets supplemented with GLN improved intestinal health similarly to A as shown by the improvements in intestinal morphology compared to pigs not provided dietary antibiotics. In addition, A increased GLP-2 gene expression and reduced mast cell counts, which indicate potential improvement in intestinal growth and reduced immune system activation. Future research should address if any additional morphological improvements or measures of gut barrier function can be elucidated at timepoints closer to onset of weaning and transport in pigs provided supplemental L-glutamine to help further explain previously observed improvements in growth performance.

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Table 3.1 Composition of nursery diets (as fed)

	Phase 1[1]			Phase 2[2]			Phase 3[3]	Phase 4[4]
Item	A[5]	GLN[6]	NA[7]	A	GLN	NA		
Ingredient, %								
Corn	30.81	31.18	31.38	37.52	37.89	38.09	51.63	57.38
SBM, 48% CP	13.95	13.95	13.95	18.00	18.00	18.00	25.65	30.70
DDGS, 7% fat	---	---	---	---	---	---	---	5.00
Soybean oil	5.00	5.00	5.00	5.00	5.00	5.00	3.00	---
Choice white grease	---	---	---	---	---	---	---	3.00
Limestone	0.79	0.79	0.79	0.74	0.74	0.74	0.86	1.33
Monocal. phos.	0.40	0.40	0.40	0.49	0.49	0.49	0.49	0.74
Vitamin premix[8]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[9]	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Selenium premix[10]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Phytase[11]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.30	0.35
Plasma protein	6.50	6.50	6.50	2.50	2.50	2.50	---	---
Blood meal	1.50	1.50	1.50	1.50	1.50	1.50	---	---
Soy concentrate	4.00	4.00	4.00	3.00	3.00	3.00	2.50	---
Fish meal	5.00	5.00	5.00	4.00	4.00	4.00	4.00	---
Dried whey	25.00	25.00	25.00	25.00	25.00	25.00	10.00	---
Lactose	5.00	5.00	5.00	---	---	---	---	---
Lysine-HCL	0.07	0.07	0.07	0.20	0.20	0.20	0.28	0.40
DL-Methionine	0.22	0.22	0.22	0.23	0.23	0.23	0.18	0.17
L-Threonine	0.04	0.04	0.04	0.09	0.09	0.09	0.12	0.14
L-Tryptophan	---	---	---	0.01	0.01	0.01	0.01	0.00
Zinc oxide	0.38	0.38	0.38	0.38	0.38	0.38	0.38	---
Copper sulphate	---	---	---	---	---	---	---	0.10
Aureomycin 50[12]	0.40	---	---	0.40	---	---	---	---
Denagard 10[13]	0.18	---	---	0.18	---	---	---	---
L-Glutamine[14]	---	0.20	---	---	0.20	---	---	---
Banminth 48[15]	---	---	---	---	---	---	---	0.10
Clarifly, 0.67%[16]	---	---	---	---	---	---	0.08	0.07
Calculated chemical composition								
ME, kcal/kg	3536	3536	3536	3510	3510	3510	3418	3396
Fat, %	7.27	7.27	7.27	7.36	7.36	7.36	5.73	5.86
CP, %	24.62	24.62	24.62	22.87	22.87	22.87	22.29	21.28
Total Lys, %	1.74	1.74	1.74	1.61	1.61	1.61	1.50	1.41
SID Lys, %	1.55	1.55	1.55	1.45	1.45	1.45	1.35	1.25
Ca, %	0.90	0.90	0.90	0.85	0.85	0.85	0.80	0.75
Total P, %	0.75	0.75	0.75	0.71	0.71	0.71	0.64	0.57
Avail. P, %	0.60	0.60	0.60	0.55	0.55	0.55	0.45	0.36

Table 3.1 continued

Analyzed chemical composition								
Summer replicate								
GE, kcal/kg	4217	4251	4173	4172	4146	4184	---	---
CP, %	24.42	25.62	23.85	22.30	22.38	22.46	22.07	22.00
Total Lys, %	1.30	1.35	1.26	1.13	1.18	1.11	---	---
Total Glu, %[17]	3.15	3.43	3.11	2.78	2.88	2.68	---	---
Chlortetracycline, ppm[18]	467	0	0	468	0	0	---	---
Spring replicate								
GE, kcal/kg	4266	4199	4079	4174	4193	4129	---	---
CP, %	25.36	26.37	22.51	22.78	23.02	24.68	22.37	21.14
Total Lys, %	1.58	1.75	1.40	1.54	1.51	1.51	---	---
Total Glu, %	3.70	4.23	3.17	3.68	3.81	3.62	---	---
Chlortetracycline, ppm	436	0	0	436	0	0	---	---

[1]Fed d 0 to 7 post-weaning and transport.
[2]Fed d 7 to 14 post-weaning and transport.
[3]Fed d 14 to 21 post-weaning and transport.
[4]Fed d 21 to 34 post-weaning and transport.
[5]Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)].
[6]Pigs provided 0.20% L-glutamine.
[7]Pigs provided no dietary antibiotics.
[8]Provided per kg of the diet: vitamin A, 6,615 IU; vitamin D3, 662 IU; vitamin E, 44 IU; vitamin K, 2.2 mg; riboflavin, 8.8 mg; pantothenic acid, 22 mg; niacin, 33 mg; vitamin B_{12}, 38.6 mg.
[9]Provided available minerals per kg of the diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15 mg; copper, 11.3 mg; iodine, 0.46 mg.
[10]Provided 0.3 ppm Se.
[11]Provided 600 FTU per kg of the diet.
[12]Aureomycin (Zoetis, Parsippany, NJ) provided 441 ppm chlortetracycline in the diet.
[13]Denagard (Elanco Animal Health, Greenfield, IN) provided 38.6 ppm tiamulin in the diet.
[14]Ajinomoto North America, Inc., Raleigh, NC.
[15]Banminth (Phibro Animal Health Corporation, Teaneck, NJ) provided 106 ppm pyrantel tartrate in the diet.
[16]Clarifly (Central Life Sciences, Schaumburg, IL) provided 5.4 ppm (Phase 3) and 4.7 ppm (Phase 4) diflubenzuron in the diet.
[17]Samples submitted to Ajinomoto for glutamic acid analysis.
[18]Samples submitted to Zoetis, Parsippany, NJ for chlortetracycline analysis.

Table 3.2 Primers used for quantitative polymerase chain reaction

Gene	Sense (5' to 3')	Antisense (5' to 3')
TNF-α[1]	CCC CCA GAA GGA AGA GTT TC	TTG GCC CCT GAA GAG GAC
Claudin-1[2]	AGA AGA TGC GGA TGG CTG TC	CCC AGA AGG CAG AGA GAA GC
Occludin[3]	ATC AAC AAA GGC AAC TCT	GCA GCA GCC ATG TAC TCT
ZO-1[4]	AAT TAT CCC ACA GGG AGC TAT TC	AGG GTT TCA CCT TTC TCC TTA TC
GLP-2[5]	ACC TTG CAG CTG ATG TAC AC	GTG TTC TCC AGG TGT GCA CG
GAPDH[6]	GAA GGT CGG AGT GAA CGG AT	CAT GGG TAG AAT CAT ACT GGA ACA

[1]Tumor necrosis factor alpha; sequence order (Ballweg et al., 2016).
[2]Sequence order (Hu et al., 2013).
[3]Sequence order (Zhang and Guo, 2009).
[4]Zonula occludens 1; sequence order (Pearce et al., 2013).
[5]Glucagon like peptide 2; sequence order (Petersen et al., 2001).
[6]Glyceraldehyde-3-Phosphate Dehydrogenase; sequence order (Yu et al., 2007).

Table 3.3 Influence of season, L-glutamine, and antibiotics on jejunum histology[1]

	Replicate		**Diet**				***P***		
Parameter	Summer[2]	Spring[3]	A[4]	GLN[5]	NA[6]	SE	D[7]	R[8]	D×R
Day 13									
Villus height, µm	239.0	236.2	238.2	248.9	225.7	12.9	0.34	0.83	0.98
Crypt depth, µm	95.9	97.8	93.8	102.2	94.4	5.9	0.32	0.73	0.97
Villus height:crypt depth	2.59	2.45	2.58	2.49	2.48	0.13	0.75	0.25	0.94
Mast cells/mm^2	353.3	319.0	372.4	325.1	311.0	25.4	0.21	0.23	0.25
Day 33									
Villus height, µm	286.3	299.2	296.9	290.6	290.9	11.9	0.87	0.24	0.85
Crypt depth, µm	102.7	119.3	110.6	107.4	115.0	3.4	0.25	0.01	0.64
Villus height:crypt depth	2.82	2.55	2.75^{x}	2.75^{x}	2.57^{y}	0.09	0.07	0.01	0.99
Mast cells/mm^2	307.1	382.1	302.3^{b}	362.0^{a}	369.6^{a}	42.7	0.05	0.11	0.29

[1]A total of 10 pens were used per dietary treatment per replicate with 1 pig per pen closest to the pen mean BW was selected for tissue collection and histology analysis.
[2]Pigs weaned and transported for 12 h during July 2016.
[3]Pigs weaned and transported for 12 h during April 2017.
[4]Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)] for 14 d post-weaning and transport and then fed common antibiotic free diets.
[5]Pigs provided 0.20% L-glutamine for 14 d post-weaning and transport and then fed common antibiotic free diets.
[6]Pigs provided no dietary antibiotics for 14 d post-weaning and transport and then fed common antibiotic free diets.
[7]Dietary treatment.
[8]Replicate.
[a,b]Letters indicate significant differences ($P \leq 0.05$) within a row and dietary treatment.
[x,y]Letters indicate tendencies ($0.05 < P \leq 0.10$) within a row and dietary treatment.

Table 3.4 Effect of dietary treatment on jejunum mRNA abundance[1]

	Replicate		Diet				*P*		
Parameter	Summer[2]	Spring[3]	A[4]	GLN[5]	NA[6]	SE	D[7]	R[8]	D×R
Day 13									
TNF-α[9]	1.09	1.59	1.35	1.49	1.18	0.315	0.55	0.04	0.48
Claudin 1	1.02	0.74	0.75	0.84	1.05	0.232	0.58	0.22	0.59
Occludin	1.86	3.84	2.38	2.55	3.62	1.470	0.29	0.01	0.76
ZO-1[10]	1.04	1.24	1.06	1.26	1.09	0.198	0.31	0.10	0.56
GLP-2[11]	1.44	2.38	1.96	2.03	1.74	0.717	0.91	0.12	0.06
Day 33									
TNF-α	1.42	1.69	1.75	1.36	1.56	0.355	0.57	0.38	0.83
Claudin 1	1.55	1.97	1.51	2.63	1.14	0.532	0.12	0.48	0.90
Occludin	2.17	2.07	2.06	2.21	2.09	0.646	0.97	0.86	0.36
ZO-1	1.07	1.20	1.28	0.97	1.17	0.197	0.25	0.40	0.28
GLP-2	2.05	2.10	3.13[a]	1.22[b]	1.89[b]	0.565	0.01	0.92	0.73

[1]A total of 10 pens were used per dietary treatment per replicate with 1 pig per pen closest to the pen mean BW was selected for gene expression analysis.
[2]Pigs weaned and transported for 12 h during July 2016.
[3]Pigs weaned and transported for 12 h during April 2017.
[4]Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)] for 14 d post-weaning and transport and then fed common antibiotic free diets.
[5]Pigs provided 0.20% L-glutamine for 14 d post-weaning and transport and then fed common antibiotic free diets.
[6]Pigs provided no dietary antibiotics for 14 d post-weaning and transport and then fed common antibiotic free diets.
[7]Dietary treatment.
[8]Replicate.
[9]Tumor necrosis factor alpha.
[10]Zonula occludens 1.
[11]Glucagon like peptide 2.
[a,b]Letters indicate significant differences ($P \leq 0.05$) within a row and dietary treatments.

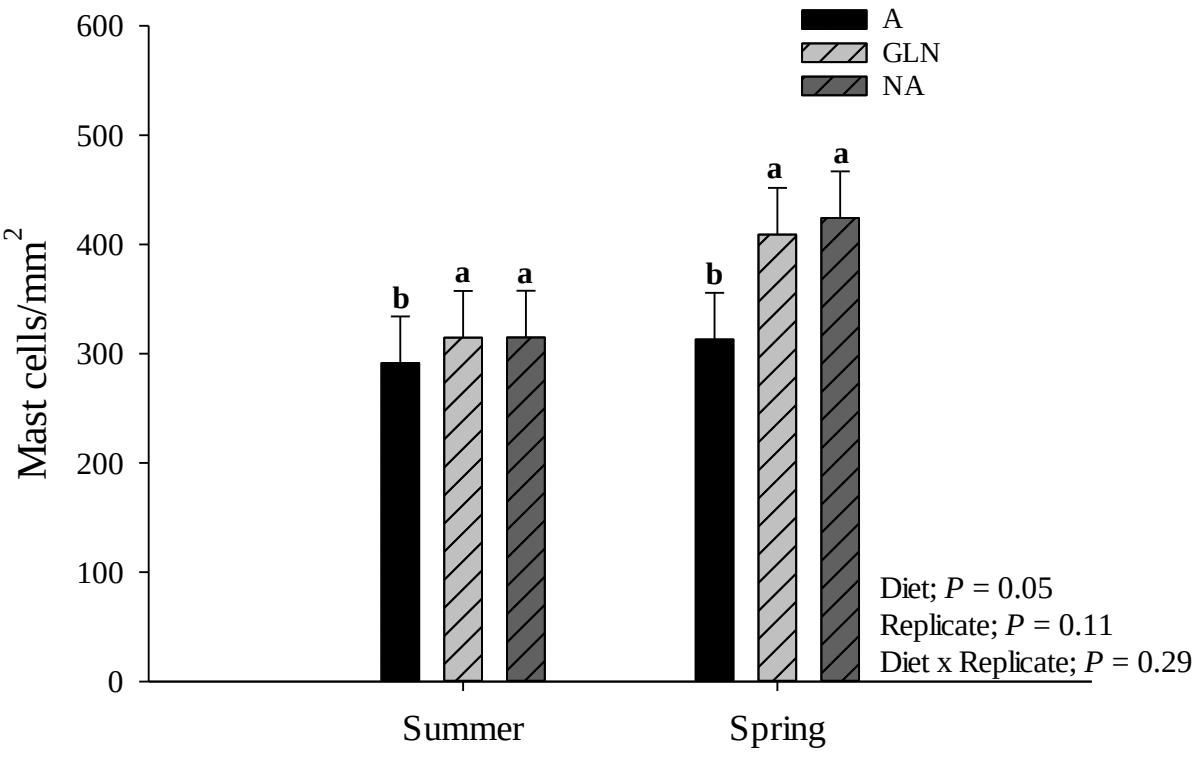

Figure 3.1 The effects of dietary treatment and replicate on mast cell/mm^2 on d 33 postweaning. Error bars indicate ± 1 SE. [a,b]Letters indicate differences between dietary treatments within replicate ($P \leq 0.05$).

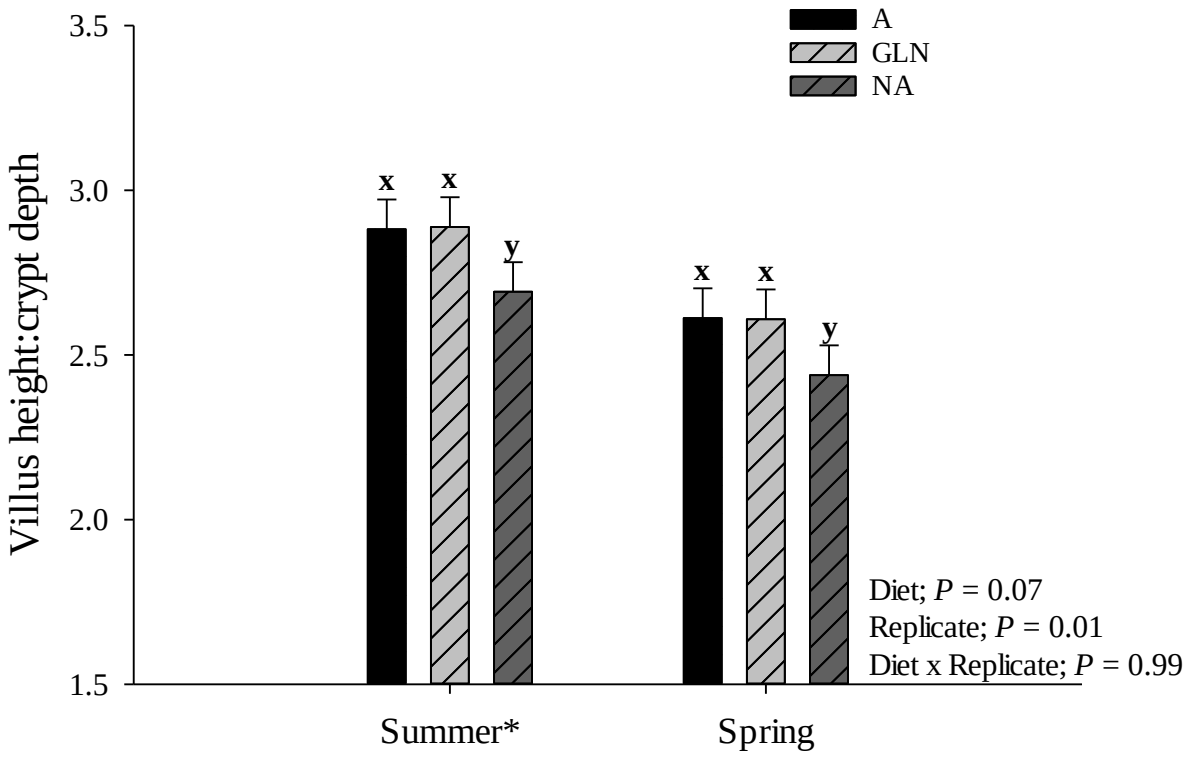

Figure 3.2 The effects of dietary treatment and replicate on villus height:crypt depth on d 33 postweaning. Error bars indicate ± 1 SE. An asterisk (*) on the x-axis indicates overall replicate differences ($P < 0.05$). ^{x,y}Letters indicate tendencies between dietary treatments within replicate ($0.05 < P \leq 0.10$).

CHAPTER 4. DETERMINING THE OPTIMAL INCLUSION LEVEL OF L-GLUTAMINE TO REPLACE DIETARY ANTIBIOTICS FOLLOWING WEANING AND TRANSPORT IN PIGS

4.1 Abstract

Previous research indicates that supplementing nursery diets with 0.20% L-glutamine (**GLN**) provides similar growth and health benefits as dietary antibiotics, but it is unknown whether greater inclusion levels will provide additional benefits. Therefore, the study objective was to evaluate the impact of replacing dietary antibiotics with increasing levels of GLN on growth performance, health status, and production costs in pigs following weaning and transport. We hypothesized that withholding dietary antibiotics would negatively impact performance and health, and that diet supplementation with 0.20% to 1.00% GLN would incrementally improve health and productivity compared to dietary antibiotics. Mixed sex pigs (N = 308; 5.64 ± 0.06 kg BW) were weaned (19.1 ± 0.2 d of age) and transported in central Indiana during the autumn of 2017. Pigs were blocked by BW and allotted to 1 of 7 dietary treatments (n = 8 pens/dietary treatment); antibiotics [**A**; chlortetracycline (441 ppm) + tiamulin (38.6 ppm)], no antibiotics or added GLN (**NA**), 0.20% GLN, 0.40% GLN, 0.60% GLN, 0.80% GLN, or 1.00% GLN fed for 14 d. On d 15 to 35, pigs were provided NA diets in two phases. Data were analyzed using PROC MIXED in SAS 9.4. Overall, ADG was reduced (P = 0.04; 6.4%) in NA pigs vs. 0.40% GLN or A pigs. Increasing GLN in the diet tended to increase (linear; P = 0.10) ADG. Average daily feed intake was reduced (P = 0.04; 6.9%) in NA pigs vs. 0.40% GLN or A pigs. Overall, d 35 BW was greater (P = 0.01) in 0.80% GLN and A pigs compared to NA, 0.20% GLN, and 0.60% GLN pigs, but no BW differences were detected between 0.80% GLN and A and 0.40% GLN and 1.00% GLN pigs. In addition, d 35 BW was greater (P = 0.01) for 0.40% GLN and 1.00% GLN compared to 0.20%

GLN. Increasing GLN in the diet tended to increase (linear; $P = 0.08$) d 35 BW. Overall, income over feed and therapeutic injectable antibiotics cost for enteric and unthrifty challenges was greater ($P = 0.02$) in 0.80% GLN pigs compared to NA, 0.20% GLN, and 0.60% GLN pigs, but no income over feed and therapeutic injectable antibiotics cost for enteric and unthrifty challenges differences were detected between 0.80% GLN pigs and 0.40% GLN, 1.00% GLN, and A pigs. In conclusion, pigs provided GLN supplementation had improved performance and health after weaning and transport compared to the NA pigs with 0.40% GLN being the optimal level while the experimental diets were fed or 0.80% GLN being the most optimal level for the duration of the trial.

Keywords: antibiotics, L-glutamine, pigs, transport, weaning

4.2 Introduction

Weaning is stressful period in a pig's life as the pig is exposed multiple stressors at once (as reviewed by Campbell et al., 2013). These stressors can include: feed and water withdrawal, mixing, thermal stress, pathogenic insults, and transport (Lewis and Berry, 2006). Oftentimes, pigs in the United States are transported following weaning to different production facilities in efforts to reduce disease transmission (Harris, 2000) and transport stress is then added to the complex of weaning stressors (Chambers and Grandin, 2001) where weight loss following transport may occur (Johnson and Lay, 2017). Historically, swine producers have used dietary antibiotics to overcome the weaning stress complex (as reviewed by Cromwell, 2005).

In recent years United States pork producers have sought out nutritional additives that reduce antibiotic use without sacrificing pig health and growth performance. For adoption of new feed technologies, pork producers thoroughly evaluate new products to ensure that they return value to their enterprise (Tokach et al., 2016), and the value needs to be derived from improved performance, reduced feed cost and mortality, or a combination of all factors and provide a positive

net return to the farm's bottom line. Previous studies determined that 0.20% L-glutamine (Ajinomoto North America, Inc., Raleigh, NC) supplementation to newly weaned and transported pigs either improved growth performance (Johnson and Lay, 2017) or equaled the growth performance and health (Duttlinger et al., 2019) of dietary antibiotics [chlortetracycline (Aureomycin, Zoetis, Parsippany, NJ) + tiamulin (Denagard, Elanco Animal Health, Greenfield, IN)]. However, no studies have been conducted to determine the optimal level of L-glutamine in swine diets to replace dietary antibiotics following weaning and transport stress and if higher inclusion levels of L-glutamine can improve growth performance and pig health or reduce production costs compared to dietary antibiotics. Therefore, study objectives were to evaluate the impact of replacing dietary antibiotics with increasing levels of L-glutamine on growth performance, health status, and production costs following weaning and transport. We hypothesized that withholding dietary antibiotics would negatively impact pig performance and health, and that diet supplementation with 0.20% to 1.00% L-glutamine would incrementally improve pig health and productivity without increased production costs compared to dietary antibiotics.

4.3 Materials and Methods

4.3.1 General

All procedures involving animal use were approved by the Purdue University Animal Care and Use Committee (protocol #1710001637), and animal care and use standards were based upon the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of Animal Science Societies, 2010). In 2 repetitions, mixed sex crossbred pigs [N = 308; 5.64 ± 0.06 kg initial BW; Duroc x (Landrace x Yorkshire)] were weaned and transported at 19.1 ± 0.2 d of age in central Indiana during the autumn of 2017. One day prior to weaning and transport, all pigs

were individually weighed, blocked by body weight, and randomly allotted to pens, and pens of pigs within BW blocks were allotted to 1 of 7 dietary treatments with 8 pens per dietary treatment (n = 4/repetition). Each pen, initially, contained 5 to 6 pigs. Dietary treatments were antibiotics [**A**; chlortetracycline (441 ppm) + tiamulin (38.6 ppm)], no antibiotics or added L-glutamine (**NA**), 0.20% L-glutamine (**0.20% GLN**), 0.40% L-glutamine (**0.40% GLN**), 0.60% L-glutamine (**0.60% GLN**), 0.80% L-glutamine (**0.80% GLN**), and 1.00% L-glutamine (**1.00% GLN**).

4.3.2 Transportation

The transportation procedure was previously described by Duttlinger et al. (2019). In brief, selected pigs were weaned from sows and placed into a gooseneck livestock trailer (2.06 × 6.05 m; Featherlite Trailers, Cresco, IA) providing 0.08 m^2 per pig. The livestock trailer was bedded with straw and wood shavings and the air inlets were adjusted based on the ambient temperature (**T_A**; National Pork Board, 2015). The T_A and relative humidity (**RH**) were measured, in 5 min intervals, by 2 data loggers (Hobo®; accuracy ±0.2°C; data logger temperature/RH; Onset®; Bourne, MA) located within the pig space of the trailer. During transport, the average trailer T_A and RH for the 2 replicates was 6.1 ± 0.1°C and 54.3 ± 0.5%, respectively. Pigs were transported without feed and water as a group in the trailer for 11 h 26 min and 825 km per repetition on average.

4.3.3 Nursery Phase

Following weaning and transport, pigs were placed in their assigned pens and provided their respective dietary treatments d 0 to 14 post-weaning in 2 phases (Table 4.1 and Table 4.2). Following the dietary treatment period, all pigs were provided common antibiotic free diets from d 14 to the end of the trial (d 35; Table 4.2). Corn-soybean meal-based diets were fed in meal form and 4 phases. Diets were formulated to meet or exceed nutrient requirements (NRC, 2012; Table

4.1 and Table 4.2). Every 7 d feeders and pigs were weighed individually to determine the response criteria of ADG, ADFI, and G:F. The nursery room where the trial was conducted was previously described by Duttlinger et al. (2019). The nursery room average daily T_A was 30.1 ± 0.1°C and 29.0 ± 0.1°C from d 0 to 14 post-weaning and d 14 to 35, respectively.

During the trial, therapeutic antibiotic administration was recorded. The research farm staff and researchers were blinded to the dietary treatments and were trained to identify pigs exhibiting clinical signs of illness. Sick pigs were then treated with therapeutic injectable antibiotics. Pig identification, pen number, product administered, date administered, dose volume, and reason for administration were recorded. Reason for therapeutic antibiotic administration was then categorized and a post-hoc analysis was conducted. Categories of illness were previously defined by Duttlinger et al., (2019) and included: enteric challenge (e.g. scours or loose watery stool), respiratory challenge (e.g. coughing, thumping, or labored breathing), lameness (e.g. carrying a limb or difficulty walking or swollen joints), un-thriftiness (e.g. BW loss, poor gain, loss of body condition, or rough hair coat), and all other treatments (e.g. side paddling associated with *Streptococcus suis* infection, skin infection, and abscess).

4.3.4 Tear Staining

Photographs were taken of the left eye of two pigs per pen (1 barrow and 1 gilt) for the assessment of tear staining as previously described by DeBoer et al. (2015). The same selected pigs were photographed immediately post-transport and at 7, 14, 21, 28, and 35 days post-weaning. To calibrate the measurement software, a laminated card printed with 1 cm × 1 cm squares was held next to the eye in each picture.

Tear staining analysis was conducted by one trained individual who was blinded to the treatments. Images were analyzed using the tracing tool in ImageJ (NIH, Bethesda, MD). The

stained area(s) associated with the direct periphery of the eye were measured (DeBoer et al., 2015). The stained area(s), reported as cm^2, were summed and the results were averaged across the two pigs per pen prior for later analysis.

4.3.5 Economic Analysis

A post-hoc economic analysis of the growth performance differences observed was conducted. The revenue was determined using the average of the reported price of feeder pigs, 18.1 kg BW basis, (USDA-Iowa Dept of Ag Market News, 2018) from 2014 to 2018. To calculate the feed costs, the average corn price ($0.1427/kg) from 2014 to 2018 as reported by USDA National Agricultural Statistic Service (2018) was utilized, the soybean meal price ($0.3977/kg) used was calculated as the average weekly price in central Illinois according to USDA Market News (2018) from 2014 to 2018, and the dried distillers grains with solubles price ($0.1500/kg) used was the average price for the eastern corn-belt as reported by the USDA Agricultural Marketing Service (2018) from 2014 to 2018. The additional ingredient costs were provided from a major U.S. swine nutrition company. Income over the variable of interest was calculated using equations previously described by Menegat et al. (2019).

