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From Crypt Depths to Carbon Dioxide Equivalents: Characterizing the Interactions between Swine Nutrition, Health and the Environment

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FROM CRYPT DEPTHS TO CARBON DIOXIDE EQUIVALENTS: CHARACTERIZING THE INTERACTIONS BETWEEN SWINE NUTRITION, HEALTH AND THE ENVIRONMENT

by

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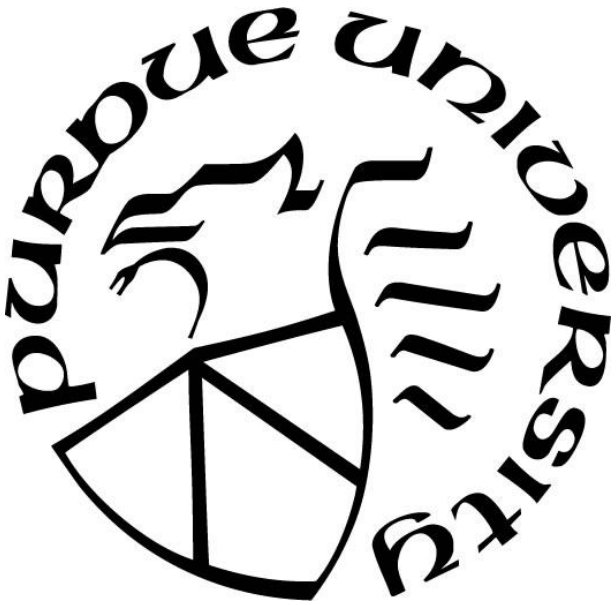

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To my parents, for teaching me to always approach my challenges with a good sense of humor and a lot of grit, and for my sister for never hesitating to be my partner in crime.

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This paper exists because I have had the best of help.

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ABSTRACT

Author: Vonderohe, Caitlin, E.. PhD
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There are many ways to measure the relative success and sustainability of a swine production system. The most economically relevant measures are growth performance and feed conversion. However, other measures such as greenhouse gas emissions, nutrient excretion, health and degree of intestinal inflammation can also be significant reflections of the interaction between nutrition, genetics and environment. Three experiments were conducted to characterize how swine nutrition and management affect the environmental footprint of swine production. The objective of experiment 1 was to determine the effect of reducing dietary crude protein and supplementing synthetic amino acids on growth performance, greenhouse gas emissions and nutrient excretion in wean to finish pigs. Three diets were fed: 1) control diet, balanced on lysine with no synthetic amino acids, 2) a diet containing a CP concentration intermediate between diets 1 and 3, balanced to meet all amino acid requirements, using synthetic amino acids where needed, and 3) a low CP diet balanced to the 7th limiting amino acid with synthetic amino acids, replacing soy proteins. Pigs fed the lower crude protein diet had reduced N excretion and NH_3 emissions, however growth performance in the pigs fed the diet balanced to the 7th limiting amino acid was reduced compared to the control. The objective of experiment 2 was to determine how antibiotic-free management affected growth performance, frequency of clinical signs, and the environmental footprint of swine production. Pigs were either reared with antibiotics or antibiotic alternatives. There were no observed differences in greenhouse gas emissions, nutrient excretion, growth performance or feed conversion, but significantly more animals were removed when reared without antibiotics than reared with antibiotics due to health concerns. The objective of experiment 3 was to evaluate and validate a novel, digital method for measuring intestinal inflammation. Intestinal tissues from pigs selected for high, mild or no allergic response to soy protein

were collected, fixed in 10% NBF, embedded and stained with Hematoxylin and Eosin, Alcian blue, and CD3+ IHC. The H and E slides were analyzed for villus height and crypt depth by two technicians in Aperio® Imagescope and Adobe Photoshop software to compare the accuracy and consistency of measures. The measures taken in Aperio® Imagescope more accurately reflected the severity of soy allergy than the measures taken in Photoshop, but Photoshop measures were more consistent between observers. The H and E, Alcian Blue and CD3+ IHC slides were colorimetrically analyzed by Aperio® Imagescope software using algorithms designed to quantify eosinophils, goblet cells and CD3+ T cells. These counts were compared to manual cell counts performed by two blinded observers, and analyzed to determine how accurately the cellular infiltrate reflected the severity of soy allergy and inter-observer variation. There was greater ($P<0.05$) inter-observer variation in cell count observations collected manually than cell counts calculated by Aperio® Imagescope software and the Aperio® Imagescope measurements did not correlate, in nearly all measures, with the manual counts. In conclusion, the methods used can significantly alter the conclusions reached about holistic animal health and the sustainability of a swine production system, therefore it is important to use a variety of methods, from intestinal histology to production system-level greenhouse gas emissions to support these conclusions.

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1 Livestock and the Environment

Livestock are fed to meet their nutrient requirements. Unfortunately it is impossible to balance diets to precisely meet nutritional requirements without exceeding at least some of them. The animals will also not use 100% of nutrients consumed. Excess nutrients are excreted in feces and urine or in gaseous form. Therefore the efficiency of livestock production is not 100% and it is important to understand how this affects the environment the animals are reared in but also the overall environmental footprint of livestock production.

Livestock production profoundly affects the environment and may significantly contribute to climate change. The magnitude of this impact varies by species and by location. Investigators have attempted to quantify this impact across species in the form of life cycle assessments (de Vries and de Boer, 2010; FAO, 2006; Guinee et al., 2002). The methodology used to determine which facets of a production system need to be included in an LCA differ among studies (de Vries and de Boer, 2010). However in 2006, the Food and Agriculture Organization of the United Nations published Livestock's Long Shadow, a highly comprehensive LCA for multiple livestock species that shaped a great deal of the public perception of the global environmental impact of livestock.

There are two primary ways that livestock affect the environment: the use of resources such as land or fossil fuels during the production life cycle and the emission or excretion of pollutants (Guinee et al., 2002). The incorporation of these aspects of the production cycle are incorporated to different degrees in different assessments of the impact of livestock production on the environment.

Livestock's Long Shadow had a much less direct criteria for inclusion in the life cycle assessments included in the report (FAO, 2006). The FAO analyzed, and included, potential greenhouse gas emissions from transport of the animals, feed ingredient production, and manure storage. Using this inclusion criteria, the FAO concluded that livestock production contributes more to global greenhouse gas (GHG) emissions than fossil fuel combustion in vehicles (FAO, 2006). However this conclusion is questionable because of the depth and breadth of the livestock assessment (it included all aspects of livestock production including the construction of livestock-related infrastructure) relative to the "transport" assessment which only included an estimate of current greenhouse gas emissions due to fuel use (FAO, 2006).

Whereas the FAO estimated that livestock contribute 18% of total anthropogenic GHG emissions, the EPA estimated that livestock actually contribute 8.3% of all U.S. GHG emissions (EPA, 2016). The difference in the estimates is likely due to the breadth of the inclusion criteria used in the FAO analysis. The EPA estimate is likely a better reflection of annual GHG contribution by livestock production (EPA, 2016; FAO, 2006).

Although there is some question about the breadth of the FAO analysis and the subsequent comparison to GHG emissions by vehicles, this report has driven a great deal of the public perception of the environmental impact of livestock production, including encouragement from some organizations to reduce animal protein consumption in favor of plant protein-based diets (Laestadius et al., 2016). However there is increasing demand for animal protein, particularly from the developing world because animal protein is nutrient dense, palatable, and consumption thereof is a status symbol (de Vries and de

Boer, 2010). Livestock remains the fastest internationally growing segment of agriculture (Steinfeld and Wassenaar, 2007).

Swine production produces fewer greenhouse gases and uses less land than beef or dairy production (de Vries and de Boer, 2010). Swine contribute approximately 0.03% of total GHG emissions due to enteric fermentation, and approximately 0.3% of total GHG emissions from manure management (EPA, 2016). However, there are additional ways that swine production affects the environment beyond greenhouse gas emissions, including downstream eutrophication of aqueous habitats due to nutrient run- off after manure application and odors associated with swine manure during storage and application as fertilizer (Dourmad and Jondreville, 2007).

The objective of this literature review is to examine how different minerals and organic molecules excreted by swine into manure affect the environment and how nutrition and management can mitigate these effects.

1.1.1 Nitrogen

1.1.1.1 Nitrogen in the Pig

Nitrogen is primarily consumed by the pig in the form of proteins, small peptides and amino acids. Chemical digestion begins in the stomach with the addition of hydrochloric acid and the zymogen pepsinogen. Pepsinogen is chemically transformed to pepsin at a low pH. The combination of low pH and pepsin simultaneously denatures the quaternary structure of the protein and cleave amide bonds, reducing proteins to small peptides. In the duodenum, pancreatic proteases are added as zymogens and subsequently activated in the lumen of the duodenum by enterokinase. Proteolytic enzymes reduce large polypeptides into di- and tripeptides and amino acids which are then absorbed by

active transport, simple diffusion and facilitated diffusion. (Yuanlong et al., 1995) It has been hypothesized that small peptides are more efficiently absorbed than amino acids (Otto et al., 2013).

One of the destinations for absorbed amino acids is to be incorporated into protein synthesis. Proteins are synthesized during cell division, for cellular maintenance, or to increase cell size. Protein synthesis is primarily under the regulation of rapamycin (mTOR), which is regulated in the presence of insulin and amino acids. In fact, the presence of glucose, amino acids and insulin increases the rate and efficiency of protein synthesis in visceral organ tissues such as the liver, pancreas and spleen, and in skeletal muscle (Yao et al., 2013).

Amino acids also feed into other cellular processes such as energy metabolism and can be bio-transformed into other amino acids. After intestinal absorption, peptides and amino acids follow portal circulation to the liver where they are sorted for their eventual fate. Some (alanine, cysteine, glutamate, histidine and lysine) are directly involved or are de-aminated then used in gluconeogenesis. Others (arginine, aspartic acid) are used in the urea cycle (Yin et al., 2013).

There are multiple pathways for protein degradation. Intracellular pathways, such as the proteasome or the lysosome are activated when there is a significant lack of essential amino acids and insulin. The calcium-activated proteolytic process is an important aspect of physiologic response to injury. After a protein has been tagged for degradation or has been degraded into its constituent parts, the amino acids may be recycled and used in other proteins or undergo trans-amination, which will result in the

eventual removal of the amino group, leaving a carbon skeleton that can be fed into an energy-generating pathway (Yin et al., 2013).

During the trans-amination process, the alpha amino group is removed from the amino acid of interest and transferred to alpha-ketoglutarate, making glutamate. The glutamate will accumulate and eventually release the nitrogen group to urea in the liver. Urea is freely filtered by the glomerulus and subsequently excreted by the kidney in urine (Yao et al., 2013). Approximately 30g protein / 100 g consumed protein is excreted in the feces as endogenous losses, and approximately 15% of consumed protein is not digested and subsequently lost in feces (Ferket et al., 2002). However, the majority of nitrogen is excreted in urine by the aforementioned processes (Otto, 2003).

1.1.1.2 Nitrogen in the Environment

1.1.1.2.1 The Nitrogen Cycle

Nitrogen is used by bacteria and plants to be incorporated into amino acids, which are then used to synthesize bacterial and plant proteins. N_2 is the relatively inert form of nitrogen that exists in the atmosphere (78%) which will be fixed by terrestrial bacterial populations or lightning strikes into molecules that will become plant proteins. Both animal waste, in the form of urea and feces, and decaying plant matter feed into this cycle to release volatile nitrogen compounds (ammonia) that can be oxidized and then returned to the atmosphere to continue the cycle. (Steinfeld and Wassenaar, 2007).

Swine fit into the nitrogen cycle because they consume and subsequently produce nitrogenous compounds. Ideally, the pig will maximize the deposition of nitrogenous compounds in the form of skeletal muscle protein, however, swine retention of nitrogen ranges from 32% (Dourmad and Jondreville, 2007) to approximately 50% (Kerr and

Easter, 2006) of intake. Swine excrete nitrogen in the form of urea which is degraded by urease, found in various bacteria in feces and soil, to ammonium. The ammonium is lost during storage or in urine puddles on pen floors when it is converted to ammonia which then volatilizes to the atmosphere. The degree of volatilization is pH and temperature dependent. (Jongbreuer and Monteny, 2001)

1.1.1.2.2 Liquid Nitrogen Pollution

In the natural world, nitrogen availability is a limiting factor in plant growth (Erisman et al., 2013). Therefore manure, which contains nitrogenous compounds, is routinely applied to fields for crops as fertilizer. Although this arrangement maximizes the possible use of the nitrogenous compounds originally fed to the pig, the use of highly nitrogenous swine manure may result in unintended environmental consequences if the manure is improperly applied and escapes the intended soil as run-off, leaches into local waterways, or volatilizes as ammonia (Steinfeld and Wassenaar, 2007).

There is a limited amount of nitrogen fixation that naturally occurs, resulting in the overwhelming majority of the world's ecosystems adapted to low levels of reactive nitrogen, resulting in low productivity but high levels of biodiversity (Erisman et al., 2013; Steinfeld and Wassenaar, 2007). Therefore, when excessive nitrogen leaches into an environment it increases the productivity and growth of different plant species; the excess nitrogen is beneficial for some species but toxic to others (Erisman et al., 2013; Kenny and Hatfield, 2008). Excessive nitrogen may also alter the microbial population of soil and/or water, therefore affecting the rest of the ecosystem (Steinfeld and Wassenaar, 2007).

When nitrogen (typically in the form of ammonium) leaches into bodies of water it can exacerbate the growth of some plant species, particularly algae. The greater amount of organic matter now present in the polluted body of water will eventually decay, depleting the oxygen present in the body of water. When a body of water becomes hypoxic, it can result in the death of other organisms present in the body of water, such as fish. (Erisman et al., 2013).

Nitrogen in the form of nitrate (NO_3) can discharge into drinking water sources after manure or fertilizer application. If excessive nitrates (greater than 10 ppm) are consumed by humans, it can lead to methemoglobinenia (blue-baby syndrome) in infants. Nitrate pollution of drinking water is also associated with abortion and stomach cancer (FAO, 2006).

1.1.1.2.3 Gaseous Nitrogen Emission in Confinement

Exposure to high concentrations (greater than 50 ppm) of ammonia (NH_3) can cause clinical pulmonary disease and ocular irritation in both humans and swine (Peterson et al., 2010). If ammonia is present at a concentration of 22 ppm, 15% fewer replacement gilts reach puberty before 28 weeks of age (Malayer et al., 1987). If ammonia is present at 25 ppm or 50 ppm it can reduce average daily gain by 6% and 12%, respectively (Peterson 2010). Ammonia present in a facility at 100 ppm is associated with a 30% reduction in average daily gain, and 9% reduced feed conversion (Peterson et al., 2010). Ammonia represents a significant occupational hazard for people who work in, and around swine buildings because lung disease, mucous membrane irritation, and watery eyes have all been reported when humans are exposed to ammonia levels of 25 ppm or higher (Schiffman et al., 2005).

Nitrous oxide (N_2O) is also a pulmonary irritant (Erisman et al., 2013). However, N_2O is not considered as great of an irritant because very little (0.0001 kg N_2O/kg N) is released during manure handling relative to other gasses that are much greater irritants (FAO, 2006).

1.1.1.2.4 Global Gaseous Nitrogen Pollution

Approximately 23 million metric tons of nitrogen in the form of ammonia was produced by domestic animals in 2006, and this number appears to remain consistent (FAO, 2006; Erisman et al., 2013). Approximately 40% of ammonium in manure topically applied as fertilizer volatilizes to ammonia (Steinfeld and Wassenaar, 2007). It is possible to reduce ammonia emissions by up to 95% with deep injection instead of topical application of manure as fertilizer (Webb et al., 2010). This ammonia is released to the atmosphere where, as ammonium, it will dissolve in the water present in the atmosphere and react with acidic compounds. This interaction results in the formation of nitrogen salts which are then deposited by rain events and can result in the same aforementioned eutrophication of terrestrial environments and bodies of water as liquid nitrogen pollution. Ammonia can also be oxidized to form NO_3 which can be deposited into drinking water and cause severe human health problems. (FAO, 2006)

In swine production, 15-20% of slurry N may be emitted as N_2O (Jongbreuer and Monteny, 2001). Nitrous oxide is an extremely potent greenhouse gas that has 300 times the global warming potential of carbon dioxide (Jongbreuer and Monteny, 2001). Nitrous oxide reacts with ozone to reduce forest productivity, which results in less carbon sequestration, resulting in greater atmospheric carbon dioxide. The increase in atmospheric carbon allows energy to remain in a climate, leading to climate change.

(Galloway et al., 2008; Erisman et al., 2013). In fact, doubling the concentration of N_2O in the atmosphere would result in an "estimated 10% decreased in the ozone layer which would in turn increase the ultraviolet radiation reaching the earth by 20%" (FAO, 2006).

1.1.2 Carbon

1.1.2.1 Carbon in the Pig

Carbon is primarily present in the atmosphere as carbon dioxide (CO_2) (EPA, 2016). Photosynthesis converts CO_2 and solar energy into carbohydrates; storing energy in the form of covalent bonds. Swine consume these covalent bonds in the form of carbohydrates, fats and amino acids present in the majority of feed ingredients. The carbon-chains are digested, absorbed, and subsequently metabolized by cellular respiration. Carbon dioxide is released as a waste product, transported to the lung, and exhaled by the animal. The exhaled CO_2 feeds back into the carbon cycle. (Shepherd, 2011)

In swine, not all organic molecules are fully digested or absorbed by the gastrointestinal tract. In some cases, carbon bonds are not accessed or hydrolyzed by mammalian enzymes. These molecules are either excreted in waste or are anaerobically fermented in the swine hindgut. Here, longer organic molecules are digested into volatile fatty acids, which can be absorbed and used as an energy source by the pig, and methane, which is released to the environment. Organic molecules excreted in fecal material can also be fermented during storage, releasing methane and other volatile organic compounds (Shepherd, 2011).

1.1.2.2 Carbon excretion in confinement

Carbon dioxide is not known to be a significant pulmonary irritant. However, excessive CO_2 present in a confined space may modulate the pulmonary inflammatory cascade in the presence of other gaseous irritants such as hydrogen sulfide (H_2S) (Schneberger et al., 2017). Methane is produced by enteric fermentation and anaerobic fermentation in stored manure. Methane is primarily considered an odorant, but is also considered an asphyxiant if it comprises greater than 85% of inhaled gas (Pickrell et al., 2012). Methane becomes an explosion risk when it is present in excess of 10-15% of total gas in a confined space (Pickerell et al., 2012).

1.1.2.3 Carbon in Global Climate

The primary contributors to the carbon cycle are human/animal respiration, decomposition of organic matter, and fire (including combustion engines) (Steinfeld and Wassenaar, 2007). Humans have significantly altered the carbon cycle by contributing to it in the form of the combustion of fossil fuels (oil and coal), the removal of carbon sinks (historic deforestation), and in the form of livestock production (Shepherd, 2011). Livestock have recently been marked as major contributors to the global carbon cycle because of the concentration of production and growth in response to demand for animal protein (de Vries and de Boer, 2010). Manure is a significant source of carbon and methane in the non-ruminant whereas enteric rumen fermentation is a more significant source of methane in the ruminant (Shepherd, 2011).

Global atmospheric CO_2 has increased by 40%, to 393.6 ppm, from pre-industrial times to 2012 (Ciais, 2013). The greater concentration of CO_2 in the atmosphere is strongly associated with the greenhouse gas effect. Carbon dioxide, among other

greenhouse gases, lingers in the atmosphere to allow energy to remain in climate systems which results in prolonged, elevated temperatures. This elevation in temperature results in climate change, which results in changes in plant growth, rising sea levels and changing patterns of disease (Shepherd, 2011).

Methane is considered 20 times more likely to trap heat energy than CO_2, primarily because it lingers in the atmosphere. Fifty percent of methane is attributed to fossil fuels, livestock production, rice cultivation, and waste management. A sizeable proportion of this emission occurs during manure storage; China has the largest country-wide emission of methane from swine manure (Steinfeld and Wassenaar, 2007).

1.1.3 Sulfur

1.1.3.1 Sulfur in the pig

There are multiple sources of sulfur in swine diets. Some of these source include sulfur-containing amino acids (Kim et al., 2015), sulfuric acid added to dried distillers grains with solubles (DDGS) during processing (Kim et al., 2015; Song et al., 2013) and exogenous sulfur-containing compounds added to the diet for various purposes, particularly in trace mineral premixes and $CuSO_4$ added as an antimicrobial (Cromwell, 2002). In the body, sulfur is incorporated into proteins and sulfur- containing molecules have been shown to have an antioxidant effect (Song et al., 2013).

1.1.3.2 Sulfur Excretion

When sulfur-containing compounds are not fully digested and absorbed, sulfur can be excreted in feces or anaerobically fermented to be released as gaseous H_2S. Sulfur can also be introduced to the manure slurry as feed wastage or as water spillover. When

sulfur-containing amino acids (Hartung and Phillips, 1994), or other sulfur containing compounds (Clark et al., 2005) are anaerobically fermented, hydrogen sulfide is released.

Hydrogen sulfide is toxic when inhaled and particularly dangerous while manipulating manure storage tanks or pits because it is heavier than air and insoluble in water. Hydrogen sulfide has been shown to cause caspase-3 mediated apoptosis of smooth muscles in-vitro. If H_2S concentrations reach 50-150 ppm, animals or humans inhaling the contaminated air can experience pulmonary edema. When H_2S concentrations are above 500 ppm, this can result in permanent neurologic damage. If H_2S reaches over 2,000 ppm, the mammalian respiratory tract will be paralyzed. (Pickerell, 2012) Beyond its properties as a pulmonary toxicant and irritant, hydrogen sulfide is also responsible for 50% of offensive odors from swine waste (Clark, 2005)

1.1.4 Phosphorus

1.1.4.1 Phosphorus in the pig

Phosphorus balance, represented by absorption in the intestine, bone resorption, and renal excretion is tightly regulated by multiple facets of the endocrine system. Parathyroid hormone and calcitriol mediated sodium-dependent active transport in the jejunum, RANK-L controlled calcium and phosphorus resorption from bone, and active transport of phosphorus in the proximal convoluted tubule of the kidney. Phosphorus is an important aspect of bone mineralization. It is also a significant player in bioenergetics, blood pH buffering, signal transduction, and cell membrane polarity. (Oster et al., 2016)

1.1.4.2 Phosphorus Excretion

The primary challenge of adding phosphorus to a diet is to balance the digestibility of the phosphorus source with the rate of deposition and degree of soft tissue

storage (Leyoutneau-Montminy et al., 2015). The degree of phosphorus absorption and deposition is dependent on animal age and degree of bone deposition, dietary calcium (Ferndandez et al., 1995), and the digestibility of the phosphorus present in feed ingredients (Oster et al., 2016).

Phytic acid is the primary storage form of phosphorus in plants commonly used as feed ingredients for swine. This affects how we supplement phosphorus to monogastrics because monogastrics do not naturally produce phytase; making the phosphorus present in phytic acid unavailable to the animal. Phytic acid freely binds minerals such as Ca and Zn, becoming phytate. However, exogenous phytase can be, and is often, supplemented to improve the digestibility of phosphorus in monogastric diets (Oster et al., 2016).

1.1.4.3 Phosphorus Pollution

When manure is applied to fields as fertilizer, phosphorus is typically held by soil particles better than nitrogen, and therefore less likely to spontaneously leach into the local environment. However, when excess phosphorus is present in applied manure or commercial fertilizer and either erosion or run-off into bodies of water occurs, the phosphorus will have a similar eutrophication effect as is seen in nitrogen pollution (FAO, 2006). Livestock excrete 60-80% of fed phosphorus, therefore manure often contains nitrogen and phosphorus in an inverse of the intended crop requirements, which further exacerbates runoff and pollution of local bodies of water (Knowlton et al., 2004)

1.1.5 Strategies Used to Reduce the Environmental Impact of Swine Production

Livestock affect the environment in different ways which can be considered in terms of production inputs and outputs. There are significant climate effects associated with land use for livestock housing and feed production. It is also important to consider

the products of livestock production beyond meat, milk, or eggs that are intentionally produced. The animals themselves produce greenhouse gases through enteric fermentation. Manure produced by the animals can directly pollute local ecosystems and manure storage is associated with toxic and greenhouse gases. (FAO, 2006)

The three primary issues surrounding livestock manure storage and application are the accumulation of excess nutrients in soil, eutrophication of bodies of water, and gas emissions (Jongbloed and Lewis, 1998). Animal waste, depending on species and nutritional status, typically "exceeds the assimilatory capacity of [local and regional] landscape" (Mallin and Cahoon, 2003). If excess nutrients accumulate in the soil, these nutrients often fail to remain in the soil and either run-off or leach into surrounding bodies of water or terrestrial ecosystems where they impact local plant and animal life. The gasses emitted by stored manure range from severely toxic (H_2S) to highly significant greenhouse gases (N_2O) (Jongbloed and Lewis 1998).

Paik (1996) presented several strategies to reduce the environmental impact of animal manure. Perhaps the most intuitive of these strategies is to reduce the amount of livestock produced, because fewer animals produce less manure. However, this strategy is in contrast with the recent growth experienced by the livestock industry (de Vries and de Boer, 2010). The human population is growing, and a great number of countries with the fastest growing populations have the greatest proportion of that population entering the middle class and therefore able to afford animal protein (de Vries and de Boer, 2010).

Another strategy described by Paik (1996) is to transport manure from nutrient-rich areas to nutrient-poor locations. Although this is plausible it has logistical shortcomings and the transport process would further contribute to greenhouse gas

emissions. The third strategy is to collect and recycle excreted minerals and/or gases to be placed back into the production system as feed or fuel. This has been used to some success in some sectors of the industry, but is still under development (Holm-Nielsen et al., 2009). Another strategy described by Paik (1996) is to continue to apply manure as fertilizer and grow crops that better utilize the nutrients present in manure to prevent leaching and run-off.

There are several ways that manure management has been used to reduce both emissions and nutrient content of manure. For example, the aeration of slurry stores to reduce anaerobic fermentation has successfully reduced the emission of H_2S (Clark et al., 2005). Other investigators have used biofiltration to remove different components of manure prior to field application (Paik, 1996). Others (Galloway et al., 2008) have successfully treated the manure to remove solids to reduce emissions (Klimont et al., 2004), precipitate phosphorus (Paik, 1996), added acids to reduce ammonia volatilization (Hartung and Phillips, 1993) and other additives to improve in-slurry digestion of solids (Petersen et al., 2010) prior to field application. Frequent pit flushing has also been shown to reduce the harmful effects of manure storage in confinement (Jongbreuer and Monteny, 2001). Finally, injecting manure into fields instead of surface application has also successfully reduced ammonia volatilization from fields and reduced surface runoff of nutrients during rain events (Jongbreuer and Monteny, 2001).

The final strategy described by Paik (1996) to decrease the impact of livestock manure on the environment is to minimize nutrient excretion by maximizing nutrient use by the animal. Although all of the previously described management strategies are logistically plausible ways to alter the effect of swine production on the environment, the

most widely explored strategy is limiting nutrient excretion by maximizing nutrient use by the animal. This effectively uses the same or similar inputs (feed, water, and infrastructure) in a more effective manner to change the composition of the farm outputs: yielding a greater concentration of the fed nutrients in the meat, milk, or eggs instead of in manure or gas.

1.1.5.1 Swine Nutrition to Reduce Emissions and Pollution

Proper husbandry and health management are two significant aspects to maximize the productivity and therefore minimize the excess nutrient excretion associated with swine production. Healthy, non-inflamed and non-stressed animals are the most efficient at using feed inputs to deposit skeletal muscle (Klasing, 1998). Beyond diet formulation, it is also important to maximize facility productivity by minimizing feed and water waste and optimal feed processing (Ferket et al., 2002).

1.1.5.1.1 Phase and Split-Sex Feeding

As a pig grows from a neonate to a market or breeding animal, the nutritional requirements of that pig change profoundly (Yin and Tan, 2010). Every aspect of the diet, from the ingredients used in diet formulation to energy and amino acid requirements can change as the animal grows. Therefore it is significantly more productive and less wasteful to feed the animal according to its requirements as opposed to a common diet throughout the production life cycle.

Amino acid requirements change throughout the animal's life depending on the primary tissue type being deposited at various parts of the animal's life. For example, newly weaned pigs will be synthesizing proteins involved in immune system development while finishing animals are more likely to deposit skeletal muscle (Yin and

Tan, 2010). Assuming similar intakes, animals that are genetically lean will require higher protein diets during phases of rapid growth. The same is true for animals treated with beta agonists and highly prolific sows. (Sutton and Richert, 2004)

The primary pollutants of concern present in swine manure are minerals that are either excreted by the animal or simply not digested by that animal. Mineral requirements, similarly to protein requirements, are dictated on what kind of tissue the animal deposits during certain life stages. Phosphorus is more readily deposited when the animal is experiencing rapid skeletal growth, therefore the metabolism of phosphorus can greatly differ in the young animal compared to older animals (Oster, 2016).

Gilts produce a leaner carcass, therefore they require greater protein than barrows at a similar body weight. Therefore, split sex feeding has the potential to reduce nitrogen wastage because greater precision can be practiced in diet formulation. (Petersen et al., 2010)

1.1.5.1.2 Use of Synthetic Amino Acids

Synthetic amino acids are highly available forms of essential amino acids that allow nutritionists to formulate diets according to the animal's amino acid requirements with great precision. It is possible to reduce nitrogen excretion by 50% with the use of synthetic amino acids and by phase feeding (Dourmad and Jondreville, 2007). Supplementation of synthetic amino acids (lysine, threonine, methionine and tryptophan) can allow nutritionists to reduce dietary crude protein by 25.8% (Sutton and Richert, 2004). However there are multiple facets to synthetic amino acid use, including how that affects nitrogen retention, swine growth performance, and how their use can affect the environment.

1.1.5.1.3 Nitrogen Balance

Nitrogen retention changes with the addition of particularly high concentrations of, synthetic amino acids with a concurrent reduction in dietary crude protein. For example, Otto et al. (2003) demonstrated that reducing dietary crude protein by greater than three percentage units reduces nitrogen retention by the pig. The reduction in crude protein reduces the number of peptides available for absorption, compared to free amino acids. Protein may be better absorbed as small peptides instead of as individual amino acids. Humans absorb approximately 33% of their protein as amino acids and 67% as small peptides (Otto et al., 2003; Yuanlong et al., 1995). Therefore the reduction in N retention observed when low CP, synthetic amino acid-supplemented diets are fed may be due to the reduction in efficiently-absorbed peptides present in the diet.

Greater nitrogen retention has been reported with the supplementation of synthetic amino acids. Kerr and Easter (1995) found that pigs fed a 12% crude protein diet with supplementary lysine, tryptophan and threonine had greater nitrogen retention than pigs fed a 16% crude protein diet with no synthetic amino acid supplementation. Fan et al., (1994) reported that apparent amino acid ileal digestibility decreases when crude protein is less than 8%, but is not affected when crude protein is reduced from 15 to 12%. This observation may be reflective of endogenous protein loss, a basal non-protein N, or non-essential amino acid requirements. Reducing crude protein from 15 to 12% was also associated with a significant reduction in urinary nitrogen excretion (Fan et al., 1994).

1.1.5.1.4 Swine Growth and Carcass Characteristics

The excessive use of reduced CP, amino acid supplemented-diets can result in a reduction in growth performance and poorer carcass characteristics (Kerr and Easter,

1995). However, the reductions in economically relevant performance measures observed in elevated use of synthetic amino acids may be due to amino acid imbalances (Maxwell et al., 2016). In fact, Yin and Tan (2012) state that the formulation of diets on standardized ileal digestibility (including synthetic amino acids) improves average daily gain and feed conversion by 20%, compared to diets formulated on total amino acid content.

1.1.5.1.5 Effect on N emission

On average, nitrogen excretion decreases by 8% for every percentage unit reduction in dietary crude protein (Sutton and Richert, 2004). Kendall et al. (1998) showed that feeding reduced crude protein, synthetic amino acid-supplemented diets with additional dietary fiber results in a 40% reduction in NH_3 and H_2S and a 30% reduction in total odors. Jones et al., (2014) reported a 45% reduction in nitrogen excretion when diets were balanced (and supplemented with synthetic amino acid) to the seventh limiting amino acid compared to diets that had no synthetic amino acid supplementation. However, this study also noted a reduction in energy digestibility in pigs fed diets high in synthetic amino acids (Jones et al., 2014).

1.1.5.1.6 Nutrient Digestibility

Swine do not produce carbohydrases that can degrade cellulose, therefore cellulose consumed by the pig is inevitably fermented by the microflora present throughout the gastrointestinal tract, particularly in the hindgut. Diets supplemented with fiber have been reported to reduce ammonia emissions, but increase methane emissions and reduced carbon digestibility (Aarnik and Vestegen, 2007).

Another strategy used by nutritionists and producers to maximize nutrient digestibility in swine and poultry is the use of exogenous enzymes. These enzymes include proteases, lipases, pentosanases, xylanases, and oligosaccharidases that may improve the degradation of various molecules for eventual absorption of components of those molecules and/or degrade molecules that could elicit an immune response in the intestine. However, it has been reported that the addition of these enzymes improves ileal digestibility but does not improve overall dry matter digestibility by more than 1%. (Yin and Tan, 2010)

1.2 Health and Growth Performance in Livestock

Some of the foremost reasons why livestock do not reach their genetic growth or production potential are immune-mediated events in response to pathogens and stress (Cromwell, 2002). The relationship between livestock health and performance was strongly established by Klasing et al., (1987) in chicks. This study established that chicks housed in sanitary conditions grew faster and consumed more feed than animals exposed to immune mediated challenges, even in the absence of clinical signs. A similar phenomenon was observed in swine by van Heugten et al. (1994).

An immune-mediated challenge can result in a 15% reduction in growth rate and a 10% reduction in feed conversion in birds (Klasing et al., 1987). Multiple authors (Williams et al., 1997, van Heugten, 1994) have shown similar effects in pigs, including significant depressions in feed intake, growth rate, and feed efficiency. In fact, Williams et al. (1997) reported that immune challenged animals expend more energy to maintain their body weight than non-challenged. Daiwen et al. (2008) used lipopolysaccharide (a component of gram negative bacterial membranes) as an immune challenge in young pigs

and observed a 43% reduction in average daily gain, a 28% reduction in feed intake, and a 21% reduction in feed efficiency. Comparatively, non-immune challenged animals have historically had a faster rate of gain, increased feed intake, and a greater proportion of skeletal muscle relative to lipid in the carcass (van Heugten et al., 1994; Williams et al., 1997; Daiwen et al., 2008).

