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DEVELOPMENTAL CHANGES IN THE PIG FROM BIRTH TO 42 DAYS POST-WEANING (1.5 – 25 KILOGRAMS BODYWEIGHT)

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DEVELOPMENTAL CHANGES IN THE PIG FROM BIRTH TO 42 DAYS POST-WEANING (1.5 – 25 KILOGRAMS BODYWEIGHT)

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in the
College of Agriculture, Food and Environment
at the University of Kentucky

By

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2019

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ABSTRACT OF THESIS

DEVELOPMENTAL CHANGES IN THE PIG FROM BIRTH TO 42 DAYS POST-WEANING (1.5 - 25 KILOGRAMS BODYWEIGHT)

This study evaluated the changes in body composition, glycogen tissue reserves, visceral organ growth, and small intestine morphology in the young pig. A total of 96 crossbred pigs were euthanized at birth (pre-suckle), days 1, 2, 3, 5, 7, 14 postpartum, weaning at day 21, and days 1, 2, 3, 5, 7, 14, 28, and 42 post-weaning. Body composition of the pig had increasing dry matter and fat, decreasing ash, calcium and phosphorus, and relatively static protein percentage over the course of the study. Liver and muscle glycogen was greatest at birth. Following birth and weaning there was a distinct decrease in the amount of liver glycogen, while there was only a clear decrease in muscle glycogen at birth. Absolute measures of the visceral organs increased in a variety of manners (linear, quadratic and/or cubic); relative measures of visceral organs responded in different manners to increasing age. In the suckling period, villous height, villous height:crypt depth ratio, and goblet cell count was greater than in the post-weaning period. Crypt depth continued to increase through the entire study. Villi measurements of the middle and distal portion of the small intestine taken via scanning electron microscope, revealed different responses to increasing age, but numerically, villi width increased, villi density, enterocyte width, and microvilli density decreased, and microvilli diameter was relatively static. Villi, on average, increased the absorptive area of the small intestine 18 fold and microvilli increased the surface area on average 400 fold. This study provided a vast amount of biometric information on the development of the young pig from birth to 42 d post weaning.

KEYWORDS: chemical composition, glycogen, visceral organs, small intestine, birth, weaning

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12/05/2019

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DEDICATION

Dedicated to my parents who always encourage me to chase my dreams no matter where they lead me, and who have given me their constant support in all I do.

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fall in love with microscopy, and always took the time to have conversation with the student who showed up to the Electron Microscope Center nearly every day for months.

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CHAPTER 1. Introduction

The modern swine producer uses management practices that are designed to maximize the growth rate and efficiencies of feed conversion of their pigs. One of these practices includes selecting pigs for genetics that result in larger litters and pigs that produce leaner carcasses at slaughter. However, selecting pigs for particular traits always has potential repercussions. For example, when selecting for overall leanness, the pork also has less intramusclular fat (i.e. marbling) which can lower value to the consumer. Leanness also results in reduced fat deposits overall in both sows and piglets (Theil et al., 2012). The decrease in fat deposits for the fetus can lead to lower energy stores for the piglet to utilize at birth. Reduced energy stores around the time of birth is an issue, as the first three days of life are the most critical for piglet survival and there are three sources of energy available to the piglet: glycogen reserves, colostrum, and milk (Theil et al., 2014). Another example of a repercussion of selecting for leaner pigs is that lean pigs have been reported to tend to have greater visceral organ (VO) mass (Pond et al., 1988; Cliplef et al., 1993), which may lead to an increase in maintenance requirements (Tess et al., 1986).

Selecting for specific traits is not a "bad thing"; however, there needs to be data for the current pig that represents a baseline to see potential areas for improvement, which requires an in-depth knowledge and understanding of pig physiology, particularly anatomy and development at a young age. A variety of aspects of development including body composition, tissue glycogen concentrations, visceral organ size and growth, and small intestine morphology and villous topography all play a crucial role in the growth of the pig during early life.

The body composition of the pig can be determined by proximate analysis of the different chemical components of the body. As the pig matures, rates of deposition for tissues, such as muscle and fat, change (Shield et al., 1983). The composition of tissues present in the body allows for insight into the pig's physiological state (Tess et al., 1986).

Glycogen, a stored form of carbohydrates, is essential particularly when the pig is a neonate, as it is a major energy source for the pig along with colostrum (Theil et al., 2014). Glycogen and colostrum are important to help prevent neonatal death within the first three days of life for the piglet (Theil et al., 2014). Glycogen is typically used to help sustain blood glucose (Voet and Voet, 2004; Xie et al., 2016) and also to maintain body temperature (Mersmann, 1974; Okai et al., 1978; Heeren et al., 2018). Stores of glycogen are replenished by adequate energy intake through consumption of colostrum and milk (Boyd et al., 1978).

Visceral organs are critical as they are necessary for digestion, absorption, metabolism, and distribution of nutrients throughout the body. Although VO do not contribute to the final value of the pig carcass, they are responsible for the conversion of dietary nutrients into pork (de Lange et al., 2003). Included in the visceral organs is the small intestine. The small intestine is where nutrient absorption mainly occurs through projections called villi. Villi are covered in a single cell-thick layer of epithelium cells. Most of these epithelium cells are enterocyte, which are the nutrient absorbing cells. The villi project into the lumen and help interact with digesta for nutrient absorption and undergo profound changes as the pig grows and as it is exposed to new foods (Skrzypek et al., 2005; Zabielski et al., 2008). The change in villi shape, from finger-like to leaf-like, typically occurs with development of the gastrointestinal tract (GIT) and with feed changes,

such as when feed changes from liquid to solid, in order to accomplish efficient nutrient absorption to novel diets (Skrzypek et al., 2005). The absorbed nutrients then are distributed throughout the body of the pig to promote growth of various tissues.