Antibiotics costs were calculated using the listed antibiotic price on a veterinary supply distributor's website on May 2, 2019 (Valley Vet Supply, Marysville, KS, USA). The antibiotic price was then multiplied by the antibiotic volume administered to determine the antibiotic cost per injection.

4.3.6 Statistics

Statistical analyses were performed as previously described (Duttlinger et al., 2019). Briefly, data were analyzed as a randomized complete block design using the PROC MIXED procedure in SAS 9.4 (SAS Institute INC., Cary, NC). The assumptions of normality of error,

homogeneity of variance, and linearity were confirmed post-hoc. Injectable antibiotic administration data were log-transformed to meet assumptions of normality; however, all log-transformed data are presented as arithmetic means for ease of interpretation. All non-transformed data are presented as LS means. Pen was the experimental unit and fixed effects included diet, week, and all interactions with replicate and block included as random effects. However, week data will not be presented or discussed as only dietary treatment effects were of interest in the present study and no dietary treatment by week interactions were observed. Pre-planned statistical comparisons were conducted for GLN, Linear; and GLN, Quadratic using the CONTRAST statement of SAS. For repeated analyses of growth performance and tear staining data, each pen's respective parameter was analyzed using repeated measures with a covariance structure selected based on goodness of fit criterion and week as the repeated effect when required. Statistical significance was defined as $P \leq 0.05$ and a tendency was defined as $0.05 < P \leq 0.10$.

4.4 Results

4.4.1 Growth Performance

4.4.1.1 Dietary Treatment Period (d 0 to 14)

When comparing the dietary treatments, ADG was reduced ($P = 0.01$; 17.7%) from d 0 to 14 in NA, 0.20% GLN, 0.60% GLN, 0.80% GLN, and 1.00% GLN pigs compared to A pigs, but no ADG differences were detected between 0.40% GLN pigs and A pigs (Table 4.3, Figure 4.1). In addition, ADG for 0.40% GLN pigs was greater ($P = 0.01$; 22.9%) compared to 0.20% GLN pigs, but no ADG differences were detected between 0.40% GLN and NA, 0.60% GLN, 0.80% GLN, and 1.00% GLN pigs (Table 4.3, Figure 4.1). Increasing GLN in the diet tended to increase (quadratic; $P = 0.08$) ADG (Table 4.3, Figure 4.1). No other ADG differences were observed ($P > 0.82$) with any comparison (Table 4.3, Figure 4.1).

Average daily feed intake was reduced (P = 0.02; 14.8%) from d 0 to 14 in 0.20% GLN, 0.60% GLN, 0.80% GLN, and 1.00% GLN pigs compared to A pigs, but no ADFI differences were detected between 0.40% GLN pigs and A pigs (Table 4.3, Figure 4.2). In addition, ADFI for 0.40% GLN pigs was greater (P = 0.02; 17.3%) compared to 0.20% GLN and 0.80% GLN pigs, but no ADFI differences were detected between 0.40% GLN pigs and NA, 0.60% GLN, and 1.00% GLN pigs (Table 4.3, Figure 4.2). No other ADFI differences were observed (P > 0.12) with any comparison (Table 4.3, Figure 4.2).

No G:F differences were observed (P > 0.16) from d 0 to 14, d 14 to 35, or from d 0 to 35 with any comparison (Table 4.3).

When comparing dietary treatments, d 14 BW tended to be reduced (P = 0.07; 7.3%) from d 0 to 14 in NA, 0.20% GLN, 0.60% GLN, 0.80% GLN, and 1.00% GLN pigs compared to A pigs, but no BW differences were detected between 0.40% GLN pigs and A pigs (Table 4.3). On d 14, BW for 0.40% GLN pigs tended to be greater (P = 0.07; 8.4%) compared to 0.20% GLN pigs, but no BW differences were detected between 0.40% GLN pigs and NA, 0.60% GLN, 0.80% GLN, and 1.00% GLN pigs (Table 4.3). No other BW differences were observed (P > 0.15) with any comparison (Table 4.3).

4.4.1.2 Common Diet Period (d 14 to 35)

When comparing the dietary treatments, ADG was greater (P = 0.05; 11.3%) from d 14 to 35 in 0.80% GLN pigs compared to NA, 0.20% GLN, and 0.60% GLN pigs, but no ADG differences were detected between 0.80% GLN and 0.40% GLN, 1.00% GLN, and A pigs (Table 4.3, Figure 4.3). Increasing GLN in the diet tended to increase (linear; P = 0.06) ADG (Table 4.3, Figure 4.3). No other ADG differences were observed (P > 0.92) with any comparison (Table 4.3, Figure 4.3).

Average daily feed intake was greater (P = 0.01; 10.3%) from d 14 to 35 in 0.80% GLN pigs compared to NA, 0.20% GLN, and 0.60% GLN pigs, but no ADFI differences were detected between 0.80% GLN pigs and 0.40% GLN, 1.00% GLN, and A pigs (Table 4.3, Figure 4.4). Increasing GLN in the diet tended to increase (linear; P = 0.06) ADG (Table 4.3, Figure 4.4). In addition, ADFI for 0.20% GLN and NA pigs was reduced (P = 0.01; 9.3%) compared to A pigs, but no ADFI differences were detected between 0.40% GLN, 0.60% GLN, and 1.00% GLN pigs and A pigs (Table 4.3, Figure 4.4). In addition, ADFI for 0.40% GLN, 0.60% GLN, and 1.00% GLN pigs was greater (P = 0.01; 9.5%) compared to 0.20% GLN pigs, but no ADFI differences were detected between 0.40% GLN, 0.60% GLN, and 1.00% GLN pigs and NA pigs (Table 4.3, Figure 4.4). Increasing GLN in the diet increased (linear; P = 0.01) ADFI (Table 4.3, Figure 4.4). No other ADFI differences were observed (P > 0.55) with any comparison (Table 4.3, Figure 4.4).

4.4.1.3 Overall Nursery Period (d 0 to 35)

Overall, ADG was reduced (P = 0.01; 8.8%) from d 0 to 35 in NA, 0.20% GLN, and 0.60% GLN pigs compared to A pigs, but no ADG differences were detected between 0.40% GLN, 0.80% GLN, and 1.00% GLN pigs and A pigs (Table 4.3, Figure 4.5). In addition, ADG for 0.40% GLN, 0.80% GLN, and 1.00% GLN pigs was greater (P = 0.01; 10.8%) compared to 0.20% GLN pigs, but no ADG differences were detected between 0.40% GLN, 0.80% GLN, and 1.00% GLN pigs when compared to NA and 0.60% GLN pigs (Table 4.3, Figure 4.5). Increasing GLN in the diet tended to increase (linear; P = 0.10) ADG (Table 4.3, Figure 4.5). No other ADG differences were observed (P > 0.44) with any comparison (Table 4.3, Figure 4.5).

Overall, ADFI was reduced (P = 0.02; 10.5%) from d 0 to 35 in 0.20% GLN pigs and NA pigs compared to A pigs, but no ADFI differences were detected between 0.40% GLN, 0.60%, 0.80% GLN, and 1.00% GLN pigs and A pigs (Table 4.3, Figure 4.6). In addition, ADFI for 0.40%

GLN, 0.80% GLN, and 1.00% GLN pigs was greater (P = 0.02; 11.1%) compared to 0.20% GLN pigs, but no ADFI differences were detected between 0.40% GLN, 0.80% GLN, and 1.00% GLN pigs vs. NA and 0.60% GLN pigs (Table 4.3, Figure 4.6). No other ADFI differences were observed (P > 0.14) with any comparison (Table 4.3, Figure 4.6).

Overall, d 35 BW was greater (P = 0.01; 6.6%) in 0.80% GLN and A pigs compared to NA, 0.20% GLN, and 0.60% GLN pigs, but no BW differences were detected between 0.80% GLN and A and 0.40% GLN pigs and 1.00% GLN pigs (Table 4.3). In addition, d 35 BW for 0.40% GLN and 1.00% GLN pigs was greater (P = 0.01; 6.8%) compared to 0.20% GLN pigs, but no BW differences were detected for 0.40% GLN and 1.00% GLN pigs compared to NA and 0.60% GLN pigs (Table 4.3). Increasing GLN in the diet tended to increase (linear; P = 0.08) d 35 BW (Table 4.3). No other BW differences were observed (P > 0.49) with any comparison (Table 4.3).

4.4.2 Treatment rate

4.4.2.1 Dietary Treatment Period (d 0 to 14)

When comparing the dietary treatments, pigs treated for lameness was greater (P = 0.02) from d 0 to 14 in 0.40% GLN (3.33 ± 0.81%) and 0.80% GLN (4.17 ± 0.81%) pigs compared to NA (0.00 ± 0.81%), 0.20% GLN (0.00 ± 0.81%), 0.60% GLN (0.00 ± 0.81%), and 1.00% GLN (0.00 ± 0.81%) pigs, but no treatment rate differences were detected between 0.40% GLN and 0.80% pigs and A pigs (Table 4.4). No additional therapeutic antibiotic treatment rate differences were observed (P > 0.15) with any comparison from d 0 to 14 (Table 4.4).

4.4.2.2 Common Diet Period (d 14 to 35)

No therapeutic antibiotic treatment rate differences were observed (P > 0.16) with any comparison during the common diet period (Table 4.4).

4.4.3 Tear Staining

No tear staining differences were observed ($P > 0.17$) post-transport, on day 14, or on day 35 with any comparison (Table 4.5).

4.4.4 Economics

Overall, revenue was greater ($P = 0.01$; 6.6%) in 0.80% GLN and A pigs compared to NA, 0.20% GLN, and 0.60% GLN pigs, but no revenue differences were detected between 0.80% GLN and A pigs and 0.40% GLN and 1.00% GLN pigs (Table 4.6). In addition, revenue for 0.40% GLN and 1.00% GLN pigs was greater ($P = 0.01$; 6.9%) compared to 0.20% GLN pigs, but no revenue differences were detected between 0.40% GLN and 1.00% GLN pigs and NA and 0.60% GLN pigs (Table 4.6). Increasing GLN in the diet tended to increase (linear; $P = 0.08$) revenue (Table 4.6). No other revenue differences were observed ($P > 0.49$) with any comparison (Table 4.6).

Overall, feed costs were greater ($P = 0.01$; 13.8%) in 0.40% GLN and A pigs compared to NA and 0.20% GLN pigs, but no feed cost differences were detected between 0.40% GLN and A pigs and 0.60% GLN, 0.80% GLN, and 1.00% GLN pigs (Table 4.6). In addition, feed costs for 0.60% GLN, 0.80% GLN, and 1.00% GLN pigs were greater ($P = 0.01$; 10.5%) compared to 0.20% GLN pigs, but no feed cost differences were detected between 0.60% GLN, 0.80% GLN, and 1.00% GLN pigs and NA pigs (Table 4.6). Increasing GLN in the diet increased (linear; $P = 0.02$) feed cost (Table 4.6). No other feed cost differences were observed ($P > 0.15$) with any comparison (Table 4.6).

Overall, income over feed cost was greater ($P = 0.01$; 6.6%) in 0.80% GLN pigs compared to NA, 0.20% GLN, and 0.60% GLN pigs, but no income over feed cost differences were detected between 0.80% GLN pigs and 0.40% GLN, 1.00% GLN, and A pigs (Table 4.6). In addition, income over feed cost for 0.20% GLN and 0.60% pigs was reduced ($P = 0.01$; 6.1%) compared to

A pigs, but no income over feed cost differences were detected between NA, 0.40% GLN, and 1.00% GLN pigs and A pigs (Table 4.6). Income over feed cost for 0.40% GLN pigs was greater (P = 0.01; 7.2%) compared to 0.20% GLN pigs, but no income over feed cost differences were detected between 0.40% GLN pigs and NA, 0.60% GLN, 0.80% GLN, 1.00% GLN, and A pigs (Table 4.6). No other income over feed cost differences were observed (P > 0.13) with any comparison (Table 4.6).

No differences for therapeutic injectable antibiotics cost for enteric and unthrifty challenges or total therapeutic injectable antibiotics cost were observed (P > 0.17) with any comparison (Table 4.6).

Overall, income over feed and therapeutic injectable antibiotics cost for enteric and unthrifty challenges was greater (P = 0.02; 6.8%) in 0.80% GLN pigs compared to NA, 0.20% GLN, and 0.60% GLN pigs, but no income over feed and therapeutic injectable antibiotics cost for enteric and unthrifty challenges differences were detected between 0.80% GLN pigs and 0.40% GLN, 1.00% GLN, and A pigs (Table 4.6). In addition, income over feed and therapeutic injectable antibiotics cost for enteric and unthrifty challenges for 0.40% GLN and A pigs was greater (P = 0.02; 7.6%) compared to 0.20% GLN pigs, but no income over feed and therapeutic injectable antibiotics cost for enteric and unthrifty challenges differences were detected for 0.40% GLN and A pigs compared to NA, 0.60% GLN, and 1.00% GLN pigs (Table 4.6). No other differences for income over feed and therapeutic injectable antibiotics cost for enteric and unthrifty challenges were observed (P > 0.13) with any comparison (Table 4.6).

Overall, income over feed and therapeutic injectable antibiotics cost was greater (P = 0.02; 7.4%) in 0.80% GLN pigs compared to 0.20% GLN and 0.60% GLN pigs, but no income over feed and therapeutic injectable antibiotics cost differences were detected between 0.80% GLN pigs

and 0.40% GLN, 1.00% GLN, and A pigs (Table 4.6). In addition, income over feed and therapeutic injectable antibiotics cost for 0.40% GLN and A pigs was greater (P = 0.02; 7.8%) compared to 0.20% GLN pigs, but no income over feed and therapeutic injectable antibiotics cost differences were detected between 0.40% GLN and A pigs and NA, 0.60% GLN, and 1.00% GLN pigs (Table 4.6). No other income over feed and therapeutic injectable antibiotics cost differences were observed (P > 0.14) with any comparison (Table 4.6).

4.5 Discussion

Supplementing swine nursery diets with 0.20% GLN may be an effective antibiotic alternative in swine nursery diets because GLN supplementation improves growth performance and health at a similar (Duttlinger et al., 2019) or greater (Johnson and Lay, 2017) level compared to A. However, it is currently unknown whether increasing GLN supplementation beyond the aforementioned 0.20% inclusion level has an additional benefit to swine health and productivity during the nursery phase of growth when compared to A. In the present study, increasing GLN supplementation levels improved ADG as indicated by a quadratic tendency for greater growth rate with increasing GLN supplementation. Furthermore, it was determined that 0.40% GLN supplementation yielded the greatest improvement in ADG amongst the GLN treatments and that supplementation at this level improved ADG to a similar level as A inclusion. These data suggest that although increasing GLN supplementation may have an additive effect on growth rate, this effect may peak at 0.40% GLN when supplemented after weaning and transport. These results were somewhat surprising considering that Wu et al. (1996) observed that 1.0% GLN inclusion is the threshold for maximum feed efficiency benefits and it was expected that this outcome would be observed in the present study. While reasons for this discrepancy are currently unknown, poorer palatability of diets containing higher inclusion levels of synthetic GLN (hypothesized from

reduced ADFI) may be a potential obstacle to feeding higher inclusion levels of GLN and future research should determine through preference testing whether higher synthetic GLN inclusion levels are a barrier to improved feed intake and subsequently growth rate. Regardless, no studies that the authors are aware of have observed an increased ADG response to increasing levels of GLN.

The mechanisms for improved growth rate with increasing levels of dietary GLN are likely multifaceted as endogenous circulating GLN levels can be reduced under stress, GLN can be an energy source for enterocytes allowing glucose to be spared for other functions such as growth, and supplemental GLN has been shown to improve intestinal barrier function (Souba et al., 1985a,b; Souba and Wilmore, 1985). Previous studies have shown that when animals undergo stressful catabolic events, skeletal muscle proteolysis is accelerated and amino acids (e.g. glutamine) are translocated into circulation (as reviewed by Souba et al., 1985a) and GLN uptake by the gastrointestinal (**GI**) tract is significantly increased (Souba et al., 1985b). Due to the increase in uptake of GLN by the GI tract and other tissues, circulating concentrations of GLN are diminished despite accelerated muscle proteolysis (Souba et al., 1985a) thus demonstrating the need for exogenous GLN supplementation to help replenish GLN stores. Glutamine is the preferred energy source of enterocytes under homeostasis (as reviewed by Souba et al., 1985a). However, under stress conditions GLN consumption by the enterocyte increases as GLN is the preferential energy source for enterocytes and glucose is released for other functions such as growth or immune system function (Souba and Wilmore, 1985). However, pigs fed GLN have reduced circulating TNF-α levels compared to NA pigs (Duttlinger et al., 2019) and less systemic inflammation may reduce immune system activation and energy requirements as a previous study has determined that the glucose requirement of the immune system is significantly increased when

the immune system is activated (Kvidera et al., 2017). When the immune system requires less energy, more energy is allowed to be shunted towards growth (NRC, 2012). In addition, GLN has been shown to improve intestinal health and barrier function through improvements in intestinal morphology and increased tight junction protein abundance (Wang et al., 2015b).

Although, ADG was improved with increasing levels of GLN supplementation, 0.20% GLN fed pigs had similar ADG with NA pigs. This finding was surprising due to previous studies found that ADG for 0.20% GLN pigs was greater compared to NA pigs (Johnson and Lay, 2017; Duttlinger et al., 2019). Although specific reasons are currently unknown, the lack of ADG improvement with 0.20% GLN supplementation in the current study may be due to insufficient levels of exogenous GLN supplementation to overcome the endogenous GLN losses following weaning and transport, thus reducing gut barrier function and limiting available glucose to be spared by the GI tract through the aforementioned mechanisms resulting in reduced growth and delayed stress recovery.

An unexpected observation in the present study was that pigs provided diets containing greater than 0.40% GLN had numerically lower ADFI from d 0 to 14 compared to 0.40% GLN with 0.80% GLN having numerically the lowest ADFI and subsequently poorer ADG compared to diets containing 0.40% to 1.00% added GLN. The ADFI reduction for pigs fed greater than 0.40% GLN is counter to previous research where pigs were fed up to 1.00% GLN (Wu et al., 1996) and 2.00% GLN (Yi et al., 2005), respectively, with no reduction in ADFI compared to control diets. However, it is possible that this reduction in ADFI may have been a result of palatability issues because ingredient and diet composition can affect dietary palatability (Dong and Pluske, 2007). The chemical senses of olfaction and taste influence voluntary feed intake which ultimately determines nutrient intake, thus highly palatable diets are important to maximize feed intake

(Jacela et al., 2010). Synthetic GLN may potentially have a disconcerting taste and when higher inclusion levels of GLN are fed palatability and subsequently feed intake may be negatively affected. Therefore, reduced palatability of synthetic GLN may a contributing factor to poorer ADFI when 0.80% GLN was fed. An alternative explanation for the poor ADFI may be that total GLN concentration in the diet (from synthetic GLN and other feed ingredients) resulted in the imbalance of other amino acids. Previous studies have shown pigs can detect amino acid imbalances within 1 h of consuming a diet and subsequently reduce feed intake as a protective mechanism to prevent against the degradation of protein within the brain (Hao et al., 2005; Gloaguen et al., 2012).

Despite the negative growth performance for higher inclusion levels of GLN when the experimental diets were fed, once the dietary treatment phase ended and all pigs were provided common diets, ADFI of pigs provided 0.60% to 1.00% GLN had a substantial improvement in ADFI (with 0.80% GLN treated pigs having the greatest improvement) and this may point towards compensatory feed intake and gain. Compensatory growth performance was previously described in pigs fed low lysine diets followed by higher lysine diets and growth performance exceeds control levels once diets are switched (Reynolds and O'Doherty, 2006). These demonstrates that pigs may compensate for reduced growth performance once diets are changed from nutrient restrictive diets or self-restricted (low ADFI) diets.

In addition to the improved ADFI and ADG for 0.80% GLN pigs during the common diet phase, ADG and ADFI increased linearly during the common diet phase with increasing GLN inclusion level and this contrasts to a previous study where no growth performance benefits to GLN were observed compared to NA pigs after experimental diets ceased (Duttlinger et al., 2019). Although the growth performance advantages observed during the common diet phase are

interesting, they are not believed to be due to the direct impact of the dietary treatments as efficacy of GLN is quite short after administration ceases (Harris et al., 2012). Rather, it is likely that the continued performance advantages observed may be due to the dietary treatments altering the intestinal health through improved intestinal morphology (Wang et al., 2015a,b; Johnson and Lay, 2017) and greater tight junction protein abundance (Wang et al., 2015b) resulting in greater growth performance due to improved nutrient absorption and intestinal barrier function.

Weaning, transport, and the associated stressors can compromise animal welfare due to the social stress of mixing, feed and water withdrawal, and pathogen insults (Chambers and Grandin, 2001; Lewis and Barry, 2006). In the present study, tear staining was used as the response criterion to measure welfare associated with postweaning and transport stress. Tear staining has been determined to be an assessment of welfare in pigs (DeBoer et al., 2015 and Telkänranta et al., 2016) where an increase in the reddish-brown to black facial stains in and around the corner of the eye can be an indicator of reduced welfare. This is because of porphyrins secreted from the Harderian gland is correlated with hypothalamic pituitary adrenal (**HPA**) axis activation (DeBoer et al., 2015). However, despite the fact that a previous study observed decreased tear staining in pigs provided GLN (Parois et al., 2018), no tear staining differences were observed in the present study. While reasons for this discrepancy are currently unclear, it may be due to the timing of the effect as pigs in the aforementioned study (Parois et al., 2018) that were provided 0.20% GLN had decreased tear staining compared to NA pigs on d 84 and 110 post weaning and transport and after the dietary treatments were only fed for 14 days post weaning and transport. Therefore, the response differential between the studies may be due to a latency response of tear staining to the dietary treatments observed in the previous study could not be replicated in the shorter 35 d present study.

Based on the growth performance and antibiotic treatment rate of present study, the production costs within each dietary treatment were estimated and it was determined that income over feed cost and income over feed and therapeutic antibiotic costs for enteric and unthrifty challenges for 0.40%, 0.80% and 1.00% GLN pigs were similar to A pigs. The purpose of the production cost data is to provide pork producers and decision makers with an assessment that encompasses the production data, injectable antibiotic cost, and diet cost differences to use as a tool when investigating technologies to reduce dietary antibiotic use. These data support that GLN supplementation can potentially be used as an alternative to dietary antibiotics without reducing income with the estimates used in the production cost analysis. The advantage in 0.80% GLN production costs are predominately derived from lower feed costs. The feed costs are lower as the 0.80% GLN had reduced ADFI from d 0-14 postweaning when the higher cost experimental diets were fed and increased ADFI when the lower cost common diets were fed thus resulting in comparable production costs to the 0.40% GLN, 1.00% GLN, and A.

4.6 Conclusion

Previous research demonstrates that 0.20% GLN supplementation may be an effective alternative to antibiotics following weaning and transport stress. Despite the improvements observed in growth performance and pig health when pigs were fed 0.20% GLN in previous studies, the question remained if pig performance would respond more positively to greater levels of dietary GLN. It was determined that pigs provided 0.40%, 0.80%, and 1.00% GLN supplementation had similar growth performance and health after weaning and transport compared to A pigs. However, 0.40% GLN supplementation in the present study was the optimal level while the experimental diets were fed and 0.80% GLN was the optimal level for the duration of the trial when considering the production costs. Future research should address the effects of the quantity

of post-weaning stressors on growth performance, intestinal function, and immune response of weaned pigs supplemented with 0.40% GLN and determine why or if this level is the threshold for use in production systems.