The reasons for this drop in performance are highly inter-related, complex, and may be pathogen- or challenge- dependent. Some of the reasons for the observed growth depression are a result of pathogen induced tissue damage. The pathogen may damage a specific organ or tissue necessary for nutrient absorption or weight gain, as seen with enteric pathogens such as salmonella damaging intestinal villi and therefore interfering with nutrient absorption (Brox et al., 2012). The pathogen may also cause systemic effects that lead to multiple organ dysfunction (Klasing et al., 1987).

The immune system can also damage non-infected tissue. This bystander damage is most often associated with the innate arm of the immune system, particularly degranulation of leukocytes such as neutrophils. This degranulation results in the release of free radicals and proteases which are, by nature, not cell-type specific and can result in significant pathology (Klasing, 2007).

Stimulation of the immune system also alters whole-body metabolism and the efficacy of nutrient use (Klasing et al., 1987). This alteration is due to the diversion of nutrients to fuel the energy- and protein- expensive immune response. It is also due to immune-mediated cytokine release and activation of different endocrine axes that modify whole-body metabolism. (Klasing, 2007)

1.2.1 Metabolic Effects of Immune Activation

During an immune challenge, nutrients are diverted from skeletal muscle to other organs, under the direction if Interleukin-1 and cortisol (Klasing et al., 1987; Cook et al., 1993). Williams et al. (1987) observed a greater incidence of fat deposition in challenged animals relative to skeletal muscle and Daiwen et al. (2008) noted a greater degree of protein turnover in LPS challenged swine. Ballmer (1991) and Williams et al. (1997) observed a greater liver and heart protein synthesis compared to skeletal muscle when animals are immune-challenged. Williams et al. (1997) also reported an increase in skeletal resorption in immune-challenged animals. Daiwen et al (2008) observed that an LPS challenge reduced nitrogen retention and apparent nitrogen digestibility by 10%. When these data were corrected for reduced intake, an LPS challenge resulted in a 46% increase in fecal nitrogen excretion (Daiwen et al., 2008).

1.2.2 The Immune System

The immune system is a uniquely functioning and wholly necessary aspect of "self-maintenance" in the body that includes a lot of cellular machinery, including leukocytes and accessory proteins. (Klasing, 2007) The immune system is also unique because it has specificity to respond to any external stimuli an organism may encounter in it's lifetime, and memory to respond to a pathogen more vigorously the second time it is encountered (Kubena and McMurray, 1996).

The immune system has two functional "arms," including the innate system that detects pathogen associated molecular patterns (PAMPs) and the adaptive immune system that identifies and detects pathogens via antigen receptors on B and T cells

(Klasing, 2007). The innate system has a much higher energetic and pathologic cost to the host than the adaptive immune system because the innate system maintains a population of antimicrobial-ready cells and proteins (Klasing, 2007; Mair et al., 2014). The innate system is also more likely to result in some form of immuno-pathology from the inflammatory cascade because it lacks the specificity of the adaptive system (Mair et al., 2014). The adaptive immune system has a lower metabolic cost and lower likelihood of causing immunopathology, but is initially ineffective when confronted with novel pathogens (Klasing, 2007).

Different species emphasize either the innate or adaptive immune system based on the species' longevity and reproductive rate. Species with shorter life-spans and high reproductive rates (such as poultry) typically rely more on the innate immune system than the adaptive. On the other end of the livestock spectrum, cattle are relatively long-lived with relatively low birthrates, maintain a symbiotic population of enteric microbes, and rely on the adaptive immune system more than poultry. (Klasing, 2007)

As mentioned, the adaptive immune system carries a lower cost than the innate immune system and represents a lower risk of immunopathology upon activation. Livestock producers and veterinarians take advantage of the memory capacity of the adaptive system with vaccination protocols. Vaccinations represent a minor stimulation of the immune system that primes the adaptive immune system to respond vigorously the next time a pathogen is encountered. The response to the second encounter with a pathogen includes a highly effective adaptive immune response with minimal tissue damage experienced by the animal (Klasing, 2007).

Unfortunately, many of the immune-mediated challenges that are most expensive for animals and producers to combat are not caused by highly virulent pathogens nor available as vaccines (Klasing, 2007). In fact, some animals will exhibit a depression in growth or feed intake and no other clinical signs. Therefore it is important to understand how the innate and adaptive immune systems affect and protect the animal.

1.2.2.1 Innate Immune System

1.2.2.1.1 Activation of the Innate System

The innate immune system is based around the recognition of molecular patterns associated with pathogens. These receptors are "evolutionarily conserved germline - encoded host censors" for the express purpose of detecting pathogen associated molecular patterns (PAMPS) expressed by pathogens and commensal flora (Mair et al., 2014). These receptors include Toll-like receptors, a family of membrane glycoproteins that recognize PAMPs and then trigger the downstream expression of NFKB which up-regulates production of pro-inflammatory cytokines and stimulates myeloid receptors that promote leukocyte differentiation (Mair et al., 2014). The largest group of PAMP receptors are nucleotide-binding oligomerization domain - receptors (NOD) that exist in cytosol (Mair et al., 2014). Other receptors include retinoic acid –inducible gene- 1 receptors that detect RNA viruses in the cytosol, DNA sensors, and membrane C-like leptin receptors (Mair et al., 2014).

After a pathogen has been recognized by a PAMP receptor, several pathways may commence to target and destroy that pathogen. The complement cascade is activated by three independent pathways using more than thirty soluble and membrane-anchored proteins (Miller and Zachary, 2016). All three pathways result in the formation of a

common membrane attack complex that will lyse pathogenic cells by punching holes in the membrane (Mair et al., 2014). Products of the complement cascade act as cytokines to perpetuate the activation of the inflammatory cascade by attracting and subsequently activating leukocytes and increasing the production of antibodies (Mair et al., 2014). At the same time, the activation and perpetuation of the clotting cascade is closely intertwined with the complement cascade which allows rapid response to potential pathogens and tissue remodeling at the site of trauma (Mair et al., 2014).

1.2.2.1.2 Signaling

Upon activation of a PAMP receptor, the complement cascade, or the clotting cascade, multiple cytokines are released to either attract more leukocytes to the damage tissue, to signal healthy tissues to perform an anti-microbial action, such as vasodilation to allow migration and localization of neutrophils, or to signal an endocrine axis to allow the immune system to fight the pathogen on a systemic basis. There are many (Mair et al., 2014) relevant cytokines involved in the activation, perpetuation and eventual relaxation of the immune system, but the majority of them are beyond the scope of this literature review.

Interleukin-1 (IL-1) has been strongly associated with performance reductions observed during health challenges. Klasing et al. (1987) observed that daily injections of IL-1 elicited reduced average daily gain, lower feed intake, and poorer feed conversion relative to saline-injected controls. Interleukin-1 stimulation is also strongly associated with anorexia observed during stress and health challenges (Cook et al., 1993).

Cortisol is released by the adrenal cortex in response to stress. Daily injections of cortisol were strongly associated with reduced feed intake, growth, and feed conversion

in chickens. Skeletal muscle resorption (cachexia) has been frequently observed in multiple species upon administration or supplementation of glucocorticoids (Klasing, 2007).

Other cytokines, such as PGE-2 and cyclooxygenase are also associated with skeletal muscle catabolism. Tumor necrosis factor-alpha (TNF-α), with IL-1, is one of the first cytokines released as part of the acute inflammatory cascade (Miller and Zachary, 2016). Tumor necrosis factor-alpha has been shown to reduce skeletal muscle protein synthesis and increase protein degradation (Williams et al., 1997).

1.2.2.1.3 Cellular Components of the Innate Immune System

Epithelial cells are the first line of "defense" as part of the immune system because they, and their connections to each other, represent an ideally impenetrable barrier for pathogen entry into the body. If a cell is damaged, or the barrier is breached, the epithelial cell will release cytokines such as TNF-α, and interferon α, β, and γ which act as chemo-attractants for leukocytes such as neutrophils. (Mair et al., 2014; Miller and Zachary, 2016).

Mast cells are involved in the mucosal response to intestinal pathogens, food borne-allergies, and probiotics. Upon binding to immunoglobulins via an Fc receptor, mast cells will degranulate, releasing histamines, leukotrienes, prostaglandins, and heparin, which in turn recruit immune cells to the area. (Mair et al., 2014)

Polymorphonuclear neutrophils are the first responding leukocyte at a site of trauma or microbial invasion. Their primary purpose is to destroy foreign material, and they accomplish this in two ways. Neutrophils can phagocytize foreign material then fuse the endosome containing that foreign material with a lysosome, thus destroying the

material with a combination of proteases, elastases, and peroxidases. Neutrophils can also secrete the contents of their cytoplasmic granules (containing antimicrobial peptides, matrix metalloproteases, elastases, myeloperoxidase) into the extracellular space, destroying the targets, but also causing significant tissue damage. (Mair et al., 2014; Brox et al., 2012)

Macrophages, like neutrophils, are synthesized in the bone marrow, but unlike neutrophils they differentiate then migrate to a tissue and further differentiate into tissue-specific macrophages such as kupffer cells, langerhans cells, and alveolar macrophages (Mair et al., 2014). At maturity they may be found in the circulating blood, but are more often associated with a tissue type (Kubena and McMurray, 1996). Macrophages are activated to maximum antimicrobial activity by IFN- γ or interleukin- 4 or 13 (Mair et al., 2014). However, other cytokines, released at the end of the inflammatory cascade, will shift macrophage activity from antimicrobial to debris-clearance (Mair et al., 2014).

Dendritic cells act as immuno-sensory cells. They sample the mucosa for potential pathogens, use PAMP-receptors to recognize foreign material as a pathogen, then transport the pathogen to lymphoid tissue where it is further sampled and the appropriate cells activated (Mair et al., 2014). Although dendritic cells have the primary purpose of presenting antigens to the tissues of the adaptive immune system, macrophages and neutrophlils can also serve as antigen presenting cells and regulate the T cell response with Prostaglandin E-2 (PGE-2; Kubena and McMurray, 1996).

Basophils express high-affinity IgE receptors, and when stimulated, release granules and inflammatory mediators (Miller and Zachary, 2016). Eosinophils have numerous intracytoplasmic granules that contain cytokines, chemokines, proteases, and

free radicals that are released in response to allergy or helminth infection. When eosinophilic granules are released, they contribute to tissue damage in the heart, lungs, skin and gastrointestinal tract (Miller and Zachary, 2016). Natural killer cells lyse tumor and virus-infected cells by releasing perforin from cytoplasmic granules (Miller and Zachary, 2016).

The proteinaceous components of the cellular phase of the innate immune system are acute phase proteins and host defense peptides. Acute phase proteins are very rapidly synthesized blood proteins in response to tissue damage and stress. Host defense peptides, including cathelicidns and defensins, have roles in wound healing and resolution of inflammation. (Mair et al., 2014)

1.2.2.1.4 Inflammation

Inflammation is a protective mechanism that results in the accumulation of fluid, electrolytes, plasma proteins, and leukocytes in extravascular tissue. The purpose is to isolate, dilute, and destroy the cause of cellular damage followed by repairing the damaged tissue.

Acute inflammation is a well ordered cascade of reactions mediated by chemoattractants, vasoactive molecules, cytokines, and antimicrobial molecules. The primary result of acute inflammation is the exudation of proteins, electrolytes, fluid, and neutrophils. The initial stage of acute inflammation is localized hyperemia facilitated by vasodilators such as prostaglandins, endothelin, and nitric oxide. This allows fluid leakage through modified endothelial junctional complexes that also contains complement components, prostaglandins, and vasoactive amines. As more fluid leaks through the endothelium, the gaps between endothelial cells widen, allowing neutrophils

and other proteins into the extravascular space. As the process continues, greater amounts of chemoattractants are released, especially closest to the nidus of tissue injury, resulting in neutrophil migration to the site of injury. Repair begins early in the inflammatory cascade and ends after the causative agent is removed. (Miller and Zachary, 2016)

Chronic inflammation results after acute inflammation fails to eliminate the source of the initial inflammation, after multiple episodes of acute inflammation, or in response to a unique characteristic of a pathogen that allows it to persist, isolate in tissue, or limit the efficacy of the immune system. Chronic inflammation is also seen in cases of autoimmunity. The characteristic cells are NK, lymphocytes, macrophages, and the B and T cells of the adaptive immune response. The response is characterized by prolonged duration with lymphocytes, macrophages, and tissue necrosis with granulation, fibrosis, and tissue repair. (Miller and Zachary, 2016)

Young pigs with high levels of chronic immune stimulation had reduced muscle accretion, increased skeletal muscle degradation, reduced average daily gain, decreased feed intake, and a greater proportion of lipid in the carcass (Williams et al., 1997). If a pathogen or area of damage is unresolvable, the body will wall it off to isolate the damaged area from the rest of the body. The extent of the damage from lesions associated with chronic inflammation depends on the location of the lesion and the extent of tissue involvement (Miller and Zachary, 2016)

1.2.2.1.5 Adaptive Immune System

Components of the adaptive immune system, B and T cells, develop in the bone marrow and thymus, respectively. B and T cells express surface receptors that allow them to be activated by a specific foreign molecule or antigen. Activation of the adaptive

immune system requires contact with antigen presenting cells via adhesion molecules, or the binding of adhesion cytokines or soluble immune products (Kubena and McMurray, 1996). Upon activation, B cells proliferate and differentiate into plasma cells that produce antigen-specific antibodies which, in turn, activate the complement system and coat antigens to facilitate uptake by phagocytes (Kubena and McMurray, 1996). There are multiple types of T cells that will differentiate after activation, ranging from CD8+ T cells that are cytotoxic, to suppressor T cells that downregulate the cytotoxic response, and Th2 T cells that assist in B cell proliferation and plasma cell differentiation (Kubena and McMurray, 1996).

1.2.3 Measures of Swine Health

The most medically intuitive, and specific measure of swine health is the observation of clinical signs in a swine herd. The severity and presentation of these clinical signs are determined by the signalment, the pathogen, and the duration of infection. Signalment, clinical signs, and associated lesions allow a veterinarian to diagnose, treat the affected animals, and protect the rest of the herd. (Jackson and Cockcroft, 2002)

Unfortunately, pigs may be immunologically stressed in the absence of definitive clinical signs. In that case, the most economically relevant and non-invasive measure of swine health and stress is growth performance, particularly feed intake, growth rate, and feed conversion (Patience et al., 2015). It has been well established (Williams et al., 1997; van Heugten et al., 1994) that many of the cytokines and hormones released as inflammatory mediators directly result in a reduction in feed intake, and therefore growth.

Feed efficiency is a highly complicated, sensitive, but not a specific measure of health status. Growth and feed efficiency have multiple determining factors, including environment, plane of nutrition, genetics and health (Patience et al., 2015). However, animals that are immunologically stressed will have poorer feed efficiency than their non-stressed counterparts because the immune response diverts resources that would be used to deposit skeletal muscle proteins to fuel the innate and adaptive immune response and to repair damage caused by the pathogen and immune systems (Patience et al., 2015).

Investigators have used other behavioral and physiologic measures to quantify reactions to stressors, particularly the unique physiologic, environmental, nutritional, and immunologic stressors that surround weaning. These measures range in degree of invasiveness from infrared measures of skin temperature to histopathologic examination of small intestinal morphology.

Hicks et al. (1998) noted that both the immunologic and social stresses around weaning activate the hypothalamic-pituitary-adrenal axis and noted significant elevations in serum cortisol in stressed animals. Hicks et al. (1998) also used clinical pathology measures such as serum fibrinogen and the albumin/globulin ratio with more specific measures of immune activity such as neutrophil migration and haptoglobin concentration to better characterize the immune response to social stresses. Funderburke and Serrely (1990) measured blood glucose and hepatic glycogen stores in recently weaned pigs to reflect energy intake and expenditure of weanling pigs. Søerensen and Pedersen (2015) discussed the use of infrared cameras to detect hypothalamic-mediated temperature elevations in times of immunologic stress and localized inflammation in swine.

Other investigators (McLamb et al., 2013; van Heugten, 1994) have successfully measured the expression of different pro-inflammatory cytokines in blood and tissues, such as IFN-γ, TNF-α, IL-1, IL-6 and IL-8 to reflect immune-activation. McLamb et al. (2013) measured intestinal barrier function and trans-epithelial nutrient movement using Ussing chambers after an enterotoxogenic E. coli challenge.

Researchers have also been heavily invested in correlating villus height and crypt depth to intestinal health and function for over forty years. Most early investigators noted a simultaneous reduction in villus height and crypt cell hyperplasia strongly correlated to weaning (Pluske, 1997). Pluske et al. (1997) hypothesized that the observed shortening of villi is either due to increased rate of cell loss or a reduction in cell division in crypts.

The strong correlation between villus shortening and the gastrointestinal clinical signs associated with weaning has driven a lot of investigators to measure villus height and crypt depth to reflect intestinal health and function. However, when villus height and crypt depth measurements are coupled with other measures of intestinal function and inflammation, such as PCR to detect expression of pro-inflammatory cytokines, or the use of Ussing chambers to measure active transport capacity of the tissue, the villus/crypt measures may not reflect the physiology described by other methodologies (Moeser, 2007, and Horn, 2014). For example, Li et al. (2016) noted a decrease in villus height when pigs were over-supplemented with iron, but did not observe the expected increase in local inflammatory mediators. Moeser (2007) observed no differences in histologic measures in newly weaned pigs compared to pigs that remained on the sow, despite observing significant differences in nutrient transport, barrier function, and local and systemic cortisol.

Investigators use different methods to measure villus height and crypt depth, including different criteria to choose which villi to include or exclude from measurement and different ways of performing the measurement itself. For example, Yeruva (2016) used the tallest two intact villi present in subsections of a slide, but Li (2016) used three "well oriented" villi from four subsections of each slide.

Williams et al. (2015) indicated that a reduction in villus height:crypt depth was due to uncompensated enterocyte loss. Epithelial cells are normally shed from the apex of the villus tip. New epitheliocytes are generated (under the influence of Wnt) from the crypt. During acute disease stress, epithelial cells undergo faster rates of necrosis and extrusion followed by contraction of the villus core. The core contraction is coupled with flattening of remaining epithelial cells and fusion of adjacent villi. Tumor necrosis factor is a potent stimulator of pathologic cell shedding in mice, but NFK-B may mediate epithelial cell response to acute intestinal injury. (Williams et al., 2015)

Other investigators have used different methods to reflect inflammatory processes that occur in the gastrointestinal system as a response to weaning and other pathogenic insults. These auxiliary methods include additional histologic measurements under H:E staining, the use of histopathologic scales to grade intestinal lesions, recording cell type and density present in sections, and the use of immunohistochemistry to measure cell replication and death.

The most common additional histopathologic measures taken on H:E slides, beyond villus height and crypt depth are muscle layer thickness (Meyerholtz, 2010 and Hayakawa, 2016), cellular density of the lamina propria (Kongsted, 2013), and number of intraepithelial lymphocytes (Sanchez-Cordon, 2003). Hayakawa (2016) also noted the

presence of eosinophilic cell detritus in the crypts and the loss of goblet cells from the crypt. Additionally, Meyerholtz (2010), Schweer (2016), Hayakawa (2016), and Sanchez-Cordon (2003) enlisted a pathologist to evaluate intestinal sections and grade the severity of the observed lesions based on inflammatory infiltrate, necrosis, and villus fusion.

Karhausen (2013) demonstrated that stabilization of intestinal mast cells during ischemia/reperfusion reduces systemic inflammation and subsequent injury in rodent models. Horn et al. (2014) used toluidine blue to visualize and count mast cells, but saw no differences in cell density in response to weaning-related stressors such as feed and water deprivation. Birck (2014) and Meyerholz (2010) used Alcian blue and PAS to detect mucins and evaluate goblet cell density.

Williams (2015) successfully used caspase-3 immunohistochemistry to discover an increased prevalence of apoptotic cells present at the villus tip in LPS-challenged mice compared to control animals. Gonzales et al. (2013) extensively described the use of immunohistochemistry to identify cells of different lineages and confirmed the accuracy of these stains in the pig using PCR. Other tests, including PCNA, MCM2, BrdU, and Caspase 3 were all successfully used to identify various stages of cell proliferation and apoptosis (Gonzalez, 2013).

1.2.4 Allergy

Dendritic cells have a dual role. As mentioned previously, they are primarily responsible for antigen presentation to, and activation of the adaptive immune system. However, dendritic cells present in the gut have a slightly different phenotype than systemic dendritic cells and are important for generation of regulatory cell-T cells that are important players in the down-regulation of the cytotoxic T cells response. If a dendritic

cell encounters a particular protein or carbohydrate complex in low concentrations, and often, it will signal the generation of regulatory T cells to elicit oral tolerance of that protein. This means that the immune system will not react to that molecule. The establishment of oral tolerance is important to ensure that the immune system does not stage a highly expensive and immune-pathologic reaction to carbohydrates and proteins (Perrier and Corthesy, 2010).

However, in some genetically predisposed individuals, oral tolerance is not established to specific molecules, resulting in an oral intolerance or allergy. If this occurs, the presence of the allergen in the small intestine results in acute inflammation and a significant increase in mucosal permeability. The acute permeability is both induced by the presence of the allergen and the degranulation of local mast cells (Perrier and Corthesy, 2010; Strait et al., 2011). Mast cell granules contain proteases, chymases, histamine and cytokines that, upon release, destroy epithelial tight junctions (especially occludin), cause local vasodilation, hydropic change and increase epithelial permeability (Perrier and Corthesy, 2010). This mucosal permeability results in opportunistic invasion by pathogens and the potential for local infection. The inflammation and intestinal permeability result in further activation of the inflammatory cascade which results in further tissue damage and clinical signs.

Young swine experience a significant lag in growth, coupled with diarrhea after weaning (Li et al., 1990). Weaning is often described as the most stressful time in the pig's life, and while the growth depression is expected from the stress response, investigators have shown that the growth lag and gastrointestinal distress are worsened and prolonged because of a transient hypersensitivity to soy protein (Li et al., 1990; Li et

al., 1991; Calbrix et al., 2012). Although oral tolerance to soybean meal is achieved 7-10 days after weaning, the intestinal permeability and inflammation can result in expensive, potentially fatal opportunistic infections (Engle, 1994).

The primary clinical signs observed in young pigs with soybean protein sensitivity are diarrhea and failure to gain weight. However, investigators have also observed dermatologic signs, respiratory distress, systemic anaphylaxis and death in soy-sensitive pigs (Hao et al., 2009). Other investigators have observed histologic changes such as villus blunting and crypt hyperplasia (Li et al., 1990, Li et al., 1991; Resende et al., 2016). Helm et al. (2001) reported severe passive congestion, mucosal hemorrhage, lymphocyte and macrophage invasion and submucosal edema in sensitized pigs exposed to soybean and peanut allergies.

Food allergies are of particular concern to medical professionals because the incidence of food allergies in the human population grew by 18% in children from 1997-2007. In some of these cases the food allergy is resolved as the child ages, but may persist into adulthood (Calbrix et al., 2012). The symptoms of food allergy range from gastrointestinal distress to systemic anaphylaxis and death (Strait et al., 2011). Therefore there is a great deal of interest in animal models of childhood food allergy and intolerance (Calbrix et al., 2012).

Investigators have capitalized on the similarity between the development of neonatal swine and children to explore different aspects of protein hypersensitivity. Some investigators (Helm et al., 2001; Rupa et al., 2007) have successfully induced protein allergies in young pigs with intraperitoneal injections of soybean or egg protein. Other investigators have more closely examined the naturally occurring protein sensitivity in

young pigs (Hao et al., 2009; Li et al., 1990, Li et al., 1991) to better develop a model for human sensitivity and to better manage the observed sensitivity in young pigs.

Investigators at Purdue University have selected for pigs that are genetically predisposed to have high or low sensitivity to soybean meal. Pigs were genetically selected based on response to an intradermal soy protein skin test; animals with high scores (based on the resulting wheal and flare from the localized inflammatory reaction to the soybean injection) were bred to one another and animals with little to no skin reaction were also bred to create two diverging lines (Callbrix et al., 2012). More recently, an ex-vivo study using tissue from these lines has shown that animals with high soybean sensitivity express markers of intestinal growth (GLP-2), tight junctions (occludin) and immune activity (p65/RelA) to a lower degree than pigs selected for low soybean sensitivity (Amaral et al., In Press). This may be indicative of mucosal inflammation and increased intestinal permeability.

1.2.5 The Current and Historic Use of Antibiotics in Livestock Production

Antibiotics were first reported to have a beneficial effect on animal health and performance in the late 1940's. This coincided with significant increases in demand for animal protein and subsequent intensification and condensing of agriculture (Dibner et al., 2005). In swine, antibiotics have been used in three different ways: for growth promotion, pro-and metaphylaxis and therapeutically (Cromwell, 2002). From 1970-2002, 70-80% of nursery, 70-80% of grower, 50-60% finisher and 40-50% of sow feeds were estimated to contain antibiotics (Cromwell, 2002). Of these antibiotics, 82% were reported to be used to maintain or improve animal health, and 18% were used for growth promotion (AHI, 2000). The most frequently used antibiotics include tetracycline,

tylosin, and sulfamethazine, most of which target gram positive facultative anaerobes (McEwen and Fedorka-Cray, 2002).

Investigators have noted significant increases in growth rate and feed efficiency in a variety of species given low doses of antibiotics throughout their life cycle (Dibner et al., 2005). In swine, investigators reported a 16.4% improvement in average daily gain and a 6.9% increase in feed efficiency in the nursery phase (Cromwell, 2002). Other investigators observed as much as a 20% improvement in feed efficiency in young pigs given sub-therapeutic antibiotics (Allen et al., 2013). Producers also found that subtherapeutic antibiotic use reduced mortality by 50% in young pigs, with even greater reductions in high-stress facilities (Cromwell, 2002).

The response to low-dose antibiotics decreases as the animal ages. Average daily gain is improved by 10.6% and efficiency increases by 4.5% in grower pigs given antibiotics. Finishers experience a 4.2% increase in growth rate and a 2.2% improvement in feed conversion with sub-therapeutic antibiotic administration (Cromwell, 2002). Long-term subtherapeutic antibiotic use has also been strongly associated with improvements in feed intake and nitrogen digestibility, N retention, and reductions in N excretion (McEwen and Fedorka-Cray, 2002). Dritz et al. (2002) compared growth performance in pigs receiving sub-therapeutic, in-feed antibiotics to pigs reared without in-feed antibiotics. Dritz et al., (2002) found that the addition of sub-therapeutic antibiotics did not improve growth performance in finishing swine, but improved growth rate in nursery animals.

The potential mechanisms of antibiotic-associated growth promotion include a selection for growth promoting commensal species, selection for bacteria that produce

fewer growth-depressing metabolites, and they reduce the incidence of clinical and subclinical disease. (Barton et al., 2014; Dibner et al., 2005).

1.2.5.1 Selection for Growth-Promoting Commensal Species

Oral antibiotics do not enhance the growth of germ free animals, therefore one of the primary theories behind the mechanism of antibiotic growth promotion is the selection for growth promoting commensal bacteria species (Gaskins et al., 2009). The intestinal tract contains 100 trillion non-pathogenic bacteria, representing 500-1000 species. The microbiome exerts a positive regulatory force on the immune system (Noverr and Huffnagle, 2004). Antibiotics may select for GI bacteria that decrease the energetic cost of digestion, and therefore increased the amount of energy available to the animal (Allen et al., 2013). Microbiota have been implicated in having a major role in developing oral immune tolerance to some proteins (Noverr and Huffnagle, 2004).

1.2.5.2 Reduction of Growth Depressing Metabolites

Antibiotics also select for bacteria that produce fewer growth-depressing metabolites (Gaskins et al., 2002). Gut bacteria use glucose and make lactic acid, limiting the availability of glucose to host epithelial cells and lactic-acid may have local effects on the gut such as increasing the rate of peristalsis; increasing passage rate, and decreasing absorption of nutrients (McEwen and Fedorka-Cray, 2002). Amino acids are transformed to toxic metabolites such as amines, ammonia, indoles and phenols (McEwen and Fedorka-Cray, 2002), antibiotic use reduces the amount of toxic metabolites by reducing the number of bacteria that produce these metabolites. The use of antibiotics also represents a reduction in competition for nutrients in the gastrointestinal tract (Gaskins et

al., 2002). Culture-based studies show that 6% of net energy in pig's diets is lost to bacterial use (McEwen and Fedorka-Cray, 2002).

1.2.5.3 Reduction of Disease

"Antimicrobials suppress the microorganisms that are responsible for non-specific sub-clinical disease, thereby allowing pigs to more closely meet their genetic potential"(Cromwell, 2002). Antimicrobials reduce the incidence ofsub-clinical infections that contribute to immune-mediated growth suppression (Barton et al., 2014; Dibner et al., 2005; Gaskins et al., 2002, Cromwell, 2002). This is supported by the fact that young pigs and pigs exposed to disease challenges respond to antibiotic use more than healthy animals (Cromwell. 2002).

Antimicrobials are also used prophylactically and metaphylactically to prevent disease, thereby reducing the incidence of pathogen and immune-mediated growth depression (Barton, 2014). The antibiotic of choice for prophylactic use depends on the disease of interest. Ceftiofur, sulfonamides, and tetracyclines are used to control the incidence of respiratory disease. Gentamycin, apramicin, and neomycin are used to treat and limit the spread of *Clostridium perfringens*. Lincomycin, tiamulin and macrolides are commonly used to treat and control swine dysentery (*Suerpulina hyodysenteriae*) and ileitis (*Lawsonia intracellularis*) (McEwen and Fedorka-Cray, 2002).

Antibiotics are also used therapeutically to treat disease. Upon diagnosis, pigs can be individually dosed by injection or orally or group-treated provided by in-feed or in-water medication (Barton et al., 2014).

1.2.5.4 History of Antibiotic Use

Investigators reported antibiotic resistant bacterial isolates in poultry soon after antibiotic use began in livestock and poultry, particularly at long-term sub-therapeutic levels, (Dibner et al., 2005). The British were the first to discuss and recommend banning antibiotics as growth promoters, and the US started recommending exploration and antibiotic growth promoter (AGP) bans in the 1960s (Dibner et al., 2005). The Swedish were the first to institute a ban on AGPs in 1986, and subsequently observed increases in prophylactic and therapeutic use of antibiotics (McEewn and Fedorka-Cray, 2002).

Denmark ceased the use of antibiotic growth promoters in January, 2000 (Dibner et al., 2005). After the ban on sub-therapeutic use of antibiotics for the purpose of growth promotion, the total use of antibiotics dropped by 54%, but coincided with significant increases in the therapeutic use of antibiotics (Dibner et al., 2005). Producers also noted a reduction in average daily gain, an increase in mortality (Dibner et al., 2005), and an increase in the incidence of Lawsonia intracellularis (McEwen and Fedorka-Cray, 2002). However, a reduction in the use of virginiamycin and avilamycin coincided with a reduction in resistant isolates (McEwen and Fedorka-Cray, 2002).

The European Union banned the use of antibiotics as growth promoters in 2006 (Dibner et al., 2005). The United States has considered the implications of the sub-therapeutic antibiotic use for growth promotion in livestock since 1969 (GFI 209). The tenuous connection between antibiotic resistant bacteria in the human clinic and antibiotic use in livestock resulted in numerous risk assessments on the development of antibiotic resistant bacteria versus the benefit for the livestock industry (GFI 209). In 2013, the United States Food and Drug Administration released Guidance for Industry

209 and 213 that state that medically important antibiotics should be only used to enhance animal health, not for growth promotion (GFI 209 and 213).

1.2.6 Why Antibiotic Use in Livestock is a Concern

The WHO has described human pathogens resistant to antimicrobials as a "global health challenge" (Barton et al., 2014). The majority of antibiotic resistance patterns center around the use of antibiotics in human hospitals (Rajic et al., 2006). However, there are multiple points of interaction between humans and livestock that would allow humans to be exposed to antibiotic resistant bacteria or resistant genes present in and around livestock (Stanton, 2012). This exposure can be a direct interaction with antibiotic resistant bacteria, antibiotics or metabolites while working directly with livestock, encountering contaminated slurry applied to fields, washed into coastal waters or into drinking water. Humans can also be exposed to antibiotics in food animal products in the unlikely event of a producer not respecting an antibiotic withdrawal time. (Wellington et al., 2013)

In the absence of antibiotics acting as a selection pressure, bacteria will exchange and express antibiotic resistant genes, but these are biologically costly. However in the presence of an antimicrobial to act as a selection pressure, these genes are biologically beneficial. Some of the species naturally present in the microbiome may be resistant to antibiotics in the absence of antibiotic exposure. These create a pool that can be selected upon if the animal is given an antibiotic (Stanton, 2012). The use of different antibiotics selects for different patterns of resistance in different bacterial species (Stanton, 2012).

The initial theory explaining the emergence of vancomycin-resistance enterococci in Europe was the use of Avoparicin as a growth promotant in swine. However, the

isolation of vancomycin-resistant enterococci from American farms, where Avoparicin has never been used, adds questions to this theory (Barton, 2014). Swine have been shown to carry Campylobacter coli and C. jejuni, significant causes of zoonotic diarrheal disease (WHO, 2016; Barton, 2014). Isolates of C. coli, resistant to macrolides have emerged, likely due to the routine and prolonged use of tylosin (Barton, 2014).

Some authors are not concerned about the emergence of increasing numbers of antibiotic resistant salmonella isolates observed in agriculture. However, there are an increasing number of multidrug resistance salmonella infections presenting in humans that can be linked to the food chain (Barton et al., 2014).

There is a very large variety and degree of resistance, particularly to tetracyclines in E. coli subspecies. The emergence of multidrug resistant strains, especially to second and third generation Cephalosporins is of greatest concern. Although Methicillin resistant staphylococcus aureus (MRSA) is a major concern in human medicine, there is limited evidence that the use of antibiotics in livestock result in MRSA infections in humans (Barton, 2014). Isolates of Streptococcus suis resistant to macrolides and tetracyclines have also been observed in manure (Barton, 2014).