In this thesis project, body composition from birth through six weeks post-weaning was evaluated by proximate analysis of a ground carcass composite. Glycogen levels were assessed for both the liver and skeletal muscles to evaluate the major glycogen stores in the body. All visceral organ size was measured at all times of collection. To capture the morphology of the small intestine, sections at 25%, 50%, and 75% of total length, were collected and subjected to both light microscopy and scanning electron microscopy to generate 2-D and 3-D images, respectively, to fully detail all features of the small intestine, including: villi, crypts, enterocytes, and microvilli, during development. This study has the potential to highlight critical time periods of significant change that can be subjected to future research studies to improve overall pig performance. Therefore, the overarching objective of this study was to assess these anatomical and compositional measures from birth through 25 kg through frequent collection over this critical timeline, to produce detailed documentation of the development of the modern pig after decades of genetic selection have altered some aspects of the pig.

CHAPTER 2. Literature Review

2.1 Introduction

The world population, as of 2019, is 7.7 billion people, which is 1,860 times the 4 million people that the population was 12 millennia ago (Roser et al., 2019). Earth has hosted a total of around 108 billion people over the course of its time, which means that today's population constitutes 6.5% of all the people that have ever lived on the earth (Roser et al., 2019). If it assumed that the people living on earth today are between the ages of 0 and 100, then today's population constitutes 6.5% of all people who have ever lived on earth, but exists during only 0.83% of all the time that people have been living on earth. It is predicted that by 2100, there will be approximately 10.9 billion people in the world (Roser et al., 2019). With the increase of the human population, there has been an associated increase in the need for, and production of, food. In particular, pork production has grown 4-5 fold from the 1960s to 2014 (Ritchie and Roser, 2019). However, even with the increase in food production that has already occurred, there is still a potential lingering problem, that being to continue to produce more and more food to feed the perpetually increasing human population.

Pork is the most widely eaten meat in the world, representing 36% of meat consumption (FAO, 2014). Enhancing pig performance through improved growth rates and efficiency of converting plant nutrients into animal protein can be an answer to further increase pork production. In order to further improve pig performance, an in-depth knowledge of the anatomy and development of the "modern" pig is needed to identify areas for improvement.

2.2 Pig Development

2.2.1 *Body Composition*

Swine producers focus on maximizing the value of the pig carcass for the meat industry. Value of the carcass depends on the percentages of muscle, fat, and bone in the body and the distribution of each throughout the carcass (Fortin et al., 1987). In general, the composition of the pig is roughly 70% carcass and 30% noncarcass (Wiseman, 2006). However, there are many factors that are involved in the development of pigs including: genetics, sex, nutrition, environment, age, and all their corresponding interactions (Wagner et al., 1999).

In general, muscle and bone are deposited first followed by fat. As the pig matures, fat deposition rates increase, while muscle and bone deposition rate decreases (Shields et al., 1983). Also, as bodyweight (BW) continues to increase, daily metabolizable energy intake increases and body protein deposition (g/day) decreases as the pig approaches 125 kg BW (NRC, 2012). Mature pigs generally have a body composition that is approximately: 60% water, 16% protein, 20% fat, and 4% ash or mineral matter (Lloyd et al., 1978).

2.2.1.1 Bone/Ash

Bones provide structure to animals through the skeletal system, and act as a reservoir for minerals, including calcium and phosphorus (Cheeke and Dierenfeld, 2010). Bone also plays a role in organ protection and structure for the animal. The majority of the calcium, 99%, and phosphorus, 80%, are located in bones and teeth (Lloyd et al., 1978;

Cheeke and Dierenfeld, 2010), and 70% of total ash in the body is attributed to calcium and phosphorus (Lloyd et al., 1978).

The process of bone formation begins with the calcification of an organic matrix that consists chiefly of a protein collagen (Cheeke and Dierenfeld, 2010; Lloyd et al., 1978). Two different kinds of tissue encompass the composition of bones: compact (the exterior part of the bone) and cancellous (the interior part of the bone which is constantly being renewed) (Gilbert, 1966). Each bone also has a periosteum layer (a fibrous membrane that covers the bone), marrow (a substance similar to fat), arteries, veins, nerves, and a lymphatic system that pass through the compact bone to the marrow (Gilbert, 1966). Catabolism, as well as anabolism, are continuous metabolic processes keeping bones in a dynamic state known as remodeling, where osteoblasts are responsible for bone anabolism and osteoclasts are responsible for bone catabolism (Cheeke and Dierenfeld, 2010). Parathyroid hormone inhibits synthesis done by osteoblasts and also stimulates resorption by osteoclasts (Voet and Voet, 2004).

As previously mentioned, bones are comprised of minerals such as calcium and phosphorus, and those minerals can be mobilized by the body to remain in mineral homeostasis and to be involved metabolically (Lloyd et al., 1978; Wiseman, 2006; Cheeke and Dierenfeld, 2010). Both calcium and phosphorus are critical in all living cells (Lloyd et al., 1978; Pettey, 2004). Because the nutritional role of calcium in the body is closely related to phosphorus, the two minerals are usually considered together. The proportion of calcium to phosphorus weight is relatively constant at a ratio of 2:1 (Lloyd et al., 1978; Pettey, 2004).