4.7 References

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Table 4.1 Composition of phase 1[1] nursery diets

Item	NA[2]	0.20% GLN[3]	0.40% GLN[4]	0.60% GLN[5]	0.80% GLN[6]	1.00% GLN[7]	A[8]
Ingredient, % as fed							
Corn	30.32	30.32	30.32	30.32	30.32	30.32	30.32
SBM, 48% CP	14.00	14.00	14.00	14.00	14.00	14.00	14.00
Soybean oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Limestone	0.79	0.79	0.79	0.79	0.79	0.79	0.79
Monocalcium phosphate	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin premix[9]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[10]	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Selenium premix[11]	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Phytase[12]	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Plasma protein	6.50	6.50	6.50	6.50	6.50	6.50	6.50
Spray dried blood meal	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Soy concentrate	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Select menhaden fish meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Dried whey	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Lactose	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Lysine-HCl	0.07	0.07	0.07	0.07	0.07	0.07	0.07
DL-methionine	0.22	0.22	0.22	0.22	0.22	0.22	0.22
L-threonine	0.04	0.04	0.04	0.04	0.04	0.04	0.04
L-tryptophan	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Zinc oxide	0.375	0.375	0.375	0.375	0.375	0.375	0.375
Corn starch	1.00	0.80	0.60	0.40	0.20	---	0.43
Aureomycin 50[13]	---	---	---	---	---	---	0.40
Denagard 10[14]	---	---	---	---	---	---	0.18
L-glutamine[15]	---	0.20	0.40	0.60	0.80	1.00	---
Calculated chemical composition							
ME, kcal/kg	3542	3542	3542	3542	3542	3542	3542
Fat, %	7.23	7.23	7.23	7.23	7.23	7.23	7.23
CP, %	24.56	24.56	24.56	24.56	24.56	24.56	24.56
SID Lys, %	1.55	1.55	1.55	1.55	1.55	1.55	1.55
Ca, %	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Total P, %	0.74	0.74	0.74	0.74	0.74	0.74	0.74
Avail. P, %	0.56	0.56	0.56	0.56	0.56	0.56	0.56
Analyzed chemical composition							
GE, kcal/kg	4195	4173	4205	4207	4208	4238	4187
CP, %	23.58	23.76	24.36	24.27	24.67	25.07	23.84
Total Lys, %	1.57	1.59	1.58	1.57	1.55	1.61	1.56
Total Glu, %[16]	3.53	3.78	3.94	4.08	4.26	4.46	3.58
Chlortetracycline, ppm[17]	0	0	0	0	0	0	386
Tiamulin, ppm[18]	0	0	0	0	0	0	33.1

[1]Fed d 0 to 7 post-weaning and transport

Table 4.1 continued

[2]Pigs provided no dietary antibiotics and 0.00% added L-glutamine
[3]Pigs provided 0.20% L-glutamine
[4]Pigs provided 0.40% L-glutamine
[5]Pigs provided 0.60% L-glutamine
[6]Pigs provided 0.80% L-glutamine
[7]Pigs provided 1.00% L-glutamine
[8]Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)]
[9]Provided per kilogram of the diet: vitamin A, 6,615 IU; vitamin D3, 662 IU; vitamin E, 44 IU; vitamin K, 2.2 mg; riboflavin, 8.8 mg; pantothenic acid, 22 mg; niacin, 33 mg; vitamin B_{12}, 38.6 mg
[10]Provided available minerals per kilogram of the diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15 mg; copper, 11.3 mg; iodine, 0.46 mg
[11]Provided 0.3 ppm Se
[12]Provided 600 FTU per kg of the diet
[13]Aureomycin (Zoetis, Parsippany, NJ) provided 441 ppm chlortetracycline in the diet
[14]Denagard (Elanco Animal Health, Greenfield, IN) provided 38.6 ppm tiamulin in the diet
[15]Ajinomoto North America, Inc., Raleigh, NC
[16]Samples submitted to Ajinomoto for glutamic acid analysis
[17]Samples submitted to Zoetis, Parsippany, NJ for chlortetracycline analysis
[18]Samples submitted to Elanco Animal Health, Greenfield, IN for tiamulin analysis

Table 4.2 Composition of phase 2, 3, and 4 nursery diets

Item	Phase 2[1]							Phase 3[2]	Phase 4[3]
	NA[4]	0.20% GLN[5]	0.40% GLN[6]	0.60% GLN[7]	0.80% GLN[8]	1.00% GLN[9]	A[10]		
Ingredient, % as fed									
Corn	37.04	37.04	37.04	37.04	37.04	37.04	37.04	51.63	57.38
SBM, 48% CP	18.05	18.05	18.05	18.05	18.05	18.05	18.05	25.65	30.70
Dried distillers grain with solubles	---	---	---	---	---	---	---	---	5.00
Soybean oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	3.00	---
Choice white grease	---	---	---	---	---	---	---	---	3.00
Limestone	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.86	1.33
Monocalcium phosphate	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.49	0.74
Vitamin premix[11]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[12]	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125	0.125
Selenium premix[13]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Phytase[14]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.30	0.35
Plasma protein	2.50	2.50	2.50	2.50	2.50	2.50	2.50	---	---
Spray dried blood meal	1.50	1.50	1.50	1.50	1.50	1.50	1.50	---	---
Soy concentrate	3.00	3.00	3.00	3.00	3.00	3.00	3.00	2.50	---
Select menhaden fish meal	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	---
Dried whey	25.00	25.00	25.00	25.00	25.00	25.00	25.00	10.00	---
Lysine-HCl	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.28	0.40
DL-Methionine	0.23	0.23	0.23	0.23	0.23	0.23	0.23	0.18	0.17
L-Threonine	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.12	0.14
L-Tryptophan	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.010	---
Zinc oxide	0.375	0.375	0.375	0.375	0.375	0.375	0.375	0.375	---
Copper sulphate								---	0.10
Corn starch	1.00	0.80	0.60	0.40	0.20	---	0.43		
Aureomycin 50[15]	---	---	---	---	---	---	0.40	---	---
Denagard 10[16]	---	---	---	---	---	---	0.18	---	---
L-glutamine[17]	---	0.20	0.40	0.60	0.80	1.00	---	---	---
Banminth 48[18]	---	---	---	---	---	---	---	---	0.10
Clarifly, 0.67%[19]	---	---	---	---	---	---	---	0.08	0.07
Calculated chemical composition									
ME, kcal/kg	3516	3516	3516	3516	3516	3516	3516	3418	3396
Fat, %	7.32	7.32	7.32	7.32	7.32	7.32	7.32	5.73	5.86
CP, %	22.82	22.82	22.82	22.82	22.82	22.82	22.82	22.29	21.28
SID Lys, %	1.45	1.45	1.45	1.45	1.45	1.45	1.45	1.35	1.25
Ca, %	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.80	0.75
Total P, %	0.70	0.70	0.70	0.70	0.70	0.70	0.70	0.64	0.57
Avail. P, %	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.45	0.36
Analyzed chemical composition									
GE, kcal/kg	4197	4174	4199	4185	4200	4208	4179	4102	4109
CP, %	22.35	22.32	23.12	22.92	23.11	23.36	22.32	---	---
Total Lys, %	1.52	1.52	1.54	1.50	1.53	1.52	1.53	---	---
Total Glu, %[20]	3.50	3.70	3.91	4.00	4.22	4.42	3.56	---	---
Chlortetracycline, ppm[21]	0	0	0	0	0	0	386	---	---
Tiamulin, ppm[22]	0	0	0	0	0	0	39.0	---	---

[1]Fed d 7 to 14 post-weaning and transport
[2]Fed d 14 to 21 post-weaning and transport
[3]Fed d 21 to 35 post-weaning and transport
[4]Pigs provided no dietary antibiotics and 0.00% L-glutamine

Table 4.2 continued

[5]Pigs provided 0.20% L-glutamine
[6]Pigs provided 0.40% L-glutamine
[7]Pigs provided 0.60% L-glutamine
[8]Pigs provided 0.80% L-glutamine
[9]Pigs provided 1.00% L-glutamine
[10]Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)]
[11]Provided per kilogram of the diet: vitamin A, 6,615 IU; vitamin D3, 662 IU; vitamin E, 44 IU; vitamin K, 2.2 mg; riboflavin, 8.8 mg; pantothenic acid, 22 mg; niacin, 33 mg; vitamin B_{12}, 38.6 mg
[12]Provided available minerals per kilogram of the diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15 mg; copper, 11.3 mg; iodine, 0.46 mg
[13]Provided 0.3 ppm Se
[14]Provided 600 FTU per kg of the diet
[15]Aureomycin (Zoetis, Parsippany, NJ) provided 441 ppm chlortetracycline in the diet
[16]Denagard (Elanco Animal Health, Greenfield, IN) provided 38.6 ppm tiamulin in the diet
[17]Ajinomoto North America, Inc., Raleigh, NC
[18]Banminth (Phibro Animal Health Corporation, Teaneck, NJ) provided 106 ppm pyrantel tartrate in the diet
[19]Clarifly (Central Life Sciences, Schaumburg, IL) provided 5.4 ppm (Phase 3) and 4.7 ppm (Phase 4) diflubenzuron in the diet
[20]Samples submitted to Ajinomoto for glutamic acid analysis
[21]Samples submitted to Zoetis, Parsippany, NJ for chlortetracycline analysis
[22]Samples submitted to Elanco Animal Health, Greenfield, IN for tiamulin analysis

Table 4.3 Effects of dietary treatment on nursery growth performance in pigs.[1]

Parameter	Dietary Treatments[2]							SE	*P*		
	NA[3]	0.20% GLN[4]	0.40% GLN[5]	0.60% GLN[6]	0.80% GLN[7]	1.00% GLN[8]	A[9]		D[10]	GLN, Linear	GLN, Quadratic
Day 0 to 14											
Initial BW, kg	5.63	5.63	5.66	5.63	5.64	5.63	5.66	0.14	1.00	1.00	0.91
ADG, g	229^{ab}	210^{a}	258^{bc}	233^{ab}	225^{ab}	231^{ab}	274^{c}	15	0.01	0.82	0.08
ADFI, g	282^{abc}	260^{a}	308^{bc}	275^{ab}	265^{a}	273^{ab}	315^{c}	17	0.02	0.46	0.12
G:F	0.86	0.84	0.90	0.88	0.88	0.87	0.92	0.03	0.18	0.32	0.19
Day 14 BW, kg	8.75^{xy}	8.49^{x}	9.20^{yz}	8.81^{xy}	8.72^{xy}	8.81^{xy}	9.40^{z}	0.29	0.07	0.82	0.15
Day 14 to 35											
ADG, g	406^{a}	389^{a}	422^{ab}	399^{a}	443^{b}	416^{ab}	417^{ab}	17	0.05	0.06	0.92
ADFI, g	608^{ab}	578^{a}	640^{bcd}	621^{bc}	664^{d}	637^{bcd}	654^{cd}	21	0.01	0.01	0.55
G:F	0.67	0.68	0.66	0.64	0.67	0.66	0.64	0.02	0.34	0.32	0.16
Day 0 to 35											
ADG, g	335^{ab}	317^{a}	356^{bc}	333^{ab}	356^{bc}	342^{bc}	360^{c}	12	0.01	0.10	0.44
ADFI, g	478^{ab}	451^{a}	508^{bc}	483^{abc}	504^{bc}	491^{bc}	519^{c}	20	0.02	0.14	0.34
G:F	0.74	0.74	0.75	0.74	0.76	0.74	0.75	0.02	0.96	0.89	0.93
Day 35 BW, kg	17.26^{ab}	16.66^{a}	18.04^{bc}	17.20^{ab}	18.17^{c}	17.55^{bc}	18.16^{c}	0.43	0.01	0.08	0.49

[1]A total of 308 pigs (initial BW, 5.64 ± 0.06 kg) were used with 5 to 6 pigs per pen with 8 pens per dietary treatment
[2]Pigs provided dietary treatments for 14 d post-weaning and transport and then fed common antibiotic free diets
[3]Pigs provided no dietary antibiotics and 0.00% L-glutamine
[4]Pigs provided 0.20% L-glutamine
[5]Pigs provided 0.40% L-glutamine
[6]Pigs provided 0.60% L-glutamine
[7]Pigs provided 0.80% L-glutamine
[8]Pigs provided 1.00% L-glutamine
[9]Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)]
[10]Dietary treatment
[a,b,c,d]Letters indicate differences ($P \leq 0.05$) within a row
[x,y,z]Letters indicate tendencies ($0.05 < P \leq 0.10$) within a row

Table 4.4 Effects of dietary treatment on therapeutic antibiotic treatment rate during the nursery period in pigs.[1]

	Dietary Treatments[2]								*P*		
Parameter	NA[3]	0.20% GLN[4]	0.40% GLN[5]	0.60% GLN[6]	0.80% GLN[7]	1.00% GLN[8]	A[9]	SE	D[10]	GLN, Linear	GLN, Quadratic
Day 0 to 14											
Enteric[11]	5.83	6.25	1.25	8.75	1.25	6.25	0.00	1.26	0.15	0.92	0.59
Lameness[12]	0.00[a]	0.00[a]	3.33[b]	0.00[a]	4.17[b]	0.00[a]	2.29[ab]	0.81	0.02	0.34	0.33
Unthrifty[13]	1.04	0.00	2.50	0.00	4.58	1.04	2.29	0.81	0.20	0.32	0.77
Respiratory[14]	0.00	0.00	0.00	0.00	0.00	0.00	1.04	0.59	0.44	1.00	1.00
Other[15]	0.00	2.50	0.00	0.00	0.00	2.29	0.00	0.67	0.19	0.35	0.18
Day 14 to 35											
Enteric	5.19	5.26	1.05	8.25	2.22	5.56	0.00	1.21	0.93	0.56	0.51
Lameness	0.00	0.00	2.81	0.00	3.70	0.00	1.93	0.62	0.19	0.59	0.42
Unthrifty	0.93	0.00	2.11	0.00	4.07	0.93	1.93	0.89	0.73	0.16	0.91
Respiratory	0.00	0.88	0.00	0.00	0.00	0.00	0.88	0.71	0.35	0.55	0.65
Other	0.00	2.11	0.00	0.00	0.00	2.04	0.00	0.56	0.43	0.36	0.64

[1]A total of 308 pigs (initial BW, 5.64 ± 0.06 kg) were used with 5 to 6 pigs per pen with 8 pens per dietary treatment
[2]Pigs provided dietary treatments for 14 d post-weaning and transport and then fed common antibiotic free diets
[3]Pigs provided no dietary antibiotics and 0.00% L-glutamine
[4]Pigs provided 0.20% L-glutamine
[5]Pigs provided 0.40% L-glutamine
[6]Pigs provided 0.60% L-glutamine
[7]Pigs provided 0.80% L-glutamine
[8]Pigs provided 1.00% L-glutamine
[9]Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)]
[10]Dietary treatment
[11]Percent of pigs within pen treated with therapeutic antibiotics for enteric challenge
[12]Percent of pigs within pen treated with therapeutic antibiotics for lameness
[13]Percent of pigs within pen treated with therapeutic antibiotics for un-thriftiness
[14]Percent of pigs within pen treated with therapeutic antibiotics for respiratory challenge
[15]Percent of pigs within pen treated with therapeutic antibiotics for all other conditions
[a,b]Letters indicate differences ($P \leq 0.05$) within a row

Table 4.5 Effect of dietary treatment on tear staining in pigs.[1]

	Dietary Treatments[2]								*P*		
Tear stain area, cm^2	NA[3]	0.20% GLN[4]	0.40% GLN[5]	0.60% GLN[6]	0.80% GLN[7]	1.00% GLN[8]	A[9]	SE	D[10]	GLN, Linear	GLN, Quadratic
Post-transport	0.06	0.07	0.08	0.10	0.05	0.10	0.09	0.03	0.27	0.23	0.38
Day 14	0.15	0.19	0.22	0.21	0.18	0.28	0.17	0.05	0.62	0.17	0.81
Day 35	0.35	0.39	0.39	0.30	0.34	0.43	0.26	0.07	0.70	0.86	0.57

[1]A total of 308 pigs (initial BW, 5.64 ± 0.06 kg) were used with 5 to 6 pigs per pen with 8 pens per dietary treatment
[2]Pigs provided dietary treatments for 14 d post-weaning and transport and then fed common antibiotic free diets
[3]Pigs provided no dietary antibiotics and 0.00% L-glutamine
[4]Pigs provided 0.20% L-glutamine
[5]Pigs provided 0.40% L-glutamine
[6]Pigs provided 0.60% L-glutamine
[7]Pigs provided 0.80% L-glutamine
[8]Pigs provided 1.00% L-glutamine
[9]Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)]
[10]Dietary treatment

Table 4.6 Effects of dietary treatment on production costs in pigs.[1]

Parameter	Dietary Treatments[2]							SE	*P*		
	NA[3]	0.20% GLN[4]	0.40% GLN[5]	0.60% GLN[6]	0.80% GLN[7]	1.00% GLN[8]	A[9]		D[10]	GLN, Linear	GLN, Quadratic
Day 0 to 35											
Revenue, $/pig[16]	61.46^{ab}	59.30^{a}	64.25^{bc}	61.23^{ab}	64.70^{c}	62.48^{bc}	64.66^{c}	3.90	0.01	0.08	0.49
Feed cost, $/pig	8.21^{ab}	7.78^{a}	9.02^{c}	8.52^{bc}	8.75^{bc}	8.80^{bc}	9.17^{c}	0.68	0.01	0.02	0.15
Income over feed cost, $/pig	53.25^{abc}	51.52^{a}	55.23^{bcd}	52.71^{ab}	55.96^{d}	53.68^{abcd}	55.49^{cd}	3.29	0.01	0.13	0.65
Therapeutic injectable antibiotics cost for enteric and unthrifty challenges, $/pig	0.55	0.41	0.23	0.38	0.38	0.35	0.46	0.27	0.72	0.37	0.21
Income over feed and therapeutic injectable antibiotics for enteric and unthrifty challenges, $/pig	52.70^{ab}	51.11^{a}	55.00^{bc}	52.33^{ab}	55.58^{c}	53.33^{abc}	55.02^{bc}	3.44	0.02	0.13	0.57
Therapeutic injectable antibiotics cost total, $/pig	0.56	0.55	0.28	0.42	0.52	0.42	0.52	0.28	0.79	0.58	0.17
Income over feed and therapeutic injectable antibiotics, $/pig	52.69^{abc}	50.97^{a}	54.95^{bc}	52.29^{ab}	55.43^{c}	53.27^{abc}	54.97^{bc}	3.44	0.02	0.14	0.56

[1]A total of 308 pigs (initial BW, 5.64 ± 0.06 kg) were used with 5 to 6 pigs per pen with 8 pens per dietary treatment
[2]Pigs provided dietary treatments for 14 d post-weaning and transport and then fed common antibiotic free diets
[3]Pigs provided no dietary antibiotics and 0.00% L-glutamine
[4]Pigs provided 0.20% L-glutamine
[5]Pigs provided 0.40% L-glutamine
[6]Pigs provided 0.60% L-glutamine
[7]Pigs provided 0.80% L-glutamine
[8]Pigs provided 1.00% L-glutamine
[9]Pigs provided dietary antibiotics [chlortetracycline (441 ppm) + tiamulin (38.6 ppm)]
[10]Dietary treatment
[11]Feeder pig value of $3.56/kg. Source: NW_LS255 National Direct Delivered Feeder Pig Report; average price from 2014-2018.
[a,b,c,d]Letters indicate differences ($P \leq 0.05$) within a row

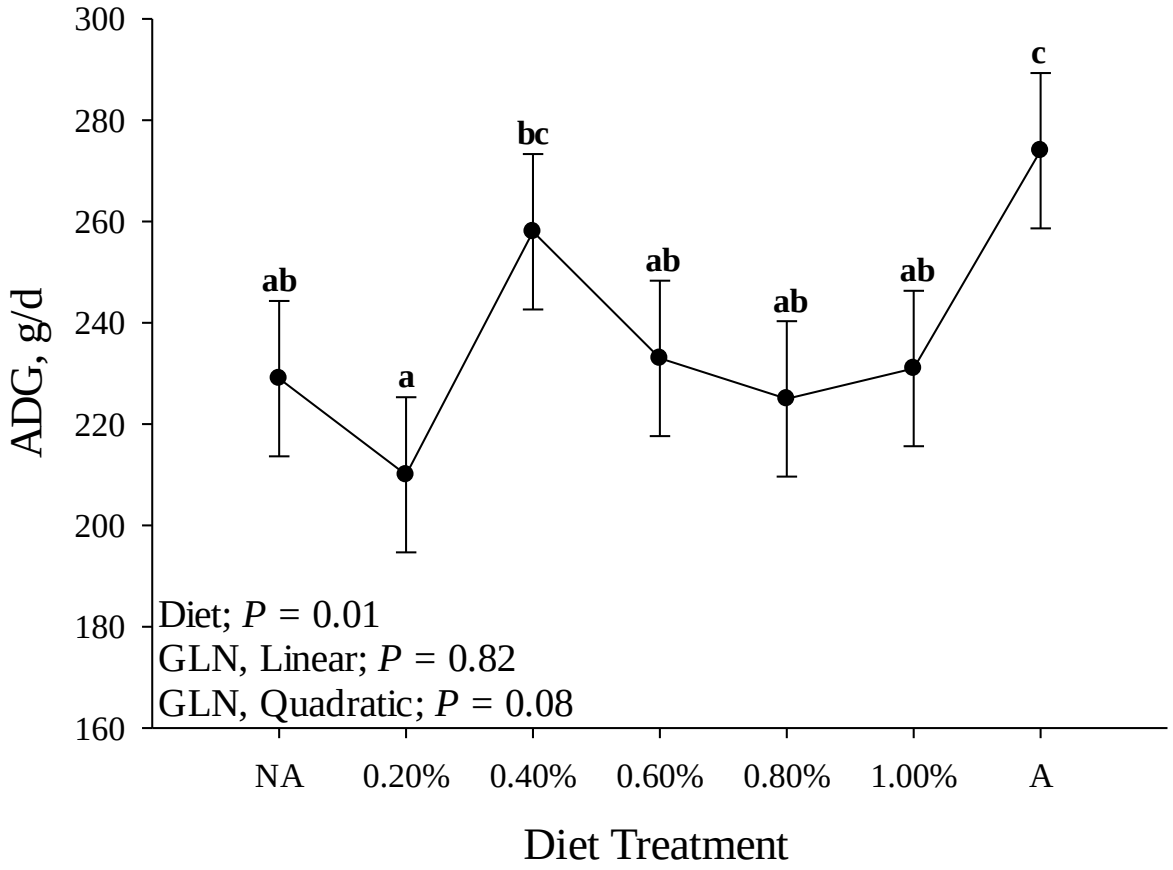

Figure 4.1 The effects of dietary treatment on ADG from d 0 to 14 postweaning. Error bars indicate ± 1 SE. [a,b]Letters indicate differences between treatments ($P \leq 0.05$).

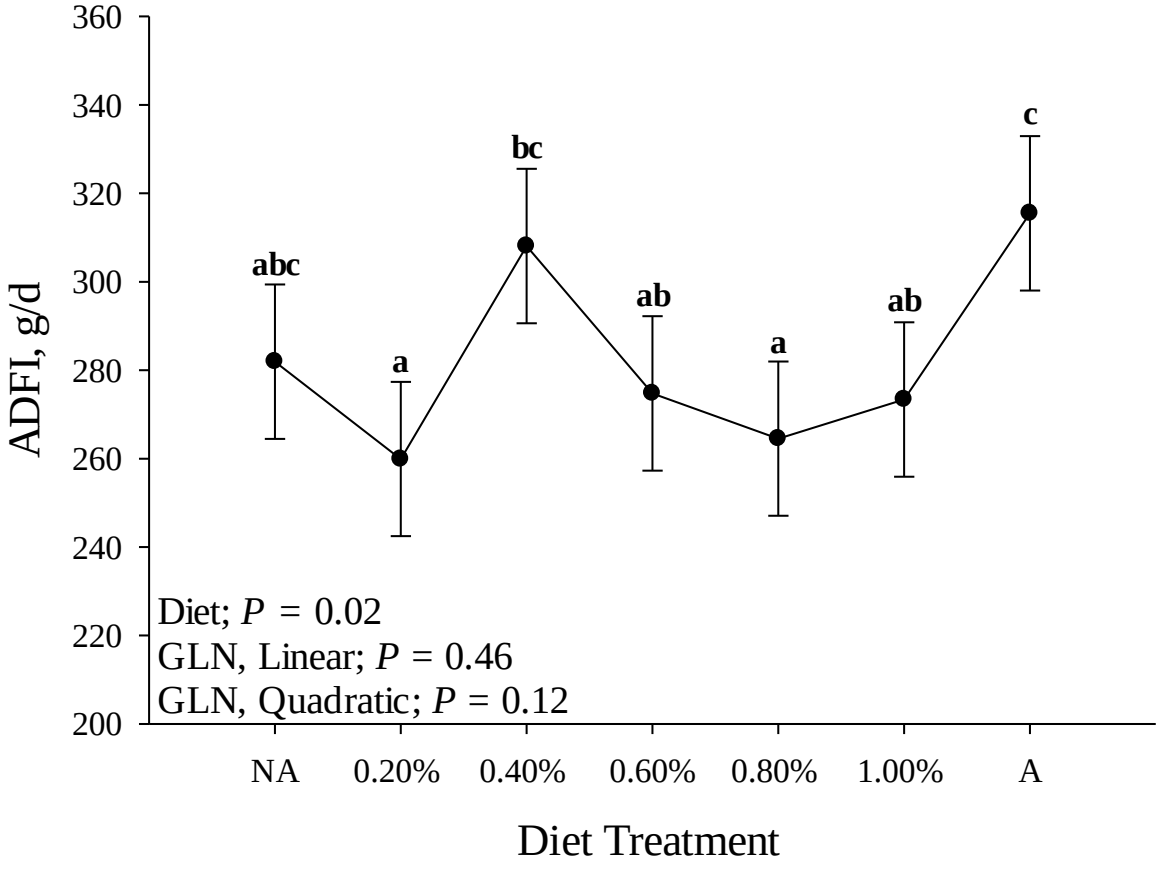

Figure 4.2 The effects of dietary treatment on ADFI from d 0 to 14 postweaning. Error bars indicate ± 1 SE. [a,b]Letters indicate differences between treatments ($P \leq 0.05$).

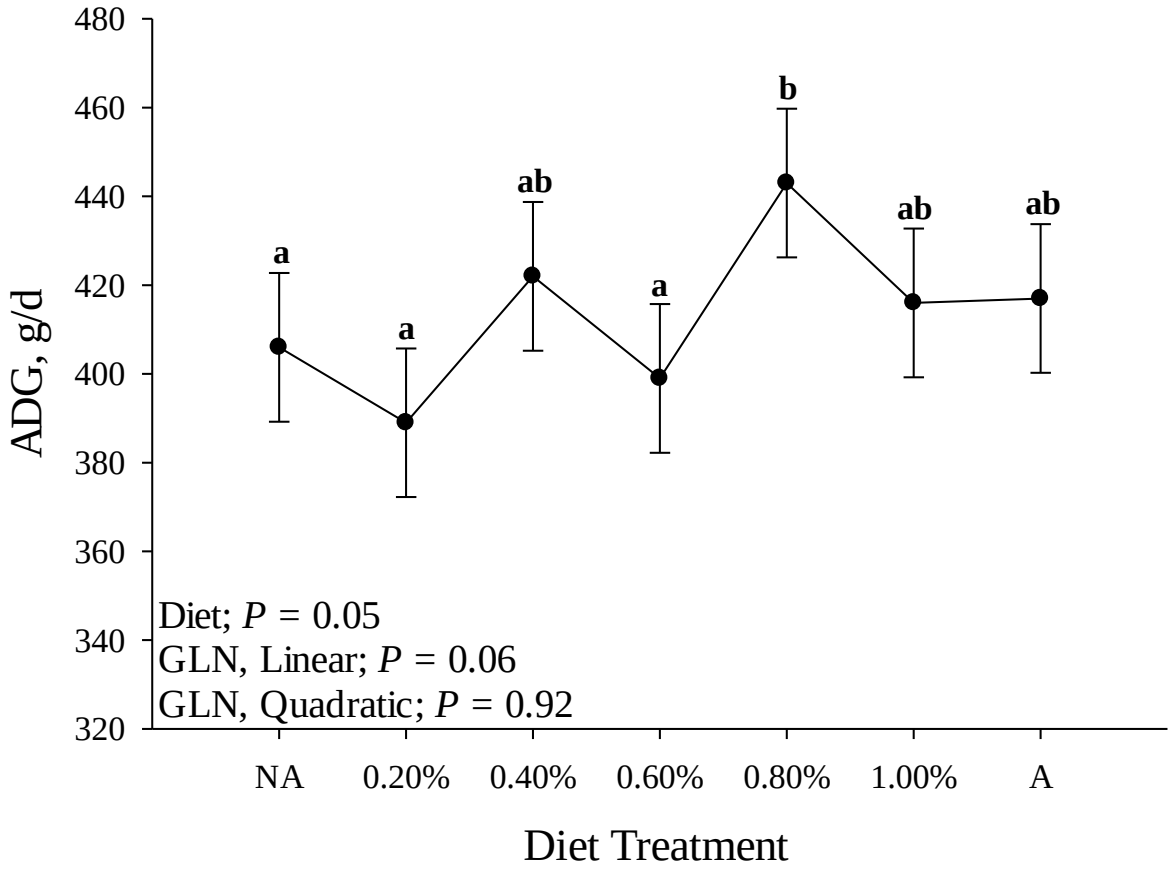

Figure 4.3 The effects of dietary treatment on ADG from d 14 to 35 postweaning. Error bars indicate ± 1 SE. [a,b]Letters indicate differences between treatments ($P \leq 0.05$).

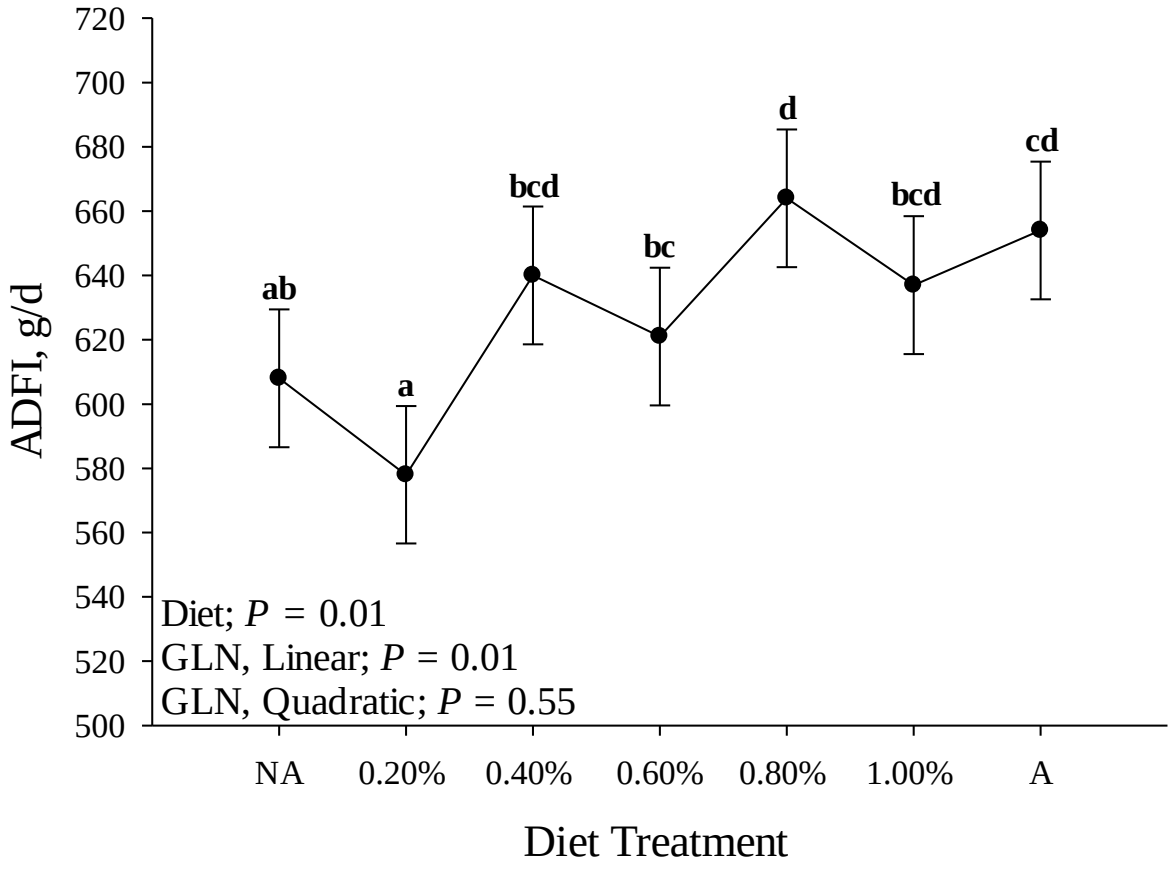

Figure 4.4 The effects of dietary treatment on ADFI from d 14 to 35 postweaning. Error bars indicate ± 1 SE. [a,b]Letters indicate differences between treatments ($P \leq 0.05$).