The greatest concern associated with antibiotic use in humans and animals is the emergence of extended spectrum beta lactamase (ESBL) and AMP-C producing bacteria. Extended spectrum beta lactamase-producing bacteria are of serious concern because they undermine the therapeutic advantage of penicillins and beta lactamase inhibitors (Wellington et al., 2013). Investigators believe these resistant isolates emerged due to the use of Ceftiofur and Cefalcinome (Barton et al., 2014). This has driven human medical professionals to prescribe imbipenem and maropenem instead of third generation

cephalosporins and quinolones. This change in prescription patterns has coincided with an increase in isolates containing carbapenemase, further limiting effective therapeutics (Wellington et al., 2013).

It is important to also note that antibiotic resistance occurs in the absence of antibiotics. The University of Kentucky has maintained a herd of antibiotic free swine since 1972. In the first several years after antibiotic withdrawal, fecal coliforms resistant to tetracycline dropped from 90% to 50%. However, since 1972, 30-70% of fecal coliforms are resistant to tetracycline even though there has been no exposure to antibiotics. The proportion of resistant isolates are dependent on animal age, stress, and housing. (Cromwell, 2002)

1.2.7 Why a Ban on Antibiotics is Concerning

The issue of antibiotic use in livestock is complex. Some scientists, and particularly members of the general public, firmly believe that the human health risk of antibiotic resistant bacteria should drive legislation to fully ban the use of antibiotics in livestock. Other investigators, producers, and veterinarians believe that antibiotics should continue to be available for use in livestock to incentivize antibiotic development, maximize production efficiency, reduce and treat the incidence of infectious disease in animals, and allow the producer and veterinarian to maximize animal welfare by preventing and treating disease.

There has been no new development of antibiotics to treat gram (-) bacteria in over 40 years, with a simultaneous 75% reduction in research and development efforts for novel antibiotic production (Stanton, 2012). The livestock industry consumes a significant amount of antibiotics, therefore significant limits in antibiotic use would drive

commerce further away from this aspect of the pharmaceutical industry, further reducing the incentive to develop novel antibiotics.

The withdrawal of antibiotics in swine production is of greatest concern in the early nursery. Here there is a fine balance between immune system development, inoculation of the gut with commensal bacteria, and infection with enteric pathogens (DeBusser et al., 2011). There is also a risk of increased infectious disease. For example, the removal of antibiotic growth promotants from swine in Denmark coincided with an increase in zoonotic salmonella and campylobacter infections (Kil and Stein, 2010). The removal of antibiotics from livestock represents a significant risk to welfare of swine because it limits the capability of the veterinarian and producer to prevent and treat disease and the associated pain and suffering(McEwen and Fedorka-Cray, 2002).

1.2.8 The Future of Antibiotic Use in Livestock

Guidance for Industry 209 and 213 create a framework to legislate the judicious use of medically important antibiotics. Guidance 209 defined judicious use as limiting antibiotic use to only treat and prevent disease. Guidance 213 and 209 recommend the relabeling of the use of antibiotics to be under veterinary oversight and consultation. The American Veterinary Medical Association expands on these recommendations for the veterinarian. The use of any prescription pharmaceuticals must meet all requirements of the veterinarian-client-patient relationship, meaning that in order to prescribe antimicrobials, the veterinarian must be familiar with the animals that the medication will be for and deem that the use of the pharmaceutical is necessary (AVMA, 2017). This legislation removes medically important antibiotics from the market as growth promoters,

creating new opportunities for producers to maintain swine health and performance with other methods and management strategies.

The strategies that can be used to maintain swine health and performance in the absence of antibiotics fall into two categories: health management and the use of antibiotic alternatives (McEwen and Fedorka-Cray, 2002). Some of the management strategies include increasing weaning ages and using reduced crude protein-amino acid supplemented diets in the early nursery.(Kil and Stein, 2010). Other investigators (McEwen and Fedorka-Cray, 2002; Allen et al., 2013) have recommended using vaccination and biosecurity protocols to limit disease.

The primary issue with a lot of antibiotic alternatives is the fact that the GI tract is a very complicated environment that contains a highly complex ecosystem of bacteria that act as commensal species. There is also a great deal of inconsistency in the results of studies exploring the efficacy of antibiotic alternatives because of animal choice, experimental design and the fact that we have a limited understanding of how these antibiotic alternatives work in the complicated environment of the GI tract (Allen et al., 2013).

Direct-fed microbials are living cells, usually lactobacillus, streptococcus, bacillus, or bifidobacterium that are non-pathogenic, have a highly reduced incidence of gene transfer and antagonize pathogens, therefore benefiting the host (Allen et al., 2013). Bacillus based direct-fed microbial was associated with an improvement in feed efficiency in the finisher phase and changed manure consistency to reduce solids for greater ease of pen cleaning and improved application as fertilizer (Davis et al., 2008). A

direct-fed microbial increased ADG after a salmonella challenge and reduced Salmonella shedding (Walsh et al., 2012).

Inorganic acids reduce the pH of the environment and reduce the incidence of feed spoilage, therefore reducing pathogen survivability in the gut, reducing disease (Allen et al., 2013). The use of organic acids have been reported to stimulate epithelial cell production and reduce the shedding of haemolytic E. coli when water pH was reduced to 4, however this pH of water correlated with a reduction in water intake (De Busser et al., 2011).

Essential oils are derived from aromatic plants and represent part of the natural pharmacopeia, particularly in tropical and Mediterranean climates. They have been shown to have natural antibacterial, antifungal and insecticidal effects (Bakkali et al., 2008). Essential oils, such as oregano have been consistently shown to be cytotoxic because they penetrate bacterial membranes, causing a great deal of unregulated permeability and the subsequent loss of ions, proteins and other macromolecules. This cytotoxicity has been observed in gram positive and gram negative bacteria (Lambert et al., 2001). Some essential oils also have been shown to have antiviral properties against enveloped and non-enveloped viruses (Giling et al., 2014).

Copper sulfate, supplemented at levels above the copper requirement has been shown to be toxic to streptococci (Jensen, 1998). Copper acts on bacteria more than it acts on the morphology and physiology of the animal, indicated by the fact that copper supplementation was strongly associated with shorter villi in germ free animals, but taller, more slender villi with greater surface area in conventional animals (Jensen, 1998).

Copper sulfate use is associated with reduced lactic acid bacteria and lactobacilli and reduced coliforms in the large intestine (Hojberg et al., 2005).

Zinc helps maintain the epithelial barrier; it reduces ulceration and has a protective effect against TNF-alpha mediated disruption of the epithelial layer (Roselli et al., 2005). Zinc helps maintain tight junction integrity and is associated with increased villus height (Roselli et al., 2005). Zinc has also been shown to reduce bacterial adhesion, but has a lesser bacteriotoxic effect than other supplements, such as copper (Roselli et al., 2005). High doses of ZnO have been associated with increased diversity of fecal coliforms and a ten-fold reduction in bacteria in the stomach and ileum with significant reductions in urease-producing bacteria (Hojberg et al., 2005). However, the use of Cu and Zn as in-feed antimicrobials has been implicated in the selection of resistant isolates of enterococci in China (Barton et al., 2014, Zhou et al., 2012). Zinc oxide use is now under a prescription in parts of the E.U. due to the potential environmental impact of pharmacological levels of Zn being fed and the resultant Zn content of manure that is to be applied to land (McEwen and Fedorka-Cray, 2002).

1.3 Conclusion

Swine are not 100% efficient animals, therefore swine production results in the excretion of nutrients in liquid and gaseous form that can negatively impact the environment. The success and sustainability of a swine operation can be measured by the growth performance, health, and environmental footprint of the operation. Nutrition, genetics, and health management have significantly reduced the greenhouse gas emissions, reduced the number of resources used, and significantly improved the rate of growth in the last 50 years. There are opportunities to further reduce the environmental

impact of swine production with nutrition and health management. There are also opportunities to improve the ways that health and productivity are measured, particularly using histology. Therefore the objective of this dissertation is to examine how nutrition and health management affect the environmental impact of swine production.

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CHAPTER 2. THE EFFECT OF REDUCED CP, SYNTHETIC AMINO ACID SUPPLEMENTED DIETS ON GROWTH PERFORMANCE, NUTRIENT EXCRETION, AND AIR EMISSIONS IN WEAN TO FINISH SWINE

2.1 Abstract

Seven hundred twenty, mixed sex pigs were placed in 12 rooms at the Purdue Swine Environmental Research Building to measure the effect of reduced CP, AA-supplemented diets on growth performance and carcass characteristics. Pigs were blocked by BW and gender and allotted to room and pen with 10 mixed-sex pigs/pen. Pigs were fed a 9-phase, wean-finish feeding program and weighed at the start and end of each dietary phase. Within each phase, control pigs consumed corn-soybean meal – 20% DDGS based diets containing no synthetic amino acids. The 2X diet was balanced using synthetic AAs to the seventh limiting AA and represented the lowest CP diet. The 1X diet was formulated using synthetic AA to meet a CP value halfway between the control and 2X diets. Diets were formulated to identical NE concentrations and balanced to meet SID AA requirements (NRC, 2012). Pit vacuum samples were collected at the end of each growth phase and frozen at -20°C for subsequent analyses. Gas emissions were monitored by sampling every two hours. Data were analyzed using the GLM procedure in SAS and treatment means were separated using the Tukey procedure in SAS. Pigs fed the Control and 1X diet consistently grew faster ($P < 0.005$), had greater feed efficiency ($P < 0.001$), and were 4 kg heavier at market weight ($P < 0.001$) than animals fed the 2X diet. There were no consistent effects of diet on ADFI throughout the project. Carcass data were analyzed for sex, diet and sex*diet effects. Reductions in dietary crude protein resulted in a linear reduction in ammonium N excretion per kg of BW gain in Nursery

(Control=8.6 g/kg gain, 1X=7.2 g/kg gain, 2X=5.5 g/kg gain; $P<0.0001$) and Grow-Finish (Control=18.0 g/kg gain, 1X=14.3 kg/kg gain, 2X=10.1 g/kg gain; $P<0.0001$) phases. Reductions in dietary CP, with synthetic amino acid supplementation also resulted in a linear reduction in total N excreted per kg gain in the Grow-Finish phase (Control=18.5 g/kg gain, 1X=14.9 g/kg gain, 2X=13.1 g/kg gain; $P<0.0001$) and overall (Control=17.4 g/kg gain, 1X=15.4 g/kg gain, 2X=13.1g/kg gain; $P = 0.0009$). Total mineral excretion (Ash) per kg gain was reduced in the 1X and 2X diets compared to the control (Control=39.6 g/kg gain, 1X=36.0 g/kg gain, 2X=33.4 g/kg gain; $P = 0.0046$). There was no effect of diet on total manure volume or P excreted per kg gain. These results indicate that reductions in dietary crude protein of ~3 and 5%-units from wean-finish result in reductions of total N excretion of 11.7 and 24.4%, respectively. Overall, the reduced performance and carcass characteristics observed in pigs fed the 2X diets indicates an inaccurate estimate of amino acid requirements (NRC, 2012) or ratios to lysine in this extremely low CP diet.

2.2 Introduction

In the natural world, the limited amount of fixed nitrogen sources has selected for very limited productivity, but high biodiversity in naturally occurring plant-life (Erisman et al., 2013). When nitrogen leaches from fields after swine manure application, it can cause downstream acidification and eutrophication of bodies of water (Erisman et al., 2013; Steinfeld and Wassenaar, 2007). Excessive nitrogen can also result in downstream homogenization of plant populations because nitrogen can be phytotoxic in high doses and subsequently selects for nitrogen-tolerant species (Erisman, 2013; Kenny and Hatfield, 2008).

Inorganic nitrogen (as nitrate) can also discharge into drinking water resources after field application of manure. Excessive nitrates in drinking water can cause methemoglobinemia, abortion, and is associated with stomach cancer (FAO, 2006). Human health risks, coupled with environmental damage associated with excessive nitrogen, has driven interest in strategies to reduce nitrogen excretion in swine.

Producers have successfully reduced nitrogen excretion from swine with dietary inclusion of synthetic amino acids. Investigators have reported an 8.5% reduction in N excretion for every one percentage-unit reduction in dietary crude protein (CP) (Sutton and Richert, 2004). However, other investigators have noted a reduction in growth performance and energy retention with excessive dietary CP reduction (Kerr and Easter, 1995; Jones et al., 2014). Therefore, the objective of this experiment was to determine the effect of using high levels of synthetic amino acids to balance diets to the seventh limiting amino acid on growth performance and manure characteristics in wean-to-finish swine.

2.3 Materials and Methods

Seven hundred twenty mixed sex pigs (Duroc X (York x Landrace)) were placed in twelve rooms at the Purdue Swine Environmental Research Building (SERB) to investigate the effects of feeding a reduced CP diet with supplemental synthetic amino acids on growth performance and environmental footprint. Each room contained 6 pens with 10 pigs per pen, and 2 manure pits (1.8 m deep) under sets of 3 pens, allowing for quantitative collection of manure. Pigs were blocked by source (Sow vs. Gilt farm), BW, and sex and randomly assigned to room and diet. All procedures were approved by the Purdue University Animal Care and Use Committee (PACUC# 1117000447).

2.3.1 Diets

Each room was assigned one of three dietary treatments (4 rooms per diet). The control diet was formulated to meet the first limiting amino acid requirement and had no synthetic amino acids with the exception of methionine in nursery phases 1-3. The 2X diet was balanced to the 7th limiting amino acid, with synthetic amino acids used to meet the pigs' requirement for the first 5-6 limiting amino acids. The 1X diet was formulated to have a crude protein concentration equidistant between the control and 2X diets. The result was that the 1X and 2X diets contained a stepwise reduction (~3 and 5 percentage units, respectively) in crude protein from the control diet. During nursery phases 1-3, and then for nursery phase 4 to finisher 2, soybean concentrate and soybean meal, respectively were reduced from the control diet as synthetic amino acids were increased in the 1X and 2X diets. Ractopamine (Paylean®, Greenfield, IN) was added in finisher 2 diets, and therefore dietary CP could only be reduced to 16% in the 2X diet to meet Paylean® feeding label requirements.

Diets (Tables 2.1-2.9) were formulated to meet or exceed all nutritional requirements (NRC, 2012), and were fed in 9 dietary phases (4 nursery phases, 3 grower phases and 2 finisher phases). Nursery 1 and 2 were 7d, Nursery 3 and 4 were 14d, and the remaining dietary phases were 21d in length. Diets were balanced to meet or exceed NRC (2012) standardized ileal digestible (SID) amino acid ratios to SID lysine. Net energy was kept constant across treatments and synthetic amino acids were added as needed. Synthetic amino acids used as needed to meet limiting amino acid requirements included lysine, methionine, threonine, tryptophan, isoleucine and valine.

2.3.2 Performance Data Collection

Pigs had ad libitum access to feed and water via 2 single space feeders and 2 nipple waterers and were housed at 0.84 m^2/pig. Pigs and feeders were weighed at each diet change (d0, 7, 14, 28, 42, 63, 84, 105, 126, and prior to market). Pigs in the heaviest replicates were marketed at d 139 and pigs on the light replicates were marketed on d 147 in an attempt to better mimic industry practices and have a similar time at market among treatments.

Morbidity and mortality were recorded daily. When deemed necessary, pigs were administered injectable antibiotics and treatments were recorded. Animals of the same sex within a pen were tattooed with a common pen number so that carcass data could be collected from the packing plant (Tyson Foods Inc., Logansport IN). Aerial gas concentrations were determined every 2 h from each room and coupled with continuous airflow rates to determine daily gas emissions from each room.

2.3.3 Sample Collection and Analysis

Samples of feed were ground through a 1 mm screen using a Wiley mill (Thomas Scientific, Swedsboro, NJ) for subsequent analyses. Feed was analyzed for dry matter (DM), ash, total Kjeldahl Nitrogen (TN), Carbon (C) and total Phosphorus (P) in the Purdue Animal Sciences Nutrition Laboratory and for amino acid concentration by the University of Missouri Experiment Station Laboratory.

Manure volume was calculated at each diet change by using manure depth measurements in each pit. Manure pit samples were collected at the end of the nursery, grower, and finisher phases using a vacuum sampler. All feed and manure samples were analyzed in duplicate and required to be within 5% of each other to accept the values.

Samples were homogenized and a sub-sample was frozen (-20°C) for subsequent analyses. In addition to the vacuum samples, at the end of the experiment, manure pits were emptied into a small Slurry Store, mixed, and a representative subsample was collected and frozen (-20°C) for subsequent analyses. These manure slurry samples were analyzed for pH, DM, ash, total N (TN), ammonium N (AmmN), C and P.

Carbon was measured on feed and slurry using a Flash EA 1112 Series Nitrogen-Carbon Analyzer (CE Elantech, Inc. Lakewood, NJ). Dry matter was measured following a 12 h drying period at 100°C, and ashing occurred over 8 h at 600°C in a muffle furnace (Thermo Fisher Scientific, Inc. Waltham, MA). Total N and AmmN were measured using the micro-kjeldahl procedure (Bremmer and Keeney, 1965). Manure pH was measured using an Orion 310 basic PerpHecT® LogR pH meter (Thermo Fisher Scientific Inc. Waltham, MA). Phosphorus was measured colorimetrically in feed and feces following a nitric-percholoric acid digestion (Murphy and Riley, 1962).

2.3.4 Statistical Analysis

Data were analyzed using the GLM procedure of SAS, with pen as the experimental unit for growth performance and carcass data, pit for manure data, and room for emission data. Linear and quadratic responses were compared using single degree of freedom contrasts for decreasing dietary CP concentration. Gas emissions were analyzed using the Mixed procedure in SAS with fixed effects of day and treatment. A P value ≤ 0.05 indicated a significant difference where P values between $0.05 < P \leq 0.10$ were considered a trend.

2.4 Results

2.4.1.1 Feed Composition

Analyzed content of the feed was generally similar to calculated values (Tables 2.10-18). A stepwise reduction in CP was achieved in each dietary phase.

2.4.1.2 Average Daily Gain

Average daily gain (ADG; Table 2.19) was similar across treatments for the first 7d post-weaning (P=0.2123). However, in the second week post-weaning, pigs fed the Control and 1X diets grew faster compared to pigs fed the 2X diet (P=0.011). There were no differences in growth rate in the third nursery phase (P=0.1126), but in the fourth nursery phase, animals fed the 2X diet grew slower than pigs fed the 1X and Control diets (P=0.0012). Differences in growth rate in the second and fourth nursery phases drove an overall reduction in average daily gain for pigs fed the 2X diet compared to those fed the Control and 1X diets (P=0.0001) during the nursery period.

Pigs fed the Control diet grew faster than those fed the 1X and 2X diets in the first grower phase (d105-mkt; P<0.0001). In the second grower phase, pigs fed the 1X diet grew the fastest, followed by Control-fed pigs, and then pigs fed the 2X diet (P<0.0001). There were no differences in ADG in the third grower phase (P=0.5611), but differences in growth rate in the early grower phases resulted in higher ADG for pigs fed the Control and 1X diet compared to 2X-fed pigs for the overall grower period (d 42-105; P=0.0002).

Pigs fed the 1X and 2X diets grew faster than control-fed pigs in the finisher 1 phase (d 105-126; P=0.0006), but this pattern reversed during finisher 2 (d 126-140), where Control-fed animals grew faster than both 1X- and 2X-fed pigs (P=0.0052).

Therefore, there were no differences in ADG for the overall finisher phase (d105-mkt; *P*=0.9804).

For the entire wean-to-finish study, pigs fed the 2X diet grew 3.2% slower than pigs fed the Control or 1X diets (*P*=0.0003).

2.4.1.3 Body Weight

There were not differences in mean body weight observed at d 0, 7, 14 or 28 d post weaning (*P*>0.05). At 42 d post weaning, pigs fed the Control and 1X diets were heavier than pigs fed the 2X diets (*P*= 0.0059). The 2X pigs were also lighter than 1X and Control pigs at d 63, 84, 105, 126, and were marketed approximately 4 kg lighter than pigs fed the 1X and Control diets (*P*<0.05).

2.4.1.4 Average Daily Feed Intake

Pigs fed the 1X diet tended to consume more feed (Table 2.19) during the first 7d post-weaning than Control-fed pigs with pigs fed the 2X diet consuming an intermediate amount (*P*=0.0679). There was no difference in average daily feed intake (ADFI) during the second week post-weaning (*P*=0.2558). However, in the third nursery phase (d 14-28), pigs fed the 1X diet consumed the greatest quantity of feed, followed by Control-fed pigs and then pigs fed the 2X diet (*P*<0.0001). This pattern tended to continue during the final nursery phase (d 28-42; *P*=0.0584). For the overall nursery period (d 0-42), pigs fed the Control and 1X diets consumed more feed than pigs fed the 2X diet (*P*=0.0228).

In the grower 1 phase, pigs fed the 2X and Control diets consumed more feed than pigs fed the 1X diet (*P*=0.0272). There were no differences in ADFI among treatments in the latter two grower phases (*P*=0.9689; *P*=0.4573) or for the overall grower period (d 42-105; *P*=0.7484).

During the first finisher phase (d 105-126), pigs fed the 1X diet consumed more feed than pigs fed the Control diet, with pigs fed the 2X diet consuming an intermediate amount (P=0.0448). However, no differences were observed in feed intake in the late finisher phase (P=0.7251), or for the overall finisher period (P=0.2426).

For the entire wean-to-finish period, no differences in ADFI were observed (P=0.6383) among dietary treatments.

2.4.1.5 Feed Efficiency

There was no difference in feed conversion in the first two weeks post-weaning (Table 2.19; Week 1: P=0.8742; Week 2: P=0.0871). During the third nursery phase, pigs fed the 2X diet had a greater Gain:Feed (G:F) compared to Control- and 1X-fed pigs (P<0.0001). However, this reversed in the final nursery phase where we observed higher G:F for Control- and 1X-fed pigs compared to 2X-fed pigs (P=0.0012). As a result there was no difference in feed efficiency among treatments for the overall nursery period (P=0.2927).

In the early grower period, all 3 treatments were different in feed efficiency, with pigs fed the Control diet having the highest G:F, followed by pigs-fed the 1X diet, and then pigs fed the 2X diet (P<0.0001). The top two switched rank during the second grower phase with pigs fed the 1X diet having the highest G:F, followed by Control-fed pigs, and then pigs fed the 2X diet (P=0.0012). In the final grower phase no differences in feed conversion were observed among dietary treatments (P=0.4699). For the overall grower period (d 42-105), pigs fed the 1X and Control diets had greater G:F compared to pigs fed the 2X diet (P<0.0001).

There was no difference in feed efficiency among diets in the early finisher phase (P=0.2662). However, in the late finisher, pigs fed the Control diets had a greater G:F compared to both 1X- and 2X-fed pigs (P=0.0046*).*

Overall, from wean to finish, pigs fed the Control and 1X diets had greater Gain:Feed compared to pigs fed the 2X diet (P<0.001).

2.4.1.6 Pig Health

Tracking pig health and therapeutic treatments throughout the study (Table 2.20), an overall decrease in frequency of unhealthy pigs fed the 2X diet compared to the Control fed pigs (P<0.05) was observed. This resulted in less therapeutic treatments (injections) being administered during the grower period and overall to pigs fed the 2X diets compared to Control-fed pigs with the 1X-fed pigs being intermediate (P<0.05).

2.4.1.7 Carcass Characteristics

There were no differences among treatments for carcass fat depth (P>0.05) (Table 2.21). Pigs fed the 2X diet had smaller loin depths (P = 0.0124) compared to 1X- and Control-fed pigs and tended to have lower percent lean than pigs fed the 1X diet (P = 0.0628). However, pigs fed the 2X diet held an advantage for base meat price compared to 1X-fed pigs (P=0.0461). Pigs fed the 1X diet had greater carcass yield (P=0.0072) than pigs fed the Control diet and greater carcass grade premium (P=0.0236) than pigs fed the 2X diet, but also had a larger sort loss (P=0.0254) than pigs fed the 2X diet. Pigs fed the 1X diet had heavier carcass weights (P=0.0330) than pigs fed the 2X diets with Control-fed pigs being intermediate. When all summed together, final carcass price per kg was not affected by dietary treatment (P=1882). When final carcass price was combined with

average carcass weights, pigs fed the 1X diets had the greatest revenue per pig ($163.72), followed by the Control-fed pigs ($162.08), and lastly the 2X-fed pigs ($160.73).

2.4.1.8 Manure

Overall there was a numerical reduction in manure volume excreted per kg gain by 2X-fed pigs compared to Control- and 1X-fed pigs (Table 2.22; *Linear P*=0.1324). There was no difference observed among treatments in dry matter excreted per kg gain (*P*=0.2994) or phosphorus excretion per kg gain (*P*=0.2610). Pigs fed the Control diet excreted a greater quantity of ash per kg of gain compared to both 1X- and 2X-fed pigs (*P*= 0.0046). Manure pH tended (*P*=0.0738) to go down linearly from Control- to 2X-fed pigs. Pigs fed the 2X diet excreted less ammonium N compared to Control-fed pigs, with the 1X-fed pigs excreting an intermediate quantity per kg of gain (*P*=0.0025). Reducing dietary CP by ~3 (1X) and 5 (2X) percentage units resulted in an 11.7 and 24.4 % reduction in total N excretion, respectively (*P*=0.0009).

2.4.1.9 Emissions

Daily and cumulative CO_2, N_2O, H_2S, and methane (CH_4) emissions (Table 2.23 and 2.24) were similar across dietary treatments for all growth periods and overall (*P*>0.05). The only exception was, in the first week post-weaning, pigs fed the 1X and 2X diets emitted less daily N_2O than pigs fed the control diet (*P*=0.0052).

There were no differences in daily NH_3 emissions (Table 2.23) across treatments (*P*>0.05) until the fourth nursery phase where pigs fed the 1X and 2X diets produced less NH_3 per day than pigs fed the Control diet (*P*=0.0093). The same pattern of emission continued in the first grower phase, with the Control-fed pigs producing more NH_3 than both the 1X- and 2X-fed pigs (*P*=0.0008). In the second grower phase, pigs fed the 2X

diet emitted a smaller quantity of NH_3 per day than the pigs fed the Control diet, with the 1X-fed pigs being intermediate (P=0.0079). There were no differences in daily NH_3 emissions in the third grower phase (P=0.1309) and first finisher phase (P=0.2054). In the second finisher phase there was a tendency for pigs fed the 1X and 2X diets to produce less NH_3 than Control fed pigs until d 140 (P=0.0867) and from d 140-147 pigs fed the 2X diet produced less NH_3 than those fed the Control diet, with pigs fed the 1X diet producing an intermediate amount (P= 0.0495).

Cumulatively, no differences in NH_3 emissions (Table 2.24) were observed (P>0.05) until the end of the second grower phase (d 84), where pigs fed the Control diet emitted a greater mass of NH_3 per animal unit than 2X-fed pigs, with 1X-fed pigs being intermediate (P=0.0234). Pigs fed the 2X diet continued to produce less cumulative NH_3 than Control-fed pigs, with 1X-fed pigs being intermediate, in the third grower phase (P=0.0057). By the end of the first finisher phase (d 126), Control-fed pigs produced a greater cumulative amount of NH_3 per AU than both the 1X- and 2X-fed pigs (P=0.0014). This pattern of emission persisted until all pigs were marketed (P=0.0038), where the 2X pigs produced 56% less total NH_3 than pigs fed the Control diet. This means that for every 1%-unit reduction in CP (from the Control to 2X diet) we observed approximately a 1 kg reduction of NH_3 produced per AU.

2.5 Discussion

Performance data shows a consistent, significant reduction in average daily gain, in pigs fed the 2X diet,which had a two-fold reduction in crude protein with the greatest proportion of synthetic amino acids compared to the control (which contained no synthetic amino acid supplementation) or 1X diets (intermediated level of crude protein

and amino acid inclusion). Other authors (Yin and Tan, 2012; Kerr and Easter, 1995; Jones et al., 2014) have noted reductions in performance, carcass characteristics, and energy retention in extremely low crude protein, amino acid-supplemented diets. We believe that this may be due to amino acid imbalances resulting from inconsistency in amino acid content and availability in feed ingredients, potentially improper amino acid ratios for the seventh to the tenth limiting amino acids, and inefficiencies in absorption.

Soybean meal is the vegetable protein source of choice in livestock feed because it has a relatively consistent and well-studied amino acid composition. In this study, as dietary crude protein was reduced, the concentration of soybean meal included in the diet was also reduced. As soybean meal was reduced, a greater proportion of dietary crude protein came from ingredients such as corn and corn DDGS. Corn DDGS have been reported to have highly variable amino acid concentrations and availabilities (Stein et al., 2006).

The analyzed values of amino acid content largely corresponded with calculated values. However, in some cases analyzed values were less than calculated values, especially in the 2X diet in the grower period. The 2X diet analyzed amino acid concentrations were consistently lower than calculated values which corresponded to a drop in average daily gain in 2X-fed pigs compared to the control animals. A similar situation was observed in N4, G1 and G2. In both finisher phases, we observed differences in average daily gain, but no consistent, significant differences in analyzed versus calculated amino acid content. The observed differences in analyzed versus calculated values were small, but may explain some of the performance patterns observed in pigs fed the 2X diet. This highlights the variability of amino acid content in feed

ingredients, particularly DDGs, as a significant issue with precision diet formulation in diets balanced to the 5-7th limiting amino acid. It is also important to note that the analysis reflects total amino acid concentrations in the diet, not the digestibility of these amino acids, therefore it is also plausible that amino acids were present in the feed ingredients, but not accessible to the animal.

Human and rodent studies have reported that proteins are absorbed more efficiently as small peptides than as amino acids (Otto et al., 2003; Pan et al., 1996). We did not look at the digestibility of amino acids in this study, so it is entirely possible that the animals fed the 1X diet had an advantage over the animals fed the 2X diet because they had a combination of small peptides and synthetics to absorb and deposit as muscle.

It is also possible that diets were imbalanced at the lesser-limiting amino acids. Changes in availability and differences in analyzed values could have created an imbalance, particularly at the seventh or eighth limiting amino acid, that led to the growth deficit in 2X-fed pigs. Maxwell et al. (2016), noted that when dispensable synthetic amino acids (histidine) were added to low crude protein diets of grow/finish swine, growth performance was maintained compared to a higher CP control, therefore it is plausible that we could have improved growth performance of 2X-fed pigs by feeding synthetic histidine. It is also highly plausible that the 2X reduction in crude protein resulted in an imbalance in non-essential amino acids. Although the pig can synthesize non-essential amino acids, the rate of synthesis and availability of precursors could limit the amount of these amino acids in the body, limiting growth (Hou and Wu, 2017).

It must also be noted that we observed reduced performance in conjunction with reduced clinically sick pigs fed the 2X diet. We also noted reduced frequency of

individual medical treatments in 2X-fed pigs compared to control animals. The reduction of dietary crude protein has been proposed as a health management tool in young animals by Kil and Stein (2010). Crude protein raises the pH of the stomach which facilitates pathogen survival and dissemination to the rest of the gastrointestinal tract (Kil and Stein, 2010). Other authors (Nyachoti et al., 2006) hypothesize that low crude protein, amino acid supplemented diets benefit the health of animals because the high digestibility of the synthetic amino acids reduces the production of toxic metabolites by microbial digestion of protein in the distal small intestine.

We expected an approximately 8.5% reduction in N excretion per %-unit reduction in dietary crude protein (Sutton and Richert, 2004). On average, CP was reduced 2.7% and 5.4%-units, respectively for pigs fed the 1X and 2X diets compared to the control. We observed an 11.74% and 24.45% reduction in TN excretion in manure produced by pigs fed the 1X and 2X diets, respectively. This resulted in approximately a 4.5% reduction in total N excretion per %-unit reduction in dietary crude protein. This reduction is lower than expected but may be reflective of the fact that the last several diets had higher crude protein because the animals were fed ractopamine. It is also important to note that the aforementioned 8.5% estimate reflects the expected N reduction on fresh manure, but in this case, TN was measured on stored manure. Ammonium volatilizes to ammonia during manure storage (Erisman, 2013), therefore the increased ammonia measured in control-fed rooms is from the volatilization of nitrogen in manure.

In conclusion, feeding high levels of synthetic amino acids to balance diets to the seventh limiting amino acid has a positive effect on reducing nitrogen excretion, may

have positive health effects but can decrease growth performance. Similar negative effects on energy retention (Jones et al., 2014), carcass characteristics and growth (Kerr and Easter, 1995; Yin and Tan, 2012) have been reported. It is possible that the observed reductions in performance are due to variability in the availability of essential and non-essential amino acids in feed ingredients or an imbalance in less-limiting essential amino acids.

2.6 Tables

Table 2.1 Nursery Phase 1 Diets

	Diets		
Ingredient, %	Control	1X	2X
Corn, yellow dent	27.955	31.528	35.160
Soybean meal, 47.5% CP	15.000	15.000	15.000
Soybean protein concentrate	8.067	4.614	0.997
Soybean oil	4.723	4.353	3.934
Plasma, spray-dried	6.500	6.500	6.500
Blood meal, spray-dried	1.000	1.000	1.000
Whey, dried	25.000	25.000	25.000
Fish meal, menhaden	4.000	4.000	4.000
Lactose	5.000	5.000	5.000
Limestone	1.139	1.152	1.165
Monocalcium phosphate	0.053	0.089	0.127
Salt	0.250	0.250	0.250
Vitamin premix[2]	0.250	0.250	0.250
Trace mineral premix[3]	0.175	0.175	0.175
Phytase[4]	0.100	0.100	0.100
Carbadox	0.250	0.250	0.250
Zinc Oxide	0.375	0.375	0.375
L-lysine HCl	0.000	0.166	0.339
DL-methionine	0.113	0.148	0.198
L-threonine	0.000	0.000	0.074
L-tryptophan	0.000	0.000	0.005
L-isoleucine	0.000	0.000	0.010
L-valine	0.000	0.000	0.040

[1] Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion; 2X diets had the lowest CP, highest SAA inclusion; 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.
[2]Vitamin premix provided per kilogram of the diet: vitamin A, 6615 IU; vitamin D_3, 662; vitamin E, 44.1 IU; vitamin K, 2.21 mg; vitamin B_{12}, 38.6 µg; riboflavin, 8.8 mg; pantothenic acid, 22.1 mg; niacin, 33.08 mg.
[3]TM premix supplies the following per kg of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15.03 mg; copper, 11.3 mg; iodine, 0.46 mg; selenium, 0.30 mg.
[4]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health – Dupont, St. Louis, MO).
[5] Carbadox (Mecadox® 10, Phibro Animal Health, Ridgefield Park, NJ) provided at 55 ppm.