2.2.1.1 Skeletal Muscle

Muscle fibers are formed by the end of the second trimester of gestation (Du et al., 2017). Postnatal muscle growth is due to hypertrophy, but the degree of myofibril hypertrophy depends on the number of myofibrils present, which is determined in the prenatal period (Zhu et al., 2006; Rekiel et al., 2015).

During early and mid-gestation, maternal nutrient restriction can negatively affect the development of skeletal muscle, which can have long-term consequences because skeletal muscle has a lower priority of development during gestation than other muscle like the heart (Zhu et al., 2006). In pigs, myogenesis is a two-stage process in which primary muscle fibers develop in the first stage from days 35 to 55 gestation, and the secondary fibers are formed in the second generation of fibers from gestation day 55 to 95 (Rekiel et al., 2015). The number of primary fibers that are present is genetically determined, and each primary fiber can be surrounded by roughly 20 secondary fibers (Rekiel et al., 2015).

Skeletal muscle is categorized as striated muscle (Cheeke and Dierenfeld, 2010), which have a functional unit called the sarcomere that contracts or relaxes allowing movement to occur (Lloyd et al., 1978). The general composition of fresh skeletal muscle is as follows: 75% water, 20% protein, 3% fat, and 3% ash plus other substances such as carbohydrates (Lloyd et al., 1978). The amount of protein in muscle of approximately 20% is rather consistent in trimmed lean muscle (de Lange et al., 2003).

2.2.1.2 Adipose Tissues/Fat

Adipocytes begin forming around day 69 of gestation (McPherson et al., 2004; Du et al., 2017), and on through early weaning. To accumulate fats (visceral, subcutaneous, and inter and intramuscular), a large amount of nutrients is required, which in turn reduces the feed efficiency of the animal (Du et al., 2017). The order of formation of adipocytes is the following: visceral, subcutaneous, intermuscular, and intramuscular (Du et al., 2017).

At birth, the newborn pig has around 1-2% fat (Elliot and Lodge, 1977; Dauncey et al., 1981). The fat in the neonatal pig is called brown fat for its brown color. Brown fat is mainly used by the body to produce heat and keep the neonate warm, whereas white fat is typically used as a fat-storing organ (Heeren et al., 2018). Brown adipose tissue is known for being able to process large amounts of uncoupling protein 1, a protein homodimer that acts as a channel that regulates the permeability of protons located in the inner mitochondrial membrane (Voet and Voet, 2004). When uncoupling protein 1 is activated, it enables the free flow of protons across the inner membrane of the mitochondria resulting in rapid dissipation of chemical energy that comes off as heat to the animal (Symonds, 2013). As the pig gets older after birth, brown fat diminishes while white fat becomes the dominant fat in the body.

Subcutaneous backfat is the largest fat deposit at 60 to 70% while intermuscular fat is roughly 24% (Kouba et al., 1999). Marbling, or intramuscular fat, is sought after in production animals because of its contribution to consumer acceptance of the product while visceral, subcutaneous, and intermuscular fat, are not desired by consumers and therefore have a low commercial value (Du et al., 2017). To reduce the amount of undesired fat

present in the animal, there is selection for animals to have a high lean:fat ratio; however, this often results in a decrease in the marbling as well (Du et al., 2017).

2.2.1.3 Body composition of the pig

The main application of the measurements of body composition for pigs is to determine the nutrient requirement (Miller et al., 1991). The sequential order of development in farm animals is bone, muscle, and then fat tissue (Shields et al., 1983). Prior to weaning, water and ash percentages decline while the percentage of protein and fat increase; however; during this time all components in the body do increase quantitatively with protein and water representing the majority of the body components (Shields et al., 1983). It should be noted that while general relationships between development of the body remain the same as shown in Figure 2.1, progression in genetic selection processes have shifted the curves so that accretion of the different chemical components occurs at a much faster rate (Miller et al., 1991).