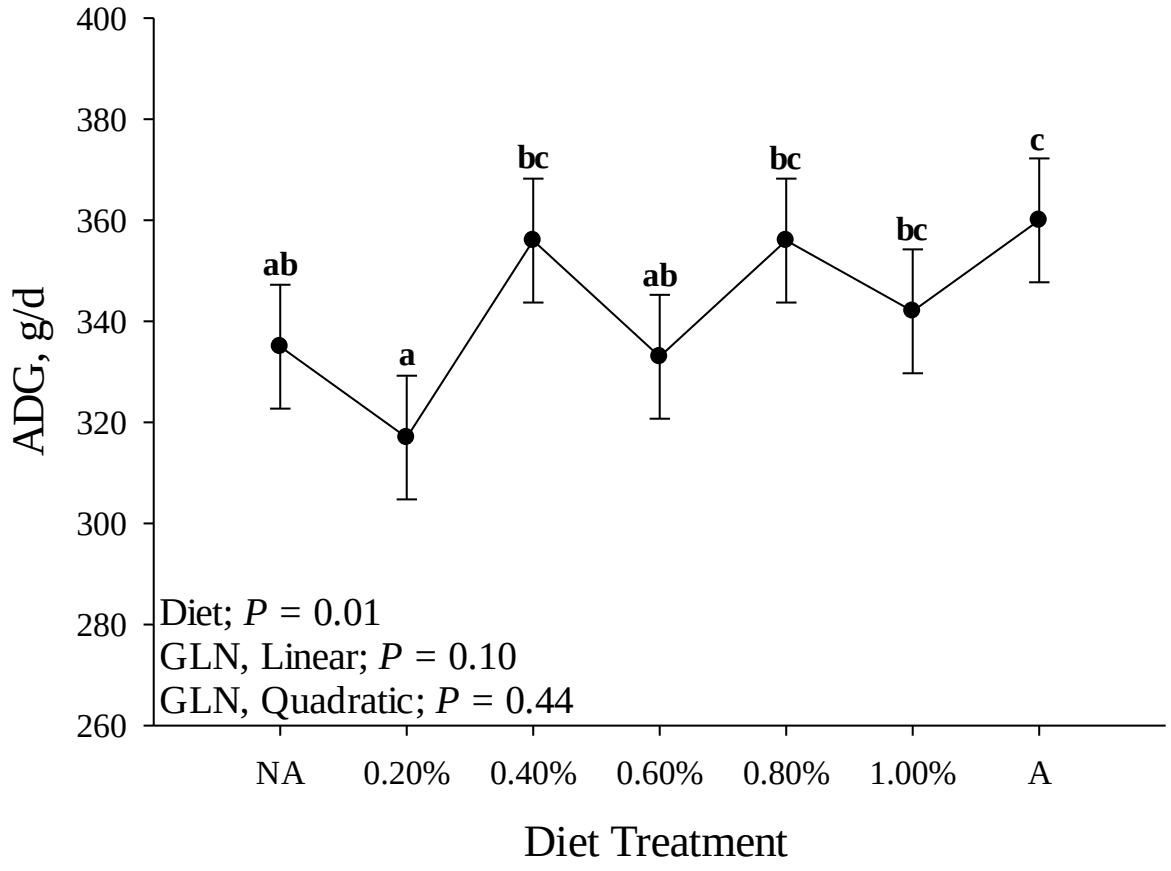

Figure 4.5 The effects of dietary treatment on ADG from d 0 to 35 postweaning. Error bars indicate ± 1 SE. [a,b]Letters indicate differences between treatments ($P \leq 0.05$).

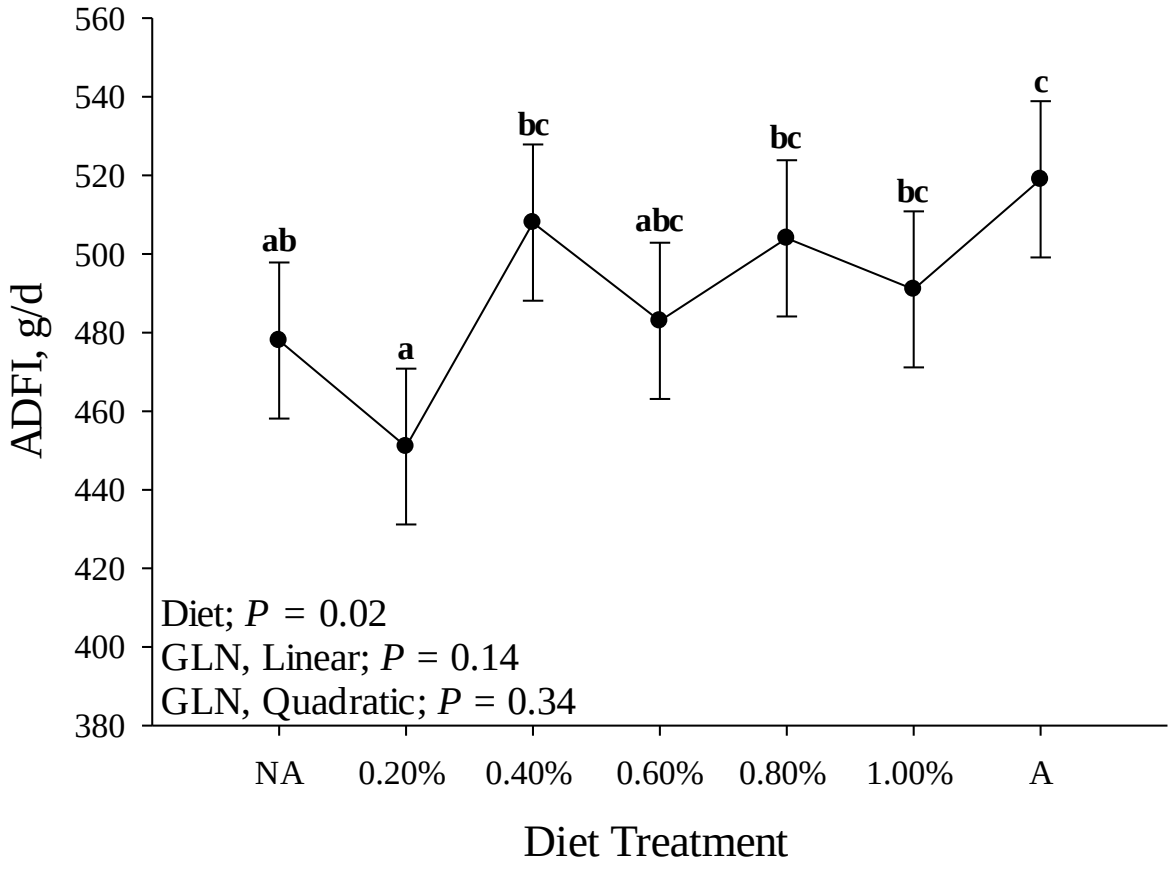

Figure 4.6 The effects of dietary treatment on ADFI from d 0 to 35 postweaning. Error bars indicate ± 1 SE. [a,b]Letters indicate differences between treatments ($P \leq 0.05$).

CHAPTER 5. THE PREDICTED GROWTH PERFORMANCE, TISSUE ACCRETION RATES, NUTRITIONAL REQUIREMENTS AND PRODUCTION COST DIFFERENCES OF PIGS REARED IN DIFFERENT REPLICATE SEASONS

5.1 Abstract

Health challenges in swine herds negatively impact swine growth rate and performance. Therefore, the objective was to quantify the impact of differences in rearing conditions through post hoc analysis on growth performance, tissue accretion rates, and production economics in pigs during different replicates (summer or spring). We hypothesized that pigs reared under health challenged conditions would have decreased growth performance and tissue accretion rates resulting in increased production costs compared to pigs reared with less health challenges. Mixed sex pigs (N = 480; 5.62 ± 0.06 kg BW) were weaned and transported approximately 12 h at 18.4 ± 0.2 d of age in central Indiana. The trial was conducted in two replicates; summer replicate (July 2016 to January 2017) and spring replicate (April 2017 to September 2017). Pigs were blocked by BW and allotted to 1 of 3 dietary treatments [n = 10 pens/dietary treatment/replicate (8 pigs/pen)]; antibiotics [**A**; chlortetracycline (441 ppm) + tiamulin (38.6 ppm)], no antibiotics (**NA**), or 0.20% L-glutamine (**GLN**) fed for the first 14 d. On d 15 to 34 and d 0 to 125 during the grow-finish period, pigs were provided common NA diets. The listing of the dietary treatments is for descriptive purposes and will not be discussed. Data were analyzed using PROC MIXED and PROC NLMIXED in SAS 9.4. Therapeutic injectable antibiotics cost was reduced (P = 0.01; 246.7%) and income over feed and therapeutic injectable antibiotics cost was greater (P = 0.01; 23.1%) in the spring replicate compared to the summer replicate during the grow-finish phase. Predicted ADG was greater ($P \leq 0.05$) in spring replicate barrows compared to the summer replicate barrows during the ranges of 22 to 38 and 119 to 177 days of age, respectively. Spring

replicate gilts had greater ADG ($P \leq 0.05$) compared to summer replicate gilts during the ranges of 22 to 47 and 112 to 177 days of age, respectively. The maximum empty body protein accretion rate for the summer replicate gilts and the spring replicate gilts was 145 and 156 g/d, respectively. In conclusion, the spring replicate, which had improved health through reduced antibiotic costs, had greater growth performance, protein accretion rates, and improved income over feed and antibiotic costs compared to the health challenged summer replicate. These data indicate that health differences between groups of pigs can have profound impact on growth performance, tissue accretion rates, and production costs.

Keywords: growth, health, modeling, pigs, season

5.2 Introduction

Swine producers work to provide pigs with good environmental conditions so that they have the opportunity to perform as close to their genetic potential as possible while balancing facility costs with productivity (Wastell et al., 2018). However, the conditions under which pigs are reared can be compromised by health challenges in swine herds and result in reduced growth performance and increased production costs (Williams et al., 1997c; Haden et al., 2012). For example, it is estimated that Porcine Reproductive and Respiratory Syndrome (**PRRS**), alone, costs the U.S. swine industry $664 million per year due to productivity losses (Holtkamp et al., 2013), and swine influenza virus infection coupled with *Mycoplasma hyopneumoniae* (**M. hyo**) infection has been estimated to cost $10.12/pig (Haden et al., 2012). To further complicate disease challenges, following viral respiratory disease, secondary bacterial infections often follow (Brockmeier et al., 2002). Despite the use of therapeutic antibiotics, when animals become sick many negative effects can occur including: decreased growth performance, increased mortality and morbidity, increased medication costs, and the loss of revenue through the sale of lighter BW

pigs (Heo et al., 2013; Schweer et al., 2017; Cornelison et al., 2018). Therefore, it is imperative that pork producers take all reasonable precautions to ensure the health of their herd.

In a previous study, Duttlinger et al. (2019) determined that spring weaned pigs had increased growth performance, increased final BW, and decreased therapeutic antibiotic usage compared to summer weaned pigs. Although these research findings in performance and reduced antibiotic usage are intriguing, the performance differences between pigs weaned in these seasons are not fully understood. Therefore, the study objective was to quantify, through post hoc analysis, the impact of weaning season on growth performance, tissue accretion rates, nutritional requirements and production economics in pigs weaned during different seasonal replicates (summer or spring). We hypothesized that summer weaned pigs would have decreased growth performance and tissue accretion rates resulting in increased production costs compared to spring weaned pigs.

5.3 Materials and Methods

5.3.1 General

All procedures involving animal use were approved by the Purdue University Animal Care and Use Committee (protocol #1603001385), and animal care and use standards were based upon the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (Federation of Animal Science Societies, 2010). All live animal methods have been previously described in great detail by Duttlinger et al. (2019). Briefly, crossbred pigs consisting of barrows and gilts [N = 480; 5.62 ± 0.06 kg initial BW; Duroc x (Landrace x Yorkshire)] were weaned and transported approximately 12 h at 18.4 ± 0.2 d of age in central Indiana. The trial was conducted in two replicates; summer replicate (from July 2016 to January 2017) and spring replicate (from April 2017 to September 2017 with identical genetics). The terms summer replicate and spring replicate

refer only to the time of year in which the pigs were weaned and transported. On the day prior to weaning and transport, pigs were weighed individually, blocked by BW, and randomly allotted to pens (initially 8 pigs/pen), and pens of pigs within BW blocks were allotted to 1 of 3 dietary treatments with 10 pens per dietary treatment per replicate season (n = 30 pens/replicate). Dietary treatments were antibiotics [**A**; chlortetracycline (441 ppm) + tiamulin (38.6 ppm)], no antibiotics (**NA**), or 0.20% L-glutamine (**GLN**) from d 0 to 14 post-weaning and transport followed by NA diets fed to all pigs from d 14 post-weaning to the end of the study (d 159 post-weaning). The listing of the dietary treatments is for descriptive purposes only to provide clarity; this paper will only focus on replicate differences as dietary treatment differences are described in great detail by Duttlinger et al. (2019).

5.3.2 Nursery Phase

The nursery room where the trial was conducted and the research procedures during the initial 34 d of the trial was conducted was previously described by Duttlinger et al. (2019). In brief, pigs were placed in their assigned pens, following weaning and transport, and fed their respective dietary treatments from d 0 to 14 in 2 phases (Table 5.1). Following the dietary treatment period, all pigs were provided common antibiotic free diets from d 14 to 34 (end of the nursery phase; Table 5.1). During the nursery phase, corn-soybean meal-based diets were fed in 4 phases in meal form and were formulated to meet or exceed nutrient requirements (NRC, 2012; Table 5.1). Feeders and pigs were individually weighed every 7 d to determine the response criteria of ADG, ADFI, and G:F during the nursery phase.

5.3.3 Grow-Finish Phase

On day 34, all pigs were moved to the grow-finish facility for the remainder of the trial and pen integrity was maintained. The same grow-finish facility was used for both summer and spring

replicate. Common NA diets were corn-soybean meal-DDGS-based diets provided in meal form to meet or exceed nutrient requirements (NRC, 2012) in six phases during the grow-finish period (Table 5.2). Pigs and feeders were weighed every 21 d during the grow-finish period to determine the response criteria of ADG, ADFI, and G:F. Serial ultrasound scanning was performed on 2 pigs per pen (1 barrow and 1 gilt) every 21 days beginning 21 d post-placement in the grow-finish facility to determine backfat (**BF**) depth and loin eye area (**LEA**). The ultrasound image was taken approximately at the 10th rib perpendicular to the spine using an Aloka model 500V (Aloka Co., Ltd., Tokyo, Japan) ultrasound fitted with an Aloka 3.5 MHz (Aloka Co., Ltd., Tokyo, Japan) probe. ImageJ software (NIH, Bethesda, MD) was used to measure backfat depth and longissimus area. The grow-finish facility where the final 125 d of the trial was conducted was previously described by Duttlinger et al. (2019).

5.3.4 Economic Analysis

A post-hoc economic analysis of the growth performance differences observed was conducted. The average prices were determined from price reports from 2014 to 2018. Revenue was determined using the average of the reported price of feeder pigs, 18.1 kg BW basis, (USDA-Iowa Dept of Ag Market News, 2018). To calculate the feed cost, the average corn price ($0.1427/kg) as reported by USDA National Agricultural Statistic Service (2018) was utilized, the soybean meal price ($0.3977/kg) used was calculated as the average weekly price of central Illinois according to USDA Market News (2018), and the dried distillers grains with solubles price ($0.1500/kg) used was the average price for the eastern corn-belt as reported by USDA Agricultural Marketing Service (2018). The additional ingredient costs were provided from a major U.S. swine nutrition company. Income over the variable of interest was calculated using equations previously described by Menegat et al. (2019).

Antibiotic cost (injectable and water administered) was calculated using the listed antibiotic price on a veterinary supply distributor's website on May 2, 2019 (Valley Vet Supply, Marysville, KS, USA). For injectable antibiotics, the antibiotic price was then multiplied by the antibiotic volume administered to determine the antibiotic cost per injection. For water administered antibiotics, water intake was estimated using 2.64 water:feed, kg:kg as reported by Brumm et al. (2000) to calculate the volume of antibiotic consumed when delivered via the waterers.

5.3.5 Statistics

Statistical analyses were performed as previously described (Duttlinger et al., 2019). Briefly, data were analyzed as a randomized complete block design using the PROC MIXED procedure in SAS 9.4 (SAS Institute INC., Cary, NC). The assumptions of normality of error, homogeneity of variance, and linearity were confirmed post-hoc. Pen was the experimental unit and fixed effects included season replicate with BW block included as a random effect. Statistical significance was defined as $P \leq 0.05$ and a tendency was defined as $0.05 < P \leq 0.10$.

5.3.5.1 Body Weight Growth Function

The BW growth function was performed as previously described (Schinckel et al., 2009a). Briefly, the BW data were fit to a generalized Michaelis - Menten (GMM) equation (Lopez et al., 2000). The equation has two alternative forms, $WT_{i,t} = [(WT_o\ K^C) + (\ WF\ t^C)]/(K^C + t^C)$ or $WT_{i,t} = WT_o + [(WF- WT_o)\ (t/K)^C)]/[1 + (t/K)^C)]$ where WF is mean mature BW, WT_o is the mean birth BW, t is days of age, K is a parameter equal to the days of age in which one-half WF is achieved and C is a unit less parameter related to changes in proportional growth and shape of the growth curves (Lopez et al., 2000; Schinckel et al., 2009a). Actual birth BW was unknown, therefore in this function, each pig's birth BW (WT_{io}) was estimated using the following equation: 1.6 kg +

0.5(weaning weight (kg) – mean weaning weight (kg)) (Schinckel et al., 2009a). The function was then evaluated using the PROC NLMIXED procedure in SAS 9.4 (SAS Institute INC., Cary, NC). The model included the fixed effect of season replicate and sex. Daily live weight gain (ADG) was determined as the derivation of the BW function on time (ADG = ∂WT / ∂T) (Schinckel et al., 2009a). To test for differences between predicted models, statistics were calculated using a student t-test with the predicted variation between animals in each subgroup.

5.3.5.2 Average Daily Feed Intake Function

The ADFI function was performed as previously described (Schinckel et al., 2009c). Briefly, the following nonlinear function model was used to describe the relationship of ADFI to BW or age, $ADFI_{i,\,x} = C\,(1 - \exp(-Kx^{A})) + e_{i,\,t}$, (Bridges et al., 1986 and Schinckel et al., 2009c). Here x is the age (d) or BW (kg) of the i^{th} animal, C is the average mature ADFI, *K* is the exponential growth decay constant and C is the kinetic order constant. Since the exponential decay parameter was close to zero, the model was reparamaterized ($K' = \log K$)) with the form, $ADFI_{i,\,x} = WF\,(1 - \exp(-\exp(K')x^{A}))$ (Schinckel et al., 2009c). In this function, the BW and age in which the rate in which ADFI changes from being increasing to decreasing relative to BW or age is called the inflection point (IP) and is equal to ADFI times F where F = 1- exp((1/A) – 1). The age at the $IP = (A\,M / (A-1))^{(-1/A)}$ (Schinckel et al., 2009c).

The ADFI data is shown only by replicate as feed intake data was collected on a pen basis and pens were mixed sex. The feed intake data including the values of each ADFI function parameter, IP variables and other predicted or actual values were fit to a mixed model using the PROC NLMIXED procedure in SAS 9.4 (SAS Institute INC., Cary, NC). The model included the fixed effect of season replicate. To test for differences between predicted models, statistics were calculated using a student t-test with the predicted variation between animals in each subgroup.

5.3.5.3 Gain:Feed Function

The G:F predictive model was calculated using the ADG and ADFI data that was produced from the functions outlined above. Average daily gain was divided by ADFI for a given day or BW to produce the G:F curves based on age and BW, respectively.

5.3.5.4 Backfat and Loin Eye Area Functions

The BF and LEA functions were performed as previously described (Schinckel et al., 2009b). Briefly, the serial ultrasonic backfat depth and loin eye area measurements were fit to alternative functions of BW using the NLMIXED procedure of SAS (SAS Institute Inc., Cary, NC). The functions included: allometric function ($Y = A\ BW^B$) and mixed model allometric function. A mixed model allometric function has the form of $Y = (A + a_i)\ BW^B$, where a_i is a pig specific random effect with variance σ_a^2 (Schinckel et al., 2009b). The alternative mixed models were evaluated based upon AIC values. The R^2 values were calculated as the squared correlations of the predicted and actual observations. The RSD was calculated with the equation

$$RSD = [\sum_{t=1}^{T}\sum_{i=1}^{I}\left(e_{i,t}\right)^2/(n-p)]^{1/2}$$

where $e_{i,t}$ is the residual value of the i^{th} pig at age t, n is the number of observations, and p is the number of parameters in the model (Schinckel et al., 2009b). Based upon AIC and R^2 values the mixed model allometric function provided the best fit to the serial ultrasonic BF and LEA data. Ultrasonic BF and LEA were predicted for each pig at five BW's (46.7, 64.6, 83.5, 102.5 and 121.5 kg BW) by the inclusion of each pig's random effect (a_i) in the sex-specific exponential function. The predicted values of BF and LEA were fit to a model including the fixed effects of season replicate.

5.3.5.5 Body Component Functions

Equations that utilized BW, BF, and LEA were used to predict empty body protein mass (**EBP**, kg), empty body lipid mass (**EBL**, kg), carcass fat-free lean mass (**FFLN**, lg), and total carcass fat mass (**TOTFAT**, kg). Different equations were used to predict body components at different weight ranges: 18 to 23, 23 to 32, 32 to 40, 40 to 54, 54 to 79, 79 to 100, 100 to 132, and 132 kg and up. The equations were developed from a previous trial in which pigs were serially scanned and harvested (Thompson et al., 1996 and Wagner et al., 1999). Data were fit to allometric ($EBP = aX^b$), augmented allometric [$EBP = aX^b(700 - X)^c$], and exponential ($EBL = \exp(b_0+b_1x + b_2x^2 + b_3x^3)$) functions ($x$) of BW (Wagner et al., 1999) to predict body components. The b_3 coefficient of the exponential function was deleted if $P > 0.10$. Exponential functions minimized the RSD values; as a result, these equations were used for all curves. Daily body component accretion rates were determined as the product of the derivatives of two functions by $\partial C/\partial T = ((\partial C/\partial BW) \; x \; (\partial BW/\partial T))$, (Schinckel and De Lange 1996), where C is the body component mass.

5.3.5.6 Daily Net Energy Intake

Daily NE intake was predicted as NE intake, Kcal/d = ($0.179BW^{0.60}$ + (5.6863 × Empty body protein accretion, kg/d) + (9.509 × Empty body lipid accretion, kg/d)) × 1,000, where $0.179BW^{0.60}$ is the NE required for maintenance (Noblet et al., 1999), and 5.6863 and 9.509 are the energy content of empty body protein accretion and empty body lipid accretion, respectively (Noblet et al., 1999).

5.3.5.7 Net Energy Maintenance Requirement

Net energy required for maintenance was predicted as $NE_{maintenance} = NE_{intake} - (NE_{protein} + NE_{lipid})$, where $NE_{intake} = NE_{diet} \times ADFI_{prediction}$; $NE_{protein}$ = (5.6863 × Empty body protein accretion, g/d) and NE_{lipid} = (9.509 × Empty body lipid accretion, g/d) (Noblet et al., 1999).

5.3.5.8 Standardized Ileal Digestible Lysine Requirement

The standardized ileal digestible (**SID**) lysine requirement was estimated as SID lysine (g/d) = gastrointestinal tract (**GIT**) losses, g/d + integument losses, g/d/{0.75 + [0.0002 × (maximum empty body protein accretion − 147.7)]}, where maximum empty body protein accretion is an estimate of the maximal protein deposition (g/d) of the pig (NRC, 2012). The GIT losses were calculated as: basal endogenous GIT lysine losses (g/d) = feed intake (g/d) × (0.417/1,000) × 0.88 × 1.1, where 0.88 accounts for the dry matter content of the feed, and the 1.1 accounts for the 10% of GIT losses, which occur in the large intestine relative to those recovered at the ileum (NRC, 2012). Feed intake for each sex within replicate was estimated as follows: $ADFI_{barrows} = 1.03 \times ADFI_{replicate}$ and $ADFI_{gilt} = 0.97 \times ADFI_{replicate}$. The integument losses are predicted as follows: integument losses (g/d) = $0.0045 \times (BW, kg)^{0.75}$ (NRC, 2012).

5.3.5.9 Standardized Ileal Digestible Lysine Requirement:Net Energy Intake

The SID Lysine:NE (g/Mcal) was predicted using the SID lysine (g/d) and NE intake (Mcal/d = kcal/d × 1,000) data that were calculated from the predictive equations outlined above. Standardized ileal digestible lysine was divided by NE intake for a given BW to produce the SID Lysine:NE curve based on BW.

5.3.5.10 Standardized Ileal Digestible Lysine Requirement as Percent of Diet

The SID lysine requirement as percent of diet was predicted using the SID Lysine:NE (g/Mcal) that was calculated from the predictive equation outlined above. Standardized ileal digestible lysine:NE (g/Mcal) was multiplied by $Diet_{NE}$ for a given dietary phase (Mcal/g of diet) multiplied by 100 to produce the SID lysine requirement as percent of diet curve based on BW.

5.4 Results

5.4.1 Closeout Data

The closeout information is presented as a percent of population within replicate for descriptive purposes only as a characterization of health differences between replicates (Table 5.3). The full value pig percentage was 82.29% and 92.00% during the summer and spring replicates, respectively (Table 5.3). The percentage of lightweight culls/morbidity was 7.43% and 2.86% during the summer and spring replicates, respectively (Table 5.3). The mortality percentage was 9.14% and 2.86% during the summer and spring replicates, respectively (Table 5.3). The percentage of culls for navel hernias was 1.14% and 2.29% during the summer and spring replicates, respectively (Table 5.3).

5.4.2 Economics

5.4.2.1 Nursery Period

Income over nursery feed cost was greater (P = 0.05; 3.0%) in the spring replicate compared to the summer replicate (Table 5.4). Therapeutic injectable antibiotic costs for enteric and unthrifty challenges was reduced (P = 0.01; 500.0%) in the spring replicate compared to the summer replicate (Table 5.4). Income over feed and therapeutic injectable antibiotics cost for enteric and unthrifty challenges was greater (P = 0.04; 3.3%) in the spring replicate compared to the summer replicate (Table 5.4). Income over total feed and all therapeutic injectable antibiotics cost was greater (P = 0.05; 3.0%) in the spring replicate compared to the summer replicate (Table 5.4). No nursery period revenue, diet cost, or therapeutic antibiotics cost differences were observed (P > 0.11) between replicates (Table 5.4).

5.4.2.2 Grow-finish Period

Grow-finish revenue was greater ($P = 0.01$; 11.0%) as were diet cost ($P = 0.03$; 3.4%) and income over feed cost ($P = 0.01$; 16.8%) in the spring replicate compared to the summer replicate (Table 5.4). Therapeutic injectable antibiotics cost was reduced ($P = 0.01$; 246.7%) in the spring replicate compared to the summer replicate (Table 5.4). Income over feed and therapeutic injectable antibiotics cost was greater ($P = 0.01$; 23.1%) in the spring replicate compared to the summer replicate (Table 5.4). Income over feed and therapeutic injectable and water-delivered antibiotics cost (**IOFAC**) for enteric and unthrifty challenges was greater ($P = 0.01$; 24.5%) in the spring replicate compared to the summer replicate (Table 5.4). No therapeutic water-delivered antibiotics cost differences were observed ($P > 0.45$) between replicates (Table 5.4).