Table 2.2 Phase 2 Nursery Diets

	Diets[1]		
Ingredient, %	Control	1X	2X
Corn, yellow dent	34.069	37.901	41.791
Soybean meal, 47.5% CP	18.000	18.000	18.000
Soybean protein concentrate	8.039	4.339	0.444
Soybean oil	4.910	4.492	4.039
Plasma, spray-dried	2.500	2.500	2.500
Blood meal, spray-dried	1.000	1.000	1.000
Whey, dried	25.000	25.000	25.000
Fish meal, menhaden	4.000	4.000	4.000
Limestone	0.987	1.013	1.028
Monocalcium phosphate	0.000	0.010	0.051
Salt	0.250	0.250	0.250
Vitamin premix[2]	0.250	0.250	0.250
Trace mineral premix[3]	0.175	0.175	0.175
Phytase[4]	0.100	0.100	0.100
Carbadox[5]	0.250	0.250	0.250
Zinc Oxide	0.375	0.375	0.375
L-lysine HCl	0.000	0.177	0.365
DL-methionine	0.072	0.125	0.183
L-threonine	0.000	0.001	0.095
L-tryptophan	0.000	0.017	0.048
L-isoleucine	0.000	0.000	0.0001
L-valine	0.000	0.000	0.033

[1] Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion; 2X diets had the lowest CP, highest SAA inclusion; 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.
[2]Vitamin premix provided per kilogram of the diet: vitamin A, 6615 IU; vitamin D_3, 662; vitamin E, 44.1 IU; vitamin K, 2.21 mg; vitamin B_{12}, 38.6 µg; riboflavin, 8.8 mg; pantothenic acid, 22.1 mg; niacin, 33.08 mg.
[3]TM premix supplies the following per kg of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15.03 mg; copper, 11.3 mg; iodine, 0.46 mg; selenium, 0.30 mg.
[4]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health – Dupont, St. Louis, MO).
[5] Carbadox (Mecadox® 10, Phibro Animal Health, Ridgefield Park, NJ) provided at 55 ppm.

Table 2.3 Nursery Phase 3 Diets

	Diets[1]		
Ingredient, %	Control	1X	2X
Corn, yellow dent	39.532	43.588	47.740
Soybean meal, 47.5% CP	20.000	20.000	20.000
Soybean protein concentrate	11.209	7.280	3.140
Soybean oil	2.500	2.072	1.591
Corn DDGS, 7% oil	10.000	10.000	10.000
Whey, dried	10.000	10.000	10.000
Fish meal, menhaden	4.000	4.000	4.000
Limestone	0.909	0.924	0.939
Monocalcium phosphate	0.148	0.189	0.233
Salt	0.465	0.468	0.471
Vitamin premix[2]	0.250	0.250	0.250
Trace mineral premix[3]	0.175	0.175	0.175
Phytase[4]	0.100	0.100	0.100
Carbadox (22 g/kg)[5]	0.250	0.250	0.250
Zinc Oxide	0.375	0.375	0.375
L-lysine HCl	0.000	0.176	0.375
DL-methionine	0.061	0.119	0.181
L-threonine	0.000	0.000	0.070
L-tryptophan	0.000	0.010	0.043
L-valine	0.000	0.000	0.042

[1] Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion; 2X diets had the lowest CP, highest SAA inclusion; 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.

[2]Vitamin premix provided per kilogram of the diet: vitamin A, 6615 IU; vitamin D_3, 662; vitamin E, 44.1 IU; vitamin K, 2.21 mg; vitamin B_{12}, 38.6 µg; riboflavin, 8.8 mg; pantothenic acid, 22.1 mg; niacin, 33.08 mg.

[3]TM premix supplies the following per kg of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15.03 mg; copper, 11.3 mg; iodine, 0.46 mg; selenium, 0.30 mg.

[4]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health – Dupont, St. Louis, MO).

[5]Carbadox (Mecadox® 10, Phibro Animal Health, Ridgefield Park, NJ) provided at 55 ppm.

Table 2.4 Nursery Phase 4 Diets

	Diets[1]		
Ingredient, %	Control	1X	2X
Corn, yellow dent	46.130	56.324	66.920
Soybean meal, 47.5% CP	31.143	21.843	11.965
Corn DDGS, 7% oil	15.000	15.000	15.000
Choice white grease	4.711	3.387	1.899
Limestone	1.090	1.133	1.177
Monocalcium phosphate	0.776	0.849	0.928
Salt	0.350	0.350	0.350
Vitamin premix[2]	0.250	0.250	0.250
Trace mineral premix[3]	0.175	0.175	0.175
Phytase[4]	0.100	0.100	0.100
Carbadox[5]	0.250	0.250	0.250
L-lysine HCl	0.000	0.295	0.608
DL-methionine	0.000	0.004	0.087
L-threonine	0.000	0.016	0.151
L-tryptophan	0.000	0.000	0.052
L-valine	0.000	0.000	0.041
L-isoleucine	0.000	0.000	0.021

[1] Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion; 2X diets had the lowest CP, highest SAA inclusion; 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.
[2]Vitamin premix provided per kilogram of the diet: vitamin A, 6615 IU; vitamin D_3, 662; vitamin E, 44.1 IU; vitamin K, 2.21 mg; vitamin B_{12}, 38.6 µg; riboflavin, 8.8 mg; pantothenic acid, 22.1 mg; niacin, 33.08 mg.
[3]TM premix supplies the following per kg of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15.03 mg; copper, 11.3 mg; iodine, 0.46 mg; selenium, 0.30 mg.
[4]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health – Dupont, St. Louis, MO).
[5]Carbadox (Mecadox® 10, Phibro Animal Health, Ridgefield Park, NJ) provided at 55 ppm.

Table 2.5 Grower Phase 1 Diets

	Diets[1]		
Ingredient, %	CTL	1X	2X
Corn, yellow dent	48.185	59.062	69.837
Soybean meal, 47.5% CP	28.085	18.318	8.286
Corn DDGS, 7% oil	18.000	18.000	18.000
Choice white grease	2.994	1.545	0.039
Limestone	1.031	0.976	1.022
Monocalcium phosphate	0.703	0.780	0.860
Salt	0.350	0.350	0.350
Trace mineral premix[3]	0.175	0.175	0.175
Vitamin premix[2]	0.250	0.250	0.250
Phytase[4]	0.100	0.100	0.100
Lincomycin[5]	0.100	0.100	0.100
L-lysine HCl	0.000	0.309	0.627
DL-methionine	0.000	0.000	0.065
L-threonine	0.000	0.007	0.144
L-tryptophan	0.000	0.004	0.058
L-valine	0.000	0.000	0.033
L-isoleucine	0.000	0.000	0.030

[1] Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion; 2X diets had the lowest CP, highest SAA inclusion; 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.
[2]Vitamin premix provided per kilogram of the diet: vitamin A, 6615 IU; vitamin D_3, 662; vitamin E, 44.1 IU; vitamin K, 2.21 mg; vitamin B_{12}, 38.6 µg; riboflavin, 8.8 mg; pantothenic acid, 22.1 mg; niacin, 33.08 mg.
[3]TM premix supplies the following per kg of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15.03 mg; copper, 11.3 mg; iodine, 0.46 mg; selenium, 0.30 mg.
[4]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health – Dupont, St. Louis, MO).
[5] Lincomycin (Linocmix, Zoetis, Parsippany, NJ) provided at 110,000 ppm

Table 2.6 Grower Phase 2 Diets

	Diets[1]		
Ingredient, %	Control	1X	2X
Corn, yellow dent	47.698	58.054	69.114
Soybean meal, 47.5% CP	25.919	16.374	6.156
Corn DDGS, 7% oil	20.000	20.000	20.000
Choice white grease	3.925	2.596	1.000
Limestone	0.907	1.015	0.996
Monocalcium phosphate	0.734	0.809	0.891
Salt	0.350	0.350	0.350
Vitamin premix[2]	0.150	0.150	0.150
Trace mineral premix[3]	0.090	0.090	0.090
Phytase[4]	0.100	0.100	0.100
Lincomycin[5]	0.040	0.040	0.040
Rabon larvacide[6]	0.038	0.038	0.038
L-lysine HCl	0.000	0.303	0.627
DL-methionine	0.000	0.000	0.063
L-threonine	0.000	0.011	0.151
L-tryptophan	0.000	0.020	0.076
L-valine	0.000	0.000	0.076
L-isoleucine	0.000	0.000	0.032

[1] Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion; 2X diets had the lowest CP, highest SAA inclusion; 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.
[2]Vitamin premix provided per kilogram of the diet: vitamin A, 3969 IU; vitamin D_3, 397; vitamin E, 26.46 IU; vitamin K, 1.32 mg; vitamin B_{12}, 23.15 µg; riboflavin, 5.29 mg; pantothenic acid, 13.23 mg; niacin, 19.85 mg.
[3]TM premix supplies the following per kg of diet: iron, 87.3 mg; zinc, 87.3 mg; manganese, 10.82 mg; copper, 8.14 g; iodine, 0.33 mg, selenium, 0.30 mg.
[4]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health – Dupont, St. Louis, MO).
[5] Lincomycin (Linocmix, Zoetis, Parsippany, NJ) provided at 110,000 ppm
[6]Rabon Lavacide (Bayer Animal Health, Leverkusen, Germany)

Table 2.7 Grower Phase 3 Diets

	Diets[1]		
Ingredient, %	Control	1X	2X
Corn, yellow dent	49.797	60.302	71.277
Soybean meal, 47.5% CP	23.922	14.337	4.067
Corn DDGS, 7% oil	20.000	20.000	20.000
Choice white grease	3.920	2.547	1.000
Limestone	0.968	1.012	1.058
Monocalcium phosphate	0.574	0.650	0.732
Salt	0.350	0.350	0.350
Vitamin premix[2]	0.150	0.150	0.150
Trace mineral premix[3]	0.090	0.090	0.090
Phytase[4]	0.100	0.100	0.100
Lincomycin[5]	0.040	0.040	0.040
Rabon larvacide[6]	0.038	0.038	0.038
L-lysine HCl	0.000	0.304	0.630
DL-methionine	0.000	0.000	0.063
L-threonine	0.000	0.008	0.149
L-tryptophan	0.000	0.022	0.078
L-valine	0.000	0.000	0.075
L-isoleucine	0.000	0.000	0.054

[1] Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion; 2X diets had the lowest CP, highest SAA inclusion; 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.
[2]Vitamin premix provided per kilogram of the diet: vitamin A, 3969 IU; vitamin D_3, 397; vitamin E, 26.46 IU; vitamin K, 1.32 mg; vitamin B_{12}, 23.15 μg; riboflavin, 5.29 mg; pantothenic acid, 13.23 mg; niacin, 19.85 mg.
[3]TM premix supplies the following per kg of diet: iron, 87.3 mg; zinc, 87.3 mg; manganese, 10.8 mg; copper, 8.2 g; iodine, 0.3 mg, selenium, 0.3 mg.
[4]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health – Dupont, St. Louis, MO).
[5]Lincomycin (Linocmix, Zoetis, Parsippany, NJ) provided at 110,000 ppm
[6]Rabon Lavacide (Bayer Animal Health, Leverkusen, Germany)

Table 2.8 Finisher Phase 1 Diets

	Diets[1]		
Ingredient, %	CTL	1X	2X
Corn, yellow dent	67.895	76.391	85.274
Soybean meal, 47.5% CP	21.619	13.849	5.526
Corn DDGS, 7% oil	5.000	5.000	5.000
Choice white grease	3.382	2.271	1.000
Limestone	0.942	0.977	1.024
Monocalcium phosphate	0.462	0.524	0.571
Salt	0.300	0.300	0.300
Vitamin premix[2]	0.100	0.100	0.100
Trace mineral premix[3]	0.100	0.100	0.075
Phytase[4]	0.100	0.100	0.100
Tylosin (88 g/lb)[5]	0.025	0.025	0.025
Rabon larvacide[6]	0.050	0.050	0.050
L-lysine HCl	0.000	0.246	0.510
DL-methionine	0.000	0.000	0.065
L-threonine	0.000	0.034	0.148
L-tryptophan	0.000	0.008	0.053
L-valine	0.000	0.000	0.078
L-isoleucine	0.000	0.000	0.051

[1] Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion. 2X diets had the lowest CP, highest SAA inclusion. 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.
[2]Vitamin premix provided per kilogram of the diet: vitamin A, 2640 IU; vitamin D_3, 264; vitamin E, 17.6 IU; vitamin K, 0.88 mg; vitamin B_{12}, 15.4 µg; riboflavin, 3.52 mg; pantothenic acid, 8.8 mg; niacin, 13.2 mg.
[3]TM premix supplies the following per kg of diet: iron, 72.75 mg; zinc, 72.75 mg; manganese, 9.01 mg; copper, 6.78 g; iodine, 0.27 mg, selenium, 0.15 mg.
[4]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Anaimal Health – Dupont).
[5]Tylosin (Tylan, Elanco, Greenfield, IN) provided at 193,600 ppm
[6]Rabon Lavacide (Bayer Animal Health, Leverkusen, Germany)

Table 2.9 Finisher Phase 2 Diets

	Diets[1]		
Ingredient, %	Control	1X	2X
Corn, yellow dent	60.390	67.352	74.530
Soybean meal, 47.5% CP	29.537	23.164	16.466
Corn DDGS, 7% oil	5.000	5.000	5.000
Choice white grease	2.904	1.992	1.000
Limestone	0.949	0.978	1.008
Monocalcium phosphate	0.395	0.445	0.499
Salt	0.300	0.300	0.300
Vitamin premix[2]	0.100	0.100	0.100
Trace mineral premix[3]	0.100	0.100	0.075
Phytase[4]	0.100	0.100	0.100
Rabon larvacide[5]	0.050	0.050	0.050
Paylean (5.5 g/kg)[6]	0.150	0.150	0.150
L-lysine HCl	0.000	0.202	0.414
DL-methionine	0.000	0.012	0.070
L-threonine	0.000	0.031	0.123
L-tryptophan	0.000	0.000	0.032
L-valine	0.000	0.000	0.035

[1] Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion. 2X diets had the lowest CP, highest SAA inclusion. 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.
[2]Vitamin premix provided per kilogram of the diet: vitamin A, 2640 IU; vitamin D_3, 264; vitamin E, 17.6 IU; vitamin K, 0.88 mg; vitamin B_{12}, 15.4 µg; riboflavin, 3.52 mg; pantothenic acid, 8.8 mg; niacin, 13.2 mg.
[3] TM premix supplies the following per kg of diet: iron, 72.75 mg; zinc, 72.75 mg; manganese, 9.01 mg; copper, 6.78 g; iodine, 0.27 mg, selenium, 0.15 mg.
[4]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Anaimal Health – Dupont).
[5]Rabon Lavacide (Bayer Animal Health, Leverkusen, Germany)
[6]Ractopamine HCl (Paylean-9®, Elanco Animal Health, Greenfield, IN) provided at 6.0 ppm in the diets.

Table 2.10 Analyzed and calculated nutrient content of nursery phase 1 diets

	Diet[1]					
	Control		1X		2X	
Nutrient, %	Analyzed	Calculated	Analyzed	Calculated	Analyzed	Calculated
Lysine	1.76	1.80	1.74	1.80	1.81	1.79
Threonine	1.15	1.21	1.07	1.13	1.09	1.11
Methionine	0.52	0.50	0.49	0.51	0.49	0.54
Met + Cysteine	0.96	1.02	0.91	1.00	0.88	0.99
Tryptophan	0.32	0.36	0.31	0.33	0.32	0.31
Isoleucine	1.03	1.11	0.96	1.01	0.88	0.94
Valine	1.42	1.39	1.32	1.28	1.27	1.18
Arginine	1.52	1.64	1.39	1.45	1.29	1.25
Histidine	0.71	0.75	0.67	0.70	0.61	0.64
Leucine	2.28	2.31	2.17	2.16	2.03	2.00
Phenylalanine	1.26	1.28	1.18	1.17	1.09	1.07
Tyrosine	2.05	2.22	1.91	2.04	1.81	1.85
Crude Protein	25.36	26.16	23.48	24.43	22.41	22.70
Phosphorus	0.68	0.66	0.64	0.65	0.68	0.64
Carbon	40.79		41.57		40.20	
Dry Matter	78.53		78.72		79.15	

[1] Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion. 2X diets had the lowest CP, highest SAA inclusion. 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.

Table 2.11 Analyzed and calculated nutrient content of nursery phase 2 diets

	Diet[1]					
	Control		1X		2X	
Nutrient, %	Analyzed	Calculated	Analyzed	Calculated	Analyzed	Calculated
Lysine	1.56	1.63	1.68	1.63	1.54	1.62
Threonine	0.97	1.10	0.97	1.01	0.87	1.00
Methionine	0.41	0.46	0.47	0.49	0.44	0.52
Met + Cysteine	0.77	0.90	0.83	0.90	0.73	0.90
Tryptophan	0.32	0.33	0.30	0.32	0.30	0.31
Isoleucine	0.97	1.09	0.97	0.97	0.77	0.86
Valine	1.24	1.28	1.22	1.17	1.04	1.08
Arginine	1.38	1.58	1.39	1.38	1.09	1.17
Histidine	0.65	0.70	0.65	0.64	0.53	0.58
Leucine	2.05	2.17	2.06	2.01	1.74	1.85
Phenylalanine	1.13	1.20	1.13	1.08	0.90	0.97
Tyrosine	1.81	2.07	1.82	1.87	1.46	1.67
Crude Protein	24.44	24.92	22.62	23.08	19.11	21.25
Phosphorus	0.68	0.63	0.65	0.63	0.62	0.60
Carbon	41.14		40.47		39.44	
Dry Matter	79.41		79.15		77.25	

[1] Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion. 2X diets had the lowest CP, highest SAA inclusion. 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.

Table 2.12 Analyzed and calculated nutrient content of nursery phase 3 diets

	Diet[1]					
	Control		1X		2X	
Nutrient, %	Analyzed	Calculated	Analyzed	Calculated	Analyzed	Calculated
Lysine	1.52	1.55	1.47	1.54	1.57	1.53
Threonine	0.99	1.07	0.89	0.97	0.88	0.94
Methionine	0.46	0.45	0.45	0.48	0.52	0.51
Met + Cysteine	0.82	0.83	0.76	0.83	0.84	0.83
Tryptophan	0.29	0.30	0.28	0.28	0.27	0.27
Isoleucine	1.09	1.08	0.97	0.96	0.89	0.83
Valine	1.25	1.30	1.11	1.19	1.08	1.10
Arginine	1.58	1.66	1.39	1.45	1.27	1.23
Histidine	0.67	0.64	0.60	0.58	0.56	0.52
Leucine	2.21	2.01	2.03	1.84	1.91	1.66
Phenylalanine	1.26	1.15	1.13	1.03	1.03	0.91
Tyrosine	2.00	2.00	0.69	1.79	1.65	1.58
Crude Protein	25.15	26.52	24.19	24.56	22.53	22.59
Phosphorus	0.69	0.64	0.68	0.63	0.63	0.62
Carbon	40.20		40.30		39.66	
Dry Matter	80.33		81.75		79.62	

[1] Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion. 2X diets had the lowest CP, highest SAA inclusion. 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.

Table 2.13 Analyzed and calculated nutrient content of nursery phase 4 diets

	Diet[1]					
	Control		1X		2X	
Nutrient, %	Analyzed	Calculated	Analyzed	Calculated	Analyzed	Calculated
Lysine	1.18	1.20	1.22	1.18	1.11	1.15
Threonine	0.82	0.86	0.75	0.73	0.64	0.71
Methionine	0.33	0.37	0.31	0.33	0.31	0.36
Met + Cysteine	0.65	0.74	0.61	0.65	0.56	0.63
Tryptophan	0.24	0.26	0.23	0.20	0.18	0.20
Isoleucine	0.90	0.95	0.80	0.78	0.59	0.62
Valine	1.06	1.09	0.93	0.92	0.74	0.77
Arginine	1.36	1.42	1.18	1.13	0.80	0.83
Histidine	0.59	0.60	0.52	0.51	0.39	0.41
Leucine	1.97	2.07	1.84	1.83	1.48	1.57
Phenylalanine	1.12	1.10	0.99	0.92	0.73	0.73
Tyrosine	1.80	1.95	1.59	1.63	1.18	1.28
Crude Protein	22.57	22.87	19.13	19.59	15.22	16.31
Phosphorus	0.68	0.63	0.69	0.61	0.65	0.58
Carbon	41.54		39.50		38.95	
Dry Matter	80.89		82.07		82.21	

[1] Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion. 2X diets had the lowest CP, highest SAA inclusion. 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.

Table 2.14 Analyzed and calculated nutrient content of grower phase 1 diets

	Diet[1]					
	Control		1X		2X	
Nutrient, %	Analyzed	Calculated	Analyzed	Calculated	Analyzed	Calculated
Lysine	1.14	1.14	1.10	1.12	1.05	1.09
Threonine	0.80	0.83	0.69	0.69	0.63	0.67
Methionine	0.32	0.37	0.30	0.32	0.33	0.34
Met + Cysteine	0.64	0.73	0.59	0.63	0.58	0.59
Tryptophan	0.23	0.25	0.20	0.19	0.17	0.19
Isoleucine	0.86	0.92	0.74	0.74	0.56	0.58
Valine	1.01	1.06	0.89	0.89	0.70	0.73
Arginine	1.28	1.35	1.04	1.05	0.70	0.74
Histidine	0.56	0.59	0.49	0.49	0.38	0.39
Leucine	1.96	2.07	1.80	1.82	1.46	1.56
Phenylalanine	1.06	1.08	0.93	0.88	0.69	0.69
Tyrosine	1.71	1.90	1.48	1.56	1.08	1.21
Crude Protein	23.02	22.43	19.04	19.00	15.02	15.66
Phosphorus	0.70	0.62	0.72	0.60	0.66	0.58
Carbon	40.26		39.93		38.47	
Dry Matter	82.06		82.21		82.26	

[1] Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion. 2X diets had the lowest CP, highest SAA inclusion. 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.

Table 2.15 Analyzed and calculated nutrient content of grower phase 2 diets

	Diet[1]					
	Control		1X		2X	
Nutrient, %	Analyzed	Calculated	Analyzed	Calculated	Analyzed	Calculated
Lysine	1.05	1.09	1.04	1.07	0.96	1.04
Threonine	0.75	0.81	0.65	0.68	0.59	0.66
Methionine	0.30	0.36	0.29	0.32	0.31	0.33
Met + Cysteine	0.60	0.72	0.57	0.62	0.56	0.58
Tryptophan	0.24	0.24	0.19	0.20	0.17	0.19
Isoleucine	0.81	0.89	0.68	0.72	0.54	0.56
Valine	0.96	1.04	0.83	0.86	0.71	0.75
Arginine	1.18	1.30	0.94	1.00	0.67	0.69
Histidine	0.54	0.57	0.47	0.48	0.37	0.37
Leucine	1.86	2.05	1.71	1.80	1.46	1.54
Phenylalanine	1.02	1.05	0.88	0.86	0.67	0.66
Tyrosine	1.64	1.86	1.40	1.52	1.07	1.16
Crude Protein	20.69	21.93	17.21	18.57	14.47	15.21
Phosphorus	0.69	0.63	0.67	0.60	0.65	0.58
Carbon	40.47		39.94		38.62	
Dry Matter	86.52		86.98		87.05	

[1] Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion. 2X diets had the lowest CP, highest SAA inclusion. 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.

Table 2.16 Analyzed and calculated nutrient content of grower phase 3 diets

	Diet[1]					
	Control		1X		2X	
Nutrient, %	Analyzed	Calculated	Analyzed	Calculated	Analyzed	Calculated
Lysine	1.05	1.04	1.05	1.01	0.91	0.99
Threonine	0.76	0.78	0.65	0.64	0.58	0.62
Methionine	0.31	0.35	0.29	0.31	0.23	0.32
Met + Cysteine	0.61	0.69	0.57	0.60	0.44	0.55
Tryptophan	0.22	0.22	0.21	0.19	0.20	0.18
Isoleucine	0.87	0.86	0.73	0.68	0.52	0.54
Valine	1.00	1.00	0.86	0.83	0.70	0.71
Arginine	1.21	1.24	0.99	0.94	0.57	0.63
Histidine	0.54	0.55	0.46	0.45	0.32	0.35
Leucine	1.93	2.00	1.73	1.75	1.33	1.48
Phenylalanine	0.99	1.01	0.85	0.82	0.57	0.62
Tyrosine	1.62	1.79	1.37	1.45	0.95	1.09
Crude Protein	21.50	21.16	18.24	17.79	15.29	14.41
Phosphorus	0.63	0.58	0.62	0.56	0.61	0.54
Carbon	40.19		39.35		38.86	
Dry Matter	85.43		85.37		85.15	

[1] Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion. 2X diets had the lowest CP, highest SAA inclusion. 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.

Table 2.17 Analyzed and calculated nutrient content of finisher phase 1 diets

	Diet[1]					
	Control		1X		2X	
Nutrient, %	Analyzed	Calculated	Analyzed	Calculated	Analyzed	Calculated
Lysine	0.83	0.88	0.84	0.86	0.82	0.84
Threonine	0.58	0.65	0.52	0.56	0.49	0.54
Methionine	0.24	0.29	0.22	0.25	0.27	0.27
Met + Cysteine	0.48	0.59	0.44	0.51	0.45	0.49
Tryptophan	0.19	0.19	0.16	0.15	0.17	0.15
Isoleucine	0.66	0.71	0.56	0.56	0.45	0.46
Valine	0.76	0.82	0.64	0.68	0.54	0.60
Arginine	0.93	1.06	0.75	0.82	0.51	0.56
Histidine	0.41	0.47	0.35	0.39	0.27	0.30
Leucine	1.45	1.62	1.29	1.42	1.10	1.20
Phenylalanine	0.76	0.84	0.65	0.69	0.50	0.52
Tyrosine	1.21	1.46	1.02	1.19	0.80	0.89
Crude Protein	18.42	17.32	16.33	14.60	13.56	11.89
Phosphorus	0.48	0.48	0.47	0.46	0.43	0.44
Carbon	41.92		40.00		40.04	
Dry Matter	85.03		84.37		85.41	

[1] Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion. 2X diets had the lowest CP, highest SAA inclusion. 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.

Table 2.18 Analyzed and calculated nutrient content of finisher phase 2 diets

	Diet[1]					
	Control		1X		2X	
Nutrient, %	Analyzed	Calculated	Analyzed	Calculated	Analyzed	Calculated
Lysine	1.09	1.10	1.04	1.08	1.05	1.06
Threonine	0.73	0.77	0.64	0.70	0.65	0.69
Methionine	0.30	0.33	0.27	0.31	0.32	0.33
Met + Cysteine	0.59	0.68	0.53	0.63	0.58	0.61
Tryptophan	0.24	0.24	0.20	0.20	0.18	0.19
Isoleucine	0.87	0.86	0.73	0.74	0.65	0.61
Valine	0.97	0.97	0.82	0.85	0.76	0.76
Arginine	1.23	1.31	1.00	1.11	0.89	0.90
Histidine	0.51	0.55	0.44	0.48	0.39	0.42
Leucine	1.76	1.84	1.57	1.67	1.42	1.50
Phenylalanine	0.97	1.00	0.83	0.88	0.74	0.74
Tyrosine	1.55	1.75	1.31	1.52	1.20	1.29
Crude Protein	22.71	20.46	19.29	18.23	18.08	16.00
Phosphorus	0.47	0.50	0.48	0.48	0.49	0.47
Carbon	40.95		40.99		40.80	
Dry Matter	82.95		82.37		82.99	

1 Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion. 2X diets had the lowest CP, highest SAA inclusion. 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets

Table 2.19 The effects of lowering dietary crude protein and adding synthetic amino acids on growth performance

	Diet[1,3]				Diet	Diet	Diet
	Control	1X	2X	MSE	P-Value	Linear P<	Quad. P<
Pig BW, kg							
d0	6.22	6.20	6.19	0.6908	0.9920	0.8655	0.9720
d7	7.42	7.55	7.45	0.7717	0.8407	0.9182	0.5635
d14	9.90	10.19	9.87	0.8781	0.3865	0.9059	0.1712
d28	16.88	17.39	16.82	1.1783	0.1891	0.8577	0.0704
d42	26.44[a]	26.70[a]	25.14[b]	1.7250	0.0059	0.0114	0.0405
d63	43.82[a]	42.60[a]	40.70[b]	2.4695	0.0002	<0.0001	0.5840
d84	64.88[a]	64.47[a]	61.15[b]	2.9240	<0.0001	<0.0001	0.0515
d105	87.17[a]	86.99[a]	83.29[b]	3.2197	<0.0001	<0.0001	0.0320
d126	108.65[a]	110.16[a]	105.79[b]	3.3309	0.0001	0.0042	0.0008
Market[2]	129.01[a]	129.03[a]	125.08[b]	3.9212	0.0008	0.0008	0.0527
ADG, kg							
d0-7	0.171	0.192	0.179	0.0410	0.2123	0.4950	0.1050
d7-14	0.353[b]	0.377[a]	0.345[b]	0.0362	0.0111	0.4708	0.0036
d14-28	0.498	0.514	0.496	0.0322	0.1126	0.8117	0.0386
d28-42	0.683[a]	0.664[a]	0.592[b]	0.0507	0.0012	0.2416	<0.0001

Table 2.19 continued

ADG, kg							
d42-63	0.827[a]	0.757[b]	0.740[b]	0.0500	<0.0001	<0.0001	0.0359
d63-84	1.003[b]	1.035[a]	0.978[c]	0.0409	<0.0001	0.0258	<0.0001
d84-105	1.060	1.072	1.053	0.0599	0.5611	0.6838	0.3215
d105-126	1.023[b]	1.103[a]	1.071[a]	0.0682	0.0006	0.0168	0.0018
d126-Mkt[2]	1.242[a]	1.146[b]	1.171[b]	0.1020	0.0052	0.0140	0.0182
Nursery, d0-42	0.481[a]	0.488[a]	0.451[b]	0.0296	0.0001	0.0008	0.0049
Grower, d42-105	0.963[a]	0.957[a]	0.922[b]	0.0337	0.0002	<0.0001	0.1087
Finisher, d105-mkt[2]	1.118	1.115	1.115	0.0563	0.9804	0.7828	0.7771
Overall, d0-Mkt[2]	0.861[a]	0.862[a]	0.834[b]	0.0254	0.0003	0.0004	0.0361
ADFI, kg							
d0-7	0.177	0.197	0.188	0.0283	0.0679	0.1804	0.0563
d7-14	0.417	0.436	0.410	0.0552	0.2558	0.6790	0.1110
d14-28	0.720[b]	0.762[a]	0.661[c]	0.0601	<0.0001	0.0012	<0.0001
d28-42	1.089[ab]	1.106[a]	1.047[b]	0.0866	0.0584	0.0945	0.0848

Table 2.19 Continued

ADFI, kg							
d42-63	1.679[a]	1.601[b]	1.649[a]	0.0977	0.0272	0.5607	0.0101
d63-84	2.295	2.303	2.299	0.1293	0.9689	0.6471	0.9807
d84-105	2.846	2.902	2.899	0.1934	0.4573	0.5642	0.7021
d105-126	3.242[b]	3.367[a]	3.335[ab]	0.1749	0.0448	0.0762	0.0747
d126-Mkt[2]	3.121	3.134	3.160	0.1694	0.7251	0.4319	0.8875
Nursery, d0-42	0.679[a]	0.705[a]	0.670[b]	0.0446	0.0228	0.0124	0.0003
Grower, d42-105	2.278	2.267	2.291	0.1101	0.7484	0.6774	0.5256
Finisher, d105-Mkt[2]	3.196	3.294	3.254	0.1371	0.2426	0.1977	0.2769
Overall, d0-Mkt[2]	2.048	2.070	2.061	0.0814	0.6383	0.5785	0.4445
Gain:Feed							
d0-7	0.956	0.970	0.953	0.1199	0.8742	0.6821	0.7530
d7-14	0.854	0.866	0.810	0.0896	0.0871	0.9214	0.7478
d14-28	0.698[b]	0.689[b]	0.770[a]	0.0647	<0.0001	0.0003	0.0073
d28-42	0.629[a]	0.603[a]	0.572[b]	0.0507	0.0012	0.0003	0.8120

Table 2.19 Continued

Gain:Feed							
d42-63	0.492[a]	0.474[b]	0.447[c]	0.0253	<0.0001	<0.0001	0.6234
d63-84	0.438[b]	0.453[a]	0.425[c]	0.0242	0.0012	0.0355	0.0006
d84-105	0.372	0.371	0.364	0.0248	0.4699	0.2486	0.6796
d105-126	0.316	0.328	0.322	0.0259	0.2662	0.4083	0.1612
d126-Mkt[2]	0.393[a]	0.366[b]	0.371[b]	0.0287	0.0046	0.0027	0.0192
Nursery, d0-42	0.687	0.673	0.678	0.0314	0.2927	0.3387	0.2139
Grower, d42-105	0.423[a]	0.420[a]	0.403[b]	0.0155	<0.0001	<0.0001	0.0286
Finisher, d105-mkt[2]	0.350	0.344	0.343	0.0203	0.4308	0.2102	0.5623
Overall, d0-Mkt[2]	0.421[a]	0.414[a]	0.405[b]	0.0122	0.0001	0.0001	0.3238

[1]Values with different superscripts, within a row are different ($P < 0.05$)

[2]Mkt = market. Pigs were marketed at d140 or d147

[3] Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion; 2X diets had the lowest CP, highest SAA inclusion; 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.