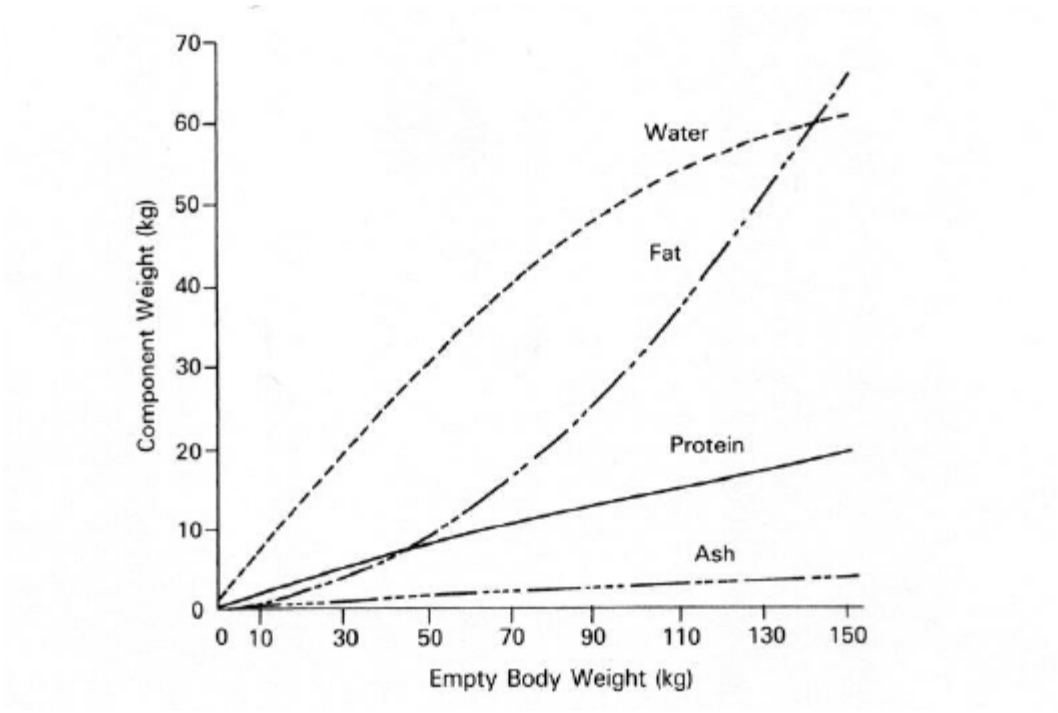

Figure 2.1 Development patterns for chemical components in growing swine from birth through 145 kg; figure taken from Shields et al., 1983.

When considering factors that can influence body composition, one of the biggest factors to consider is the genetic background of the animal (Herpin et al., 1993). For example, the fetal pig is receives nutrients from the sow during the gestation period, but the sow's gestational diet was determined by Herpin et al. (1993) to not have much effect on the body composition of the piglet, but rather that genetic selection plays a larger role in differences in chemical composition of piglets. Another example of this was reported by Newcomb et al. (1991) and Pettigrew (1981) who looked into feeding a higher lipid diet to gestating sows to increase the glycogen content of the piglet so that more energy would be available to the piglet at birth, but there was not a significant increase in neonate glycogen. However, sows that tend to have a higher backfat thickness have piglets that possess a higher lipid content at birth and are therefore more resilient against the cold and have fewer

postnatal losses than conventional pigs that are selected for leanness (Herpin et al., 1993). It has also been documented that the change in piglet chemical composition is dependent on the energy level intake of the sow (Noblet et al., 1987), as this affects the milk composition, and in turn the average daily gain of the piglet.

During the process of weaning, not only is the piglet taken away from the sow, but they are often mixed with other litters when they are moved to a new pen (Lallès et al., 2007). It is not uncommon that at this time piglets usually experience low and variable feed and water intake (Pluske et al., 1997). The reduction in feed intake can lead to undernourishment and affect the growth of the pig (Lallès et al., 2004) and increase its use of energy reserves.

As previously mentioned, there are many factors that need to be considered when looking at the body composition of a pig including: genetic background, sex of the pig, temperature, diet, and weaning age (Miller et al., 1991). When a pig is weaned early, it can have reduced fat stores during the first week after weaning, while a pig that is weaned late can have an increase in its fat stores (Miller et al., 1991). A pig that is weaned early can also have an increased water:protein ratio (Miller et al., 1991).

Water and fat increase in a curvilinear fashion, but the two chemical components increase inverse to one another as BW increases (Shields et al., 1983). Protein and ash quantitively increase in linearly as BW increases (Shields et al., 1983). The maximum rate of protein deposition is dependent on the genetic potential of the individual animal (Shields et al., 1983). The typical composition of adult mammals is approximately: 60% water, 16% protein, 20% fat, and 4% ash (Lloyd et al., 1978). Carbohydrates are not a structural part of the body, and the amount of carbohydrates in the body can vary from hour to hour (Lloyd

et al., 1978). Therefore, 1% of the body is typically set aside to account for the carbohydrates that are present, mainly in the form of liver glycogen but also as muscle glycogen and blood sugar (Maynard and Loosli, 1962; Lloyd et al., 1978).

2.2.2 *Visceral Organs*

Visceral organs (VO), similar to the skeletal bones, are indispensable for the function of the living animal, yet provide no additional value to the carcass (de Lange et al., 2003). The majority of organ development that the fetal pig undergoes occurs after the 60th day of gestation (McPherson et al., 2004; McPherson-McCassidy, 2003; Ullrey et al., 1965); however, between the time of birth and weaning is the most dramatic change in body development. The VO of the young pig increase at different rates, but all VO absolute weight increase with an increase in body weight and age (Doornenbal and Tong, 1981). Select organs, the heart, lung, and spleen tend to grow in a linear fashion while other organs have differing degrees of curvilinearity to their growth (Doornenbal and Tong, 1981).

Weights of VO, particularly their relative weights in relation to the body weight of the animal, are evaluated in nutritional, biological, and medical studies (Doornenbal and Tong 1981). The development of VO yields information on the physiological state of the animal, which also illuminates nutrient requirements of the animal (Shields et al., 1983). The absolute weight of VO does not provide much information on its own, so there is a common practice to look at the change of the relative weight of the VO in relation to body weight. By relating the VO weight to the total body weight, the relative importance of visceral organs can be compared across a longitudinal study. Also, having documented

information about the weight of VO in relation to BW allows for future studies to have information about the VO development at a particular BW as well as age.

As mentioned above, understanding the development of VO can lead to knowledge about nutrient requirements. For example, the genetically selected leaner pig tends to have larger VO (Pond et al., 1988; Cliplef et al., 1993). Visceral organs are very energy demanding and are responsible for a large proportion of the body energy expenditure (Anugwa et al., 1989; Nyachoti et al., 2000). Larger organs require more nutrients, leading to the conclusion that large organ size relative to body weight results in a higher maintenance requirement for that pig (Tess et al., 1986). In addition to the composition of the pig influencing the size of VO, the diet that is fed to the pig can also affect the relative size of VO. For instance, high fiber diets have been known to result in larger VO, particularly the gastrointestinal tract (Jørgensen et al., 1985; de Lange et al., 2003). A final thing to consider, the maturity, sex, environment, breed, and interaction between all mentioned factors play a role in growth and development (Wagner et al., 1999; de Lange et al., 2003).