5.4.3 Predicted Functions

5.4.3.1 Body Weight

The predicted BW curves based on days of age for replicate and sex increased as days of age increased (Figure 5.1). Predicted BW was greater ($P \leq 0.05$) in spring replicate barrows compared to the summer replicate barrows during the ranges of 24 to 52 and 160 to 177 days of age, respectively (Table 5.5; Figure 5.1). Spring replicate gilts had greater BW ($P \leq 0.05$) compared to summer replicate gilts during the ranges of 22 to 76 and 137 to 177 days of age, respectively (Table 5.5; Figure 5.1). Predicted BW was greater ($P \leq 0.05$) in spring replicate gilts compared to the summer replicate barrows during the ranges of 23 to 65 and 166 to 177 days of age, respectively (Table 5.5; Figure 5.1). Spring replicate barrows had greater BW ($P \leq 0.05$) compared to summer replicate gilts during the ranges of 23 to 60 and 137 to 177 days of age, respectively (Table 5.5; Figure 5.1). No other predicted BW differences were detected ($P > 0.05$) with any comparison (Table 5.5; Figure 5.1).

5.4.3.2 Average Daily Gain

The predicted ADG curve for summer replicate barrows increased as BW (Figure 5.2) and days of age (Figure 5.3) increased until 70.5 kg and 120 days of age, respectively, and then decreased until the end of the study. The predicted ADG curve for summer replicate gilts increased as BW (Figure 5.2) and days of age (Figure 5.3) increased until 67.8 kg and 117 days of age, respectively, and then decreased until the end of the study. The predicted ADG curve for spring replicate barrows increased as BW (Figure 5.2) and days of age (Figure 5.3) increased until the end of the study at 118.6 kg and 177 days of age, respectively. The predicted ADG curve for spring replicate gilts increased as BW (Figure 5.2) and days of age (Figure 5.3) increased until 113.9 kg and 171 days of age, respectively, and then decreased until the end of the study.

Predicted ADG was greater ($P \leq 0.05$) in spring replicate barrows compared to the summer replicate barrows during the ranges of 22 to 38 and 119 to 177 days of age, respectively (Table 5.5; Figure 5.3). However, spring replicate barrows had decreased ADG ($P \leq 0.05$) compared to summer replicate barrows during the range of 59 to 90 days of age (Table 5.5; Figure 5.3). Spring replicate gilts had greater ADG ($P \leq 0.05$) compared to summer replicate gilts during the ranges of 22 to 47 and 112 to 177 days of age, respectively (Table 5.5; Figure 5.3). Predicted ADG was greater ($P \leq 0.05$) in spring replicate gilts compared to the summer replicate barrows during the ranges of 22 to 44 and 126 to 177 days of age, respectively (Table 5.5; Figure 5.3). However, spring replicate gilts had decreased ADG ($P \leq 0.05$) compared to summer replicate barrows during the range of 71 to 90 days of age (Table 5.5; Figure 5.3). Spring replicate barrows had greater ADG ($P \leq 0.05$) compared to summer replicate gilts during the ranges of 22 to 41 and 107 to 177 days of age, respectively (Table 5.5; Figure 5.3). No other predicted ADG differences were detected ($P > 0.05$) with any comparison (Table 5.5; Figure 5.3).

5.4.3.3 Average Daily Feed Intake

The predicted ADFI based on BW (Figure 5.4) and days of age (Figure 5.5) for each season replicate increased as BW and days of age increased, respectively. Predicted ADFI was greater ($P \leq 0.05$) in spring replicate compared to the summer replicate during the ranges of 21 to 46 and 133 to 177 days of age, respectively (Table 5.5; Figure 5.5). However, spring replicate had decreased ADFI ($P \leq 0.05$) compared to summer replicate during the range of 57 to 111 days of age (Table 5.5; Figure 5.5). No other predicted ADFI differences were detected ($P > 0.05$) with any comparison (Table 5.5; Figure 5.5).

5.4.3.4 Gain:Feed

The predicted G:F curves for the summer replicate decreased as BW (Figure 5.6) and days of age (Figure 5.7) increased with the poorest G:F of 0.26 at 116.4 kg of BW and 177 days of age, respectively. The predicted G:F curves for the spring replicate decreased as BW (Figure 5.6) and days of age (Figure 5.7) increased with the poorest G:F of 0.31 at 125.8 kg of BW and 177 days of age, respectively.

5.4.3.5 Loin Eye Area

The predicted loin eye area for the summer replicate barrows increased as BW increased with the greatest loin eye area of 42.7 cm^2 at 118.6 kg of BW (Figure 5.8). The predicted loin eye area for the summer replicate gilts increased as BW increased with the greatest loin eye area of 41.9 cm^2 at 114.2 kg of BW (Figure 5.8). The predicted loin eye area for the spring replicate barrows increased as BW increased with the greatest loin eye area of 45.1 cm^2 at 126.4 kg of BW (Figure 5.8). The predicted loin eye area for the spring replicate gilts increased as BW increased with the greatest loin eye area of 45.3 cm^2 at 125.1 kg of BW (Figure 5.8).

5.4.3.6 Backfat Depth

The predicted backfat depth for the summer replicate barrows increased as BW increased with the greatest backfat depth of 1.94 cm at 118.6 kg of BW (Figure 5.9). The predicted backfat depth for the summer replicate gilts increased as BW increased with the greatest backfat depth of 1.58 cm at 114.2 kg of BW (Figure 5.9). The predicted backfat depth for the spring replicate barrows increased as BW increased with the greatest backfat depth of 1.99 cm at 126.4 kg of BW (Figure 5.9). The predicted backfat depth for the spring replicate gilts increased as BW increased with the greatest backfat depth of 1.73 cm at 125.1 kg of BW (Figure 5.9).

5.4.3.7 Empty Body Protein Accretion

Predicted EBP accretion rates for summer replicate barrows increased as BW increased until 61.6 kg of BW with a maximum EBP accretion rate of 157 g/d and then EBP accretion decreased until the end of the study where the EBP accretion rate was 93 g/d at 118.6 kg (Figure 5.10). Predicted EBP accretion rates for summer replicate gilts increased as BW increased until 64.3 kg of BW with a maximum EBP accretion rate of 145 g/d and then EBP accretion decreased until the end of the study where the EBP accretion rate was 99 g/d at 114.2 kg (Figure 5.10). Predicted EBP accretion rates for spring replicate barrows increased as BW increased until an inflection point at 67.1 kg of BW with a maximum EBP accretion rate of 160 g/d (Figure 5.10). Predicted EBP accretion rates for spring replicate barrows then decreased as BW increased until an inflection point at 117.2 kg of BW with a EBP accretion rate of 124 g/d (Figure 5.10). Then, the predicted EBP gain rates for spring replicate barrows increased as BW increased until the end of the study at 126.4 kg of BW with a maximum EBP gain rate of 128 g/d (Figure 5.10). Predicted EBP accretion rates for spring replicate gilts increased as BW increased until 76.9 kg of BW with

a maximum EBP accretion rate of 156 g/d and then EBP accretion decreased until the end of the study where the EBP accretion rate was 134 g/d at 125.1 kg (Figure 5.10).

5.4.3.8 Empty Body Lipid Accretion

Predicted EBL accretion rates for summer replicate barrows increased as BW increased with a maximum EBL accretion rate of 401 g/d at 118.6 kg of BW (Figure 5.11). Predicted EBL accretion rates for summer replicate gilts increased as BW increased with a maximum EBL accretion rate of 316 g/d at 114.2 kg of BW (Figure 5.11). Predicted EBL accretion rates for spring replicate barrows increased as BW increased with a maximum EBL accretion rate of 593 g/d at 126.4 kg of BW (Figure 5.11). Predicted EBL accretion rates for spring replicate gilts increased as BW increased with a maximum EBL accretion rate of 435 g/d at 125.1 kg of BW (Figure 5.11).

5.4.3.9 Carcass Fat-free Lean Gain

The predicted FFLN gain curve for summer replicate barrows increased as BW increased until 61.6 kg of BW with a maximum FFLN gain rate of 389 g/d and then FFLN gain decreased until the end of the study where the FFLN gain rate was 246 g/d at 118.6 kg (Figure 5.12). The predicted FFLN gain curve for summer replicate gilts increased as BW increased until 61.7 kg of BW with a maximum FFLN gain rate of 362 g/d and then FFLN gain decreased until the end of the study where the FFLN gain rate was 274 g/d at 114.2 kg (Figure 5.12). The predicted FFLN gain for spring replicate barrows increased as BW increased until an inflection point at 69.9 kg of BW with a maximum FFLN gain rate of 396 g/d (Figure 5.12). The predicted FFLN gain for spring replicate barrows, then, decreased as BW increased until an inflection point at 104.0 kg of BW with a minimum FFLN gain rate of 365 g/d (Figure 5.12). Then, the predicted FFLN gain curve for spring replicate barrows increased as BW increased until the end of the study at 126.4 kg of BW with a maximum FFLN gain rate of 422 g/d (Figure 5.12). The predicted FFLN gain curve

for spring replicate gilts increased as BW increased until 80.6 kg of BW with a maximum FFLN gain rate of 399 g/d and then FFLN gain decreased until the end of the study where the FFLN gain rate was 369 g/d at 125.1 kg (Figure 5.12).

5.4.3.10 Total Carcass Fat Percent

The predicted TOTFAT percentages for the summer replicate barrows decreased as BW increased until 38.6 kg of BW with a minimum TOTFAT percentage of 13.7% and then TOTFAT percentage increased as BW increased until the end of the study where the TOTFAT percentage was 30.4% at 118.6 kg (Figure 5.13). The predicted TOTFAT percentages for the summer replicate gilts decreased as BW increased until 49.1 kg of BW with a minimum TOTFAT percentage of 14.3% and then TOTFAT percentage increased as BW increased until the end of the study where the TOTFAT percentage was 28.7% at 114.2 kg (Figure 5.13). The predicted TOTFAT percentages for the spring replicate barrows decreased as BW increased until 47.9 kg of BW with a minimum TOTFAT percentage of 15.1% and then TOTFAT percentage increased as BW increased until the end of the study where the TOTFAT percentage was 31.2% at 126.4 kg (Figure 5.13). The predicted TOTFAT percentages for the spring replicate gilts decreased as BW increased until 51.1 kg of BW with a minimum TOTFAT percentage of 15.3% and then TOTFAT percentage increased as BW increased until the end of the study where the TOTFAT percentage was 29.9% at 125.1 kg (Figure 5.13).

5.4.3.11 Net Energy Intake

The predicted net energy intake curve for summer replicate barrows increased as BW increased with a maximum net energy intake of 7,482 kcal/d at 118.6 kg of BW (Figure 5.14). The predicted net energy intake curve for summer replicate gilts increased as BW increased with a maximum net energy intake of 6,639 kcal/d at 114.2 kg of BW (Figure 5.14). The predicted net

energy intake curve for spring replicate barrows increased as BW increased with a maximum net energy intake of 9,629 kcal/d at 126.4 kg of BW (Figure 5.14). The predicted net energy intake curve for spring replicate gilts increased as BW increased with a maximum net energy intake of 8,139 kcal/d at 125.1 kg of BW (Figure 5.14).

5.4.3.12 Net Energy Intake Required for Maintenance

The predicted $NE_{maintenace}$ intake curve for summer replicate barrows increased as BW increased until 84.7 kg of BW with a maximum $NE_{maintenace}$ intake of 3,934 kcal/d and then, generally, $NE_{maintenace}$ intake decreased as BW increased until the end of the study where the $NE_{maintenace}$ intake was 3,426 kcal/d at 118.6 kg (Figure 5.15). The predicted $NE_{maintenace}$ intake curve for summer replicate gilts increased as BW increased until an inflection point at 82.1 kg of BW with a maximum $NE_{maintenace}$ intake of 3,747 kcal/d (Figure 5.15). The predicted $NE_{maintenace}$ intake curve for summer replicate gilts then remained relatively constant until the end of the study where the $NE_{maintenace}$ intake was 3,747 kcal/d at 114.2 kg (Figure 5.15). The predicted $NE_{maintenace}$ intake curve for spring replicate barrows increased as BW increased until 85.2 kg of BW with a maximum $NE_{maintenace}$ intake of 3,914 kcal/d and then, generally, $NE_{maintenace}$ intake decreased as BW increased until the end of the study where the $NE_{maintenace}$ intake was 2,443 kcal/d at 126.4 kg (Figure 5.15). The predicted $NE_{maintenace}$ intake curve for spring replicate gilts increased as BW increased until 85.3 kg of BW with a maximum $NE_{maintenace}$ intake of 3,547 kcal/d and then, generally, $NE_{maintenace}$ intake decreased slightly as BW increased until the end of the study where the $NE_{maintenace}$ intake was 3,400 kcal/d at 125.1 kg (Figure 5.15).

5.4.3.13 Standardized Ileal Digestible Lysine Requirement

The predicted SID lysine requirement curve for summer replicate barrows increased as BW increased until 65.1 kg of BW with a maximum SID lysine requirement rate of 18.5 g/d and then

the SID lysine requirement decreased until the end of the study where the SID lysine requirement was 12.7 g/d at 118.6 kg (Figure 5.16). The predicted SID lysine requirement curve for summer replicate gilts increased as BW increased until 68.5 kg of BW with a maximum SID lysine requirement rate of 17.2 g/d and then the SID lysine requirement decreased until the end of the study where the SID lysine requirement was 13.3 g/d at 114.2 kg (Figure 5.16). The predicted SID lysine requirement curve for spring replicate barrows increased as BW increased until an inflection point at 71.8 kg of BW with a maximum SID lysine requirement rate of 19.1 g/d (Figure 5.16). The predicted SID lysine requirement curve for spring replicate barrows then decreased as BW increased until an inflection point at 114.2 kg of BW with a SID lysine requirement of 16.5 g/d (Figure 5.16). Then, the predicted SID lysine requirement curve for spring replicate barrows increased as BW increased until the end of the study at 126.4 kg of BW with a SID lysine requirement of 17.3 g/d (Figure 5.16). The predicted SID lysine requirement curve for spring replicate gilts increased as BW increased until 84.4 kg of BW with a maximum SID lysine requirement rate of 19.0 g/d and then the SID lysine requirement decreased slightly until the end of the study where the SID lysine requirement was 18.0 g/d at 125.1 kg (Figure 5.16).

5.4.3.14 Standardized Ileal Digestible Lysine Requirement:Net Energy Intake

The predicted SID lysine:NE curve for summer replicate barrows increased as BW increased until 36.2 kg of BW with a maximum SID lysine:NE of 4.12 g/Mcal and then the SID lysine:NE decreased until the end of the study where the SID lysine:NE was 1.69 g/Mcal at 118.6 kg (Figure 5.17). The predicted SID lysine:NE curve for summer replicate gilts increased as BW increased until 34.0 kg of BW with a maximum SID lysine:NE of 4.08 g/Mcal and then the SID lysine:NE decreased until the end of the study where the SID lysine:NE was 2.00 g/Mcal at 114.2 kg (Figure 5.17). The predicted SID lysine:NE ratio for spring replicate barrows increased as BW

increased until 39.9 kg of BW with a maximum SID lysine:NE of 4.06 g/Mcal and then the SID lysine:NE decreased until the end of the study where the SID lysine:NE was 1.79 g/Mcal at 126.4 kg (Figure 5.17). The predicted SID lysine:NE ratio for spring replicate gilts increased as BW increased until 38.3 kg of BW with a maximum SID lysine:NE of 3.92 g/Mcal and then the SID lysine:NE decreased until the end of the study where the SID lysine:NE was 2.21 g/Mcal at 125.1 kg (Figure 5.17).

5.4.3.15 Standardized Ileal Digestible Lysine Requirement as Percent of Diet

The predicted SID lysine requirement as percent of diet curve for summer replicate barrows increased as BW increased until 36.2 kg of BW with a maximum SID lysine requirement of 1.03% and then the SID lysine requirement decreased until the end of the study where the SID lysine requirement was 0.44% at 118.6 kg (Figure 5.18). The predicted SID lysine requirement as percent of diet curve for summer replicate gilts increased as BW increased until 34.0 kg of BW with a maximum SID lysine requirement of 1.02% and then the SID lysine requirement decreased until the end of the study where the SID lysine requirement was 0.52% at 114.2 kg (Figure 5.18). The predicted SID lysine requirement as percent of diet curve for spring replicate barrows increased as BW increased until 39.9 kg of BW with a maximum SID lysine requirement of 1.02% and then the SID lysine requirement decreased until the end of the study where the SID lysine requirement was 0.47% at 126.4 kg (Figure 5.18). The predicted SID lysine requirement as percent of diet curve for spring replicate gilts increased as BW increased until 38.3 kg of BW with a maximum SID lysine requirement of 0.98% and then the SID lysine requirement decreased until the end of the study where the SID lysine requirement was 0.58% at 125.1 kg (Figure 5.18).

5.5 Discussion

Pork producers work to protect the health and wellbeing of their animals; however, pigs occasionally become ill (National Pork Board, 2008). We have previously reported spring weaned pigs had improved growth performance, final BW, HCW, carcass characteristics and decreased therapeutic antibiotic usage for respiratory challenges compared to the summer weaned pigs during the grow-finish phase (Duttlinger et al., 2019). Conversely, when injectable therapeutic antibiotic use increases, this can be an indicator of illness (American Association of Swine Veterinarians, 2014). Therefore, the performance advantages observed by Duttlinger et al. (2019) in spring weaned pigs compared to summer weaned pigs may be due to reduced environmental pathogens resulting in less chronic immune system activation and improved health (Williams et al., 1997a,b,c). Alternatively, previous research (Johnson et al., 2020 in press) demonstrates that pigs gestated under heat stress (**HS**) conditions have increase immune system activation in response to an LPS challenge. Therefore, it is possible that summer weaned pigs that were gestated during the summer may have been more susceptible to disease during the growing-finishing phase of production compared to spring weaned pigs that were gestated during the winter. Regardless of the specific reasons why summer weaned pigs were under greater disease pressure, the present study, through post-hoc analysis, evaluated the impacts of health status on the production costs, growth curves, and tissue accretion rates between two season replicates in a natural infection challenge simulating commercial conditions. The spring weaned replicate, in the present study, had greater IOFAC ($16.55/pig) compared to the summer weaned replicate. Much of the advantage in IOFAC for the spring weaned replicate is due to improved revenue ($14.89/pig) as a result of greater hot carcass weight (**HCW**; 5.02 kg) in the previous study and reduced therapeutic injectable antibiotics cost ($3.70/pig) and offset by an increase in diet cost of the spring weaned replicate ($1.98/pig) compared to the summer weaned replicate driven from the increased feed

needs to reach the greater HCW (Duttlinger et al., 2019). Previous studies have estimated losses attributed to respiratory health challenges to be $8.49 to $29.82/pig in a natural disease challenge (Cornelison et al., 2018) and $10.49/pig in an experimentally controlled PRRS challenge (Schweer et al., 2017). Furthermore, a benefit-cost analysis to eliminate *Mycoplasma hyopneumoniae* from a production system was estimated to be $7.00/pig (Silva et al., 2019). Even though the present and previous studies utilize different cost and revenue estimates coupled with experimental results to estimate the financial impact of health challenges, the costs associated with and lost opportunity due to a health challenge is significant to pork producers.

Several previous studies have utilized different strategies to expose pigs to an environmental stressor or health challenge (e.g. pathogen exposure, vaccination, lipopolysaccharide (**LPS**) injection, sanitation of housing differences, etc.) with the effort to activate the immune system and subsequently measured decreased growth performance following the challenge exposure compared to control pigs not exposed to the challenge (Schinckel et al., 1995; Williams et al., 1997a,b,c; Holck et al. 1998; Rakhshandeh and de Lange, 2012; Curry et al., 2017; Kvidera et al., 2017; Schweer et al., 2017). However, fewer studies have modeled animal growth curve responses to a naturally-occurring health challenge. In the present study, from d 126 to the end of the study (d 177), the spring weaned replicate ADG was greater than the summer weaned replicate. The magnitude of differences also continued to increase from d 126 to the end of the study as the growth rate of the summer weaned replicate decreased and the spring weaned replicate growth rate slowed. The ADG of the spring weaned barrows was 34.2% greater than the summer weaned barrows on d 177. The spring weaned replicate's advantage in ADG was partially due to improvements in ADFI and G:F. The ADFI of the spring weaned replicate was greater from d 133 to the end of the study compared to the summer weaned replicate with the spring weaned

replicate having 13.4% greater ADFI compared to the summer weaned replicate on d 177. In addition, the spring weaned replicate had greater G:F compared to the summer weaned replicate during the grow-finish phase (Duttlinger et al., 2019) with spring weaned replicate having 19.5% greater G:F compared to the summer weaned replicate. The G:F advantage of the spring weaned replicate is consistent with previous work where pigs from sows gestated under thermoneutral (**TN**) conditions had improved G:F compared to pigs from sows gestated under HS conditions (Johnson et al., 2015) and may be due to increased maintenance costs for pigs gestated under HS conditions (Chapel et al., 2017). The difference in feed efficiency in pigs gestated under different thermal conditions may partially explain why the summer weaned replicate had poorer feed efficiency than the spring weaned replicate as the summer weaned replicate was gestated under increased temperatures during the summer compared to the spring weaned replicate gestated during the winter. The difference in magnitude of ADG growth rate contributes to the differences observed in the BW growth curve where the BW curves are different from d 166 to the end of the trial between replicates regardless of sex. These results in the present study are consistent with previous studies where the modeled growth rate of pigs exposed to fewer challenges had greater rate of ADG compared to challenged pigs (Holck et al., 1998; Schinckel et al. 2002; Hamilton et al., 2003). The challenges in the previous studies that pigs were exposed to were decreased sanitation (Holck et al., 1998), reduced biosecurity management with a continuous flow barn (Schinckel et al., 2002), and reduced pen space allowance (Hamilton et al., 2003).

In the present study, empty body protein accretion increased then decreased as BW weight increased. The shape of the curve is consistent with previous studies (Schinckel et al. 2002; Hamilton et al., 2003). At the end of the trial, on d 177 in the present study, the spring weaned barrows had 37.6% greater empty body protein accretion than the summer weaned barrows. In

addition, the spring weaned barrows in the present study had increasing empty body protein accretion from 117.2 kg of BW till the end of the study compared to the summer weaned barrows where the summer weaned barrows' protein accretion rate peaked at 61.6 kg and then decreased for the remainder of the trial. The increasing protein accretion at the end of the trial for the spring weaned barrows is most likely due to the feeding of ractopamine as it is well documented that ractopamine increases growth and protein accretion (Schinckel et al., 2000; Schinckel et al., 2003). Conversely, the lack of increased protein accretion due to ractopamine for the summer weaned barrows may be due to increased amino acid and energy requirements by other tissues and the immune system due to the health challenges thus limiting protein accretion and growth (Curry et al., 2017; Kvidera et al., 2017). In addition, pigs that were gestated under HS conditions have reduced protein accretion compared to pigs gestated under TN conditions (Johnson et al., 2015). The effect of gestation thermal environment on lean accretion may be due to differences in nutrient partitioning with HS conditions causing a constraint in lean tissue synthesis (Johnson et al., 2015) and may partially explain the decreased protein accretion of the summer weaned replicate gestated during the summer. In a previous study, pigs that were reared with unrestricted floor space had predicted protein accretion rates that were greater than pigs reared with restricted floor space for the entire test period (Hamilton et al., 2003). The previous study's results suggest that when a stressor is applied to pigs, pigs may not be able to maximize protein deposition due to the challenge applied. In previous studies, PRRS challenged pigs (Schweer et al., 2017) and porcine epidemic diarrhea virus challenged pigs (Curry et al., 2017) had reduced lean accretion rates compared to non-challenged controls predicted by dual-energy X-ray absorptiometry. These results from the aforementioned studies illustrate that pigs exposed to health challenges may have attenuated carcass muscle and protein accretion rates relative to non-challenged cohorts.

Mechanisms behind the growth and protein accretion rate differences between seasonal replicates in the present study are likely multifaceted. Previous work classically illustrated that pigs with higher chronic immune system activation had greater T lymphocyte CD4+:CD8+ ratios and greater alpha-1-acyglycoprotein concentrations indicating that immune cells were releasing more antigens resulting in greater cytokine release and greater immune response (Williams et al., 1997c). An additional previous study determined that an activated immune system has increased glucose requirement (Kvidera et al., 2017) and health challenged pigs may repartition glucose away from growth toward the immune system. Besides changes in immune system energy requirements, alterations in muscle physiology may be another mechanism that modifies growth and protein accretion rates as a previous study found that PRRS infected pigs had increased muscle myostatin mRNA potentially negatively affecting skeletal muscle growth (Escobar et al., 2004).

Daily lysine requirements, between challenged and non-challenged pigs are different (Williams et al., 1997c; Schinckel et al., 2002). Previous research has shown that pigs with low immune system activation have increased lysine requirements due to an increased capacity for more protein accretion and lean deposition (Williams et al., 1997c). In the present study, the shape of the curve for the SID lysine requirement is very similar to the shape of the empty body protein accretion curve where the curves increase and then decrease as BW increases. At the end of the trial, on d 177 in the present study, the spring weaned barrows had 36.2% greater SID lysine requirement compared to the summer weaned barrows. These results suggest that lysine requirements may be different between pigs with different health statuses due to differences in carcass muscle growth rates. In addition to health status differences between the seasonal replicates, the aforementioned study by Johnson et al. (2015) determined that pigs gestated under HS conditions have reduced protein accretion compared to pigs gestated under TN conditions and it

is tempting to speculate that pigs gestated under HS conditions would have reduced lysine requirement compared to its TN counterparts. Similar to the empty body protein accretion, the spring weaned barrows in the present study had an increasing SID lysine requirement from 114.2 kg of BW till the end of the study compared to the summer weaned barrows where the summer weaned barrows' SID lysine requirement plateaus at 65.1 kg and then decreases for the remainder of the trial. The increasing SID requirement for the spring weaned barrows is likely due to ractopamine as previous studies have determined that lysine requirements are increased in pigs fed ractopamine to allow for the increased lean accretion (Schinckel et al., 2000; Schinckel et al., 2003). The lysine shape of lean accretion models and SID lysine requirement models are similar in shape due to skeletal muscle being high in protein and lysine content (Williams et al., 1997c; Schinckel et al., 2002). Due to the composition of carcass muscle, the lysine requirement of health challenged pigs is reduced compared to nonchallenged control pigs due to reduced rates of tissue and protein accretion (Williams et al., 1997c; Schinckel et al., 2002). Production systems need to understand how management, health, genetics, and environment can impact nutrient requirements to be able to optimize nutrition for their animals and effectively utilize technologies like ractopamine.

The present study demonstrates that animals housed under different conditions, gestated under different conditions, and exposed to different environmental pathogens and stressors can have very different growth rates and tissue accretion rates. Granted, the present study is a post hoc analysis of a previous study that is not a controlled health challenge and pigs were reared and gestated at different times of year where the ambient temperature could bias growth rate. However, these differences in rates of growth and increased antibiotic costs to combat the environmental health challenges resulted in large differences in production costs. The production cost estimates

can be used as a tool by commercial pork producers when they are investigating the implementation of new management, nutrition, and biosecurity protocols and potential return on investment. Resent work has shown that animals exposed to disease pathogens (PRRS) can respond to different nutritional strategies to help animals recover more quickly post-infection (Rochell et al., 2015; Smith et al., 2019). Future work should continue to focus on nutritional strategies to help offset the negative effects of health challenges and further determine if the promising nutritional interventions can positively alter tissue accretion rates post-infection.