Table 2.20 The effects of lowering dietary crude protein and adding synthetic amino acids on the frequency of observed unhealthy pigs and of therapeutic treatments

	Diet[1,3]				
	Control	1X	2X	MSE	*P*<
Frequency of Unhealthy Pigs[2, %]					
Nursery Period	0.0224	0.0189	0.0177	0.0040	0.2734
Grower Period	0.0116	0.0102	0.0095	0.0018	0.3100
Finisher Period	0.0030	0.0052	0.0036	0.0031	0.6148
Overall	0.0142[a]	0.0125[ab]	0.0115[b]	0.0015	0.0978
Frequency of Treatment by Injection, %					
Nursery Period	0.0357	0.0314	0.0281	0.0077	0.4177
Grower Period	0.0195[a]	0.0142[ab]	0.0115[b]	0.0039	0.0515
Finisher Period	0.0043	0.0076	0.0060	0.0044	0.5797
Overall	0.0231[a]	0.0193[ab]	0.0165[b]	0.0034	0.0638

[1]Values with different superscripts, within a row are different (P < 0.05)

[2.]Pigs were considered "unhealthy" if they appeared clinically sick or unthrifty.

[3]Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion; 2X diets had the lowest CP, highest SAA inclusion; 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.

Table 2.21 The effects of lowering dietary crude protein and adding synthetic amino acids on carcass characteristics

Carcass Characteristics[3]

Fat Depth (mm)

	Diet[1,2]				MSE	P Value		
Sex	CTRL	1X	2X	Mean		Diet	Sex	Diet*Sex
B	22.9	22.3	22.7	22.6	2.1901	.6539	<0.0001	0.5748
G	18.4	18.8	19.4	18.9		Dt. Linear	Dt. Quadratic	
Mean	20.7	20.5	21.1			.5055	.5258	

Loin Depth (mm)

	Diet[1,2]				MSE	P Value		
Sex	CTRL	1X	2X	Mean		Diet	Sex	Diet*Sex
B	75.1	75.0	73.0	74.4	2.4881	0.0124	0.2426	0.6930
G	75.1	76.1	73.9	75.1		Dt. Linear	Dt. Quadratic	
Mean	75.1[a]	75.5[a]	73.5[b]			0.0203	0.0507	

Percent Lean (%)

	Diet[1,2]				MSE	P Value		
Sex	CTRL	1X	2X	Mean		Diet	Sex	Diet*Sex
B	55.100	55.233	54.825	55.052	0.7118	0.0628	<0.0001	0.9315
G	56.141	56.316	55.758	56.072		Dt. Linear	Dt. Quadratic	
Mean	55.620[ab]	55.775[a]	55.219[b]			0.1140	0.0779	

Base Meat Price ($/kg carcass)

	Diet[1,2]				MSE	P Value		
Sex	CTRL	1X	2X	Mean		Diet	Sex	Diet*Sex
B	1.5439	1.5418	1.5714	1.5524	0.0332	0.0461	0.0061	0.4573
G	1.5785	1.5634	1.5819	1.5746		Dt. Linear	Dt. Quadratic	
Mean	1.5612[ab]	1.5526[b]	1.5767[a]			0.1129	0.05035	

Table 2.21 Cont…

Carcass Yield (%)

	Diet[1,2]				MSE	P Value		
Sex	CTRL	1X	2X	Mean		Diet	Sex	Diet*Sex
B	74.845	76.102	75.322	75.423	1.6443	0.0072	0.4871	0.8359
G	74.820	76.644	75.619	75.694		Dt. Linear	Dt. Quadratic	
Mean	74.83[b]	76.37[b]	75.47[ab]			0.1836	0.0041	

Grade Premium ($/kg carcass)

	Diet[1,2]				MSE	P Value		
Sex	CTRL	1X	2X	Mean		Diet	Sex	Diet*Sex
B	0.147	0.151	0.134	0.145	0.0193	0.0236	0.1536	0.5918
G	0.147	0.160	0.145	0.151		Dt. Linear	Dt. Quadratic	
Mean	0.147[ab]	0.155[a]	0.140[b]			0.2088	0.0144	

Sort Loss ($/kg carcass)

	Diet[1,2]				MSE	P Value		
Sex	CTRL	1X	2X	Mean		Diet	Sex	Diet*Sex
B	0.034	0.047	0.024	0.0190	0.0214	.0254	0.0019	0.6621
G	0.018	0.025	0.013	0.0353		Dt. Linear	Dt. Quadratic	
Mean	0.026[ab]	0.036[a]	0.018[b]			0.2092	0.0156	

Total Carcass Value ($/kg carcass)

	Diet[1,2]				MSE	P Value		
Sex	CTRL	1X	2X	Mean		Diet	Sex	Diet*Sex
B	1.656	1.646	1.682	1.662	0.0484	0.1882	0.0002	0.7178
G	1.707	1.699	1.714	1.707		Dt. Linear	Dt. Quadratic	
Mean	1.681	1.672	1.698			0.2512	0.1533	

Table 2.21 Cont…

Carcass Weight (kg)

	Diet[1,2]				MSE	P Value		
Sex	CTRL	1X	2X	Mean		Diet	Sex	Diet*Sex
B	98.64	98.03	95.04	97.23	4.2146	0.0330	0.0737	0.1782
G	94.20	97.80	94.28	95.43		Dt. Linear	Dt. Quadratic	
Mean	96.42[ab]	97.92[a]	94.66[b]			0.1524	0.0274	

[1]Values with different superscripts, within a row are different ($P < 0.05$)

[2]Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion; 2X diets had the lowest CP, highest SAA inclusion; 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.

[3] n=24 per trt, n= 36 per sex

Table 2.22 The effects of lowering dietary crude protein and adding synthetic amino acids on manure generation and nutrient excretion.

	Diet[1,2]				P Values		
Amount per kg of gain[3]	Control	1X	2X	MSE	Diet	Lin. Diet	Quad. Diet
Manure Vol. (L/kg gain)	7.70	7.32	6.80	1.1523	0.3107	0.1324	0.8835
Dry Matter (g/kg gain)	368.69	308.90	350.37	76.6574	0.2994	0.6375	0.1421
Ash (g/kg gain)	87.172[a]	79.35[b]	73.57[b]	7.2894	0.0046	0.0012	0.7485
P (g/kg gain)	5.61	5.42	4.94	0.8212	0.2610	0.1282	0.3866
AmmN (g/kg gain)	32.26[a]	27.81[ab]	23.20[b]	4.5014	0.0025	0.0006	0.9674
Total N (g/kg gain)	38.40[a]	33.89[b]	29.01[c]	4.1894	0.0009	0.0002	0.9199
pH	7.3175	7.2638	6.9000	0.4438	0.1480	0.0738	0.4290

[1]Values with different superscripts, within a row are different ($P < 0.05$)

[2]Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion; 2X diets had the lowest CP, highest SAA inclusion; 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.

[3]Represents analysis of manure, water and feed wastage that accumulated in deep pits.

Table 2.23 The effect of lowering dietary crude protein and adding synthetic amino acids on average daily gas emission per animal unit

	Diet[1,2]				Diet
	Control	1X	2X	SE	P Value
NH_3 (g/AU*d)					
d7-14	37.7947	31.4391	30.7483	11.745	0.8966
d14-28	75.5529	60.1691	44.056	11.745	0.7136
d28-42	89.6702[a]	55.4752[b]	37.8793[b]	11.745	0.0093
d42-63	98.7767[a]	55.8345[b]	33.7137[b]	11.745	0.0008
d63-84	81.6927[a]	52.9978[ab]	28.1012[b]	11.745	0.0079
d84-105	60.4668	48.2051	26.857	11.745	0.1309
d105-126	51.6286	35.8223	21.7323	11.745	0.2054
d126-140	61.526	36.9393	24.7669	11.745	0.0867
d140-147[3]	46.1848[a]	32.7844[ab]	20.1072[b]	13.755	0.0495
CO_2 (kg/AU*d)					
d7-14	22.4663	23.8614	22.6152	1.3093	0.9921
d14-28	19.4748	22.6143	18.907	1.3093	0.7859
d28-42	13.7070	13.6436	13.6646	1.3093	0.7893
d42-63	10.9773	10.7008	11.6088	1.3093	0.8640
d63-84	10.4551	10.5582	11.1669	1.3093	0.8734
d84-105	8.7131	9.2452	9.4531	1.3093	0.8173
d105-126	7.3462	7.5299	7.9395	1.3093	0.7842
d126-140	7.0229	7.1088	7.5009	1.3093	0.7653
d140-147[3]	5.1141	5.3319	6.153	1.7869	0.8696
N_2O (mg/AU*d)					
d7-14	2251.33[a]	1439.84[b]	1767.57[b]	170.82	0.0052
d14-28	2120.92	1934.01	1656.10	170.82	0.1614
d28-42	1324.58	1155.15	1215.33	170.82	0.7773
d42-63	926.34	860.91	915.00	170.82	0.9590
d63-84	656.76	661.08	726.71	170.82	0.9488
d84-105	556.59	593.8	611.55	170.82	0.9734
d105-126	507.14	521.38	562.54	170.82	0.9720
d126-140	487.66	496.32	550.29	170.82	0.9613
d140-147[3]	382.76	371.67	449.54	231.77	0.9674

Table 2.23 cont…

H_2S (g/AU*d)					
d7-14	0.5547	0.9252	0.5719	0.9839	0.9995
d14-28	1.3521	1.4638	1.6758	0.9839	0.9988
d28-42	1.6821	2.2683	2.5664	0.9839	0.9869
d42-63	3.1720	4.5976	6.2488	0.9839	0.7564
d63-84	4.5169	4.7948	9.5766	0.9839	0.2097
d84-105	6.2820	7.0185	7.5446	0.9839	0.1386
d105-126	6.7945	6.3862	6.5296	0.9839	0.1477
d126-140	4.2564	4.0052	3.9687	0.9839	0.1566
d140-147[3]	3.5629	3.2426	4.3974	1.2008	0.1551
CH_4 (g/AU*d)					
d63- 84	167.26	234.48	192.52	28.7387	0.8017
d84-105	148.36	223.47	164.55	28.7387	0.3806
d105-126	132.24	165.26	182.35	28.7387	0.2576
d126-140	110.16	147.32	197.97	28.7387	0.1549
d140-147[3]	110.97	115.17	208.79	32.9955	0.1572

[1]Values with different superscripts, within a row are different ($P < 0.05$)

[2]Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion; 2X diets had the lowest CP, highest SAA inclusion; 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.

Table 2.24 The effect of lowering dietary crude protein and adding synthetic amino acids on cumulative gas emission per animal unit.

	Diet[1,2]				Diet
	Control	1X	2X	SE	P Value
NH_3 (g/AU)					
d7-14	302.36	251.51	245.99	938.05	0.9989
d7- 28	1360.10	1093.88	862.77	938.05	0.9321
d7-42	2610.48	1870.53	1393.08	938.05	0.6515
d7-63	4689.79	3043.06	2101.07	938.05	0.1502
d7- 84	6405.34[a]	4156.01[ab]	2691.19[b]	938.05	0.0234
d7-105	7675.14[a]	5168.32[ab]	3255.19[b]	938.05	0.0057
d7- 126	8759.33[a]	5920.58[b]	3711.57[b]	938.05	0.0014
d7-140	9620.70[a]	6437.73[b]	4058.76[b]	938.05	0.0004
CO_2 (kg/AU)					
d7-14	179.73	190.89	180.92	69.02	0.9921
d7- 28	452.38	507.49	445.62	69.02	0.7859
d7-42	644.28	698.50	636.92	69.02	0.7893
d7-63	874.80	923.22	880.71	69.02	0.864
d7- 84	1094.35	1144.94	115.21	69.02	0.8734
d7-105	1277.33	1339.09	1313.73	69.02	0.8173
d7- 126	1431.60	1497.22	1480.46	69.02	0.7842
d7-140	1529.92	1596.74	1585.47	69.02	0.7653
N_2O (mg/AU)					
d7-14	18011	11519	14141	9002.10	0.8769
d7- 28	47704	38595	37326	9002.10	0.6750
d7-42	66248	54767	54340	9002.10	0.5722
d7-63	85701	72846	73555	9002.10	0.5283
d7- 84	99493	86729	88816	9002.10	0.5636
d7-105	111181	99198	101659	9002.10	0.6123
d7- 126	121831	110147	113472	9002.10	0.6413
d7-140	128658	117096	121176	9002.10	0.6560
H_2S (g/AU)					
d7-14	4.43	7.40	4.57	77.13	0.9995
d7- 28	23.36	27.89	28.03	77.13	0.9988
d7-42	46.91	59.64	63.96	77.13	0.9869
d7-63	113.53	156.20	195.19	77.13	0.7564
d7- 84	208.38	256.89	369.30	77.13	0.2097
d7-105	340.31	404.28	554.74	77.13	0.1386
d7- 126	482.99	538.18	691.86	77.13	0.1477
d7-140	542.58	594.25	747.42	77.13	0.1566

Table 2.24 cont…

CH_4 (g/AU)					
d63-84	3694.07	5153.01	4222.78	1565.48	0.8017
d63-105	6809.66	9845.81	7678.43	1565.48	0.3806
d63-126	9586.62	13316.00	11508.00	1565.48	0.2576
d63-140	11129.00	15379.00	14279.00	1565.48	0.1549

[1]Values with different superscripts, within a row are different ($P < 0.05$)

[2]Control diets represent the highest CP (crude protein) with no SAA (synthetic amino acid) inclusion; 2X diets had the lowest CP, highest SAA inclusion; 1X diets were formulated to have an intermediate, equidistant CP to Control and 2X diets.

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CHAPTER 3. COMPARISON OF THE EFFECTS OF AN ANTIOBIOTIC-FREE AND A CONVENTIONAL WEAN-TO-FINISH MANAGEMENT ON SWINE GROWTH PERFORMANCE, MANURE GENERATION AND GAS EMISSIONS

3.1 Abstract

Seven hundred twenty-four, mixed sex pigs were placed in 11 rooms at the Purdue Swine Environmental Research Building to determine the effects of rearing pigs without antibiotics on growth performance and environmental footprint. Pigs were blocked by BW and gender and allotted to room and pen with 10/11 mixed-sex pigs/pen. Control pigs consumed diets containing antibiotics at subtherapeutic levels and were treated with injectable antibiotics when deemed necessary. Antibiotic-free animals consumed diets with alternatives to antibiotics and received no injectable antibiotics. If sick animals did not respond to antibiotic alternatives, they were removed from the experiment. Pigs were weighed at the start and end of each dietary phase, and mortality and morbidity were recorded daily. Manure was collected by vacuum sampling at phase changes and greenhouse gases were measured. Data were analyzed using the GLM procedure in SAS. During the nursery phase, control pigs grew faster ($P<0.02$; 0.455 vs 0.432 kg/d), and consumed more feed ($P<0.05$; 0.735 vs. 0.707 kg/d) than antibiotic free animals, resulting in similar G:F. Similar ADG, ADFI and G:F were observed throughout the grower phases, and therefore the increased BW of control-fed pigs was maintained and tended (P=0.06) to be heavier at the start of the finisher phases (86.0 vs. 84.5 kg). However, antibiotic-free animals grew 3% faster ($P<0.1$) and had 6% better G:F (P<0.001) in the finisher phases. As a result, there was no overall effect ($P>0.10$) of

treatment on ADG, ADFI, or G:F. Thirty antibiotic-free animals (8.3%) were removed from the study compared to 11 control (3.0%). There were no differences (P>0.10) observed in greenhouse gas emissions or nutrient excretion in manure. In conclusion, antibiotic-free management can yield a similar growth performance with a similar environmental footprint to conventional systems, but the limited disease treatment options may limit the number of pigs marketed under this management system.

3.2 Introduction

Antibiotics have been widely used in different facets of livestock production since the 1950s (Cromwell, 2002). The use of antibiotics has fallen into three categories: pro- and meta-phylaxis, therapeutic treatment of disease, and growth promotion (Barton, 2014). However, recent concerns about antibiotic resistance in both animal and human clinical settings has driven the growth of niche markets for antibiotic-free protein and legislation limiting the use of antibiotics in animal agriculture.

The FDA recently released Guidance for the Industry 209 and 213 to create a framework to limit the use of medically important antibiotics in animal agriculture. Guidance 209 defines growth promotion as an injudicious use of medically important antibiotics and will prevent the use of antibiotics for growth promotion by removing over the counter access to antibiotics. The veterinarian will be responsible for the producer's access to medically important antibiotics for the purpose of preventing and treating disease.

Although the initial use of antibiotics for growth promotion was based on anecdotal data, a great deal of data has established the benefits of antibiotic use

(Cromwell, 2002), but we have only recently begun to fathom how the modern production system will fare without access to over-the-counter antibiotics. There are many aspects of livestock production that may be affected by the new legislation including swine growth performance, swine health, and the environmental impact of swine production, which includes nutrient excretion in manure and gas emissions.

According to the EPA, swine are responsible for 0.35% of total anthropogenic greenhouse gas emissions (EPA, 2016). Swine health is also highly affected by gasses produced enterically and by stored manure (Peterson et al., 2010; Schneberger et al., 2017). Swine manure is also considered a potential pollutant because mismanagement of manure application can lead to N:P imbalances in soil and potential algae overgrowth in bodies of water (Erisman et al., 2013). Therefore it is highly important to understand how the removal of antibiotics and the use of alternatives affects the environmental impact of swine production.

The objective of this study was to compare the effects of rearing pigs with and without antibiotics on swine growth performance, gas emissions, and nutrient excretion.

3.3 Materials and Methods

All procedures were approved by the Purdue University Animal Care and Use Committee (PACUC# 1117000447).

3.3.1 Experimental design

Seven hundred twenty-four, single-source, mixed sex weaned pigs (Duroc x (York x Landrace); Temple Genetics, Inc.; avg 21 d of age) were placed in eleven,

identical, environmentally controlled rooms to determine the impact of rearing pigs without antibiotics on growth performance and environmental footprint. Pigs were blocked by BW and sex and randomly allotted to rooms and pens. Each room contained 6 pens with 10-11 pigs per pen, and each room contained 2 deep pits (1.83 m) under sets of 3 pens, allowing for quantitative collection of manure. Pigs in each room were reared with or without antibiotics (n=6 rooms for antibiotic free and n=5 rooms for control). Control pigs received both growth promoting concentrations of in-feed antibiotics and injectable antibiotics for treatment of clinical disease; whereas antibiotic-free animals received antibiotic alternatives throughout the wean-to-finish period. Clinically ill antibiotic-free animals were removed from the study, placed in a separate off-test room, and administered antibiotic treatments when they did not respond to antibiotic alternatives. All pigs were vaccinated for porcine corona virus-2 and mycoplasma at d 21 post weaning.

3.3.2 Diets

Pigs were fed 9 dietary phases (nursery 1, 2, 3, and 4, grower 1, 2, and 3, and finisher 1 and 2). Nursery 1 and 2 were each 7d in length, nursery 3-4 were 14d and the remaining dietary phases (grower 1- finisher 2) were 21d long. Nutrient requirements were adjusted to meet the dietary requirements (NRC, 2012) in each phase, but treatments (with or without antibiotics) remained constant (Tables 3.1-3.9). Experimental diets were identical in formulation other than addition of antibiotics or antibiotic alternatives. Antibiotics in the feed were rotated throughout the phases as is common industry practice (Table 3.10). Carbadox was included in all nursery diets along with elevated levels of Zn or Cu. Chlorotetracyline was included in Grower 1 diets

followed by Lincomycin in Grower 2 and 3. Finally Tylosin was used in both finishing phases. Antibiotic alternatives were used in the antibiotic free program. Water acidification (KEM SAN®, Kemin Industries, Inc., Des Moines IA, USA) was used in the first two nursery phases followed by supplementation of a Direct-Fed Microbial (Danisco Animal Nutrition, Marlborough UK) in the remaining nursery diets and all grower diets. Similar to the antibiotic program, elevated concentrations of Zn were used in the first 3 nursery phases, followed by elevated concentrations of Cu in the final nursery phase and the first grower phase. Finally, an oregano essential oil product (Regano®, Ralco Nutriton Inc., Marshall MN, USA) was added to both finisher phases.

3.3.3 Sample Collection

Individual BW and pen feed intakes were determined at each diet phase change. Pigs were observed every morning for any clinical abnormalities or general unthriftiness. These observations were recorded and pigs were treated and/or pulled from the study based on severity of the observed disease state. Sick pigs that did not respond to antibiotic alternatives were pulled from the study for therapeutic treatments. Manure volume was calculated at each diet change by using manure depth measurements in each pit. Manure pit samples were collected at the end of the nursery, grower, and finisher phases using a vacuum sampler. Samples were homogenized and a sub-sample was frozen (-20°C) for subsequent analyses. In addition to the vacuum samples, at the end of the experiment, manure pits were emptied into a small Slurry Store, mixed, and a representative sample was collected and frozen (-20°C) for subsequent analyses. The pigs in the heavier replicates on each treatment were marketed after 145 d on test and the lighter replicates on each treatment were marketed at 153 d on test. This was done to

mimic industry practices and to obtain a similar marketing time within treatment. Animals within a pen were tattooed with a pen number so that carcass data could be collected from the packing plant (Tyson Foods Inc., Logansport IN). Gas concentrations were determined approximately every two hours from each room and coupled with air flow rates to determine daily emissions from each room.

Samples of feed were ground to pass through a 1 mm screen using a Wiley mill (Thomas Scientific, Swedsboro, NJ) for subsequent analyses. Feed was analyzed for dry matter (DM), ash, total Kjeldahl Nitrogen (TN), Carbon (C), and total Phosphorus (P) in the Purdue Animal Sciences Nutrition laboratory, and for amino acid concentration by the University of Missouri Experiment Station Laboratory. Manure samples were analyzed for pH, DM, ash, total N, ammonium N (AmmN), C, and P.

Carbon was measured in feed and slurry using a Flash EA 1112 Series Nitrogen-Carbon Analyzer (CE Elantech, Inc. Lakewood, NJ). Dry matter was measured following a 12 h drying period at 100°C and ashing occurred over 8 hr at 600°C in a muffle furnace (Thermo Fisher Scientific, Inc. Waltham, MA). Total N and AmmN were measured using the micro-kjeldahl procedure (Bremmer and Keeney, 1965). Manure pH was measured using an Orion 310 basic PerpHecT® LogR pH meter (Thermo Fisher Scientific Inc. Waltham, MA). Phosphorus was measured colorimetrically in feed and feces following perchloric acid digestion (Murphy and Riley, 1962).

3.3.4 Statistical Analysis

Data were analyzed using the GLM procedure in SAS. Pen was the experimental unit for growth performance data, which was analyzed with main effects of treatment, body weight block, their interaction and replicate. Emission data were analyzed using the

Mixed procedure in SAS with fixed effects of treatment and day. Manure pit and room were the experimental units for manure and gas data analysis, respectively. A P value $\leq$ 0.05 indicated a significant difference and P values between $0.05 < P \leq 0.10$ were considered a trend.

3.4 Results

3.4.1 Performance

In the first week post-weaning, antibiotic-free reared pigs grew faster ($P< 0.0001$) than control pigs (Table 3.11). During week 2 post-weaning antibiotic-free reared pigs tended (P=0.0735) to continue to grow faster than control pigs. However, for the remainder of the nursery period, pigs reared with antibiotics grew faster (P<0.001) than those reared without, resulting in an overall improvement (P=0.014) in ADG for pigs reared with antibiotics from d 0-42 post-weaning (0.455 kg/d vs. 0.432 kg/d). As a result of this improved growth rate, pigs reared with antibiotics tended (P<0.067) to be ~1 kg heavier at the end of the nursery period than pigs reared without antibiotics (Table 3.11).

Growth rate was not impacted (P> 0.10) by treatment during the grower phases, and therefore a tendency (P=0.061) for pigs receiving antibiotics to be heavier persisted to d 105 (86.12 kg vs. 84.50 kg, respectively).

During the first finisher phase pigs reared without antibiotics grew faster (P=0.040) than pigs reared with antibiotics. No effect of treatment on ADG was observed during the second finisher phase (P>0.10). However, the response during finisher 1 was great enough to result in a tendency (P=0.069) for an improved ADG for the entire finisher period for pigs reared without antibiotics. This improvement in growth rate was enough for antibiotic-free reared pigs to catch up in terms of BW. Therefore,

pigs were marketed at similar BW (126.0 kg for control pigs and 125.5 kg for antibiotic-free pigs).

3.4.2 Feed Consumption

In the first week post-weaning, pigs reared without antibiotics consumed more feed than pigs reared with antibiotics (Table 3.11; $P<0.0001$). Pigs consumed a similar amount of feed during the second ($P=0.647$) and third ($P=0.719$) week post-weaning. Pigs reared with antibiotics consumed a greater amount of feed than those reared without antibiotics in the fourth nursery phase (d 28-42; $P=0.0261$). However, there was no difference between treatments in average daily feed intake (ADFI) in the overall nursery period ($P=0.2602$).

Pigs reared without antibiotics consumed more feed than control pigs in the first grower phase ($P=0.0020$), but consumed a similar amount of feed in the second ($P=0.1522$) and third ($P=0.3688$) grower phases. There was no difference observed in ADFI between treatments in the overall grower period ($P=0.9650$).

Average daily feed intake was similar across treatments in the first finisher phase ($P=0.4717$). There were no differences observed in ADFI in the overall finisher period ($P=0.7191$) nor the overall wean-to-finish period ($P=0.1730$) between treatments.

3.4.3 Feed Efficiency

Although pigs reared without antibiotics consumed more feed than those reared with antibiotics, their advantage also in growth rate resulted in greater Gain:Feed than control-fed pigs in the first week post-weaning (Table 3.11; $P=0.0454$). There were no differences in feed efficiency between treatments in week 2 post-weaning ($P=0.1660$). In the third nursery phase, pigs reared with antibiotics grew faster while consuming a

similar amount of feed as antibiotic-free pigs resulting in greater Gain:Feed (P=0.0001). However, in spite of the improvement in growth rate in pigs reared with antibiotics compared to those reared without, there were no differences in feed efficiency in the fourth nursery phase (P=0.9190) and in the overall nursery period (P=0.3573).

Although growth rates were similar in the first grower phase, the difference in feed intake resulted in a higher gain:feed in pigs reared with antibiotics compared to those reared without (P<0.0001). However, efficiency was similar between treatments in the second (P=0.6416) and third (P=0.9576) grower phases. Feed efficiency was not affected by treatment in the overall grower period (P=0.2647).

In the first finisher phase, antibiotic-free pigs gained more weight while consuming similar feed, resulting in a greater G:F than the pigs reared with antibiotics (P=0.0200). There was no difference in feed conversion in the second finisher phase (P=0.1356). The improvement in feed efficiency observed in the early finisher phase in pigs reared without antibiotics resulted in a higher feed conversion in the overall finisher period (P=0.0456).

There was no difference in feed efficiency between treatments for the overall wean-to-finish period (P=0.3616).

3.4.4 Pig Health

The frequency of observed abnormal (pigs that appeared unthrifty or clinically sick; Table 3.12) pigs was lower in the second week post-weaning for Antibiotic-free pigs than Control pigs (P=0.0354). There were no differences during phase 3 of the nursery period but during phase 4 pigs reared with antibiotics had a lower unhealthy observation rate than antibiotic-free fed pigs (P=0.0280). For the overall nursery period

observation frequency of unhealthy pigs was not different (*P*=0.72) in pigs reared with or without antibiotics. The number of abnormal pigs in antibiotic-free rooms was approximately 0.5 %-units higher than pigs reared with antibiotics during the grower period (*P*= 0.0932). There was a tendency (*P*=0.0857) for pigs raised without antibiotics to have a higher incidence of unhealthy pigs in the final finishing period. However, no differences were observed during the finishing period or the overall experimental period.

Eleven Control pigs (3% of pigs started) were pulled from the study due to death (n=2) or severe clinical problems that did not respond to injectable antibiotics. Thirty-three (8.3% of pigs started) Antibiotic-free pigs were removed for treatment with antibiotics because they did not respond to antibiotic alternatives.

3.4.5 Carcass Data

Pigs were marketed at similar weights (Table 3.13) between treatments on either day 147 (heaviest block) or 153 (remaining pigs). There were no differences observed in carcass fat depth (*P*=0.4534), loin depth (*P*= 0.8411), percent lean (*P*=0.4528) or carcass yield (*P*=0.4625) between treatments. Carcass grade premium (*P*=0.2997), carcass base meat price (*P*=0.7870), and total carcass value per kg(*P*=0.5069) were also similar between pigs reared with and without antibiotics. The final value per pig of pigs raised without antibiotics was $268.70, where the average value of pigs reared with antibiotics was $270.68.

3.4.6 Manure

Pigs reared without antibiotics excreted 10 mL/day more manure, 1.7 g/d more dry matter, 2.6 g/d more ash, 90 mg/d more total P, 780 mg/d more total N and 300 mg/d more ammonia N than the pigs reared with antibiotics (Table 3.14), however these

differences were not significant (P>0.10). Treatment did not influence C excretion but pigs reared with antibiotics excreted 16.1 g/d more C than pigs reared without antibiotics (P=0.5909). However, these differences were not significant.

3.4.7 Emissions

There were no differences in daily or cumulative NH_3 emissions (P>0.10) in the wean-to-finish period (Table 3.15 and 3.16). Similarly, daily and cumulative CO_2, CH_4, and H_2S did not differ between treatments (P>0.10). Emissions per AU/day of NH_3, CH_4, and H_2S tended to peak during the grower 1 and/or 2 periods (d42-84), however CO_2 linearly declined on a per AU/day basis as the pig aged from weaning to market weight.

3.4.8 Economics

Average cost (per pig) of in-feed and injectable antibiotics and antibiotic alternatives were calculated based on feed intake and recorded use, respectively (Figure 3.17). The additional cost of antibiotic-free management to get a pig to market, including antibiotic alternatives, was $1.34 compared to the $6.21 in additional costs to get a conventional pig to market. The pigs removed from study were not marketed conventionally, nor for the same price as the pigs that remained on test. Therefore, the increased number of removals from the antibiotic-free treatment resulted in a lower ($3-4/pig) estimated average profit compared to pigs managed with antibiotics (Table 3.18).

3.5 Discussion

The FDA Guidance for Industry 209 created a logistical and legal framework for drug manufacturers to relabel antibiotics deemed medically important to no longer be

used for growth promotion; only for treatment and prevention of disease. Guidance 209 placed “medically important” antibiotics away from over-the-counter use and under a veterinarian-issued veterinary feed directive to ensure judicious use of antibiotics. The increased legislation and regulation governing antibiotic use has also driven greater scientific interest in the use of antibiotic alternatives. Investigators are examining if substances as unintuitive as oregano oil (Bakkali et al., 2008), lactobacillus spp. (Allen et al., 2013), and other “natural health remedies” may elicit a similar health and performance effect as antibiotics without the added selection for antibiotic resistance to varying success.

In this study, we compared the effect of rearing pigs with and without antibiotics on health, growth performance, carcass characteristics, nutrient excretion, and air emissions. We chose an industry- standard rotation of broad-spectrum antibiotics that were included at sub-therapeutic levels in conventionally managed pigs. Conventionally managed pigs were also administered injectable antibiotics if clinical signs were observed. The antibiotic-free animals were fed a rotation of antibiotic alternatives, designed to have similar broad-spectrum antimicrobial effects as sub-therapeutic antibiotics, or to enhance the gut immune system and natural microbiome. Antibiotic-free animals received no injectable antibiotics; if clinical signs were observed, they were noted and observed. Dexamethasone was administered as an injectable, as needed to relieve clinical signs related to respiratory disease in both treatments. Pigs were pulled from the study if clinical signs did not resolve.

Pigs reared without antibiotics received acidified water in the early nursery phase. The purpose of water acidification is to acidify the pig’s GI tract and the pig’s immediate

environment because young pigs play with nipple waterers, spraying the acidified water in their pen. The reduction in pH within and around the pig helps reduce microbial stress and pathogen shedding (De Busser et al., 2011). In the early nursery period, pigs reared without antibiotics grew faster and consumed more feed than pigs reared with antibiotics. We also observed a lower frequency of clinical signs in antibiotic-free pigs compared to the conventionally managed pigs, indicating an acceptable level of efficacy at controlling pathogens.

Water acidification or the addition of organic acids to the diet has been studied as an antibiotic alternative against salmonella challenges. The efficacy of water and diet acidification as an antibiotic alternative has varied. Gebru et al. (2010) described improvements in growth and reductions in inflammatory markers after a salmonella challenge with the use of microencapsulated organic acids. Walsh et al. (2012) noted no improvements in growth performance with organic acid administration through water. Water acidification proved to be a successful antibiotic alternative in this case because it was associated with improvements in growth performance compared to antibiotic use. However, this improvement was observed without a known health challenge. Therefore we can conclude that water acidification is beneficial for prophylactic use but may not be as effective against a health challenge.

In the mid nursery to the early grower phases, pigs reared without antibiotics received a direct-fed microbial and elevated levels of Cu and Zn. However pigs on this treatment had poorer growth rate and feed efficiency and a greater frequency of clinical signs than pigs reared with antibiotics. We hypothesize that feeding the DFM while feeding elevated levels of Zn and Cu may have reduced the beneficial effects of the

probiotic by limiting survivability in the GI tract. This highlights the challenge of probiotic use in antibiotic-free systems; antibiotic alternatives tend to be non-selective in their bacteriocidal properties, so the probiotic of interest is less likely to colonize the intestine than it is to succumb to the antibiotic alternative.

An oregano essential oil product (Regano, Ralco, Marshall, MN) was fed to pigs reared without antibiotics throughout the finisher phase, while conventionally-reared pigs received in-feed Tylosin. Pigs reared without antibiotics had improved growth rates and feed conversion in the early finisher phase compared to conventionally-raised pigs.

Oregano oil is one of several compounds known to contain carvacrol, an aromatic molecule known to have antibacterial and antioxidant properties (Gilling, et al., 2014). Because carvacrol is lipophilic it works by integrating and subsequently disrupting microbial membranes and peptidoglycan layers and causes protein damage. Oregano oil has also been shown to have antiviral activity (Gilling, 2013). Carvacrol, and therefore oregano has been reported to be effective against some common intestinal pathogens (Burt, 2004) without selecting for antibiotic resistance (Docic and Bilkei, 2003).

Tylosin is a bacteriostatic antibiotic that inhibits bacterial protein synthesis (Plumb's, 2016) whereas the oregano product has been shown to be bactericidal (Giling, 2013). Theoretically, the bactericidal nature of the oregano is a more efficient product for reducing pathogen burden and resulting in a faster growth rate.