2.2.3 *Glycogen*

2.2.3.1 Glycogen Structure

Glycogen is a stored form of carbohydrate in the body and can be found in the liver and muscle (Voet and Voet, 2004). It is a multi-branched polysaccharide (Figure 2.2) that is somewhat similar to amylopectin, a starch, and the glucose unit branches are connected by α-(1-4) and α-(1-6) bonds (NRC, 2012). In the center of the branched units is a protein core that consists of glycogenin (Ward, 2014; Voet and Voet, 2004). There are branches

that occur every 8-14 glucosyl units, which is where glycogen differs from amylopectin that branches every 24-30 residue units (Voet and Voet, 2004).

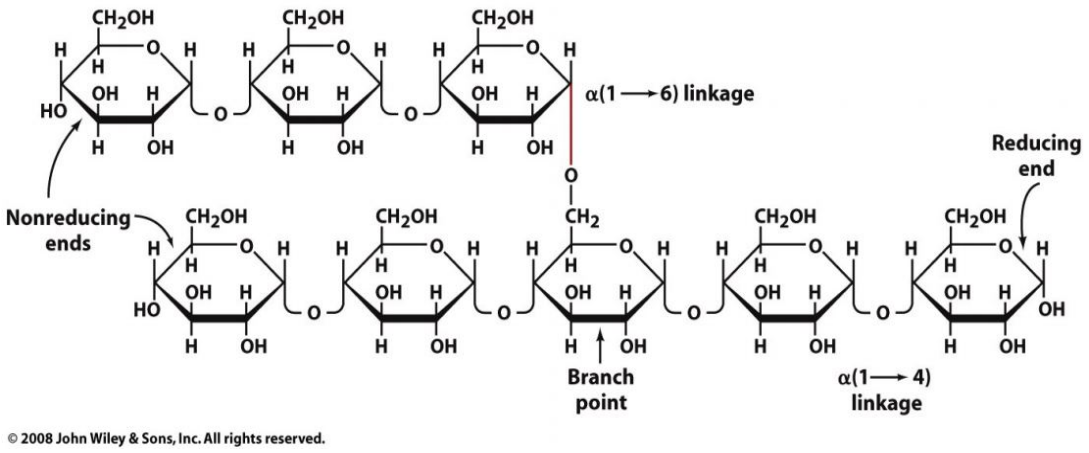

Figure 2.2 Glycogen molecular structure; figure taken from Voet and Voet, 2010.

In glycogenolysis, glycogen is broken down to glucose so that the body can utilize the now available glucose. Glucagon is a polypeptide that is released from the pancreas and stimulates the release of glucose (Voet and Voet, 2004). Glycogenolysis begins with a glycogen debranching enzyme that is responsible for exposing glucose residues to glycogen phosphorylase and phosphoglucomutase (Ward, 2014; Voet and Voet, 2004), allowing for glycogen phosphorylase, to cleave glycosidic bond on α-(1-4) branches by substitution of a phosphate group at the 1C to produce glucose-1-phosphate (Ward, 2014; Voet and Voet, 2004). Phosphoglucomutase then converts glucose-1-phosphate to glucose-6-phosphate (Figure 2.3), which can be used by the body in glycolysis to produce ATP in the muscle, or be hydrolyzed to glucose by the liver to maintain blood glucose levels in the body (Ward, 2014; Voet and Voet, 2004).