5.6 Conclusion

Growth performance and tissue accretion rates can be impacted by environmental stressors and health challenges. These stressors can alter nutrient requirements. In addition, health challenges can increase therapeutic antibiotic costs. In conclusion, health challenges in pigs can have profound negative impacts on tissue accretion rates and key economic drivers for pork producers such as reduced average daily gain, poorer feed efficiency and reduced hot carcass weight. The adverse health effects result in reduced growth performance, greater therapeutic antibiotic costs, and increased production costs ($16.62/pig) which negatively impact producer profitability.

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Table 5.1 Composition of nursery diets (as fed)

Item	**Phase 1[1]**	**Phase 2[2]**	**Phase 3[3]**	**Phase 4[4]**
Ingredient, %				
Corn[5]	30.38	38.09	51.63	57.38
SBM, 48% CP	13.95	18.00	25.65	30.70
DDGS, 7% fat	---	---	---	5.00
Soybean oil	5.00	5.00	3.00	---
Choice white grease	---	---	---	3.00
Limestone	0.79	0.74	0.86	1.33
Monocal. phos.	0.40	0.49	0.49	0.74
Vitamin premix[6]	0.25	0.25	0.25	0.25
Trace mineral premix[7]	0.13	0.13	0.13	0.13
Selenium premix[8]	0.05	0.05	0.05	0.05
Phytase[9]	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.30	0.35
Plasma protein	6.50	2.50	---	---
Blood meal	1.50	1.50	---	---
Soy concentrate	4.00	3.00	2.50	---
Fish meal	5.00	4.00	4.00	---
Dried whey	25.00	25.00	10.00	---
Lactose	5.00	---	---	---
Lysine-HCL	0.07	0.20	0.28	0.40
DL-Methionine	0.22	0.23	0.18	0.17
L-Threonine	0.04	0.09	0.12	0.14
L-Tryptophan	---	0.01	0.01	0.00
Zinc oxide	0.38	0.38	0.38	---
Copper sulphate	---	---	---	0.10
Banminth 48[10]	---	---	---	0.10
Clarifly, 0.67%[11]	---	---	0.08	0.07
Calculated chemical composition				
ME, kcal/kg	3536	3510	3418	3396
NE, kcal/kg	2772	2743	2591	2528
Fat, %	7.27	7.36	5.73	5.86
CP, %	24.62	22.87	22.29	21.28
Total Lys, %	1.74	1.61	1.50	1.41
SID Lys, %	1.55	1.45	1.35	1.25
Ca, %	0.90	0.85	0.80	0.75
Total P, %	0.75	0.71	0.64	0.57
Avail. P, %	0.60	0.55	0.45	0.36
Analyzed chemical composition				
Summer replicate				
GE, kcal/kg	4217	4172	---	---
CP, %	24.42	22.30	22.07	22.00
Total Lys, %	1.30	1.13	---	---
Spring replicate				
GE, kcal/kg	4266	4174	---	---

Table 5.1 continued

CP, %	25.36	22.78	22.37	21.14
Total Lys, %	1.58	1.54	---	---

[1]Fed d 0 to 7 post-weaning and transport.
[2]Fed d 7 to 14 post-weaning and transport.
[3]Fed d 14 to 21 post-weaning and transport.
[4]Fed d 21 to 34 post-weaning and transport.
[5]For A diet, 0.58% corn was replaced with 0.40% chlortetracycline and 0.10% tiamulin for phase 1 and 2 (Duttlinger et al., 2019). For GLN diet 0.20% corn was replaced with 0.20% L-glutamine for phase 1 and 2, respectively (Duttlinger et al., 2019).
[6]Provided per kg of the diet: vitamin A, 6,615 IU; vitamin D3, 662 IU; vitamin E, 44 IU; vitamin K, 2.2 mg; riboflavin, 8.8 mg; pantothenic acid, 22 mg; niacin, 33 mg; vitamin B_{12}, 38.6 mg.
[7]Provided available minerals per kg of the diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15 mg; copper, 11.3 mg; iodine, 0.46 mg.
[8]Provided 0.3 ppm Se.
[9]Provided 600 FTU per kg of the diet.
[10]Banminth (Phibro Animal Health Corporation, Teaneck, NJ) provided 106 ppm pyrantel tartrate in the diet.
[11]Clarifly (Central Life Sciences, Schaumburg, IL) provided 5.4 ppm (Phase 3) and 4.7 ppm (Phase 4) diflubenzuron in the diet.

Table 5.2 Composition of grow-finish diets (as fed)

Item	**Phase 1[1]**	**Phase 2[2]**	**Phase 3[3]**	**Phase 4[4]**	**Phase 5[5]**	**Phase 6[6]**
Ingredient, %						
Corn	61.47	64.65	66.40	71.10	82.38	68.67
SBM, 48% CP	23.20	16.15	9.75	5.25	4.25	15.10
DDGS, 7% fat	10.00	15.00	20.00	20.00	10.00	10.00
Choice White Grease	2.00	1.00	1.00	1.00	1.00	3.00
Limestone	1.37	1.35	1.39	1.32	1.16	1.26
Monocalcium Phosphate	0.47	0.32	0.05	0.00	0.10	0.27
Vitamin Premix	0.15[7]	0.15[7]	0.12[8]	0.12[9]	0.10[10]	0.15[7]
Trace Mineral Premix	0.10[11]	0.09[12]	0.08[13]	0.07[14]	0.05[15]	0.10[11]
Selenium Premix	0.05[16]	0.05[16]	0.05[16]	0.05[16]	0.03[17]	0.05[16]
Phytase[18]	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.35	0.35	0.30	0.30	0.25	0.30
Lysine-HCL	0.42	0.46	0.48	0.46	0.37	0.42
DL-Methionine	0.11	0.08	0.05	0.01	0.00	0.10
L-Threonine	0.130	0.130	0.120	0.105	0.095	0.160
L-Tryptophan	0.010	0.030	0.035	0.040	0.030	0.030
Paylean 2.25[19]	---	---	---	---	---	0.15
Availa Zn 120[20]	---	---	---	---	---	0.042
Clarifly, 0.67%	0.07[21]	0.09[22]	0.07[21]	0.08[23]	0.09[22]	0.10[24]
Calculated chemical composition						
ME, kcal/kg	3373	3337	3351	3359	3371	3438
NE, kcal/kg	2521	2502	2529	2558	2606	2617
Fat, %	5.29	4.69	5.06	5.15	4.73	6.40
CP, %	19.34	17.59	15.99	14.18	11.90	16.01
SID Lys, %	1.10	0.98	0.85	0.73	0.60	0.90
Ca, %	0.70	0.65	0.60	0.55	0.50	0.60
Total P, %	0.50	0.47	0.41	0.38	0.35	0.42
Avail. P, %	0.32	0.30	0.26	0.24	0.20	0.26
Analyzed chemical composition						
Summer replicate						
CP, %	19.13	18.08	14.92	14.66	11.73	15.64
Spring replicate						
CP, %	19.25	17.33	16.73	15.59	12.29	16.85

[1]Fed d 0 to 21 of the grow-finish phase.
[2]Fed d 21 to 42 of the grow-finish phase.
[3]Fed d 42 to 62 of the grow-finish phase.
[4]Fed d 62 to 83 of the grow-finish phase.
[5]Fed d 83 to 104 of the grow-finish phase.
[6]Fed d 104 to 125 of the grow-finish phase.
[7]Provided per kilogram of the diet: vitamin A, 3,969 IU; vitamin D_3, 397 IU; vitamin E, 26 IU; vitamin K, 1.3 mg; riboflavin, 5.3 mg; pantothenic acid, 13 mg; niacin, 20 mg; B_{12}, 23.2 mg.
[8]Provided per kilogram of the diet: vitamin A, 3,308 IU; vitamin D_3, 331 IU; vitamin E, 22 IU; vitamin K, 1.1 mg; riboflavin, 4.4 mg; pantothenic acid, 11 mg; niacin, 17 mg; B_{12}, 19.3 mg.

Table 5.2 continued

[9]Provided per kilogram of the diet: vitamin A, 3,175 IU; vitamin D_3, 318 IU; vitamin E, 21 IU; vitamin K, 1.1 mg; riboflavin, 4.2 mg; pantothenic acid, 11 mg; niacin, 16 mg; B_{12}, 18.5 mg.
[10]Provided per kilogram of the diet: vitamin A, 2,646 IU; vitamin D_3, 265 IU; vitamin E, 18 IU; vitamin K, 0.9 mg; riboflavin, 3.5 mg; pantothenic acid, 9 mg; niacin, 13 mg; B_{12}, 15.4 mg.
[11]Provided per available minerals kilogram of the diet: iron, 97 mg; zinc, 97 mg; manganese, 12 mg; copper, 9 mg; iodine, 0.37 mg.
[12]Provided per available minerals kilogram of the diet: iron, 87 mg; zinc, 87 mg; manganese, 11 mg; copper, 8 mg; iodine, 0.33 mg.
[13]Provided per available minerals kilogram of the diet: iron, 78 mg; zinc, 78 mg; manganese, 10 mg; copper, 7.2 mg; iodine, 0.29 mg.
[14]Provided per available minerals kilogram of the diet: iron, 68 mg; zinc, 68 mg; manganese, 8 mg; copper, 6.3 mg; iodine, 0.26 mg.
[15]Provided per available minerals kilogram of the diet: iron, 48.5 mg; zinc, 48.5 mg; manganese, 6 mg; copper, 4.5 mg; iodine, 0.18 mg.
[16]Provided 0.3 ppm Se.
[17]Provided 0.15 ppm Se.
[18]Provided 600 FTU per kg of the diet.
[19]Paylean (Elanco Animal Health, Greenfield, IN) provided 7.5 ppm ractopamine HCl in the diet.
[20]Zinpro Corporation, Eden Prairie, MN.
[21]Clarifly (Central Life Sciences, Schaumburg, IL) provided 4.7 ppm diflubenzuron in the diet.
[22]Clarifly (Central Life Sciences, Schaumburg, IL) provided 6.0 ppm diflubenzuron in the diet.
[23]Clarifly (Central Life Sciences, Schaumburg, IL) provided 5.4 ppm diflubenzuron in the diet.
[24]Clarifly (Central Life Sciences, Schaumburg, IL) provided 6.7 ppm diflubenzuron in the diet.

Table 5.3 Descriptive data of closeout information at the end of the grow-finish period at d 159 post-weaning

	Replicate	
Parameter[1]	Summer[2]	Spring[3]
Full value pigs, %[4]	82.29	92.00
Lightweight culls/morbidity, %[5]	7.43	2.86
Mortality, %	9.14	2.86
Culls for navel hernia, %	1.14	2.28

[1]Expressed as percent of population within replicate.
[2]Pigs weaned and transported for 12 h during July 2016 and marketed in Jan. 2017.
[3]Pigs weaned and transported for 12 h during April 2017 and marketed in Sept. 2017.
[4]Based on packer carcass weight discount schedule where HCW less than 73 kg are severely discounted.
[5]Pigs weighing less than 97.5 kg (live weight) at end of trial.

Table 5.4 Effect of season replicate on nursery and grow-finish production costs and revenue[1]

Parameter	Replicate		SE	***P-value***
	Summer[2]	Spring[3]		
Nursery Period (d 0 to 34 post-weaning)				
Revenue, $/pig[4]	61.24	62.74	2.64	0.11
Diet cost, $/pig	8.55	8.45	0.34	0.56
Income over feed cost, $/pig	52.68	54.29	2.31	0.05
Therapeutic injectable antibiotics cost for enteric and unthrifty challenges, $/pig	0.12	0.02	0.03	0.01
Income over feed and therapeutic injectable antibiotics for enteric and unthrifty challenges, $/pig	52.56	54.27	2.32	0.04
All therapeutic injectable antibiotics cost, $/pig	0.19	0.21	0.05	0.74
Income over feed and therapeutic injectable antibiotics, $/pig	52.49	54.08	2.32	0.05
Grow-finish period (d 34 to 159 post-weaning)				
Revenue, $/pig[5]	135.98	150.87	2.03	0.01
Diet cost, $/pig	58.90	60.88	0.64	0.03
Income over feed cost, $/pig	77.08	90.00	1.69	0.01
Therapeutic injectable antibiotics cost, $/pig	5.20	1.50	0.66	0.01
Income over feed and therapeutic injectable antibiotics, $/pig	71.88	88.50	1.88	0.01
Therapeutic water-delivered antibiotics cost, $/pig	4.23	4.29	0.05	0.45
Income over feed and therapeutic injectable and water-delivered antibiotics, $/pig	67.66	84.21	1.86	0.01

[1]A total of 10 pens were used per dietary treatment per season replicate.
[2]Pigs weaned and transported for 12 h during July 2016 and marketed in Jan. 2017.
[3]Pigs weaned and transported for 12 h during April 2017 and marketed in Sept. 2017.
[4]Feeder pig value of $3.56/kg. Source: NW_LS255 National Direct Delivered Feeder Pig Report; average price from 2014-2018.
[5]Pig value of $1.21/kg live weight. Source: LM_HG204 Iowa/Minnesota Daily Direct Prior Day Hog Report; average price from 2014-2018.

Table 5.5 Day of age ranges when each comparison was different via predictive modeling[1]

	Comparison				
Parameter	Summer[2] Barrows vs. Spring[3] Barrows	Summer Gilts vs. Spring Gilts	Summer Barrows vs. Spring Gilts	Summer Gilts vs. Spring Barrows	Summer Replicate vs. Spring Replicate
Days when BW was different[4]	24 to 52; 160 to 177	22 to 76; 137 to 177	23 to 65; 166 to 177	23 to 60; 137 to 177	---
Days when ADG was different[5]	22 to 38; 59 to 90; 119 to 177	22 to 47; 112 to 177	22 to 44; 71 to 90; 126 to 177	22 to 41; 107 to 177	---
Days when ADFI was different[6]	---	---	---	---	21 to 46; 57 to 111; 133 to 177

[1]A student t-test was utilized to test the given parameters from predictive modeling. Date ranges listed indicate significant differences ($P \leq 0.05$) within given comparison.
[2]Pigs weaned and transported for 12 h during July 2016 and marketed in Jan. 2017.
[3]Pigs weaned and transported for 12 h during April 2017 and marketed in Sept. 2017.
[4]Day of age ranges when BW was different via predictive modeling.
[5]Day of age ranges when ADG was different via predictive modeling.
[6]Day of age ranges when ADFI was different via predictive modeling.

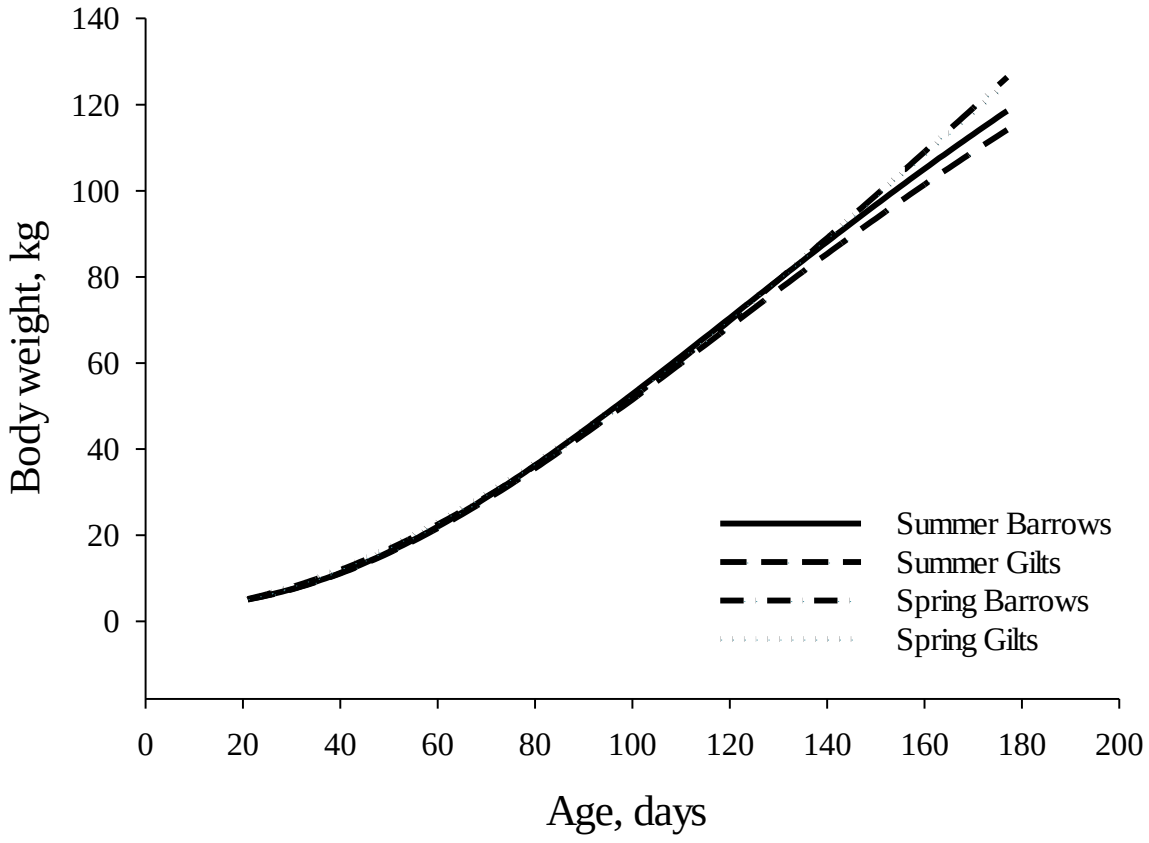

Figure 5.1 Prediction of body weight for season replicate and sex based on age. Mean BW of barrows and gilts for summer and spring replicate at each day of age predicted by a generalized Michaelis - Menten (GMM) equation; $WT_{i,t} = WT_o + [(WF- WT_o) (t/K)^C)]/[1 + (t/K)^C)]$ where WF is mean mature BW, WT_o is the mean birth BW, t is days of age, K is a parameter equal to the days of age in which one-half WF is achieved and C is a unit less parameter related to changes in proportional growth and shape of the growth curves (Lopez et al., 2000 and Schinckel et al., 2009a). (n = 30 pigs/sex/replicate).

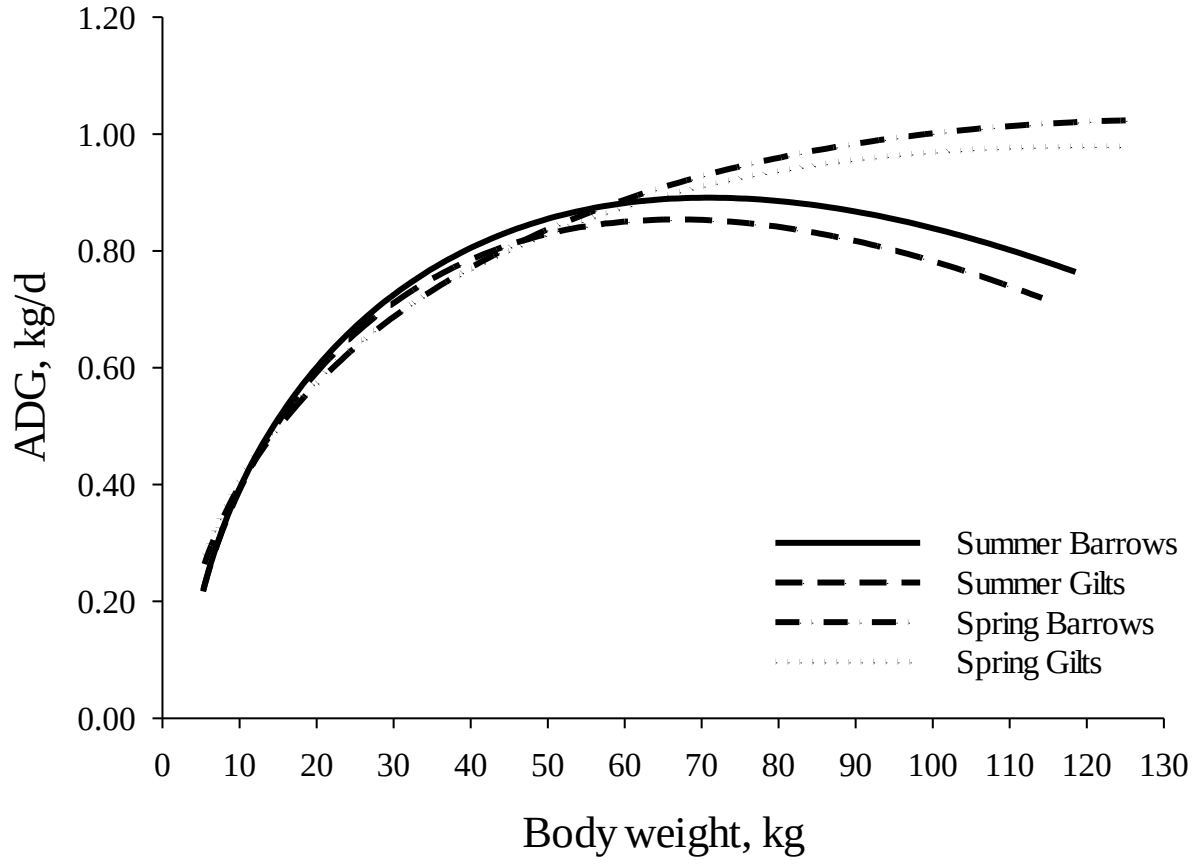

Figure 5.2 Prediction of average daily gain for season replicate and sex based on BW. Average daily gain was determined as the derivative of the BW function on time (ADG = ∂WT / ∂T) (Schinckel et al., 2009a). (n = 30 pigs/sex/replicate).

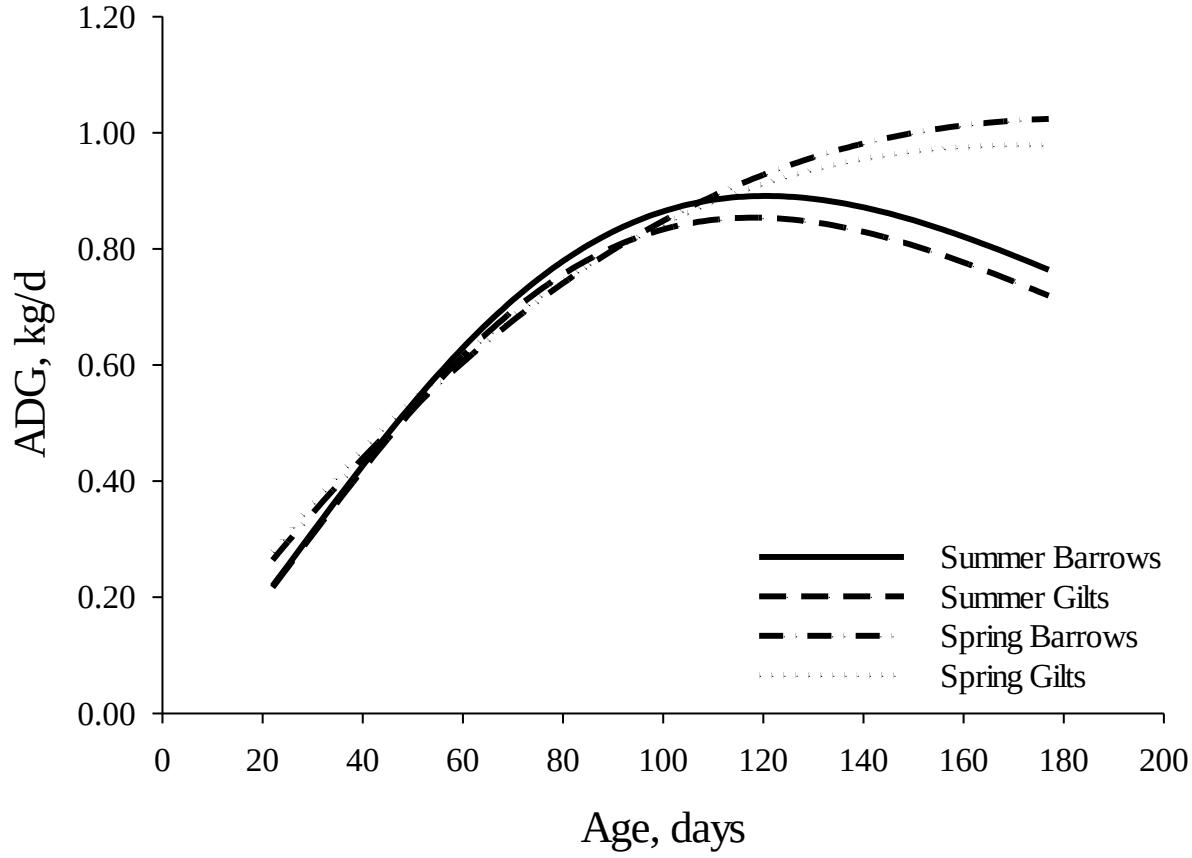

Figure 5.3 Prediction of average daily gain for season replicate and sex based on age. Average daily gain was determined as the derivation of the BW function on time (ADG = ∂WT / ∂T) (Schinckel et al., 2009a). (n = 30 pigs/sex/replicate).

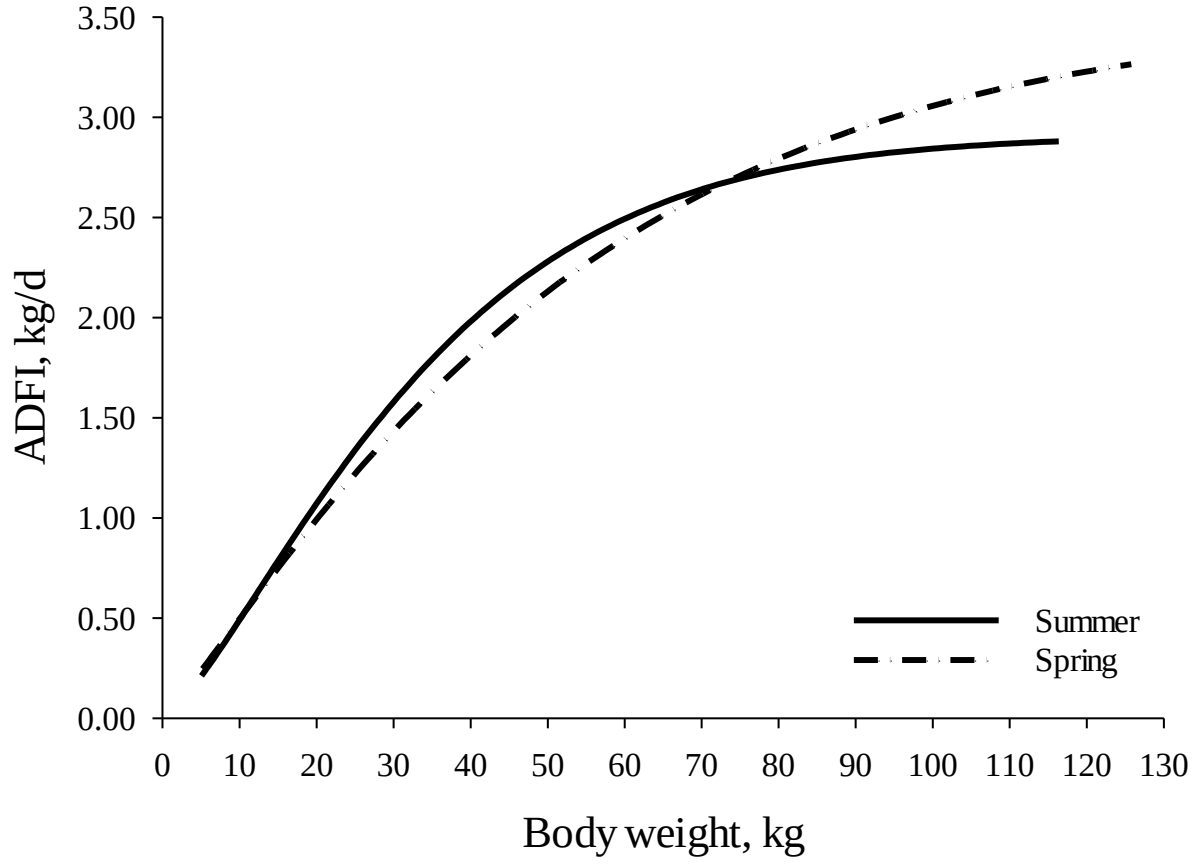

Figure 5.4 Prediction of average daily feed intake for season replicate based on BW. The following nonlinear function model was used to describe the relationship of ADFI to BW, $ADFI_{i,x} = C\ (1 - \exp(-Kx^{A})) + e_{i,t}$, where x is the BW (kg) of the i^{th} animal measured in days, C is the average mature DFI, *K* is the exponential growth decay constant and C is the kinetic order constant (Bridges et al., 1986 and Schinckel et al., 2009c). (n = 30 pens/replicate).