Pigs managed conventionally and pigs managed without antibiotics were marketed at similar weights with similar carcass characteristics, and a similar environmental footprint. There was also a similar incidence of observed clinical signs in

both sets of pigs. This indicates that antibiotic alternatives functioned at a similar level to antibiotics as prophylactics.

However, the increased number of pigs reared without antibiotics removed from the study because they exhibited clinical signs that failed to respond to treatment indicates that antibiotics are still necessary to treat disease. Essentially, while the antibiotic alternatives functioned as prophylactics similarly to antibiotics, they are not as efficacious at treating disease.

It is also important to note that although the additional cost (per pig) of rearing swine without antibiotics is less than the cost of rearing swine with antibiotics, the inability to treat sick animals necessitated that more animals reared without antibiotics needed to be marketed alternatively, for a lower price. Therefore it is important to maximize the price per carcass reared without antibiotics to economically off-set the reduced number of carcasses marketed.

Antibiotic-free is an economically feasible and environmentally sustainable method of rearing swine if animal health is aggressively managed, and there are a lack of immune challenges. However, antibiotic alternatives are not feasible options to treat disease. Therefore it is important that producers and veterinarians have access to antibiotics to treat disease.

3.6 Tables

Table 3.1 Nursery phase 1 Diets

	Diets	
Ingredient, %	Control	Antibiotic Free
Corn, yellow dent	32.200	32.590
Soybean meal, 47.5% CP	13.200	13.200
Soybean protein concentrate	4.500	4.500
Soybean oil	5.000	4.860
Plasma, spray-dried	6.500	6.500
Blood meal, spray-dried	1.500	1.500
Whey, dried	25.000	25.000
Fish meal, menhaden	4.000	4.000
Lactose	5.000	5.000
Limestone	0.730	0.730
Monocalcium phosphate	0.530	0.530
Vitamin premix[1]	0.250	0.250
Trace mineral premix[2]	0.175	0.175
Salt	0.250	0.250
Phytase[3]	0.100	0.100
L-lysine HCl	0.090	0.090
DL-methionine	0.220	0.220
L-threonine	0.040	0.040
Hemicell-HT 1.5 Enzyme[4]	0.025	0.025
Rabon larvacide[5]	0.025	0.025
Carbadox[6]	0.250	---
Zinc oxide	0.415	0.415
Calculated Composition		
NE, kcal/kg	2746.2	2746.0
CP, %	24.14	24.21
Ca, %	0.853	0.853
aP, %	0.601	0.601
P, %	1.005	1.005
Lys SID, %	1.552	1.552
Analyzed Composition		
DM, %	82.17	81.48
CP, %	26.06	27.77
P, %	0.84	0.85

[1]Vitamin premix provided per kilogram of the diet: vitamin A, 6615 IU; vitamin D_3, 662; vitamin E, 44.1 IU; vitamin K, 2.21 mg; vitamin B_{12}, 38.6 µg; riboflavin, 8.8 mg; pantothenic acid, 22.1 mg; niacin, 33.08 mg.
[2]TM premix supplies the following per kg of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15.03 mg; copper, 11.3 mg; iodine, 0.46 mg; selenium, 0.30 mg.
[3]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health – Dupont, St. Louis, MO).
[4]Hemicell HT (Elanco, Greenfield, IN)
[5]Rabon Lavacide (Bayer Animal Health, Leverkusen, Germany)
[6]Carbadox (Mecadox® 10 Phibro Animal Health, Ridgefield Park, NJ) provided at 55 ppm.

Table 3.2 Nursery phase 2 Diets

	Diets	
Ingredient, %	Control	Antibiotic Free
Corn, yellow dent	35.735	36.125
Soybean meal, 47.5% CP	18.550	18.550
Soybean protein concentrate	2.500	2.500
Soybean oil	4.000	3.860
Plasma, spray-dried	2.500	2.500
Blood meal, spray-dried	1.250	1.250
Whey, dried	28.500	28.500
Fish meal, menhaden	4.000	4.000
Limestone	0.570	0.570
Monocalcium phosphate	0.410	0.410
Vitamin premix[1]	0.250	0.250
Trace mineral premix[2]	0.175	0.175
Salt	0.250	0.250
Phytase[3]	0.100	0.100
L-lysine HCl	0.220	0.220
DL-methionine	0.230	0.230
L-threonine	0.100	0.100
L-tryptophan	0.010	0.010
Hemicell-HT 1.5 Enzyme[4]	0.025	0.025
Rabon larvacide[5]	0.025	0.025
Carbadox[6]	0.250	---
Zinc oxide	0.350	0.350
Calculated Composition		
NE, kcal/kg	2674.3	2674.1
CP, %	22.98	23.01
Ca, %	0.803	0.803
aP, %	0.496	0.496
P, %	0.838	0.838
Lys SID, %	1.501	1.502
Analyzed Composition		
DM, %	80.89	80.43
CP, %	24.43	25.90
P, %	0.73	0.74

[1]Vitamin premix provided per kilogram of the diet: vitamin A, 6615 IU; vitamin D_3, 662; vitamin E, 44.1 IU; vitamin K, 2.21 mg; vitamin B_{12}, 38.6 μg; riboflavin, 8.8 mg; pantothenic acid, 22.1 mg; niacin, 33.08 mg.
[2]TM premix supplies the following per kg of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15.03 mg; copper, 11.3 mg; iodine, 0.46 mg; selenium, 0.30 mg.
[3]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health – Dupont, St. Louis, MO).
[4]Hemicell HT (Elanco, Greenfield, IN)
[5]Rabon Lavacide (Bayer Animal Health, Leverkusen, Germany)
[6]Carbadox (Mecadox® 10 Phibro Animal Health, Ridgefield Park, NJ) provided at 55 ppm.

Table 3.3 Nursery phase 3 diets

	Diets	
Ingredient, %	Control	Antibiotic Free
Corn, yellow dent	45.656	45.903
Soybean meal, 47.5% CP	25.130	25.108
Corn DDGS, 7% oil	5.000	5.000
Soybean oil	3.000	3.000
Whey, dried	14.000	14.000
Fish meal, menhaden	4.000	4.000
Limestone	0.847	0.847
Monocalcium phosphate	0.364	0.363
Vitamin premix[1]	0.250	0.250
Trace mineral premix[2]	0.175	0.175
Salt	0.300	0.300
Phytase[3]	0.100	0.100
L-lysine HCl	0.276	0.276
DL-methionine	0.166	0.166
L-threonine	0.087	0.087
Hemicell-HT 1.5 Enzyme[4]	0.025	0.025
Carbadox[5]	0.250	---
Direct fed microbial[6]	---	0.025
Zinc oxide	0.375	0.375
Calculated Composition		
NE, kcal/kg	2692.8	2699.4
CP, %	21.75	21.76
Ca, %	0.80	0.80
aP, %	0.38	0.37
P,%	0.67	0.67
Lys SID, %	1.31	1.31
Analyzed Composition		
DM, %	83.26	83.11
CP, %	25.17	26.20
P, %	0.68	0.71

[1]Vitamin premix provided per kilogram of the diet: vitamin A, 6615 IU; vitamin D_3, 662; vitamin E, 44.1 IU; vitamin K, 2.21 mg; vitamin B_{12}, 38.6 μg; riboflavin, 8.8 mg; pantothenic acid, 22.1 mg; niacin, 33.08 mg.
[2]TM premix supplies the following per kg of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15.03 mg; copper, 11.3 mg; iodine, 0.46 mg; selenium, 0.30 mg.
[3]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health – Dupont, St. Louis, MO).
[4]Hemicell HT (Elanco, Greenfield, IN)
[5]Carbadox (Mecadox® 10 Phibro Animal Health, Ridgefield Park, NJ) provided at 55 ppm.
[6]DFM (Danisco Animal Nutrition, Marlborough UK)

Table 3.4 Nursery phase 4 diets

	Diets	
Ingredient, %	Control	Antibiotic Free
Corn, yellow dent	49.004	49.269
Soybean meal, 47.5% CP	30.066	29.999
Corn DDGS, 7% oil	15.000	15.000
Soybean oil	2.000	2.000
Limestone	1.355	1.355
Monocalcium phosphate	0.636	0.636
Vitamin premix[1]	0.250	0.250
Trace mineral premix[2]	0.175	0.175
Salt	0.350	0.350
Phytase[3]	0.100	0.100
L-lysine HCl	0.329	0.331
DL-methionine	0.200	0.200
L-threonine	0.060	0.061
Hemicell-HT 1.5 Enzyme[4]	0.025	0.025
Banmith dewormer, 48[5]	0.100	0.100
Rabon larvacide[6]	0.025	0.025
Carbadox[7]	0.250	---
Direct fed microbial[8]	---	0.025
Copper sulfate	0.075	0.100
Calculated Composition		
NE, kcal/kg	2813.8	2818.2
CP, %	23.07	23.06
Ca, %	0.75	0.75
aP, %	0.29	0.29
P,%	0.64	0.64
Lys SID, %	1.25	1.25
Analyzed Composition		
DM, %	84.11	84.52
CP, %	21.47	23.20
P, %	0.66	0.64

[1]Vitamin premix provided per kilogram of the diet: vitamin A, 6615 IU; vitamin D_3, 662; vitamin E, 44.1 IU; vitamin K, 2.21 mg; vitamin B_{12}, 38.6 μg; riboflavin, 8.8 mg; pantothenic acid, 22.1 mg; niacin, 33.08 mg.
[2]TM premix supplies the following per kg of diet: iron, 121.3 mg; zinc, 121.3 mg; manganese, 15.03 mg; copper, 11.3 mg; iodine, 0.46 mg; selenium, 0.30 mg.
[3]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health – Dupont, St. Louis, MO).
[4]Hemicell HT (Elanco, Greenfield, IN)
[5]Rabon Lavacide (Bayer Animal Health, Leverkusen, Germany)
[6]Banmith Dewormer (Phibro Animal Health, Teaneck, NJ)
[7]Carbadox (Mecadox® 10 Phibro Animal Health, Ridgefield Park, NJ) provided at 55 ppm.
[8]DFM (Danisco Animal Nutrition, Marlborough UK)

Table 3.5 Grower phase 1 diets

	Diets	
Ingredient, %	Control	Antibiotic Free
Corn, yellow dent	55.308	55.272
Soybean meal, 47.5% CP	15.259	15.270
Corn DDGS, 7% oil	25.000	25.000
Choice white grease	1.000	1.000
Limestone	1.527	1.527
Monocalcium phosphate	0.265	0.265
Vitamin premix[1]	0.150	0.150
Trace mineral premix[2]	0.140	0.140
Salt	0.350	0.350
L-lysine HCl	0.570	0.570
L-threonine	0.142	0.142
L-tryptophan	0.032	0.032
DL-methionine	0.081	0.082
Phytase[3]	0.100	0.100
Rabon larvacide[4]	0.025	0.025
Hemicell-HT 1.5 Enzyme[5]	0.025	0.025
Chlortetracycline[6]	0.050	---
Direct fed microbial[7]	---	0.025
Copper sulfate	---	0.050
Calculated Composition		
NE, kcal/kg	2633.4	2631.2
CP, %	19.64	19.64
Ca, %	0.71	0.71
aP, %	0.25	0.25
P, %	0.56	0.56
Lys SID, %	1.10	1.10
Analyzed Composition		
DM, %	83.94	83.46
CP, %	21.84	22.02
P, %	0.65	0.61

[1]Vitamin premix provided per kilogram of the diet: vitamin A, 3690 IU; vitamin D_3, 396; vitamin E, 26.4 IU; vitamin K, 1.32 mg; vitamin B_{12}, 23.1 μg; riboflavin, 5.28 mg; pantothenic acid, 13.2 mg; niacin, 19.8 mg.
[2]TM premix supplies the following per kg of diet: iron, 87.3 mg; zinc, 87.3 mg; manganese, 10.82 mg; copper, 8.136 mg; iodine, 0.33 mg; selenium, 0.30 mg.
[3]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health – Dupont, St. Louis, MO).
[4]Hemicell HT (Elanco, Greenfield, IN)
[5]Rabon Lavacide (Bayer Animal Health, Leverkusen, Germany)
[6]Chlortetracycline (CTC) provided at 55 ppm (Aureomycin 50, Alpharma Inc., Bridgewater, NJ).
[7]DFM (Danisco Animal Nutrition, Marlborough UK)

Table 3.6 Grower phase 2 diets

	Diets	
Ingredient, %	Control	Antibiotic Free
Corn, yellow dent	56.148	56.234
Soybean meal, 47.5% CP	9.622	9.611
Corn DDGS, 7% oil	30.000	30.000
Choice white grease	1.000	1.000
Limestone	1.519	1.520
Monocalcium phosphate	0.024	0.023
Vitamin premix[1]	0.150	0.150
Trace mineral premix[2]	0.140	0.140
Salt	0.350	0.350
L-lysine HCl	0.579	0.579
L-threonine	0.116	0.116
L-tryptophan	0.048	0.048
DL-methionine	0.040	0.040
Phytase[3]	0.100	0.100
Rabon larvacide[5]	0.038	0.038
Hemicell-HT 1.5 Enzyme[4]	0.025	0.025
Lincomycin[6]	0.100	---
Direct fed microbial[7]	---	0.025
Calculated Composition		
NE, kcal/kg	2453.0	2455.2
CP, %	18.42	18.43
Ca, %	0.65	0.65
aP, %	0.22	0.22
P,%	0.51	0.51
Lys SID, %	0.98	0.98
Analyzed Composition		
DM, %	83.04	83.09
CP, %	22.76	20.78
P, %	0.56	0.57

[1]Vitamin premix provided per kilogram of the diet: vitamin A, 3690 IU; vitamin D_3, 396; vitamin E, 26.4 IU; vitamin K, 1.32 mg; vitamin B_{12}, 23.1 μg; riboflavin, 5.28 mg; pantothenic acid, 13.2 mg; niacin, 19.8 mg.
[2]TM premix supplies the following per kg of diet: iron, 87.3 mg; zinc, 87.3 mg; manganese, 10.82 mg; copper, 8.136 mg; iodine, 0.33 mg; selenium, 0.30 mg.
[3]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health – Dupont).
[4]Hemicell HT (Elanco, Greenfield, IN)
[5]Rabon Lavacide (Bayer Animal Health, Leverkusen, Germany)
[6] Lincomycin (Linocmix, Zoetis, Parsippany, NJ) provided at 110 ppm
[7]DFM (Danisco Animal Nutrition, Marlborough UK)

Table 3.7 Grower phase 3 diets

	Diets	
Ingredient, %	Control	Antibiotic Free
Corn, yellow dent	59.523	59.506
Soybean meal, 47.5% CP	6.593	6.596
Corn DDGS, 7% oil	30.000	30.000
Choice white grease	1.000	1.000
Limestone	1.450	1.450
Vitamin premix[1]	0.150	0.150
Trace mineral premix[2]	0.140	0.140
Salt	0.300	0.300
L-lysine HCl	0.548	0.548
L-threonine	0.066	0.066
L-tryptophan	0.034	0.034
DL-methionine	0.028	0.028
Phytase[3]	0.080	0.080
Rabon larvacide[5]	0.038	0.038
Hemicell-HT 1.5 Enzyme[4]	0.025	0.025
Lincomycin[6]	0.025	---
Direct Fed Microbial[7]	---	0.040
Calculated Composition		
NE, kcal/kg	2455.2	2455.2
CP, %	17.18	17.18
Ca, %	0.61	0.61
aP, %	0.21	0.21
P, %	0.49	0.49
Lys SID, %	0.88	0.88
Analyzed Composition		
DM, %	82.41	82.63
CP, %	20.90	21.14
P, %	0.58	0.51

[1]Vitamin premix provided per kilogram of the diet: vitamin A, 3690 IU; vitamin D_3, 396; vitamin E, 26.4 IU; vitamin K, 1.32 mg; vitamin B_{12}, 23.1 μg; riboflavin, 5.28 mg; pantothenic acid, 13.2 mg; niacin, 19.8 mg.
[2]TM premix supplies the following per kg of diet: iron, 87.3 mg; zinc, 87.3 mg; manganese, 10.82 mg; copper, 8.136 mg; iodine, 0.33 mg; selenium, 0.30 mg.
[3]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health – Dupont).
[4]Hemicell HT (Elanco, Greenfield, IN)
[5]Rabon Lavacide (Bayer Animal Health, Leverkusen, Germany)
[6] Lincomycin (Linocmix, Zoetis, Parsippany, NJ) provided at 44 ppm
[7]DFM (Danisco Animal Nutrition, Marlborough UK)

Table 3.8 Finisher phase 1 diets

	Diets	
Ingredient, %	Control	Antibiotic Free
Corn, yellow dent	63.172	63.172
Soybean meal, 47.5% CP	3.068	3.068
Corn DDGS, 7% oil	30.000	30.000
Choice white grease	1.000	1.000
Limestone	1.373	1.373
Vitamin premix[1]	0.125	0.125
Trace mineral premix[2]	0.100	0.100
Salt	0.300	0.300
L-lysine HCl	0.533	0.533
L-threonine	0.105	0.105
L-tryptophan	0.043	0.043
Phytase[3]	0.080	0.080
Rabon larvacide[4]	0.050	0.050
Hemicell-HT 1.5 Enzyme[5]	0.025	0.025
Tylosin[6]	0.025	---
Oregano[7]	---	0.025
Calculated Composition		
NE, kcal/kg	2455.2	2455.2
CP, %	15.81	15.81
Ca, %	0.57	0.57
aP, %	0.21	0.21
P, %	0.48	0.48
Lys SID, %	0.78	0.78
Analyzed Composition		
DM, %	82.84	82.63
CP, %	18.90	18.05
P, %	0.50	0.51

[1]Premix provided per kilogram of the diet: vitamin A, 2640 IU; vitamin D_3, 264; vitamin E, 17.6 IU; vitamin K, 0.88 mg; vitamin B_{12}, 15.4 µg; riboflavin, 3.52 mg; pantothenic acid, 8.8 mg; niacin, 13.2 mg.
[2]TM premix supplies the following per kg of diet: iron, 72.7 mg; zinc, 72.7 mg; manganese, 9.0 mg; copper, 6.78 g; iodine, 0.27 mg, selenium, 0.15 mg.
[3]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health – Dupont).
[4]Rabon Lavacide (Bayer Animal Health, Leverkusen, Germany)
[5]Hemicell HT (Elanco, Greenfield, IN)
[6]Tylosin (Tylan, Elanco, Greenfield, IN) provided at 22 ppm
[7]Regano® (Ralco Nutriton Inc., Marshall MN, USA)

Table 3.9 Finisher phase 2 diets

	Diets	
Ingredient, %	Control	Antibiotic Free
Corn, yellow dent	77.146	77.146
Soybean meal, 47.5% CP	4.315	4.315
Corn DDGS, 7% oil	15.000	15.000
Choice white grease	1.000	1.000
Limestone	1.219	1.219
Monocalcium phosphate	0.114	0.114
Vitamin premix[1]	0.125	0.125
Trace mineral premix[2]	0.100	0.100
Salt	0.209	0.209
L-lysine HCl	0.434	0.434
L-threonine	0.105	0.105
L-tryptophan	0.038	0.038
DL-methionine	0.016	0.016
Phytase[3]	0.080	0.080
Rabon larvacide[4]	0.050	0.050
Hemicell-HT 1.5 Enzyme[5]	0.025	0.025
Tylosin[6]	0.025	---
Oregano[7]	---	0.025
Calculated Composition		
NE, kcal/kg	2970	2970
CP, %	13.23	13.23
Ca, %	0.53	0.53
aP, %	0.15	0.15
P, %	0.43	0.43
Lys SID, %	0.69	0.69
Analyzed Composition		
DM, %	83.27	83.28
CP, %	12.79	13.28
P, %	0.44	0.44

[1]Premix provided per kilogram of the diet: vitamin A, 2640 IU; vitamin D_3, 264; vitamin E, 17.6 IU; vitamin K, 0.88 mg; vitamin B_{12}, 15.4 µg; riboflavin, 3.52 mg; pantothenic acid, 8.8 mg; niacin, 13.2 mg.
[2]TM premix supplies the following per kg of diet: iron, 72.7 mg; zinc, 72.7 mg; manganese, 9.0 mg; copper, 6.78 g; iodine, 0.27 mg, selenium, 0.15 mg.
[3]Phytase activity level 600.1 PU/kg (Phyzyme, Danisco Animal Health – Dupont).
[4]Rabon Lavacide (Bayer Animal Health, Leverkusen, Germany)
[5]Hemicell HT (Elanco, Greenfield, IN)
[6]Tylosin (Tylan, Elanco, Greenfield, IN) provided at 22 ppm
[7]Regano® (Ralco Nutriton Inc., Marshall MN, USA)

Table 3.10 Summary of antibiotic and antibiotic alternative rotation during dietary phases

Phase	Duration/ Amt.	Control Antibiotic program	Antibiotic free program
Nursery 1	1.4 kg/hd	Carbadox (55ppm) + 2,991 ppm Zn from ZnO	Water Acid + 3,000 ppm Zn from ZnO
Nursery 2	2.5 kg/hd	Carbadox (55ppm) + 2,522 ppm Zn from ZnO	Water Acid – (13 days total) + 2,500 ppm Zn from ZnO
Nursery 3	8.2 kg/hd	Carbadox (55ppm) + 2,702 ppm Zn from ZnO	DFM + 2,700 ppm Zn from ZnO
Nursery 4	Ad lib to day 42	Carbadox + 189 ppm Cu from CuSO	DFM + 252 ppm Cu from $CuSO_4$
Grower 1	21 days	Chlortetracycline (55 ppm)	DFM + 126 ppm Cu from $CuSO_4$
Grower 2	21 days	Lincomycin (110 ppm)	DFM Plus Water Acid
Grower 3	21 days	Lincomycin (44 ppm)	DFM
Finisher 1	21 days	Tylosin (22 ppm)	Oregano
Finisher 2	21 days	Tylosin (22 ppm)	Oregano

Table 3.11 The effect of rearing pigs with and without antibiotics on growth performance

	Diet[1]			
	Control	Antibiotic Free	Root MSE	*P* value
BW, kg				
d0	6.74	6.74	0.729	0.9648
d7	6.89	7.05	0.777	0.4290
d14	8.74	9.15	1.053	0.1842
d28	15.89	15.60	1.627	0.4295
d42	25.87	24.87	2.059	0.0670
d63	44.42	43.37	2.701	0.1404
d84	64.97	63.59	3.022	0.0836
d105	86.11	84.49	3.274	0.0611
d126	105.69	105.18	3.817	0.6023
d145	121.66	121.20	3.871	0.6431
Market[2]	126.02	125.49	4.181	0.5867
ADG, kg				
d0-7	0.024	0.044	0.0219	<0.0001
d7-14	0.270	0.299	0.0624	0.0735
d14-28	0.507	0.460	0.0508	0.0008
d28-42	0.713	0.662	0.0521	0.0005
d42-63	0.883	0.880	0.0446	0.8395
d63-84	0.978	0.962	0.0444	0.1745
d84-105	1.006	0.995	0.0615	0.4715
d105-126	0.932	0.985	0.0962	0.0396
d126-mkt[2]	0.798	0.801	0.1027	0.9240
Nursery, d0-42	0.455	0.431	0.0356	0.0138
Grower, d42-105	0.956	0.946	0.0296	0.2023
Finisher, d105-mkt[2]	0.867	0.895	0.0585	0.0687
Overall, d0-mkt[2]	0.794	0.790	0.0223	0.5588
ADFI, kg				
d0-7	0.088	0.111	0.0202	<0.0001
d7-14	0.285	0.293	0.0632	0.6477
d14-28	0.691	0.701	0.1006	0.7196
d28-42	1.233	1.134	0.1667	0.0261
d42-63	1.705	1.797	0.1096	0.0020
d63-84	2.657	2.599	0.1530	0.1533
d84-105	3.023	2.993	0.1318	0.3688

Table 3.11 Cont.

d105-126	3.167	3.129	0.1982	0.4717
d126-mkt[2]	3.261	3.301	0.2083	0.5666
Nursery, d0-42	0.700	0.675	0.0834	0.2602
Grower, d42-105	2.459	2.460	0.1016	0.9650
Finisher, d105-mkt[2]	3.208	3.228	0.1634	0.7191
Overall, d0-mkt[2]	2.173	2.137	0.0772	0.1730
G:F				
d0-7	0.197	0.312	0.2158	0.0454
d7-14	0.966	1.000	0.1175	0.1660
d14-28	0.746	0.662	0.0786	0.0001
d28-42	0.586	0.589	0.0791	0.9190
d42-63	0.534	0.495	0.0285	<0.0001
d63-84	0.369	0.373	0.0271	0.6416
d84-105	0.333	0.333	0.0199	0.9576
d105-126	0.294	0.316	0.0345	0.0200
d126-mkt[2]	0.263	0.274	0.0296	0.1356
Nursery, d0-42	0.655	0.642	0.0540	0.3753
Grower, d42-105	0.390	0.386	0.0141	0.2647
Finisher, d105-mkt[2]	0.270	0.281	0.0154	0.0456
Overall, d0-mkt[2]	0.362	0.365	0.0103	0.3616

[1]The control diet represents pigs reared with in-feed and injectable antibiotics. The antibiotic free diet represents pigs reared with in-feed antibiotic alternatives.
[2]Pigs were marketed on d 145 and 153.

Table 3.12 Observed frequency of unhealthy4 pigs reared with and without antibiotics

	Diet[1]			
	Control	Antibiotic Free	MSE	*P Value*
d7-14[3]	3.6063	1.9148	0.9497	0.0354
d14-28	2.3501	2.2703	1.1469	0.9150
d28-42	0.8052	2.1484	0.7058	0.0280
d42-63	0.7650	1.4241	0.6736	0.1774
d63-84	0.7830	1.1476	0.3250	0.1320
d84-105	0.9106	1.4161	0.7254	0.3146
d105-126	0.8610	0.8578	0.4437	0.9911
d126-mkt[2]	0.2969	0.2690	0.2373	0.0857
Nursery Period	1.9895	2.1520	0.6914	0.7218
Grower Period	0.8195	1.3299	0.3953	0.0932
Finisher Period	0.5546	0.5381	0.2725	0.9266
Overall	1.0252	1.2852	0.3351	0.2686

[1]The control diet represents pigs reared with in-feed and injectable antibiotics. The antibiotic free diet represents pigs reared with in-feed antibiotic alternatives.
[2]Pigs were marketed on d 145 and 153.
[3]Week 1 post-weaning data was not collected.
[4]Pigs were considered unhealthy if they were observed to have clinical sickness or unthrifty.

Table 3.13 Comparison of carcass analysis in pigs raised with and without antibiotics

	Diet[1]			
	Control	Antibiotic Free	MSE	P value
Pigs Marketed (n)	309	359	-	-
Carcass Wt, kg	95.11	94.74	3.6707	0.6915
Loin fat depth, mm	22.10	22.46	1.8571	0.4534
Loin muscle depth, mm	64.60	64.49	2.1150	0.8411
Carcass lean, %	53.56	53.44	0.5801	0.4528
Carcass yield, %	75.50	75.83	1.7037	0.4635
Carcass base meat price, $/kg	2.7587	2.7580	0.0151	0.7870
Carcass grade premium, $/kg	0.1044	0.0987	0.0213	0.2997
Carcass sort loss, $/kg	0.0215	0.0236	0.0158	0.6026
Total carcass value, $/kg	2.8413	2.8346	0.0351	0.3909
Total Value per pig, $	270.26	268.41		

[1]The control diet represents pigs reared with in-feed and injectable antibiotics. The antibiotic free diet represents pigs reared with in-feed antibiotic alternatives.

Table 3.14 Comparison of nutrient excretion in manure from pigs raised with and without antibiotics.

	Diet[1]		MSE	*P*
	Control	Antibiotic Free		
Excretion per kg gain[2]				
Manure Volume (L)	6.14	6.40	1.0528	0.4853
DM (g)	328.59	340.90	49.5915	0.6369
Ash (g)	65.65	71.35	1.2610	0.2528
Total P (g)	4.45	4.72	0.9256	0.4296
TN (g)	31.90	34.09	4.8150	0.2742
AmmN (g)	24.91	26.20	3.8387	0.3066
Total C (g)	243.10	242.74	9.7000	0.9785
Manure pH[2]	7.3305	7.3280	0.1766	0.8382
Excretion per pig per day[2]				
Manure Volume (L)	4.79	4.80	0.71	0.7868
DM (g)	255.28	256.97	38.11	0.9466
Ash (g)	51.10	53.72	7.81	0.4625
Total P (g)	3.47	3.56	0.66	0.6140
Total N (g)	24.87	25.65	3.82	0.5424
AmmN (g)	19.41	19.71	3.10	0.5876
Total C (g)	188.00	182.90	7.02	0.5909

[1]The control diet represents pigs reared with in-feed and injectable antibiotics. The antibiotic free diet represents pigs reared with in-feed antibiotic alternatives.
[2]Represents analysis of manure, water and feed wastage that accumulated in deep pits.

Table 3.15 Comparison of the effect of raising pigs with and without antibiotics on cumulative gas emission per animal unit.

	Diet[1]				
	Control	SE	Antibiotic Free	SE	*P* Value
NH_3 (g/AU)					
d0-7	125.56	540.89	116.03	489.45	0.9896
d0-14	346.75	540.89	335.60	489.45	0.9878
d0-28	873.66	540.89	867.41	489.45	0.9932
d0-42	1537.77	540.89	1487.28	489.45	0.9449
d0-63	2602.73	540.89	2444.01	489.45	0.8280
d0-84	3658.87	540.89	3511.35	489.45	0.8400
d0-105	4587.73	540.89	4481.54	489.45	0.8844
d0-126	5341.13	540.89	5303.44	489.45	0.9588
d0-145	5885.77	540.89	5869.40	489.45	0.9821
CO_2 (kg/AU)					
d0-7	279.35	131.30	297.84	117.89	0.9168
d0-14	522.11	131.30	569.22	117.89	0.7902
d0-28	922.68	131.30	1018.03	117.89	0.5905
d0-42	1219.68	131.30	1348.08	117.89	0.4699
d0-63	1597.77	131.30	1732.88	117.89	0.4462
d0-84	1988.51	131.30	2125.75	117.89	0.4391
d0-105	2357.23	131.30	2510.15	117.89	0.3889
d0-126	2663.51	131.30	2837.86	117.89	0.3262
d0-145	2903.26	131.30	3096.87	117.89	0.276
CH_4 (g/AU)					
d0-28	1100.03	1191.69	869.33	1069.83	0.8859
d0-42	2383.43	1191.69	1943.05	1069.83	0.7843
d0-63	4571.51	1191.69	3805.97	1069.83	0.6344
d0-84	7320.54	1191.69	6069.61	1069.83	0.4379
d0-105	9903.32	1191.69	8621.76	1069.83	0.4268
d0-126	11946.00	1191.69	10765.00	1069.83	0.4641
d0-145	13331.00	1191.69	12225.00	1069.83	0.4925

Table 3.15 Cont…

H_2S (g/AU)					
d0-7	4.24	122.78	1.14	110.59	0.9851
d0-14	20.12	122.78	16.93	110.59	0.9846
d0-28	50.56	122.78	46.16	110.59	0.9788
d0-42	160.17	122.78	117.57	110.59	0.7972
d0-63	412.50	122.78	397.39	110.59	0.9274
d0-84	548.53	122.78	552.68	110.59	0.9800
d0-105	660.26	122.78	658.10	110.59	0.9896
d0-126	811.90	122.78	799.25	110.59	0.9392
d0-145	970.07	122.78	949.35	110.59	0.9005

Table 3.16 Comparison of the effect of raising pigs with and without antibiotics on daily gas emissions per animal unit

	Diet				
	Control	SE	Antibiotic Free	SE	*P*
NH_3 (g/AU*d)					
d0-7	19.72	6.648	17.199	5.816	0.9225
d7-14	33.25	6.648	31.366	5.816	0.9804
d14-28	39.29	6.648	37.986	5.816	0.9664
d28-42	49.09	6.648	44.276	5.816	0.7187
d42-63	52.37	6.648	45.558	5.816	0.5563
d63-84	51.95	6.648	50.826	5.816	0.9497
d84-105	45.89	6.648	46.199	5.816	0.8199
d105-126	37.53	6.648	39.137	5.816	0.7070
d126-145	30.32	6.648	29.787	5.816	0.8961
d145-153	35.72	7.275	27.663	6.398	0.4626
CO_2 (kg/AU*d)					
d0-7	45.04	3.086	43.983	2.085	0.7996
d7-14	34.42	3.086	38.768	2.085	0.3013
d14-28	28.35	3.086	32.058	2.085	0.3779
d28-42	20.97	3.086	23.574	2.085	0.5355
d42-63	17.74	3.086	18.323	2.085	0.8891
d63-84	18.35	3.086	18.708	2.085	0.9325
d84-105	17.30	3.086	18.303	2.085	0.8116
d105-126	14.33	3.086	15.606	2.085	0.7609
d126-145	12.36	3.086	13.632	2.085	0.7623
d145-153	11.93	2.805	11.785	3.409	0.9767
CH_4 (g/AU*d)					
d7-14	75.48	14.092	57.316	12.655	0.3415
d14-28	91.61	14.092	76.694	12.655	0.4340
d42-63	104.10	14.092	88.710	12.655	0.4186
d63-84	130.85	14.092	107.790	12.655	0.2283
d84-105	122.93	14.092	121.530	12.655	0.9413
d105-126	97.19	14.092	102.080	12.655	0.7971
d126-145	72.88	14.092	76.829	12.655	0.8356
d145-153	76.03	15.217	81.834	13.457	0.7766

Table 3.16 Cont…

H_2S (g/AU*d)					
d0-7	0.27	1.550	0.16	1.399	0.9562
d7-14	2.31	1.550	2.25	1.399	0.9781
d14-28	2.21	1.550	2.08	1.399	0.9503
d28-42	7.87	1.550	5.10	1.399	0.1882
d42-63	12.06	1.550	13.32	1.399	0.5466
d63-84	6.52	1.550	7.39	1.399	0.6772
d84-105	5.36	1.550	5.02	1.399	0.8692
d105-126	7.26	1.550	6.72	1.399	0.7953
d126-145	8.36	1.550	7.89	1.399	0.8228
d145-153[2]	6.69	1.790	6.45	1.569	0.9209

Table 3.17 Comparison of costs of in-feed and injectable antibiotics and antibiotic alternatives

Total Cost Per Pig		
	Additional Cost of in-feed treatments ($/pig)[1]	
Phase	Antibiotic Free	Control
Nursery 1	0.164123453	0.00965473
Nursery 2	0.23827999	0.01342334
Nursery 3	0.012792549	0.064933893
Nursery 4	0.020698016	0.115017224
Grower 1	0.032804402	0.058312455
Grower 2	0.047602517	1.503557283
Grower 3	0.054821821	0.684
Finisher 1	0.379457348	0.250328869
Finisher 2	0.352885995	0.240475758
	Injectable Treatments[2]	
Enrofloxacin	-	2.154143302
Tylosin	-	0.051339564
Penicilin	-	0.024224299
Ceftiofur	-	0.784191589
Lincomycin	-	0.30305296
Dexamethasone	0.041036415	0.024143302

[1]The price of water acidification was $0.1738/L water; DFM cost $0.000869/kg feed; Oregano product cost $0.00577/kg feed; Carbadox cost $0.0066/kg feed; CTC cost $0.00162/kg feed; Lincomycin cost $0.02674/kg feed when included at 110 ppm; Lincomycin cost $0.01069/kg feed when included at 44 ppm; Tylan cost $0.00375/kg feed.
[2]Injectable Enrofloxcin (Baytril, Bayer Animal Health, Leverkusen, Germany) cost $2.93/mL; Tylosin (Tylan®, Elanco Animal Health, Greenfield, IN) cost $0.16/mL; Penicillin G cost $0.072/mL; Ceftiofur (Excede®, Zoetis, Kalamazoo, MI) cost $0.662/mL; Lincomycin (Lincomix®, Zoetis, Kalamazoo, MI) cost $0.19/mL; Dexamethasone cost $0.10/mL.