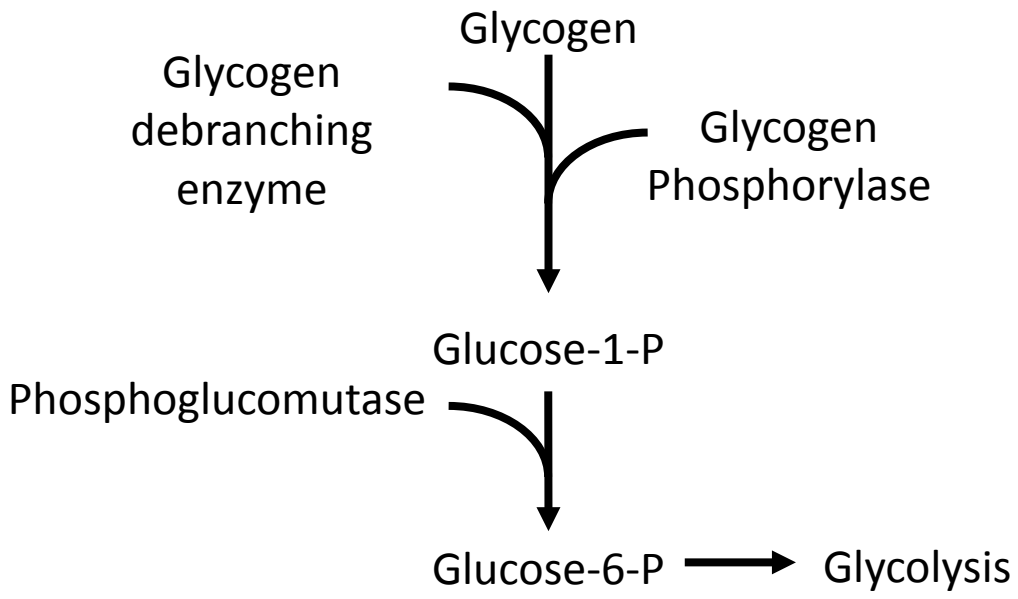

Figure 2.3 Glycogenolysis, the hydrolysis of glycogen

Glycogenesis, or the synthesis of glycogen from glucose (Figure 2.4), occurs by a separate pathway than glycogenolysis because glycogenolysis is not a reversible pathway (Voet and Voet, 2004). Insulin is a polypeptide that is released by the pancreas and stimulates the uptake of glucose by the tissues (Voet and Voet, 2004). To begin, glucose is converted to glucose-6-phosphate by glucokinase in the liver or by hexokinase in the skeletal muscle (Ward, 2014). Glucose-6-phosphate is then converted to glucose-1-phosphate by phosphoglucomutase (Ward, 2014). Glucose-1-phosphate is converted to uridine diphosphate glucose (UDPG) by the combination of uridine triphosphate (UTP) and glucose-1-phosphate (Ward, 2014; Voet and Voet, 2004). UDP-glucose-pyrophosphorylase is used as a catalyst to the previous reaction as the conversion from glucose-1-phosphate to UDPG is not thermodynamically favorable, so this additional exergonic step is required (Voet and Voet, 2004). The UPDG molecules are transferred to a C4 – OH group on a non-reducing end of glycogen, through the use of an α-(1-4)-

glycosidic bond after a glucosyl phosphorylase ion intermediate (Voet and Voet, 2004). The self-catalyzed attachment of the glucose residue to a glycogenin, a "primer" for initiation of glycogen synthesis, initiates the enzyme glycogen synthase, yielding an α-(1-4) linkage (Voet and Voet, 2004). Glycogen branching enzyme then breaks the α-(1-4)-glycosidic bond and forms α-(1-6) linkage when terminal ends of glucose residues are connected to a glycogen chain (Voet and Voet, 2004).

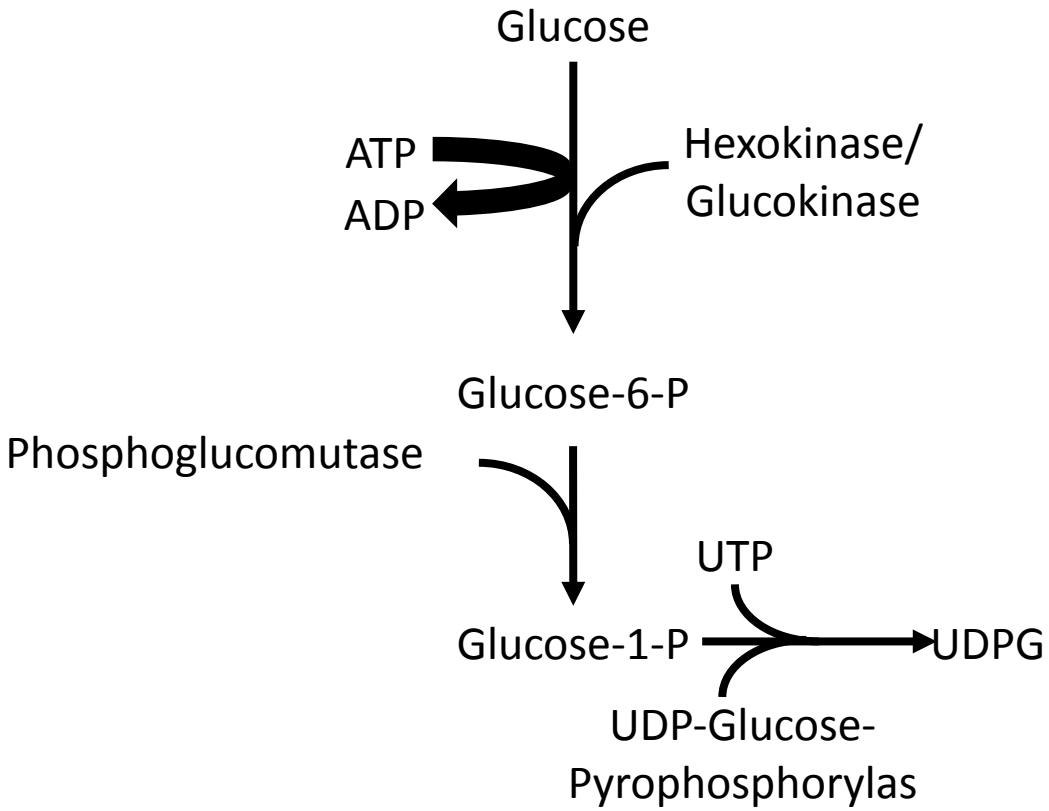

Figure 2.4 Glycogenesis, the synthesis of glycogen from glucose.

2.2.3.2 Glycogen in the pig

Glycogen deposition in the fetal pig occurs at greater rates around the last 2-4 weeks of gestation (Père, 2003). Between the 100-107th day of gestation glycogen accumulation

reaches a rate of roughly 5.5 mg/g of liver/day (Randall and L'Ecuver, 1976). Glucose is the main metabolic fuel that is utilized by the fetal pig, so when glucose is limited to the sow during gestation, general growth of the fetal pig is decreased (Ezekwe and Martin, 1978). Ezekwe and Martin (1978) concluded that glycogen stores and liver cell growth were increased when maternal glucose levels were elevated, which could be a means to increase neonate survival through the increase of liver glycogen.