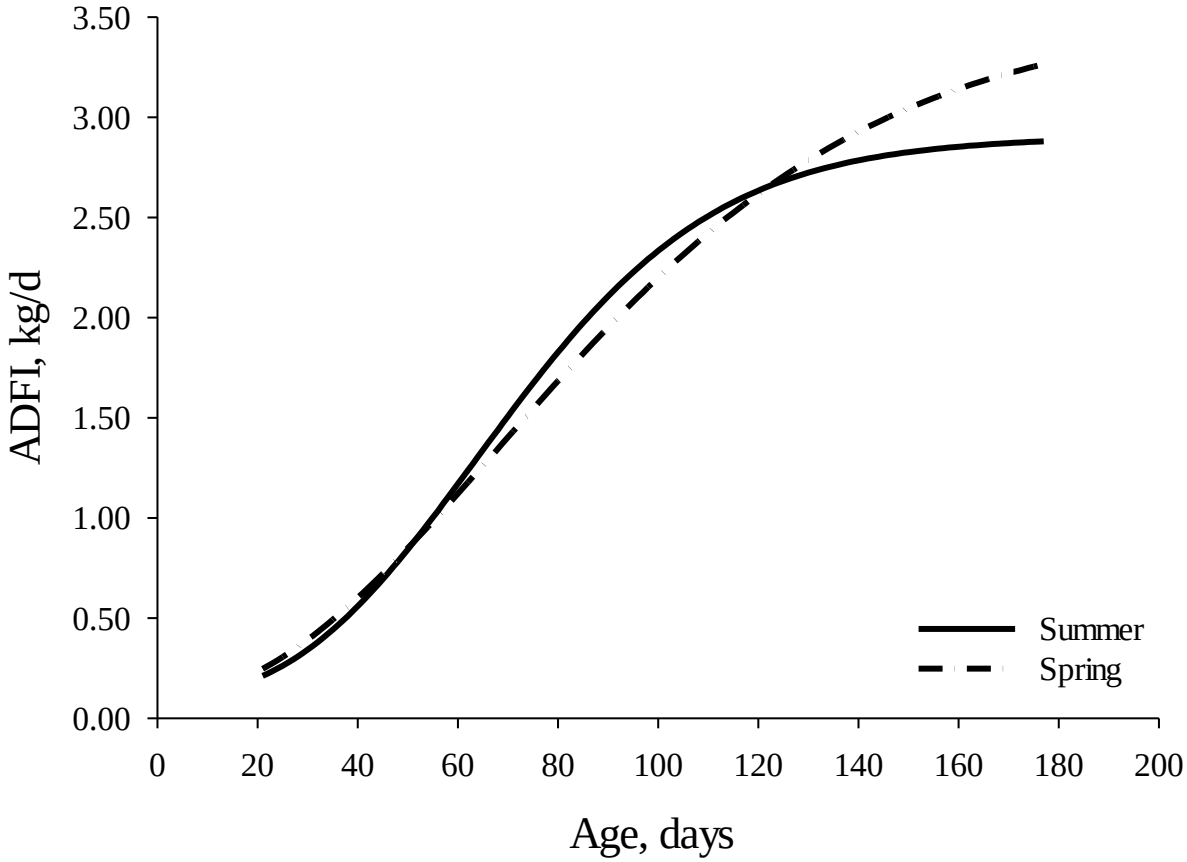

Figure 5.5 Prediction of average daily feed intake for season replicate based on age. The following nonlinear function model was used to describe the relationship of ADFI to age, $ADFI_{i,x} = C\ (1 - \exp(-Kx^{A})) + e_{i,t}$, where x is the age (d) of the i^{th} animal measured in days, C is the average mature DFI, *K* is the exponential growth decay constant and C is the kinetic order constant (Bridges et al., 1986 and Schinckel et al., 2009c). (n = 30 pens/replicate).

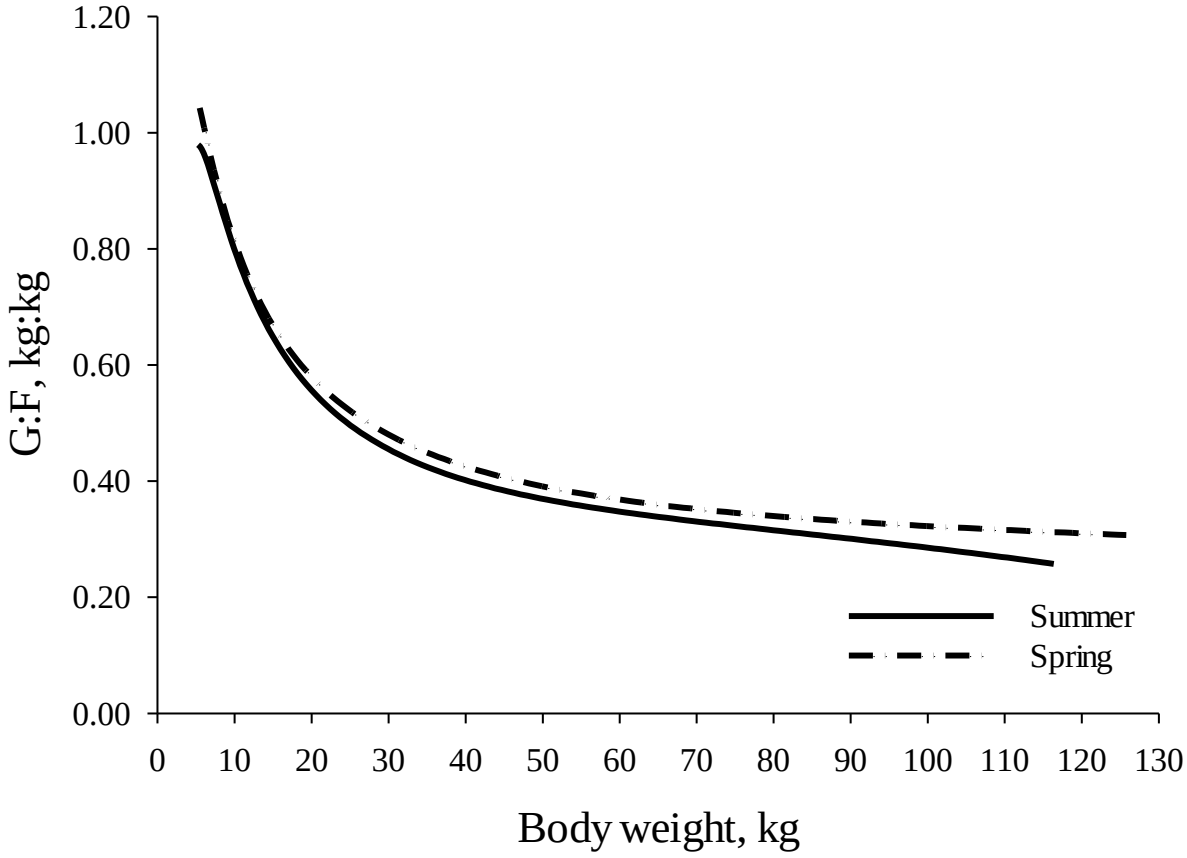

Figure 5.6 Prediction of gain:feed for season replicate based on BW. The G:F predictive model was calculated dividing ADG by ADFI for a given BW to produce the G:F curves based on BW. (n = 30 pens/replicate).

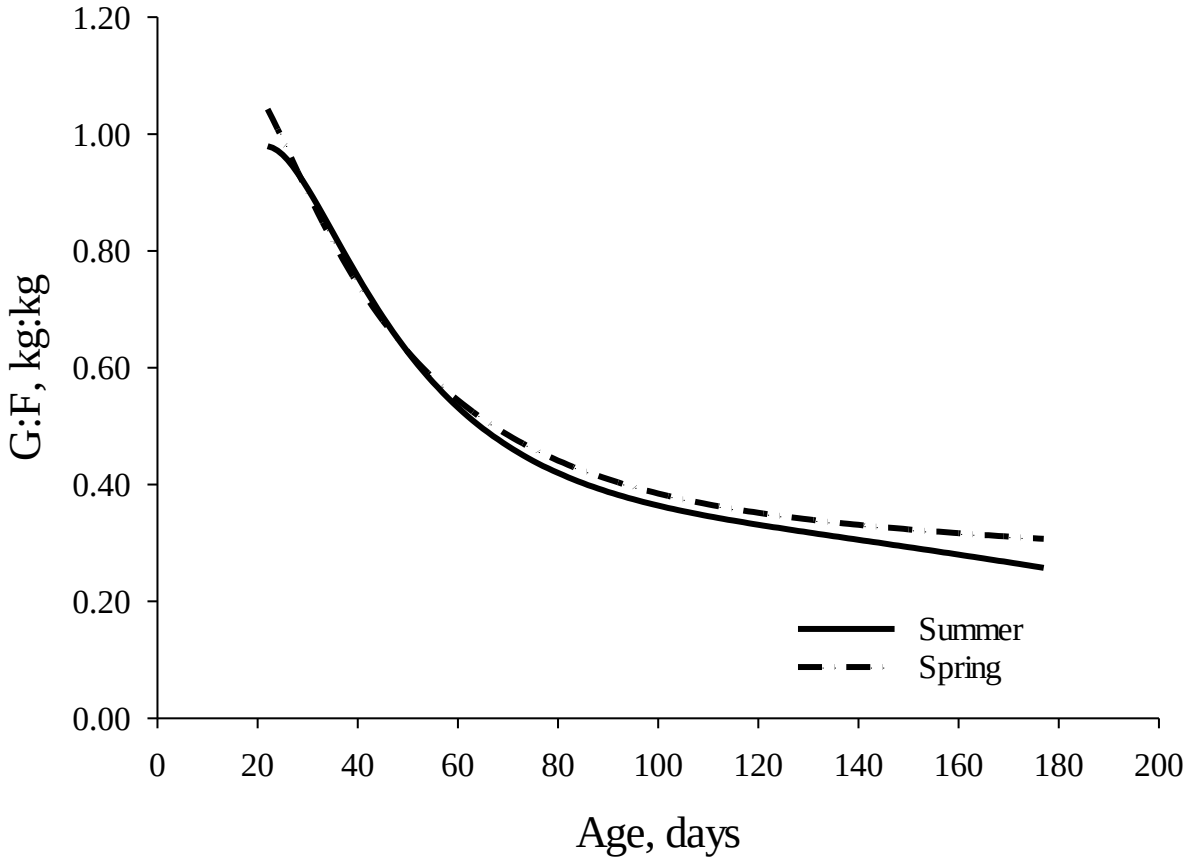

Figure 5.7 Prediction of gain:feed for season replicate based on age. The G:F predictive model was calculated dividing ADG by ADFI for a given day to produce the G:F curves based on age. (n = 30 pens/replicate).

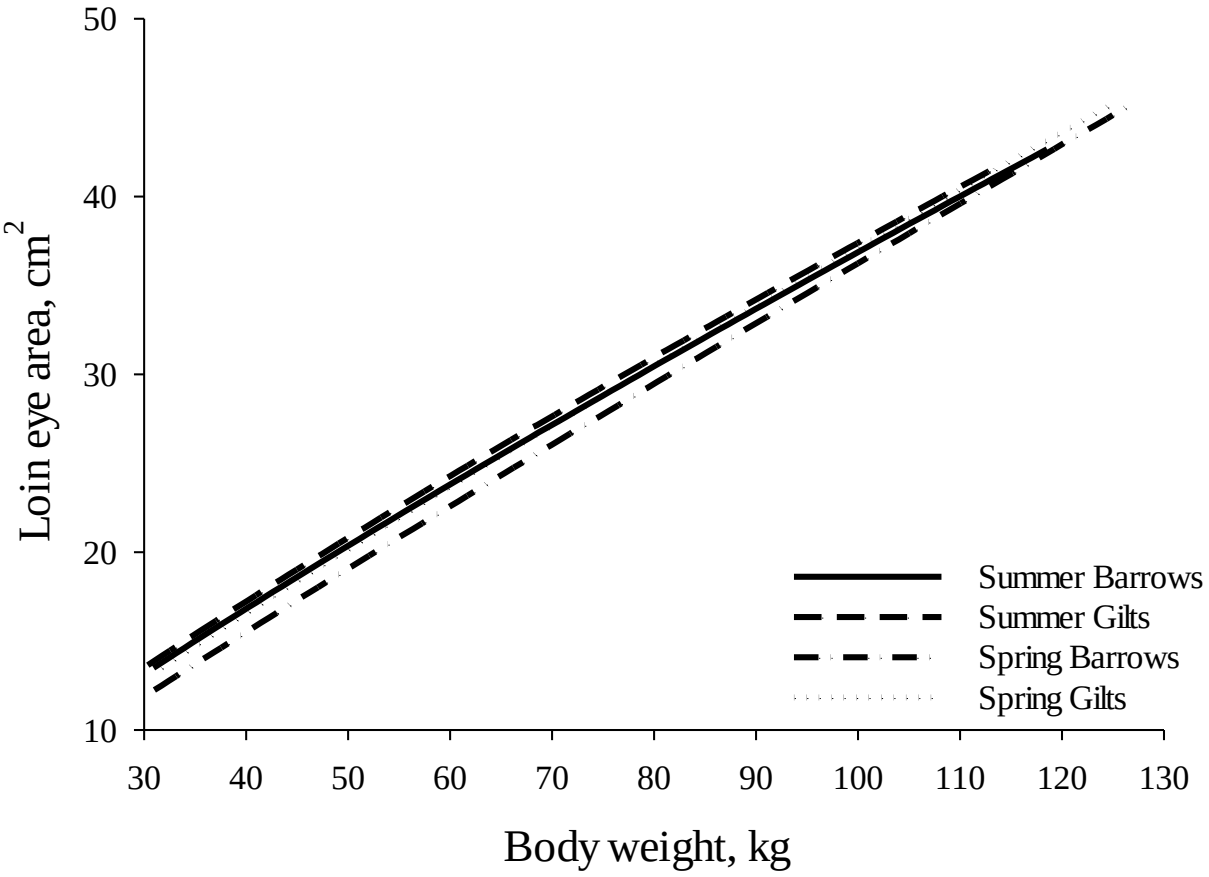

Figure 5.8 Prediction of loin eye area for season replicate and sex based on BW. The serial ultrasonic loin eye area measurements were fitted to alternative functions of BW: allometric function, ($Y = A\ BW^B$), and mixed model allometric function, $Y = (A + a_i)\ BW^B$, where a_i is a pig specific random effect with variance σ_a^2 (Schinckel et al., 2009b). The alternative mixed models were evaluated based upon AIC values. (n = 30 pigs/sex/replicate).

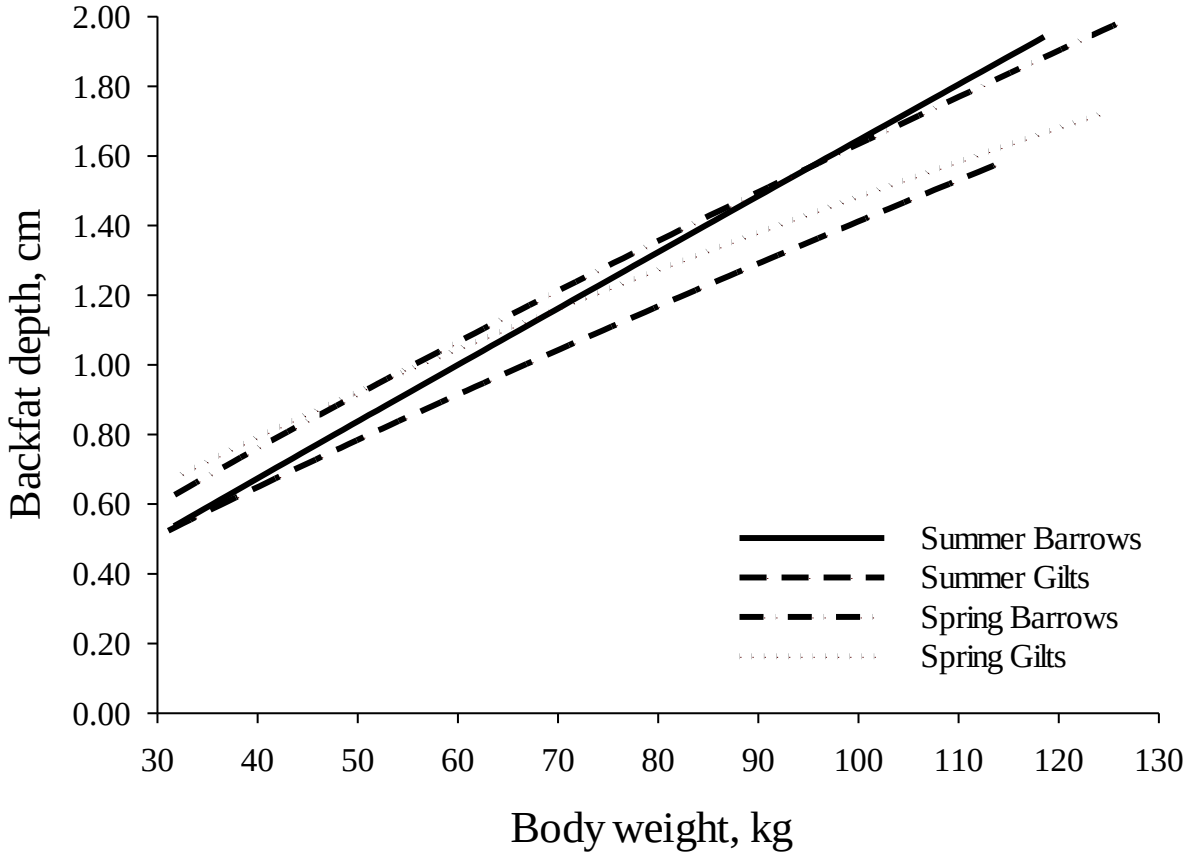

Figure 5.9 Prediction of backfat depth for season replicate and sex based on BW. The serial ultrasonic backfat depth were fitted to alternative functions of BW: allometric function, ($Y = A\ BW^B$), and mixed model allometric function, $Y = (A + a_i)\ BW^B$, where a_i is a pig specific random effect with variance σ_a^2 (Schinckel et al., 2009b). The alternative mixed models were evaluated based upon AIC values. (n = 30 pigs/sex/replicate).

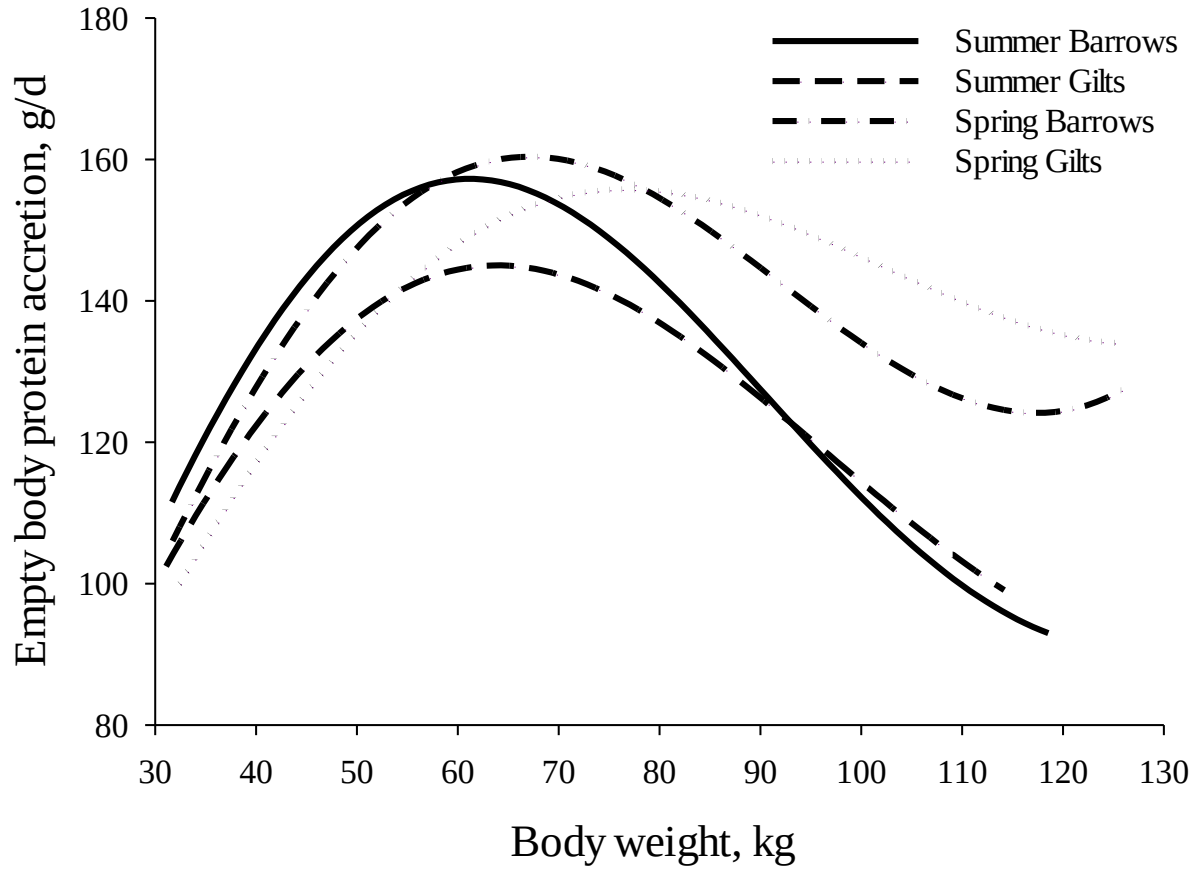

Figure 5.10 Prediction of daily empty body protein accretion rate for season replicate and sex based on BW. The exponential ($EBP = \exp(b_0+b_1x + b_2x^2 + b_3x^3)$ functions (x) of BW (Wagner et al., 1999) was used to predict empty body protein. Daily body protein accretion rates were determined as the product of the derivatives of two functions by $\partial C/\partial T = ((\partial C/\partial BW)\ x\ (\partial BW/\partial T))$, (Schinckel and De Lange 1996), where C is the body component mass. (n = 30 pigs/sex/replicate).

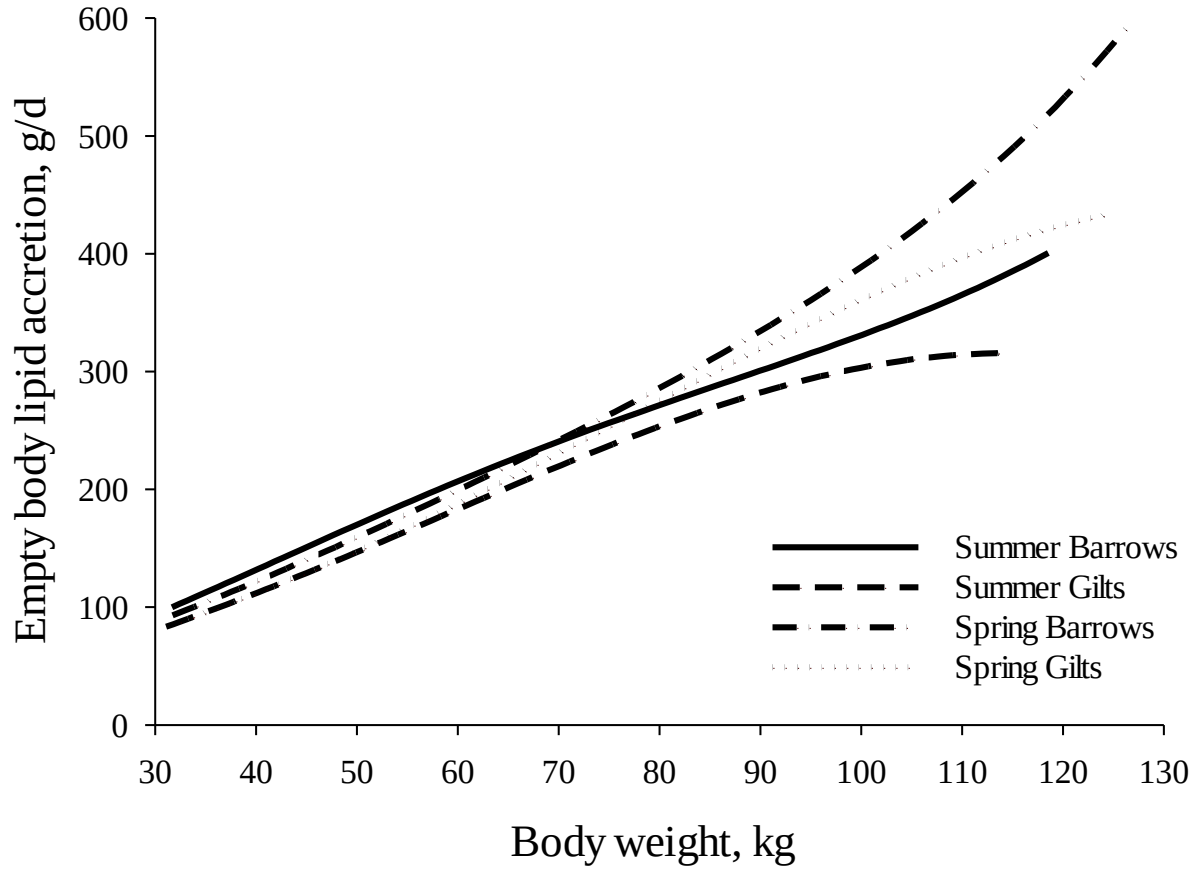

Figure 5.11 Prediction of daily empty body lipid accretion rate for season replicate and sex based on BW. The exponential ($EBL = \exp(b_0+b_1x + b_2x^2 + b_3x^3)$ functions (x) of BW (Wagner et al., 1999) was used to predict empty body lipid. Daily body lipid accretion rates were determined as the product of the derivatives of two functions by $\partial C/\partial T = ((\partial C/\partial BW)\ x\ (\partial BW/\partial T))$, (Schinckel and De Lange 1996), where C is the body component mass. (n = 30 pigs/sex/replicate).