Table 3.18 Comparison of additional costs and market prices for pigs reared with and without antibiotics

Additional Cost to Get a Pig to Market (Calculated $/Pig)			
	Control	Antibiotic Free	Difference
In-Feed Treatment	$2.94	$1.30	
Injectable Treatments	$3.27	$0.04	
Total Additional Cost	$6.21	$1.34	$4.87
Market Pricing ($/Pig)			
% Marketed	94.21	90.89	
Market Price/ Live Pig Marketed	$272.63	$270.72	
Removed Pig Price Estimate	$176.46 - 212.24	$176.46-212.24	
Final Estimated Price/Pig	$267.02 - 269.43	$259.43-261.18	$7.59 - 8.25

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CHAPTER 4. A COMPARISON OF HISTOLOGIC MEASURES OF INFLAMMATION BY DIGITAL AND MANUAL METHODS

4.1 Abstract

The gastrointestinal tract is the largest immune organ in the body and activation of the gut-associated immune system can significantly affect feed intake, nutrient absorption, and protein deposition. Therefore swine nutritionists, veterinarians and producers have a vested interest in measuring the degree of inflammation and immune activity present in the intestine. Historically, histologic measures of villus height and crypt depth have been used to reflect intestinal health; however these measures may not be accurate or holistic reflections of pathologic changes occurring in the intestine. The objective of this study was to evaluate digital methods of measuring cellular infiltrate and intestinal morphology. Three sets of tissues were selected for pathologic changes, and the degree of inflammation was graded on a semi-quantitative scale by a veterinary pathologist. Selected tissues were stained with hematoxylin and eosin, Alcian blue and immunohistochemically for CD3. Stained slides were digitally scanned with a Leica Aperio® while slide imaging scanner and analyzed digitally with ImageScope® software. Digital analysis included manual and digital eosinophil, goblet cell, and CD3 T-cell enumeration. Villus height and crypt depth were measured off of a photomicrograph in Adobe Photoshop and compared with the ImageScope® digitally scanned slide. Data were analyzed with Proc Corr and Proc Reg in SAS 9.4. Inter-observer error was calculated by comparing the standard deviation of cell counts and morphology measures reported by two separate people in the GLM procedure. There was greater inter-observer variation between manual cell counts than automated cell counts ($P<0.05$). None of the

cell counts correlated well with the pathologist's estimate of cell density and inflammation present on the slide. Only the manual and computer-based CD3+ T cell counts had a positive correlation (0.66). There was greater variation in morphology measures taken in Aperio compared to Photoshop, but none of the measures correlated with the semiquantitative pathology score. This study highlights the challenges of quantifying intestinal health based on histology measures.

4.2 Introduction

In swine, disease challenges are the primary reason animals do not reach their genetic potential for growth (Cromwell, 2002; Williams et al., 1994). Activation of the immune system, particularly in the gastrointestinal tract, profoundly affects swine performance because gut inflammation can reduce digestion and absorption, and a larger proportion of absorbed protein and energy are shunted away from skeletal muscle to support the immune response. Inflammation also results in parenchymal by-stander damage that can be nutritionally expensive to repair (Klasing, 2007).

Historically, villus height and crypt depth have been used to reflect not only the absorptive surface area of the intestine but also the immune status of the gastrointestinal tract (Pluske et al., 1977). Essentially, the taller the villi, the healthier the animal and the more nutrients it can absorb. If an animal has short, or blunted villi this animal is experiencing some kind of stressor or disease challenge that is reducing lumen surface area, increasing enterocyte turnover and reducing the energy and proteins absorbed that will be deposited as skeletal muscle (Williams et al., 2015). Villus height and crypt depth have persisted as measures of intestinal health and function because they are relatively

inexpensive to measure, do not require an in-depth understanding of intestinal anatomy and physiology, and have been well represented in animal science literature.

There are limitations to relying on villus height and crypt depth as the sole indication of intestinal health and function. The small intestine is a highly complex organ where multiple pro- and anti-inflammatory processes can occur simultaneously. An animal can be clinically ill or stressed without experiencing an appreciable reduction in villus height (Moeser, 2007). In other cases an animal may morphologically respond to a treatment, but this response will not be reflected in the balance of pro- versus anti-inflammatory cytokines expressed (Li et al., 2006). Investigators also use different methods to measure villus height and crypt depth, particularly apparent in the number of villi measured per slide. There is also a lot of variation in how investigators measure villus height and crypt depth including using light microscopy with a micrometer (Nabuurs et al., 1993), using image software with an "arbitrary line tool" to measure villi on a photomicrograph (Li et al., 2016) and using analytical software designed for light microscopy imaging studies (Yeruva et al., 2016).

Two experiments were completed to compare quantitative and semi-quantitative methods of measuring intestinal health, inflammation, and morphology. The first experiment compared colorimetric algorithm-based measures of cell density, direct cell counts completed by two blinded technicians, and a semiquantitative scale developed by a veterinary pathologist. The second experiment compared three methods of measuring intestinal morphology (two quantitative methods of measuring villus height/crypt depth and a semi-quantitative scale). We hypothesized that digital measures of cellular

infiltration and morphology would reflect differences in intestinal pathology with less variation than manual counts and morphology measures.

4.3 Materials and Methods

Pigs genetically selected for high and low soy protein allergenicity (n=8 per line) were group housed, and placed on a common, soy-free diet for 6 d immediately post-weaning (avg. 23.1 d of age). Pigs were then moved to individual pens and fed a diet containing 18% soybean meal for 13 d, followed by an intradermal injection of 0.1 mL of soy protein in the flank region of the belly. Animals were subjectively categorized as "low" or "high" reactors based on wheal and flair scores, with a score of 0 denoting no reaction and a score of 3 denoting a severe reaction. Animals were euthanized after 14 d on test and a 15 cm section of jejunum was removed 154 cm proximal to the ileocecal junction, subsectioned, fixed in 10% neutral buffered formalin prior to embedding in paraffin. The 8 animals with the lowest reactivity to soy protein were selected and classified as "low allergy" and the 8 pigs with the highest reactivity to soy protein and were selected and classified as 'high allergy."

Eight finishing pigs with no known record of soy protein sensitivity were euthanized after being group housed and fed a standard corn-soybean meal-DDGS finisher diet. Jejunal tissue was immediately collected after harvest and fixed in 10% neutral buffered formalin then embedded in paraffin. Tissues were selected to provide a range of severity of inflammatory lesions.

Sections, 4 µm thick, were stained with hematoxylin and eosin, Alcian blue and immunohistochemically for CD3 T-cells according to standard methods (Altukistani et al., 2016). Slides were scanned into Aperio light microscopy imaging software up to

200X magnification. Slides were also imaged using a camera attached to a microscope at 100X magnification.

A semiquantitative scale (Figure 4.1) was developed by a board-certified veterinary pathologist to characterize the extent of jejunal lesions and cellular infiltrate. The scale included the amount of inflammatory infiltrate present in the lamina propria and mucosa, type of inflammatory cells present and percent of goblet cells comprising the epithelial layer of the villi. The objective of the semiquantitative scale was to summarize and categorize the severity of inflammatory processes present in the gut. The higher the score, the more inflamed the intestine.

An increase in goblet cell number may reflect a chronic inflammatory process. However, a significant reduction in goblet cells is more indicative of acute or peracute inflammation, intestinal sloughing and early regeneration. The semi-quantitative scale was designed to compare acutely inflamed and non-inflamed, fully regenerated, tissue. Eosinophils and lymphocytes were included in the analysis to highlight the pathologic changes associated with acute inflammation and allergic response. Two blinded scientists corroborated semi-quantitative findings to ensure that all scores were within 1 unit, and the values were averaged for analysis.

4.3.1 Experiment 1: Digital versus manual cell infiltrate quantification

Each slide was annotated in ImageScope® software to highlight the mucosa and lamina propria and omit underlying collagen and muscle layers from inclusion in colorimetric analysis. Algorithms colorimetrically de-convoluted the slides to determine pixels from cells of interest as a proportion of total area. Cell counts were estimated by determining the mean cell surface area of 12 randomly selected cells from three different

slides. The positively stained surface area was divided by the average cell surface area and then divided by total surface area to calculate cell density in the mucosa and lamina propria. This algorithmic analysis was repeated to compare outputs for inter-technician error analysis.

Two blinded technicians independently and manually counted eosinophils, goblet cells and CD3+ T cells present in 6 representative (<0.5 mm^2) subsections of the H and E, Alcian blue, and CD3+ IHC stained slides, respectively at 200X magnification. Cells were included in the count if they contained an appropriately stained, in-tact cytoplasm with a nucleus (in CD3 and Eosinophils) or were the appropriate shape, stain and location (goblet cells).

4.3.2 Experiment 2: Measures of Morphology

Two technicians used the annotation tool to measure villus height (from crypt-villus junction to the villus tip), crypt depth (from crypt-villus junction to the deepest point of the crypt with intact epithelial cells), in 6 representative, well oriented, villi/crypt combinations per slide using ImageScope® software at 100X magnification. These values were compared to villus height and crypt depth measures taken on photomicrographs (at 100X magnification) of the same slides in Adobe Photoshop photo-editing software with an arbitrary line tool.

4.3.3 Statistical Analysis

Proc corr in SAS 9.4 (Cary, NC) was used to quantify the degree of correlation between overall semi-quantitative pathology score and villus height, crypt depth and Villus:Crypt measures taken in Aperio and Adobe Photoshop. R-square values from the regression procedure were also used to individually quantify the association between

morphology measures, cell count methods, and the semi-quantitative score of inflammation present in the slide. Inter-observer variation was measured by calculating the standard deviation among observers within software and measure type and comparing them using the GLM procedure of SAS.

4.4 Results

4.4.1 Pathology Score

Scores ranking the degree of cellular infiltration and inflammation ranged from 1-3. The total score, including lamina propria inflammation, eosinophilic enteritis and goblet cell density ranged from 4.5-7.

4.4.2 Experiment 1: Cell Counts

There was no correlation between manual or digital eosinophil counts and the pathologist's estimate of eosinophil density (Table 4.1). The manual count of eosinophils was positively and more strongly correlated with overall pathology score than the computer count (Table 4.2), with a higher R^2 from the regression equation. However, there was greater (Table 4.3; P=0.0009) variation in calculated eosinophil density due to observer in manual counts than computer counts (Figures 4.2-4.4).

The computer count of goblet cell density was more correlated with the pathologist's estimate of goblet cell density than the manual count (Table 4.1; Figure 4.5). The manual count of goblet cell density had no correlation with the overall pathology score (Table 4.2; Figure 4.6), and a low correlation with the pathologist's estimate of goblet cell density. There was also less variation in the computer measures of

goblet cell density compared to manual counts (P=0.0005). Manual and digital counts were lowly correlated (Figure 4.7).

Finally, the greatest amount of variation due to technician (Table 4.7; P<0.001*)* was observed in manual counts of the CD3 cells compared to computer estimates. However, this was the only cell measure where the human and computer-based counts had a positive correlation that tended to approach 1 (Figure 4.9) and had an R^2 of 0.465. Computer counts of CD3+ cells had as similarly poor correlation with overall pathology score than manual counts, but neither were significant (Table 4.2; Figure 4.8).

4.4.3 Experiment 2: Morphology

None of the morphology measures were highly correlated with the semiquantitative pathology score (Figures 4.10-4.12). The morphology measure with the highest correlation with the semiquantitative evaluation of inflammation present in the slide was the villus:crypt calculated based on measures taken in ImageScope® software (R^2= 0.2012) . The measure with the lowest correlation with semiquantitative score was the villus:crypt calculated based on measures taken in photoshop (R^2=0.2012). Villus height (ImageScope®: R^2= 0.1081; Photoshop: R^2= 0.0039) and crypt depth (ImageScope®: R^2= 0.0072; Photoshop: R^2= 0.00005) were poorly correlated with semi quantitative scores in both ImageScope® and Adobe Photoshop. However, Photoshop measures had significantly less variation due to technician (Tables 4.6-4.8; P < 0.0001) than measures taken in ImageScope®.

4.5 Discussion

We hypothesized that automated cell counts would more strongly correlate with the pathologist-derived semi-quantitative score than the manual counts and that there would be significantly more variation due to inter-observer error in the manual counts compared to the automated. The manual count of eosinophils had a weak correlation with the pathologist's estimate of eosinophil density, but a slightly stronger correlation with the total pathology score. The automated count of goblet cells had a stronger correlation with the pathologist's estimate of goblet cell density than the manual counts, but neither method correlated with the overall pathology score. Both the manual and the automated counts of CD3+ T-cells correlated poorly with overall pathology score but the mean counts correlated well when regressed against each other.

Eosinophils are strongly associated with allergic disease; therefore, eosinophil density should change with different levels of severity of allergic disease (Dreau et al., 1994). However, one of the challenges in this study was the fact that the slides were processed at two different times, with two different staining protocols, resulting in a different degree of eosin staining in the weanling pigs with the soy allergy compared to the finisher-aged pigs. This difference in eosin intensity made it difficult to digitally highlight eosinophils, likely resulting in the abnormally low automated counts in slides from weanling pigs with soy allergy. This, in turn, resulted in weak correlations between the automated count of eosinophils and the pathologist's evaluation of eosinophil density and the overall pathologist's evaluation of inflammation present in the tissue. Although the pathophysiology of eosinophilic enteritis is not well described in the pig, the degree of eosinophil infiltration may correlate with severity of allergic disease if slides are processed and stained consistently.

Goblet cells produce a variety of mucins that are responsible for protecting the intestinal mucosa from physical and chemical insult (Xavier and Podolsky, 2007). In fact, goblet cells have an important role in suppressing colitis (An et al., 2007). In human pathology, a reduction in goblet cell number is correlated with greater severity of celiac disease (Dickson et al., 2006).

We expected an inverse correlation between goblet cell density and the pathologist-derived goblet cell score, but the manual count of goblet cell density had a weak positive correlation with the pathologist-derived goblet cell score. The computer count of goblet cell density had a stronger, negative correlation with the pathologist's goblet cell-specific score. The computer count of goblet cell density may be a more accurate reflection of the actual goblet cell density because it reflects the conclusions drawn from the pathologist's evaluation.

This difference in density measure between the human and computer counts may be attributable to sampling differences. The automated counts measured the number of positively stained pixels in the entire lamina propria and mucosa which was then mathematically converted to cells/mm^2. Technicians randomly selected representative sub-sections of mucosa and lamina propria. It is plausible that the sub-sampling process resulted in an over-representation of areas of the mucosa with a high density of goblet cells. This highlights an important limitation of relying on manual cell counts to calculate whole-slide cell density.

CD3+ T cells include all sub-types of T cells, including helper T cells and cytotoxic T cells. An allergic disease represents an inappropriate adaptive and innate immune response to an innocuous substance. Therefore, we expected differences in the

CD3 cell density between the studied pigs that would correlate to the overall degree of inflammation observed in the slide. However, both computer and manual counts of CD3+ cells had a weak correlation with overall pathology score.

There was approximately 20x more variation between observers in manual counts of CD3+ T cells than automated counts, significantly more than counts of any other cell type in this study. The CD3+ cells are significantly smaller and more numerous than goblet cells and eosinophils and these characteristics compounded to increase the effect of sub-sampling on inter-observer error. Other investigators (Corazza et al., 2007; Fuchs and Buhmann, 2011) have noted significant differences in variability between human and computer- completed cell density and pathology-readings.

Although the CD3+ T cell counts had the greatest amount of inter-observer error, the mean computer and manual counts correlated better with each other than any of the other cell counts, indicating that should an investigator choose to use either of these methods the counts would reflect a consistent value. None of the cell counts correlated well with the overall pathology score. This indicates that the histomorphologic scale was not sensitive enough to highlight subtle differences in cell infiltrate and morphology changes, or there were not enough differences in degree of pathologic changes on the slides for the scale or counting methods to highlight.

In experiment 2, we expected to see a greater correlation between the measures taken in ImageScope® and the pathologist's evaluation of intestinal health because ImageScope® allows the technician to measure representative villi and crypts from the entirety of the slide instead of a small sub-section. The morphology measure that had the strongest correlation with the pathologist's evaluation of intestinal health was villus:crypt

calculated from measures of villus height and crypt depth taken in ImageScope®, however this correlation did not approach 1 (R^2= 0.2012.

Interestingly there were no correlations between villus height and crypt depth, taken in both ImageScope® and Adobe Photoshop, and the pathologist's inflammation score. The relatively greater correlation between villus:crypt and intestinal health is in agreement with other investigators (Corraza et al., 2007) who correlated villus:crypt with the severity of Celiac's disease.

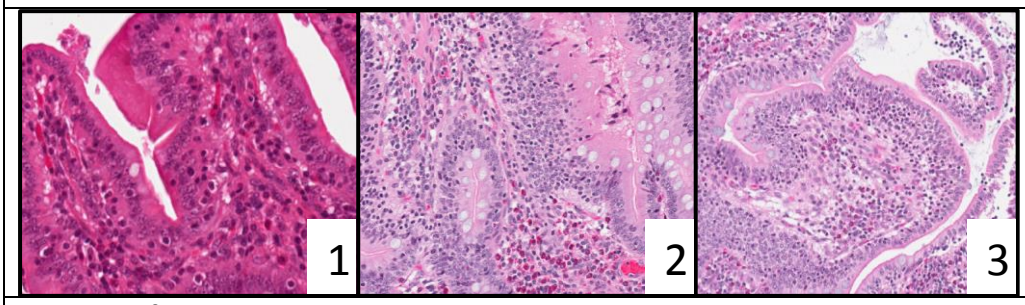
The Adobe Photoshop morphology measures were taken by two different people on the same photomicrograph, reducing the number of villi that could be selected for measurement. The ImageScope® software allowed the technician to select representative, well oriented villi from the entire slide instead of a small sub-section represented on the photomicrograph. These data indicate the importance of selecting villi from the entire slide to accurately represent the morphology of the entire slide.

The benefit of using a semi-quantitative scale to characterize intestinal inflammation is that it reflects an evaluation of the entire slide and can highlight the potential inflammatory infiltrates and morphology of interest. However in this case, only the digital goblet cell count correlated with the goblet cell-specific semiquantitative score. This highlights the challenges of using intestinal histology measures to draw specific conclusions about the inflammatory process in the gastrointestinal tract.

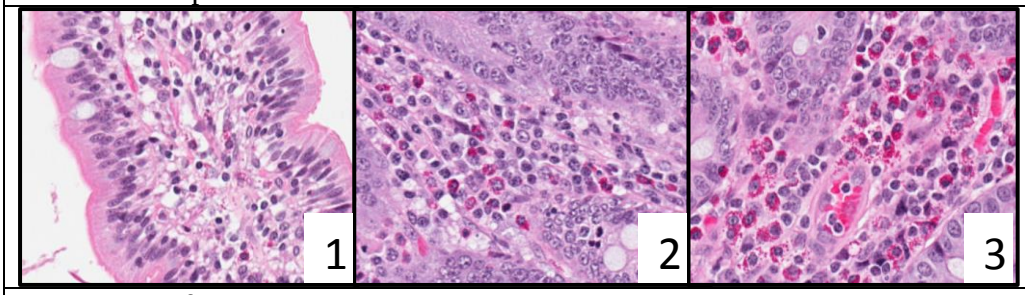
Digital histology measures provide an opportunity to mitigate the challenges of traditional histopathologic evaluation to characterize inflammation present in the small intestine. However, greater research is necessary to characterize the morphologic manifestation of localized inflammation and how that correlates to systemic health.

4.6 Tables and Figures

Inflammation in the lamina propria of the mucosa
3 = marked amounts (sheets of granulocytes expanding the width of the tip)
2 = moderate amounts (sheets of granulocytes at the base of the villous)
1 = mild amounts (multifocal scattering)
0 = none seen



Number of eosinophils
3 = high numbers of eosinophils
2 = moderate numbers of eosinophils
1 = low numbers of eosinophils
0 = no eosinophils



Percentage of goblet cells
3 = <10%
2 = 11-25%
1 = 25-50%
0 = 50% or greater

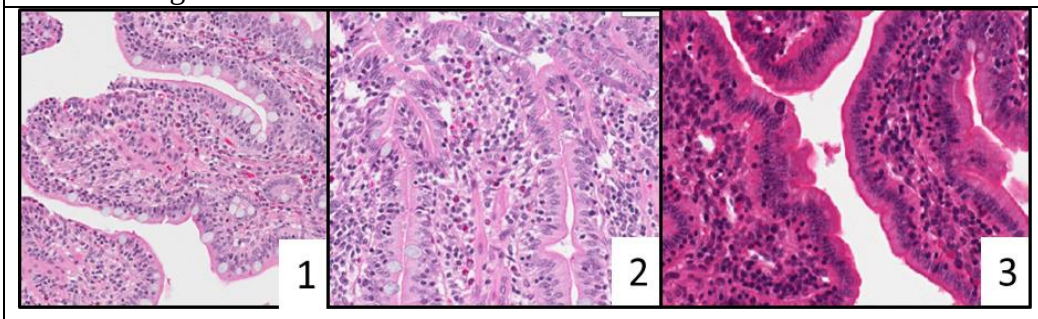


Figure 4.1 Histomorphologic Semi-quantitative Scale

Table 4.1 Correlation of Cell Counts with Pathologist's Estimate of Cell Density

Correlation with Pathologist's Estimate of Cell Density	
Computer Count of Eosinophils	-0.0934
Human Count of Eosinophils	-0.1419
Computer Count of Goblet Cells	-0.6434
Human Count of Goblet Cells	0.2819

Table 4.2 Correlation of Cell Count with Overall Pathology Score

Correlation of Cell Count with Overall Pathology Score	
Computer Count of Eosinophils	-0.0724
Human Count of Eosinophils	0.4763
Computer Count of Goblet Cells	-0.3153
Human Count of Goblet Cells	0.0220
Computer Count of CD3 T cells	-0.2333
Human Count of CD3 T cells	-0.1841

Table 4.3 Variation Due to Observer (StdDev) Eosinophil Density (Cells/mm^2)

Variation Due to Observer (StdDev) Eosinophil Density (Cells/mm^2)

	Mean of Variance	StdErr	Root MSE	P
Automated Count	53.68	21.28	106.41	0.0009
Manual Count	159.74	21.28		

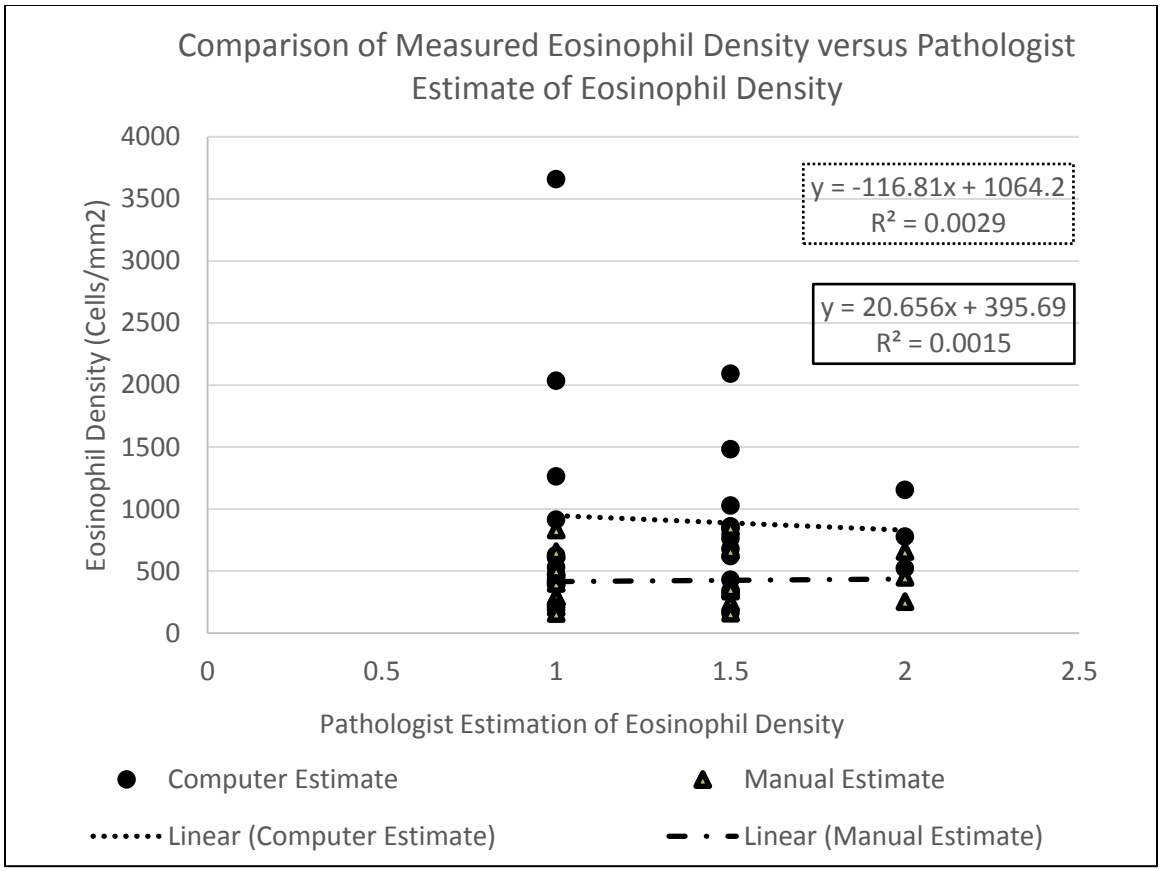


Figure 4.2 Comparison of Eosinophil Count versus Pathologist's Estimate of Eosinophil Density

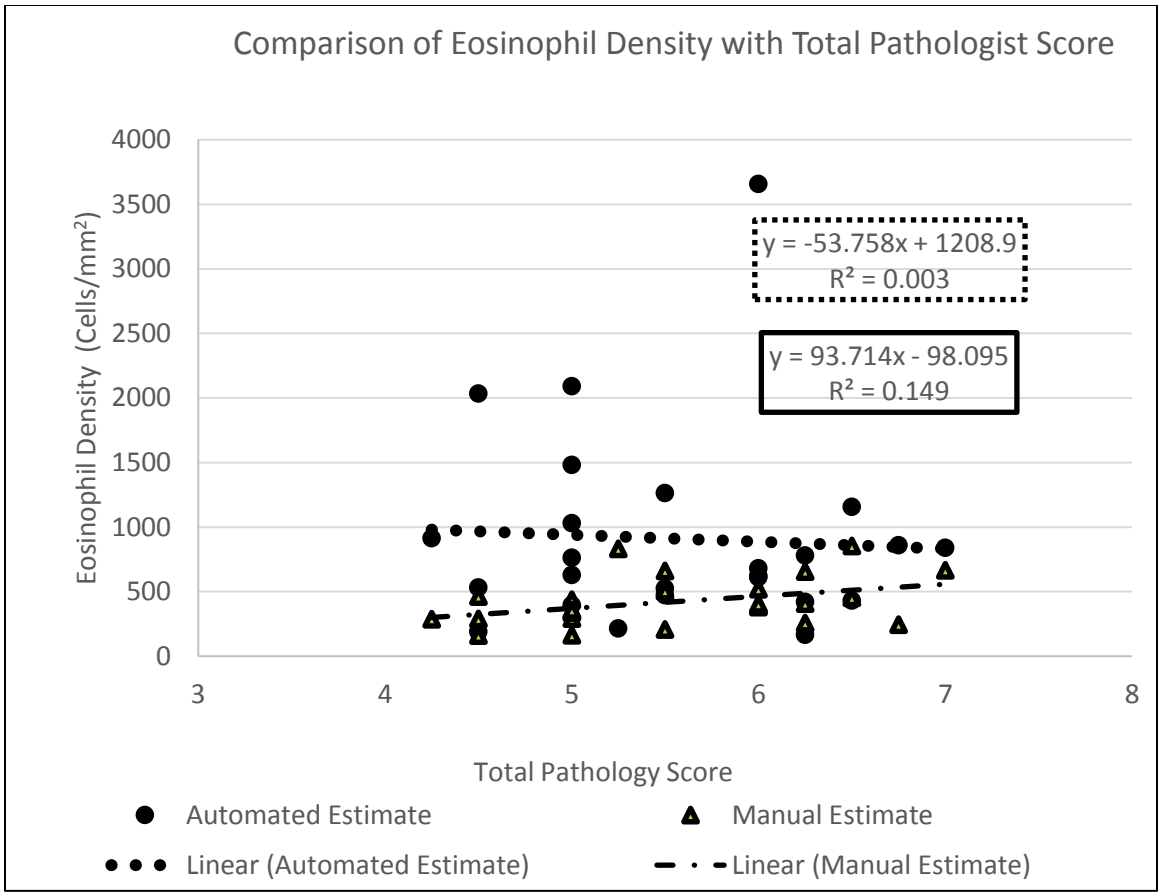


Figure 4.3 Comparison of Eosinophil Count with Total Pathology Score

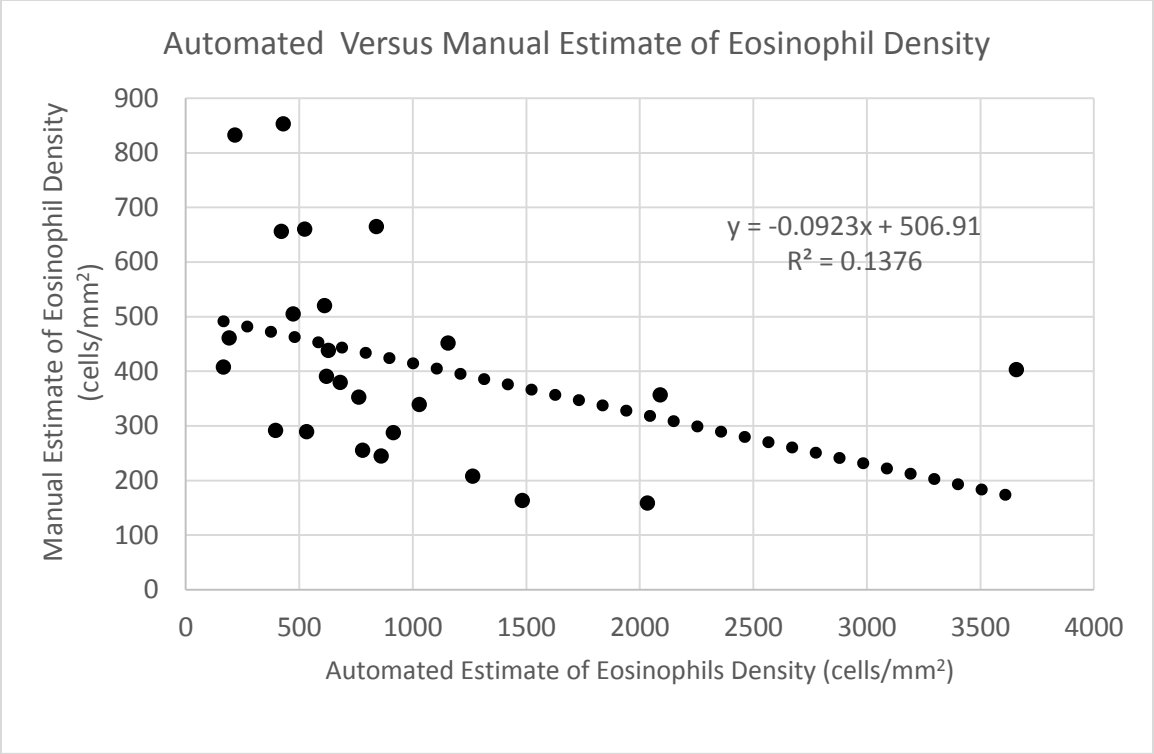


Figure 4.4 Automated Versus Manual Count of Eosinophils

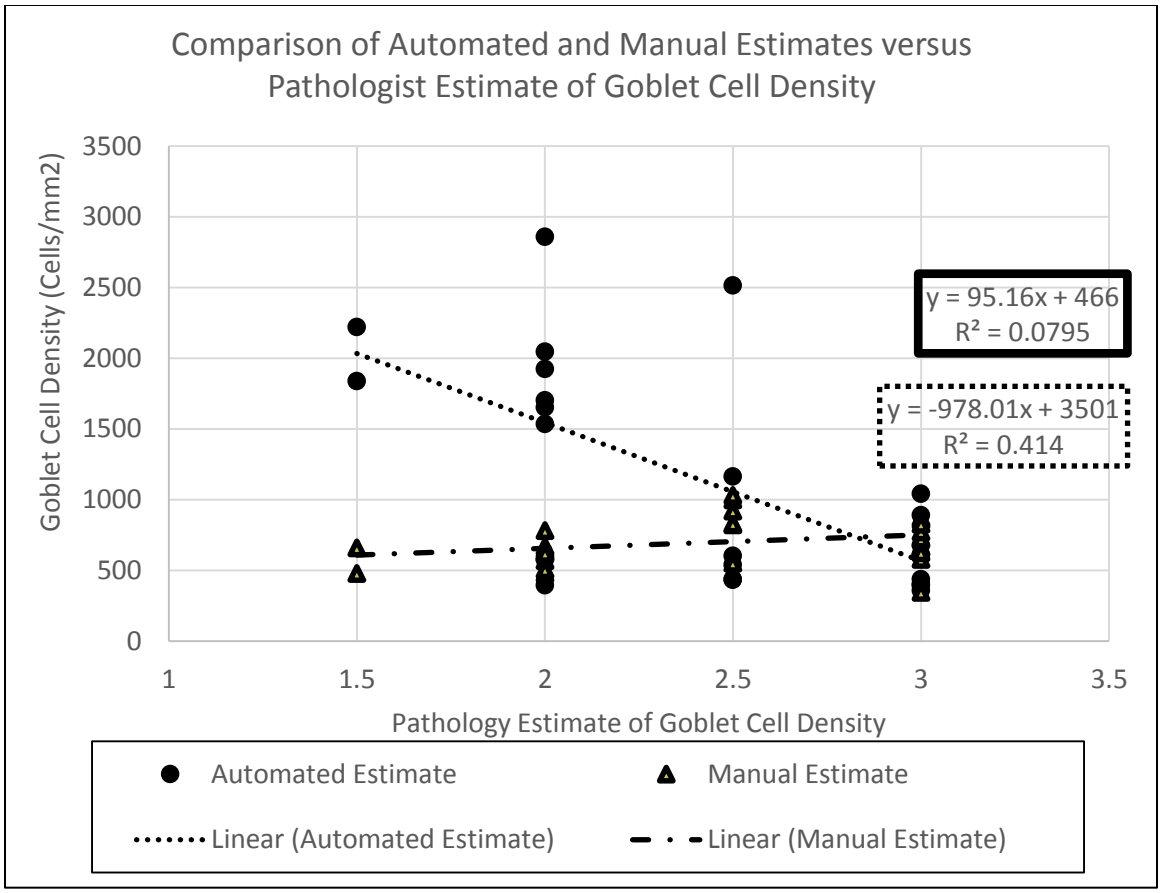


Figure 4.5 Comparison of Computer and Manual Count versus Pathologist's Evaluation of Goblet Cell Density