At birth, there is a rapid fall in the glycogen stores of the pig (Elliot and Lodge, 1977). Birth is a stressful time for the piglet as it is entering a new environment in which it must fend for itself. The piglet uses its glycogen and brown fat to produce body heat for itself to stay within its thermoneutral zone (Mersmann, 1974; Heeren et al., 2018). Typically, there is only a relatively small amount of glycogen stored in the body because lipids, or triglycerides, are the main storage form of energy (NRC, 2012). But due to the low fat-content of the pig at birth, glycogen becomes an important source of energy to the piglet at this time.

Glycogen stores are important to the neonatal pig at birth as they are a means for survival (Theil et al., 2012). Industry selection for high litter numbers and leaner pigs increases the likelihood of pre-weaning mortality as the previous traits can result in light birth weights and less fat stores in the neonatal pig (Theil et al., 2012). When the pig is still a fetus, the fetal liver has been reported to have three times the amount of glycogen stored than an adult liver, and at birth this accounts for less than 1% of the neonate's energy reserves (Mota-rojas et al., 2011).

Glycogen is one of the main sources of energy that is used by the piglet for survival and is even utilized by the fetal pig when nutrients are no longer provided by the dam

(Shelley, 1961). After birth occurs, glycogen is used by the piglet to maintain homeostasis, including blood glucose levels and thermoregulation (Elliot and Lodge, 1977; Okai et al., 1978). During the first day of life, roughly 90% of liver glycogen and up to 60% of muscle glycogen has been reported to be mobilized across a number of species (Mellor and Cockburn, 1986). During the first 24 hours postpartum, Elliot and Lodge (1977) reported that roughly 75% of the liver glycogen was utilized by the neonate pig, and approximately 41% of muscle glycogen is utilized by the neonate pig. Because glycogen stores and brown fat in the neonatal pig's body diminish rapidly through the first 12-18 hours of life (Elliot and Lodge, 1977; Boyd et al., 1978), heat lamps are typically provided in farrowing crates to provide areas of extra warmth for the piglet and to diminish the need to rely on these tissue reserves.

When the piglet begins to ingest colostrum and milk, then glycogen begins to be replenished in the liver (Theil et al., 2014). Glycogen, colostrum, and milk are the main energy sources available to the piglet that help the piglet survive the first three days of life, which are the most critical for piglet survival (Theil et al., 2014).

Low blood sugar is a trigger for glycogen hydrolysis in the liver (Voet and Voet, 2004; Xie et al., 2016), and low blood sugar can be a result of lack of food intake, which is typically a portion of the weaning stress in pigs. During the time post-weaning when the pig is not consuming feed, glycogen stores in the body are utilized to maintain function and homeostasis among physiological needs, such as maintaining blood sugar (Xie et al., 2016). Glycogen in the liver is mainly used to help maintain blood glucose levels throughout the body (Voet and Voet, 2004). Glycogen has also been reported to be found in low amounts in the small intestine (Horne and Magee, 1933). In particular glycogen has

been determined to build up in the intestinal wall and then pass into the portal blood during the absorption of carbohydrates from the digesta (Horne and Magee, 1933).

Glycogen that is stored in the skeletal muscle is utilized at a much slower rate than the glycogen that is stored in the liver and, therefore, the skeletal muscle glycogen is used by the piglet for a considerably longer amount of time than glycogen found in the liver (Elliot and Lodge, 1977; Ezekwe and Martin, 1978). Muscle will mobilize its glycogen reserves when it is in need of ATP, while liver will mobilize its reserve when blood glucose is low (Voet and Voet, 2004). Therefore, muscle utilizes its glycogen reserves to be used within that muscle itself, while the glycogen located in the liver is mobilized and distributed throughout the entire body.

There are two types of muscles that exist in the body, white and red, respectively known as fast and slow muscles (Beatty et al., 1963). The white muscles tend to move quickly and are commonly parts of the body such as limbs that undergo the majority of the body's movements and red, or slow, muscles tend to be stationary and can be found more at the animal's core (Szent-Gyorgyi, 1953). With this information, it can be concluded that the white muscles have a higher glycogen content than the red muscles (Beatty et al., 1963). Red muscles also have a lower glycogen content than white muscles because they are able to oxidize fatty acids for ATP production (Beatty et al., 1963). With more movement occurring in the white muscles, they require more glycogen for energy purposes, and white muscles also burn through their glycogen stores a lot faster than red muscles (Beatty et al., 1963). When physical activity occurs, the glycogen in the muscles is depleted during exercise; however, these stores are also quickly replenished during the recovery period that

follows the exercise period (Richter et al., 1982). Once the pig adjusts to being weaned and feed intake resumes, glycogen levels should also replenish.

Total glycogen in the skeletal muscle is roughly ten times more than that in the liver, but this can be attributed to there being larger muscle mass in the pig's body compared to the mass of the liver (Theil et al., 2014). As the pig starts to mature, the total amount of body glycogen that is found in the liver is lower than that in the skeletal muscle, resulting in the liver being the second largest reservoir of glycogen in the pig's body with skeletal muscle being the first (Okai et al., 1978). In the skeletal muscle, glycogen is used by the piglet for thermoregulation and physical activity (Elliot and Lodge, 1977; Okai et al., 1978). Glycogen is more easily mobilized than fat in the skeletal muscle, therefore glycogen is the main stored energy form in skeletal muscle instead of fat (Voet and Voet, 2004). Glycogen utilization by the skeletal muscle is typically a result of the muscle requiring ATP, and therefore the conversion of glycogen to glucose-6-phosphate, which can enter glycolysis and result in ATP production, occurs (Voet and Voet., 2004).