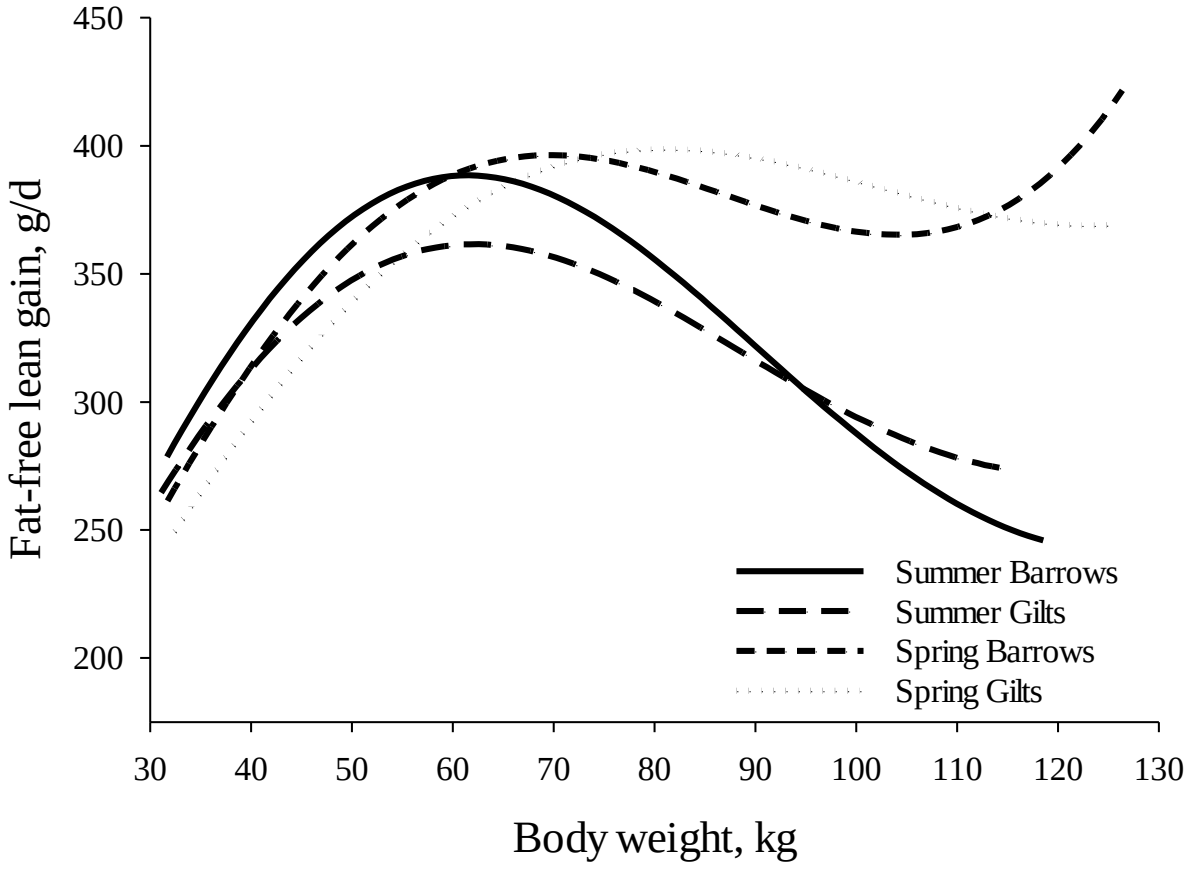

Figure 5.12 Prediction of fat-free lean gain for season replicate and sex based on BW. The exponential ($FFL = \exp(b_0+b_1x + b_2x^2 + b_3x^3)$ functions (x) of BW (Wagner et al., 1999) was used to predict fat-free lean. Daily fat-free lean accretion rates were determined as the product of the derivatives of two functions by $\partial C/\partial T = ((\partial C/\partial BW) \; x \; (\partial BW/\partial T))$, (Schinckel and De Lange 1996), where C is the body component mass. (n = 30 pigs/sex/replicate).

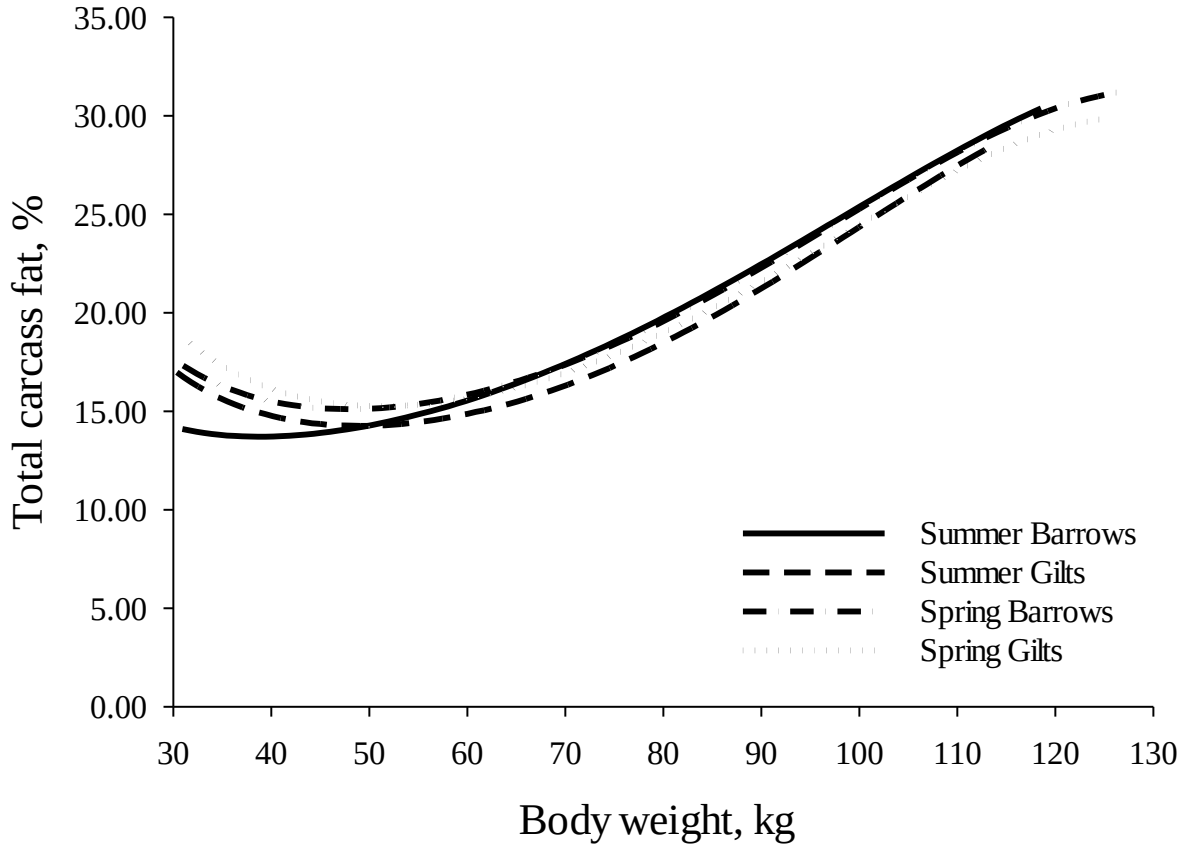

Figure 5.13 Prediction of total carcass fat percentage for season replicate and sex based on BW. The exponential ($TCF = \exp(b_0+b_1x + b_2x^2 + b_3x^3)$ functions (x) of BW (Wagner et al., 1999) was used to predict total carcass fat. Daily total carcass fat accretion rates were determined as the product of the derivatives of two functions by $\partial C/\partial T = ((\partial C/\partial BW)\ x\ (\partial BW/\partial T))$, (Schinckel and De Lange 1996), where C is the body component mass. (n = 30 pigs/sex/replicate).

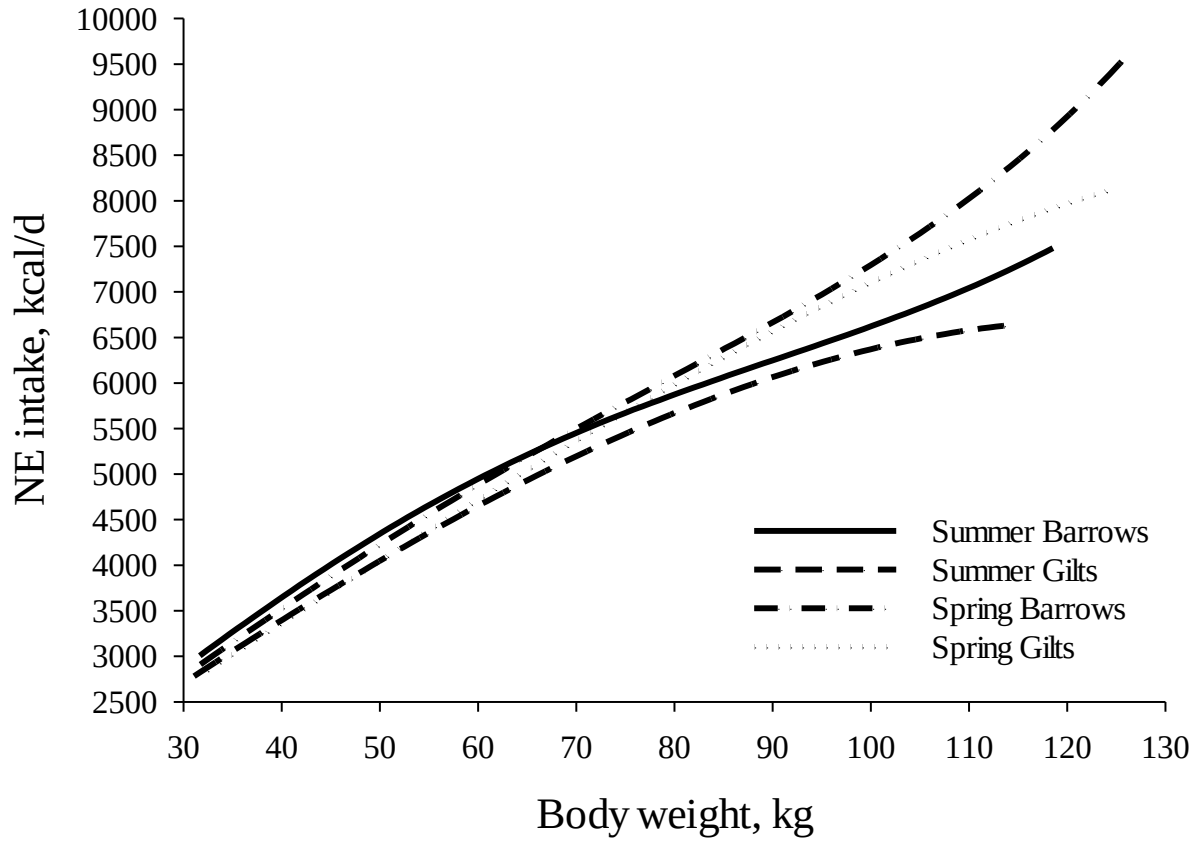

Figure 5.14 Prediction of daily net energy (**NE**) intake for season replicate and sex based on BW. Daily NE intake was predicted as NE intake, Kcal/d = ($0.179BW^{0.60}$ + (5.6863 × Empty body protein accretion, kg/d) + (9.509 × Empty body lipid accretion, kg/d)) × 1,000, where $0.179BW^{0.60}$ is the NE required for maintenance where 5.6863 and 9.509 are the energy content of empty body protein accretion and empty body lipid accretion, respectively (Noblet et al., 1999). (n = 30 pigs/sex/replicate).

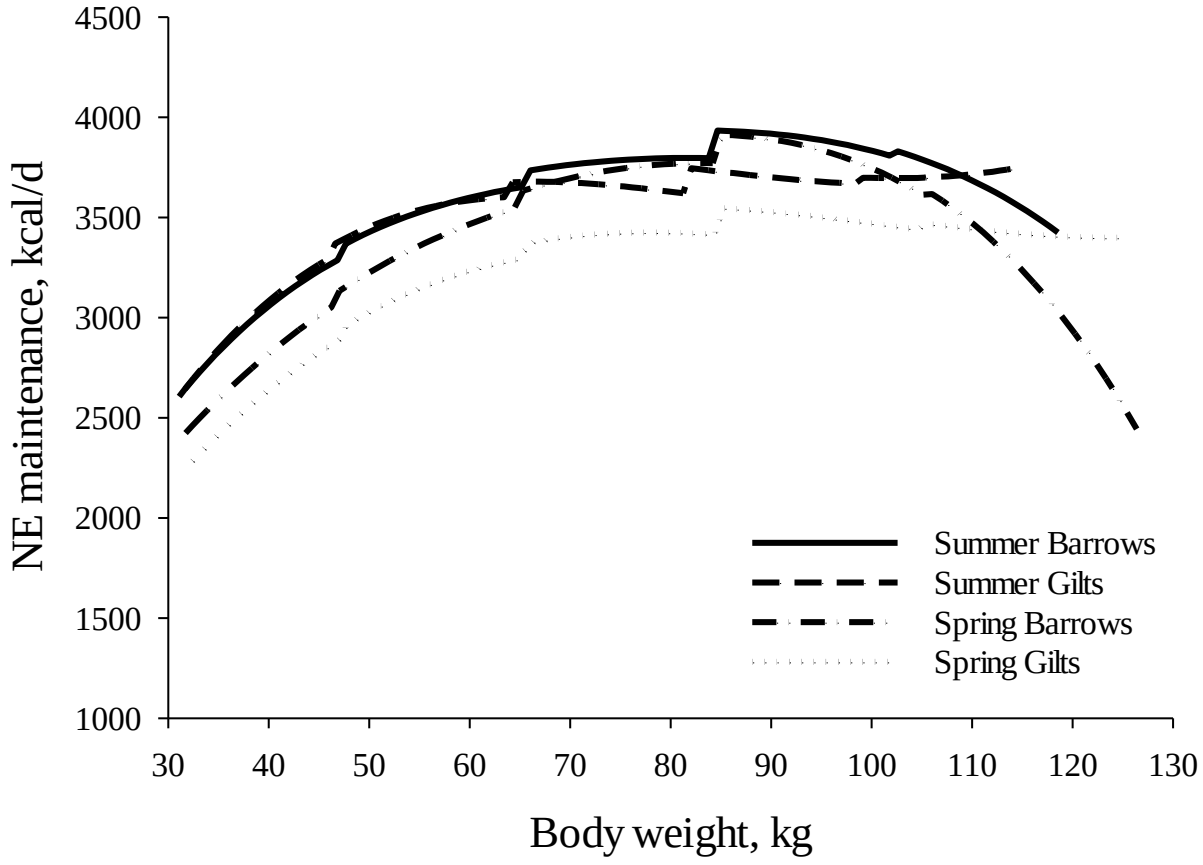

Figure 5.15 Prediction of net energy (**NE**) intake required for maintenance for season replicate and sex based on BW. Net energy required for maintenance was predicted as $NE_{maintenance} = NE_{intake} - (NE_{protein} + NE_{lipid})$, where $NE_{intake} = NE_{diet} \times ADFI_{prediction}$; $NE_{protein} = (5.6863 \times$ Empty body protein accretion, g/d) and $NE_{lipid} = (9.509 \times$ Empty body lipid accretion, g/d) (Noblet et al., 1999). (n = 30 pigs/sex/replicate).

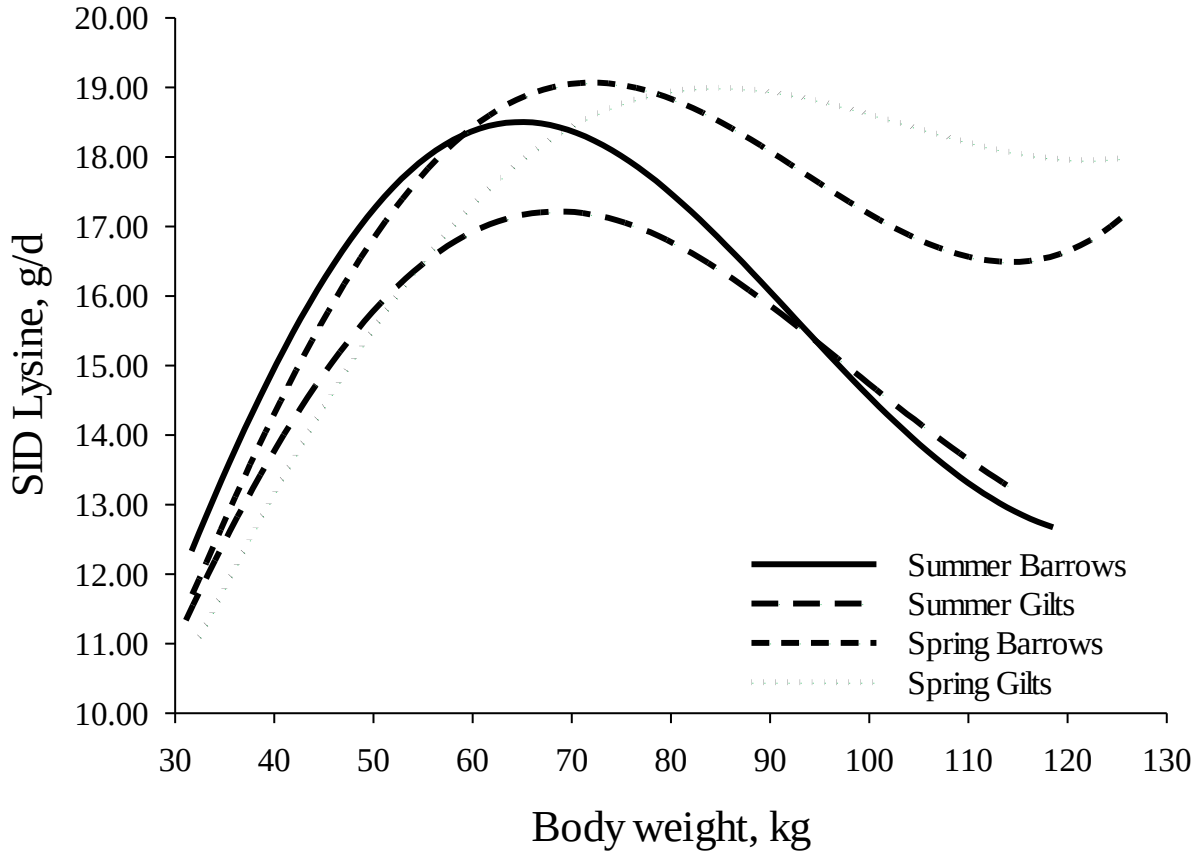

Figure 5.16 Prediction of standardized ileal digestible (**SID**) lysine requirement for season replicate and sex based on BW. The SID lysine requirement was estimated as SID lysine (g/d) = gastrointestinal tract (**GIT**) losses, g/d + integument losses, g/d/{0.75 + [0.0002 × (maximum empty body protein accretion − 147.7)]}, where maximum empty body protein accretion is an estimate of the maximal protein deposition (g/d) of the pig (NRC, 2012). The GIT losses were calculated as: basal endogenous GIT lysine losses (g/d) = feed intake (g/d) × (0.417/1,000) × 0.88 × 1.1 (NRC, 2012). Feed intake for each sex within replicate was estimated as follows: $ADFI_{barrows} = 1.03 \times ADFI_{replicate}$ and $ADFI_{gilt} = 0.97 \times ADFI_{replicate}$. The integument losses are predicted as follows: integument losses (g/d) = $0.0045 \times (BW, kg)^{0.75}$ (NRC, 2012). (n = 30 pigs/sex/replicate).

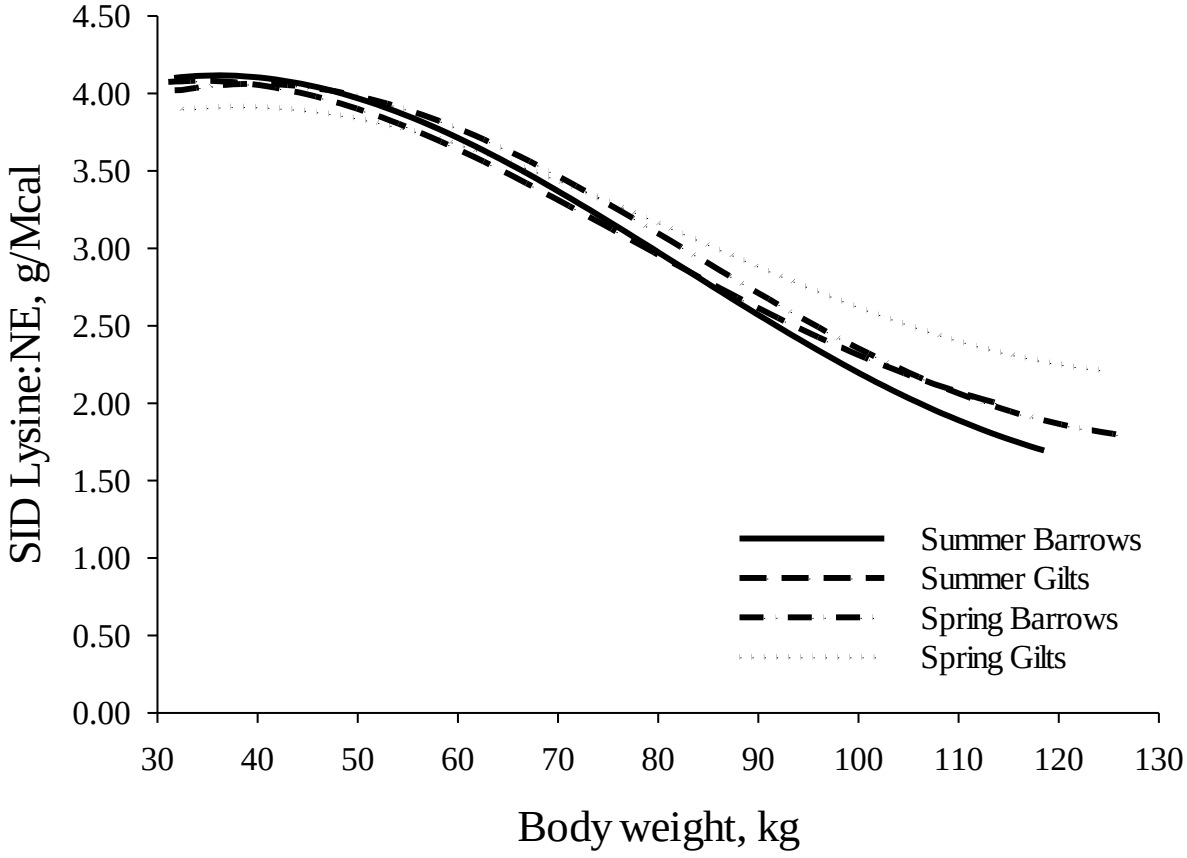

Figure 5.17 Prediction of standardized ileal digestible (**SID**) lysine requirement:Net Energy (**NE**) intake (g/Mcal) as percent of diet for season replicate and sex based on BW. The SID Lysine:NE (g/Mcal) was predicted using the SID lysine (g/d) and NE intake (Mcal/d = kcal/d × 1,000) data. Standardized ileal digestible lysine was divided by NE intake for a given BW to produce the SID Lysine:NE curve based on BW. (n = 30 pigs/sex/replicate).

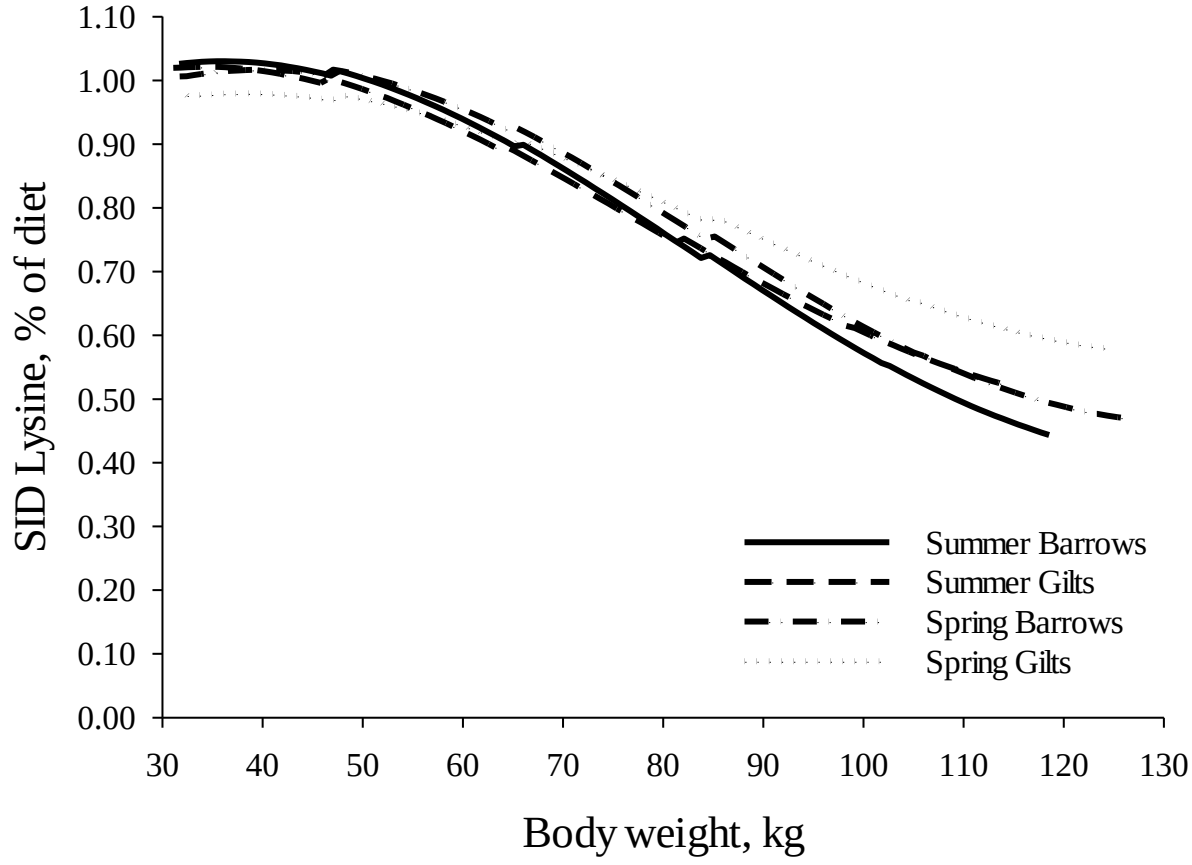

Figure 5.18 Prediction of standardized ileal digestible (**SID**) lysine requirement as percent of diet for season replicate and sex based on BW. The SID lysine requirement as percent of diet was predicted using the SID Lysine:Net Energy (**NE**) (g/Mcal), where SID lysine:NE (g/Mcal) was multiplied by $Diet_{NE}$ for a given dietary phase (Mcal/g of diet) multiplied by 100. (n = 30 pigs/sex/replicate).

CHAPTER 6. REPLACING DIETARY ANTIBIOTICS WITH L-GLUTAMINE FOLLOWING WEANING AND TRANSPORT IN SWINE: FUTURE RESEARCH

The addition of added Glutamine (**GLN**) to swine diets as a potential dietary antibiotic replacement shows great promise. Previous studies have determined that pigs fed GLN can have growth performance that equals dietary antibiotics without increased production costs. In addition, pigs fed GLN had improved intestinal health and reduced inflammation compared to pigs not fed dietary antibiotics. Future research of dietary GLN inclusion in swine diets should focus on how the magnitude of a stress response alters the dietary GLN needs of weaned pigs, how diet complexity impacts the growth response of added GLN, and continual work on the basic mechanistic understanding of how and when GLN improves gut barrier function and intestinal health.

Research in the previous chapters determined that GLN improved growth performance and intestinal health following weaning and transport stress. Future research needs to determine if stressors are additive. If it is determined that stressors are indeed additive, future work should then determine if the optimal inclusion level of GLN is altered by differences in the magnitude of stress experienced by the pig.

Since porcine epidemic diarrhea virus (**PEDV**) was first diagnosed in the United States in April 2013, swine producers have been critically evaluating potential vectors and looking for opportunities to mitigate risk of pathogen exposure. Feed ingredients have been evaluated very critically as potential pathogen vectors. This is due to several reasons including: country of origination and foreign animal diseases, raw materials and manufacturing processes, and sanitary handling of product prior to receiving ingredients in feed mills. As a result of the ingredient review

process, some U.S. swine producers have chosen to remove animal protein ingredients from swine nursery diets due to perceived risk of the raw material originating from livestock harvest facilities.

Historically, animal protein ingredients have commonly been used in swine nursery diets due to high amino acid content and high amino acid digestibility. These animal protein ingredients are now being replaced by plant derived protein sources and synthetic amino acids. Plant derived protein sources may contain reduced levels of GLN or be less digestible compared to animal derived protein sources. Future research needs to determine the appropriate GLN level in swine diets to optimize swine health and performance when alternative protein and amino acid sources are utilized in swine nursery diets.

Additional data continues to be generated about GLN attenuating weaning stress and improving intestinal health. In the previous chapters, new data has been generated to deepen the scientific community's understanding of GLN function in the intestine as it relates to reducing inflammation. Continued research is needed to better understand how GLN reduces the negative effects of corticotropin releasing factor (**CRF**) on intestinal permeability. In addition, serial tissue collection studies are needed to pinpoint when GLN improves biological markers of intestinal health so the full benefit of GLN can be documented.

As swine producers continue to be asked by consumers to produce more with less resources, improve animal welfare, and do so without dietary antibiotics; GLN is well positioned to be able to help producers standup to the headwinds and the challenge. Dietary GLN has shown great promise as being an effective alternative to dietary antibiotics. Future research will be able to help position dietary GLN as an effective tool for pork producers.