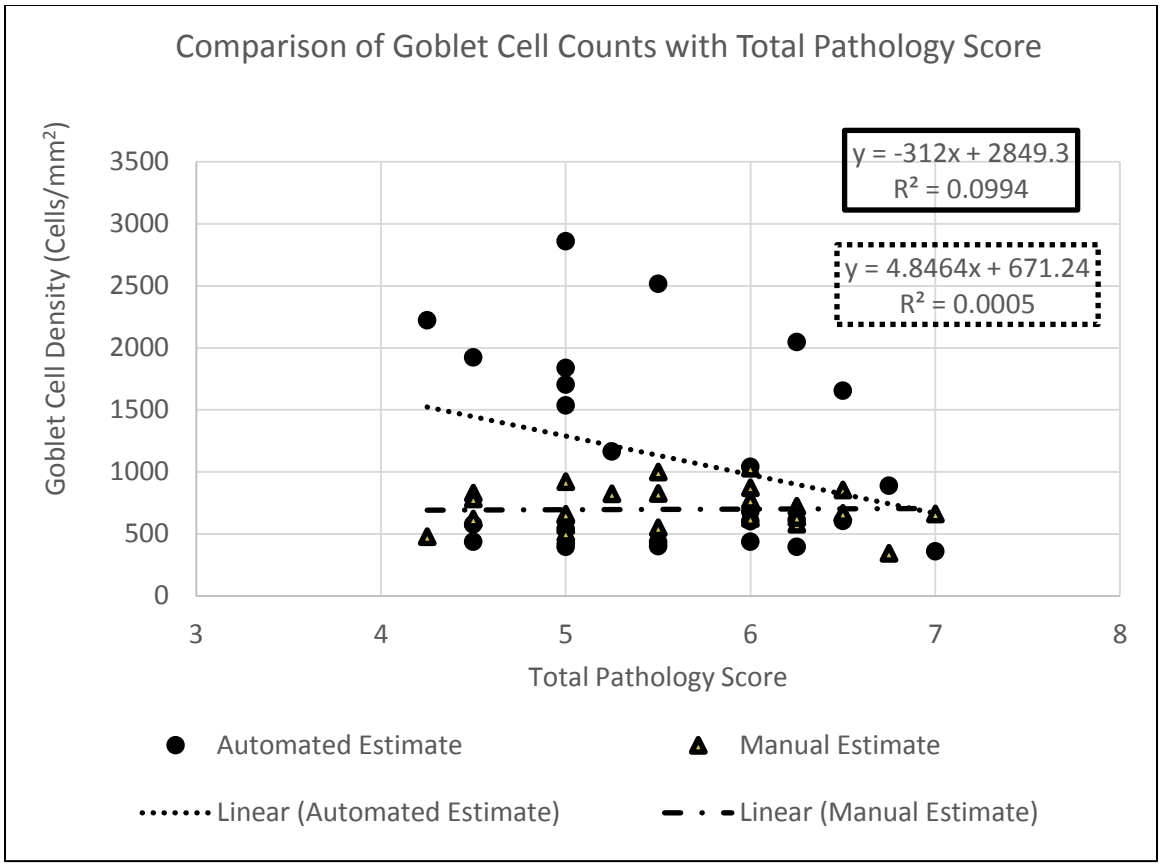


Figure 4.6 Comparison of Goblet Cell Counts with Total Pathology Score

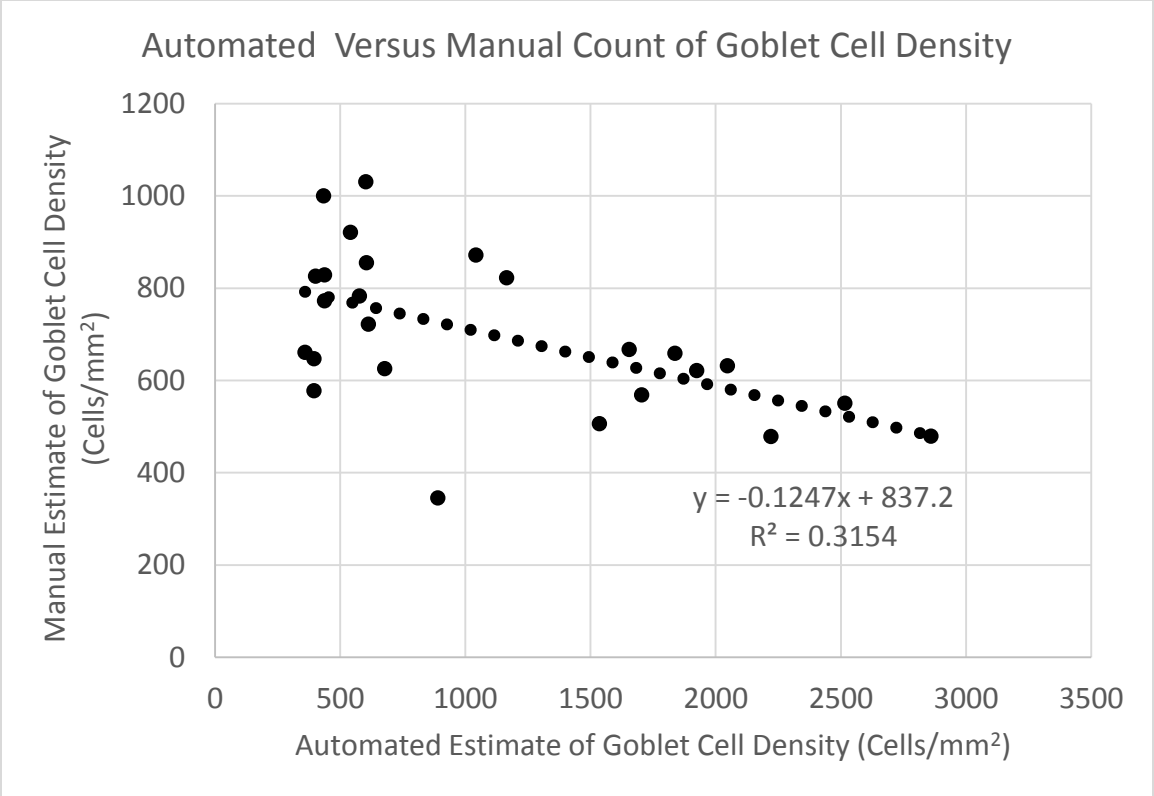


Figure 4.7 Automated Versus Manual Count of Goblet Cells

Table 4.4 Variation Due to Observer (StdDev) Goblet Cell Density (Cells/mm^2)

Variation Due to Observer (StdDev) Goblet Cell Density (Cells/mm^2)

	Mean of Variance	StdErr	Root MSE	P
Automated Count	82.49	21.80	109.02	0.0005
Manual Count	197.55	21.80		

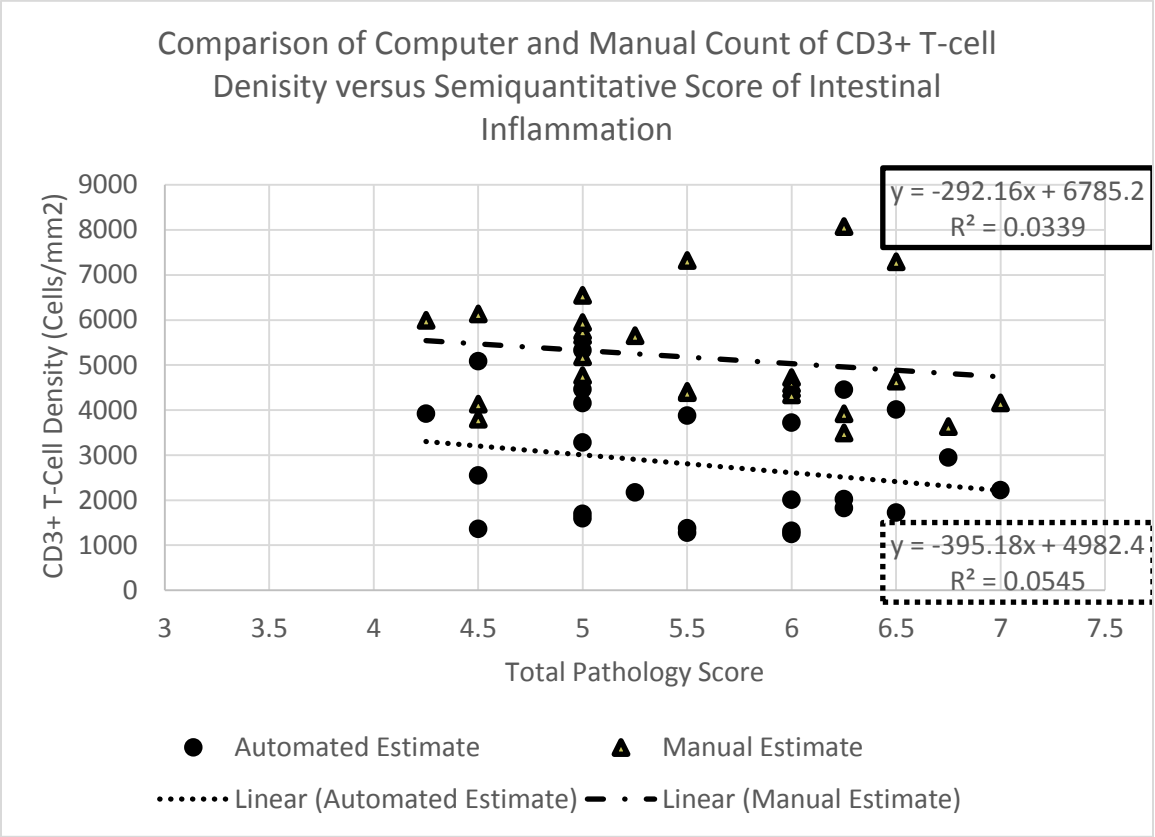


Figure 4.8 Comparison of Computer and Manual Count of CD3+ T-cell Denisity versus Pathologist's Score of Intestinal Inflammation

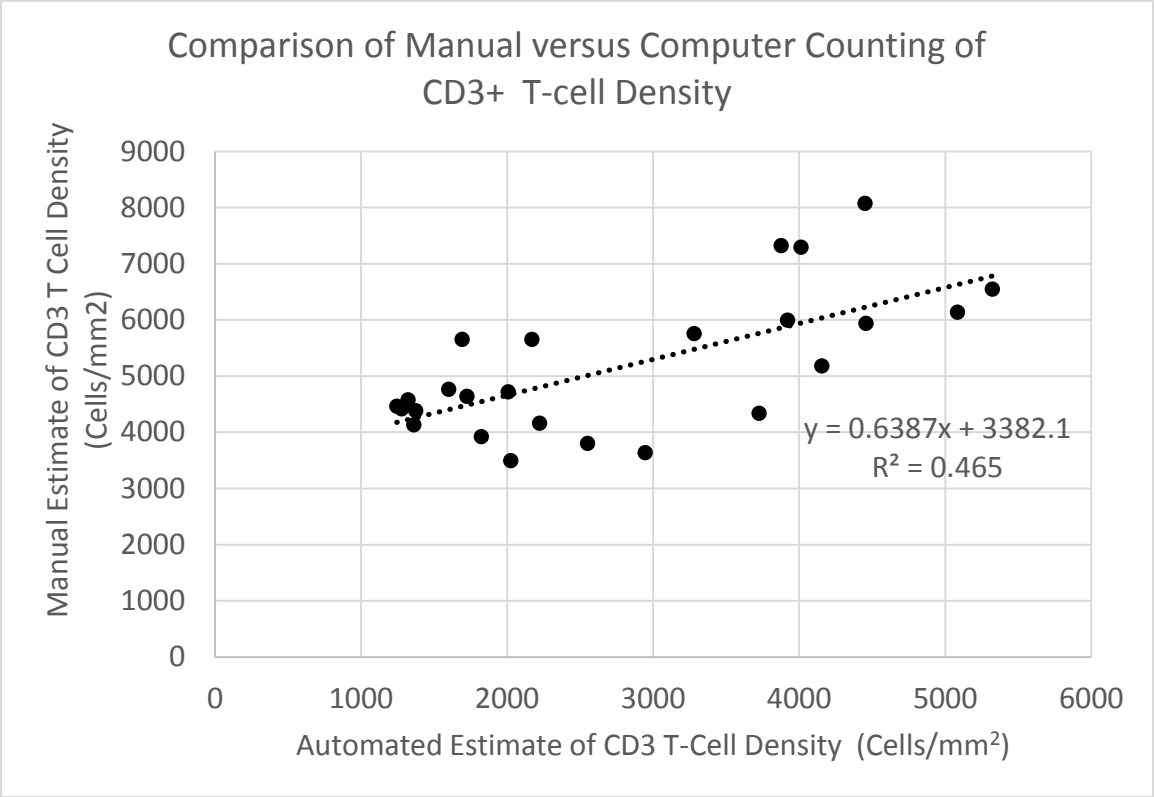


Figure 4.9 Comparison of Manual versus Computer Counting of CD3+ T-cell Density

Table 4.5 Variation Due to Observer (StdDev) CD3 Cell Density (Cells/mm^2)

Variation Due to Observer (StdDev) CD3 Cell Density (Cells/mm^2)

	Mean of Variance	StdErr	Root MSE	P
Automated Count	173.14	169.56	847.83	<0.0001
Manual Count	2384.63	169.56		

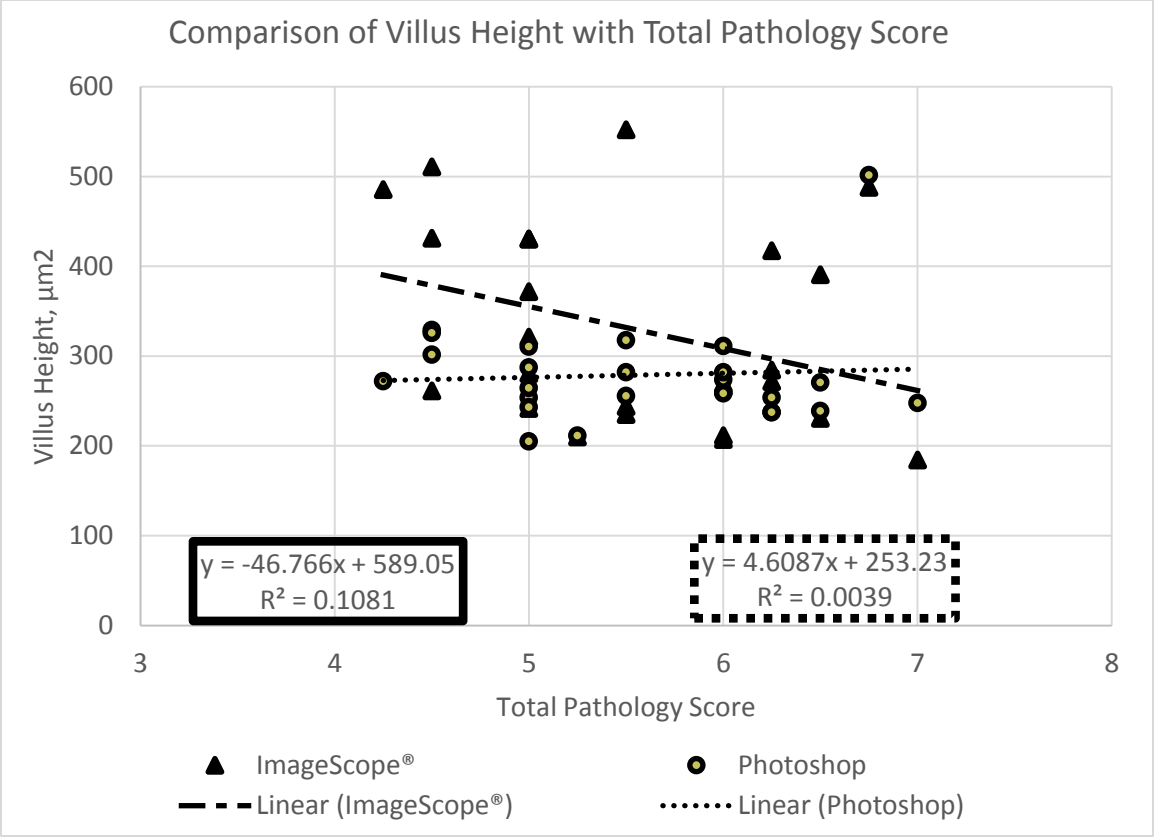


Figure 4.10 Comparison of Villus Height with Total Pathology Score

Table 4.6 Variation Due to Observer (StdDev) Villus Height (um)

Variation Due to Observer (StdDev) Villus Height (um)

	Mean of Variance	StdErr	Root MSE	P
Photoshop	16.46	4.69	23.49	<0.0001
ImageScope®	55.12	4.69		

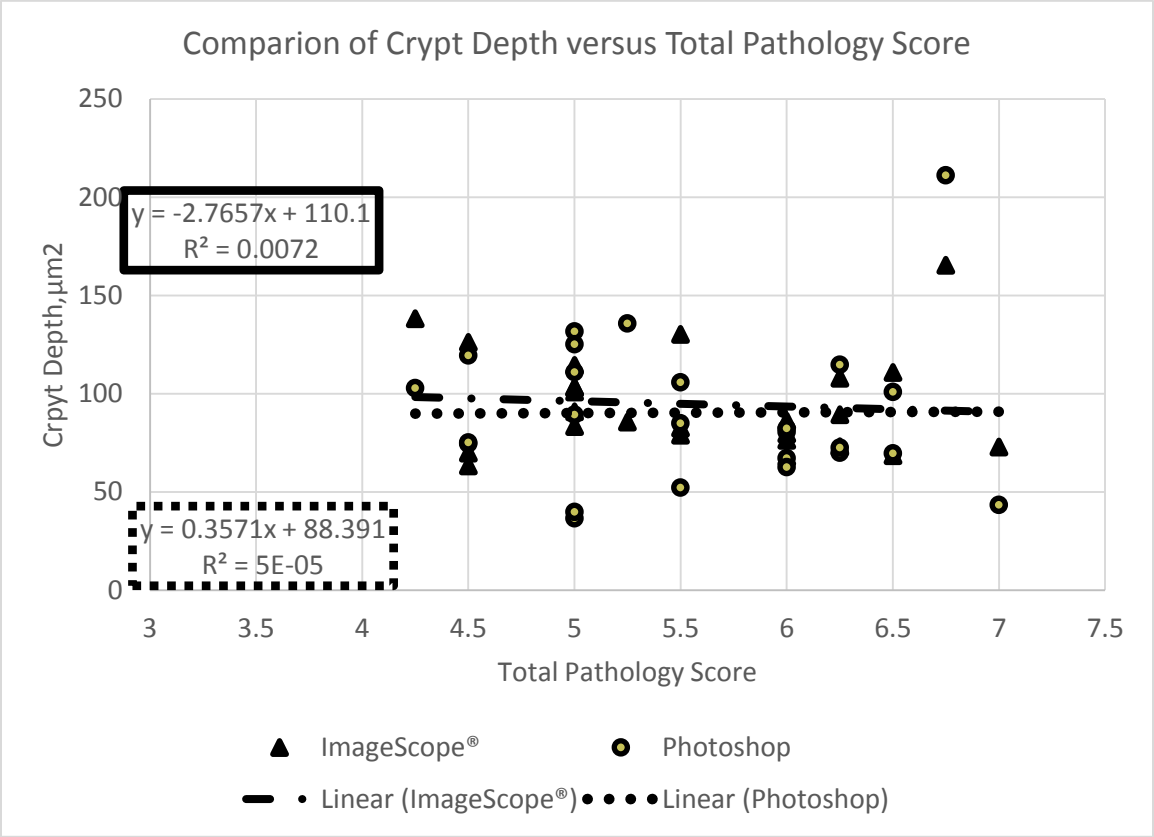


Figure 4.11 Comparion of Crypt Depth versus Total Semiquantitative Score

Table 4.7 Variation Due to Observer (StdDev) Crypt Depth

Variation Due to Observer (StdDev) Crypt Depth				
	Mean of Variance	StdErr	Root MSE	P
Photoshop	111.93	5.68	28.42	<0.0001
ImageScope®	0.39	5.68		

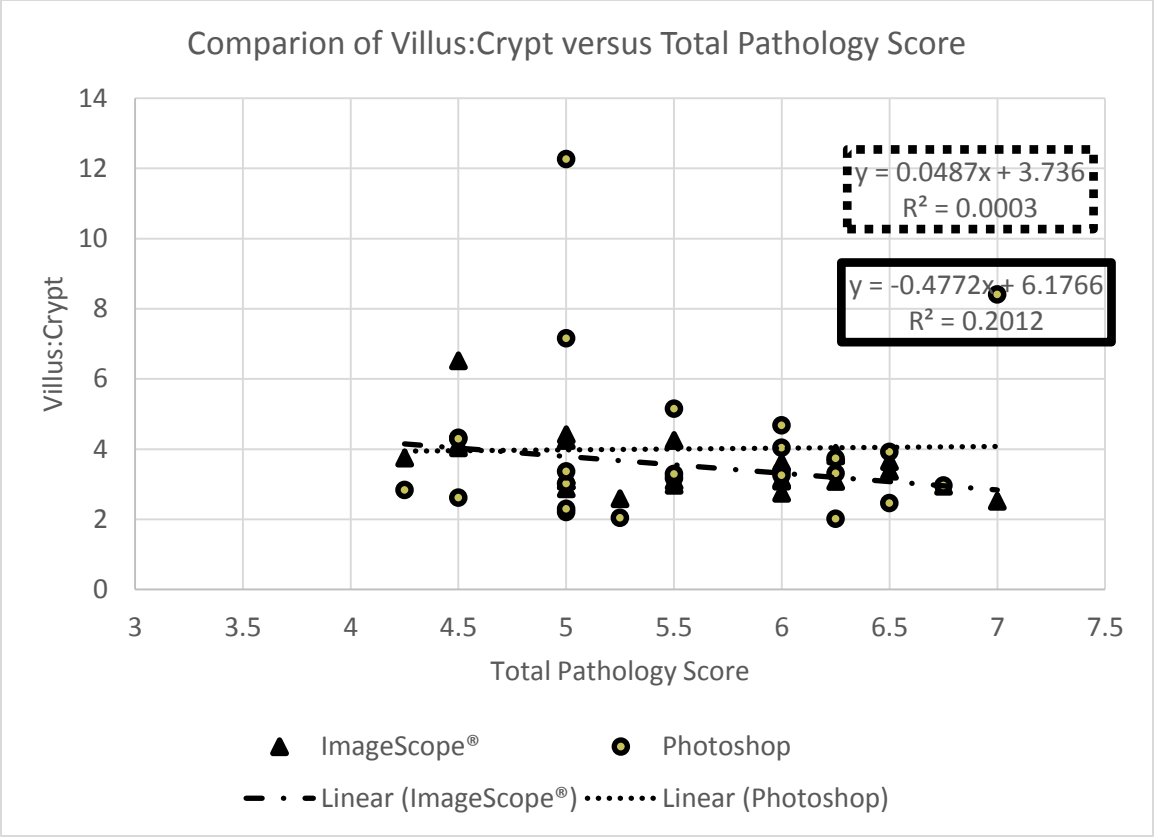


Figure 4.12 Comparion of Villus:Crypt versus Total Semiquantitative Score

Table 4.8 Variation Due to Observer (StdDev) Villus: Crypt

Variation Due to Observer (StdDev) Villus: Crypt				
	Mean of Variance	StdErr	Root MSE	P
Photoshop	1.41	5.02	25.14	<0.0001
ImageScope®	40.06	5.02		

4.7 References

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CHAPTER 5. SUMMARY

Animal agriculture is facing new challenges including public demands for greater transparency during animal rearing and harvest, antibiotic resistant bacteria, climate change and the necessity to produce more product while consuming fewer resources. Climate change is a particularly multifaceted issue, resulting in changing patterns of global disease in humans and animals, changes in the availability of feed ingredients, and the emergence of new stressors. As a result, a great deal of research has focused on the degree that animal agriculture contributes to climate change and ways to minimize this contribution.

Livestock's Long Shadow is a comprehensive evaluation of the impact of animal agriculture on the environment published by the FAO in 2006 that has shaped the public perception of the impact of livestock production on the environment. The document concluded that livestock production is a greater contributor to climate change than combustion engine vehicles. This conclusion was expanded upon by animal advocacy groups who informed the general-public that the choice to consume animal protein is more harmful to the environment than driving a car.

The life cycle analysis described in the FAO's assessment of the environmental impact of livestock production, Livestock's Long Shadow (2006) is highly inclusive; it analyzed and added every greenhouse gas-associated activity remotely related to animal agriculture and feed production. This highly inclusive analysis was compared to the estimate of greenhouse gas emissions produced by vehicles currently on the road. Since the report's release, agriculture advocacy groups have highlighted the confounding variables in the vehicle versus animal agriculture comparison citing the EPA (2016)

estimate that agriculture is only responsible for 8.3% of total greenhouse gas emissions, less than half of the estimate of livestock-specific greenhouse gas emissions calculated by Livestock's Long Shadow. However, Livestock's long shadow has fully shaped the public perception of the environmental impact of livestock production.

Regardless of the public's perception relative to the actual degree of the environmental impact of animal agriculture, livestock are not 100% efficient, produce greenhouse gases and excrete nutrients in manure that can negatively affect the environment. Management and nutrition significantly affect the efficiency of swine production in terms of the amount of pork produced relative to feed inputs. Therefore, the objective of these studies was to examine how health management and nutrition affect the environmental impact of swine production.

In the last 50 years a combination of genetics, nutrition and management have significantly reduced the environmental impact of swine production by improving animal growth efficiency, reducing resource uses such as land and feed ingredients by 78% and 34%, and outputs such as greenhouse gases and manure by 35% and 44%, respectively (Boyd and Cady, 2012). Two of the experiments in this dissertation describe ways to further mitigate the environmental impact of swine production.

The first experiment examined the effect of reduced crude protein, synthetic amino acid-supplemented diets on swine growth performance, nutrient excretion and greenhouse gas emissions. Pigs fed the diet balanced to the seventh limiting amino acid produced fewer nitrogenous wastes (NH_3 and NH_4) but had reduced growth performance than pigs fed diets balanced to the 4^{th} limiting amino acid and the control diet which contained no synthetic amino acids.

The reduction in growth performance in pigs fed diets balanced to the seventh limiting amino acid may have been due to an amino acid imbalance at the 5-7th limiting amino acids. One of the likely reasons for this imbalance are differences in the concentration and digestibility of amino acids in feed ingredients relative to calculated values. Dried distiller's grains with solubles is known to have inconsistent levels and digestibility of amino acids (Stein et al., 2006). The three diets fed in this study contained the same concentrations of DDGS, but pigs fed diets balanced to the seventh limiting amino acid received a greater proportion of intact protein from DDGS. Other studies (Maxwell et al., 2016) have reported that the NRC (2012) estimates of the 4-7th limiting, and non-essential amino acid requirements may be incorrect, resulting in depressions in growth performance in animals fed diets formulated to the seventh amino acid.

These results demonstrate further research opportunities for investigators to develop means to balance diets to the 4-7th limiting amino acid with extreme precision. Inconsistencies in amino acid concentrations and digestibility in feed ingredients, and inaccurate estimates of amino acid requirements highlight the challenges of dramatically reducing dietary crude protein and including highconcentrations of synthetic amino acids.

The second experiment examined how antibiotic-free management affects the environmental footprint of swine production. The efficiency of swine production, in terms of minimizing feed-related inputs, maximizing pork product outputs, and minimizing gas and manure outputs, is dependent on maintaining animal health. In fact, the primary reasons why a pig will not reach its genetic growth potential is immune stimulation and health challenges. Healthy animals grow faster and are more efficient, requiring less time to market, producing less waste.

Historically, producers have used sub-therapeutic levels of in-feed antibiotics to promote growth, however recent changes in legislation surrounding the availability of antibiotics and increases in demand for antibiotic-free production have made it necessary to only use antibiotics for disease treatment and prevention and increase the use of antibiotic-alternatives to maintain animal health. Therefore, this study compared the effect of rearing swine with antibiotics and antibiotic alternatives on growth performance, frequency of clinical signs, greenhouse gas emissions, and nutrient excretion in manure.

Pigs reared with antibiotics received an industry-standard rotation of sub-therapeutic antibiotics in the feed and were treated with injectable antibiotics if they presented with clinical signs. Pigs reared without antibiotics received a rotation of antibiotic alternatives in feed and water. If animals presented with clinical signs and did not respond to treatment, they were removed from the study. No differences were observed in growth performance, nutrient excretion, or greenhouse gas emissions between pigs reared with and without antibiotics. There were also no differences between frequency of clinical signs observed. However, a greater number of antibiotic-free pigs were removed from the study because they failed to respond to antibiotic alternatives.

This study highlighted the benefits and major challenges associated with rearing pigs without antibiotics. The antibiotic alternatives maintained the level of clinical signs present in the herd similar to antibiotics, however they lacked efficacy in treating clinical signs, resulting in a greater number of removals from pigs reared without antibiotics. Therefore, antibiotic-free management is a feasible option in swine production with aggressive health management and access to antibiotics for treatment of disease.

It is also important to understand the most effective and accurate ways of measuring and monitoring animal health, particularly in light of the importance of maintaining animal health to maximize sustainability and success of a swine operation. Growth rate, feed intake and feed efficiency are the most commonly used markers of animal health, but are not very specific. Intestinal morphology has been used as a reflection of intestinal health and absorptive capacity, however the methods used to measure morphology vary significantly between investigators.

The field of veterinary pathology relies on the expertise and extensive training of the board certified veterinary pathologist to evaluate, diagnose and classify tissue based on the severity of disease. However, this level of expertise may not be available to the animal scientist and these observations may not be quantifiable or easily analyzed statistically. Therefore, the objective of Experiment 3 was to use "digital pathology" to quantify the degree of inflammation and cellular infiltrate present in histology slides of intestinal tissue, and examine how this method compares to histology methods historically used to quantify intestinal health by comparing the results of each analysis and the variability between technician for consistency of observation.

Jejunal slides from pigs selected for high or low allergenicity to soy protein were compared with slides taken from pigs with no known soy allergy. The first study in this experiment examined how estimates of cell density derived from manual cell counts from sub-sections of the slide compared to whole-slide counts done by a computer. The computer counts were more consistent between observation compared to manual counts, but correlation between cell count and allergy severity differed between cell type.

We also examined how morphology measures taken in two different softwares differed due to severity of allergy and inter-observer error. When representative villi were selected and measured from the entire slide, the measure correlated more strongly with severity of intestinal disease, however there was greater inter-observer error. Conversely when the observers measured villi from the same photomicrograph, these measures had a weaker correlation with severity of allergy but had less inter-observer error.

This study indicates that the method used to measure and quantify the level of inflammation in the small intestine can significantly alter conclusions made about the degree of intestinal inflammation and systemic health of the animal. It also highlights an opportunity to use computer-based counts and representative evaluations of intestinal morphology to evaluate intestinal inflammation in swine.

In conclusion three experiments were completed to characterize how nutrition and health affect the environmental footprint of swine production. While these studies highlight the complexities that exist between animal physiology and the environment, they also strongly point toward future opportunities for more research in these areas. Better analysis to provide greater precision is needed in characterizing feed ingredients and amino acid requirements to further reduce nitrogen excretion. More effective antibiotic alternatives are needed to reduce the swine industry's reliance on antibiotics to prevent and treat disease. Finally, a better integration of pathology and immunology is needed to best characterize the significance of intestinal morphology and cellular infiltrate for diagnosis of disease.

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