2.2.4 *Gastrointestinal Tract (GIT)*

The GIT is one of the most important organs for all species, as it is the organ that is responsible for absorbing nutrients that are essential for life. Not only is the GIT responsible for absorbing nutrients, it also acts as major line of defense against dangerous pathogens as it is the largest surface area of the body that gets exposed to the outside world (Pelaseyed et al., 2014). The mucus produced from the epithelial lining on the intestine is responsible for mediating the size of particles that pass through it and protection against pathogens (Pelaseyed et al., 2014).

Within the first week of postnatal life, it has been reported that the length of the small intestine increases by 24%, there is a 15% increase in diameter, an increase in crypt depth by 24%, and a 33% increase in villi height (Skrzypek et al., 2005). Rapid growth of the small intestine before, and immediately after, birth helps to increase the absorptive capacity of the gut when the pig is born and becomes reliant on obtaining nutrients solely through absorption from the GIT. The structural changes that occur in the GIT can be used as indicators of gut development or changes that can occur in response to changes in diet (Skrzypek et al., 2005).

The gastrointestinal system of humans and pigs are both capable of processing milk produced from their mother, in particular, colostrum (Buddington et al., 2012). Though both gastrointestinal systems are able to digest similar diets, there are different brush border membrane enzyme activities (Buddington et al., 2012). The differences in brush border membrane activities leads to differences in what can and cannot be tolerated by the neonate. For example, human infants are able to tolerate maltose and sucrose, whereas pigs will develop diarrhea if these are presented to their GIT (Buddington et al., 2012). During the final weeks of gestation, there is a rapid increase in the intestinal glucose absorptive capacities, coinciding with preparation of the fetus for birth and the start of milk consumption (Buddington et al., 2012). There are definite changes in the enzymes that are present in the GIT at certain ages, specifically sucrase, lactase, isomaltase, maltase, trehalase enzymes (Miller et al., 1991). Enzyme activity of the digestive system can also give way to functional development of the GIT (Miller et al., 1991). Pluske et al. (2003) reported that villus height, crypt depth, and specific activities of lactase and sucrase have

been used as “markers” of the small intestine to determine maturation and functional development after the pig is weaned.

2.2.4.1 Intestinal Villi

Villi are the most fundamental structural features for nutrient absorption that are present in the alimentary tract (Palay and Karlin, 1959). The intestinal villi, finger-like projections, line the small intestine and increase the luminal surface area (Cera et al., 1988; McCracken et al., 1993). Villi are covered in columnar epithelium cells, which are mostly absorptive cells, as compared to secretory cells which make up the majority of the cells in the crypt (Kiela and Ghishan, 2016). Absorption of luminal nutrients occurs primarily on the apical side of the villous (Kiela and Ghishan, 2016) via the activity of enzymes and transporters of the brush border.

The intestinal villi morphology have the potential to illuminate how structure and shape can lead to function in the gut (Palay and Karlin, 1959). On the villi there are a number of cells present including secretory cells (goblet cells) and absorptive cells (enterocytes). The cells that are present on the villi are proliferated from crypt basements, and there are multiple crypts that contribute to the cells that are present on the villous (Shashikanth et al., 2017). Villi are covered with epithelium cells. Absorptive epithelium cells, or enterocytes, have a lining of luminal-facing microvilli. Microvilli, which also increase the surface area of the small intestine even more and they are covered by glycoproteins, constituting the glycocalyx, and digestive enzymes that aid with digestion (Williams et al., 2015).

During stressful events, such as weaning, there is a loss in villi height (Pluske et al., 1996; Vente-Spreeuwenberg et al., 2003). Both increased cell loss and decreased cell production are associated with villous shortening; however, the increased loss in cells from being sloughed off of the villous is associated with an increased rate of cell production from the crypt which results in the crypt deepening (Pluske et al., 1997). The trend of villi shortening with an increase in crypt depth is a commonly reported phenomenon around the time of weaning (Cera et al., 1988; Pluske et al., 1997).

2.2.4.2 Crypts

Crypts are tubular glands located at the base of villi that contain a variety of entero-endocrine cells, goblet cells, and undifferentiated cells (Stevens and Humes, 2004). Undifferentiated, or stem cells, divide in the crypt, differentiate, and then travel up the villi, with multiple crypts being responsible for epithelium renewal (Shashikanth et al., 2017). Crypts have also been suggested to be responsible for secretions of electrolytes, particularly chloride ions (Geibel et al., 2005). Intestinal cells take 2-6 d in adult mammals to travel from the crypt to the top of the villi where they are sloughed off into the lumen and are considered endogenous loss, but this loss is compensated for by stem cell mitosis located in the crypts (Williams et al., 2015).

During stressful events such as weaning, crypts increase in depth (Pluske et al., 1997). The crypts becoming deeper is reflective of the high demand for tissue synthesis to compensate for the rapid sloughing rate of cells off the villi that result in the villi shortening (Wang and Peng, 2008). The increase in depth of crypts and the decrease in height of villi during stressful events results in a villi height to crypt depth ratio (V:C ratio) that decreases. A decrease in the V:C ratio is an indicator of the pig undergoing physiological stress. When

the V:C ratio increases again, the increased ratio is associated with better nutrient absorption and therefore faster growth (Wang and Peng, 2008). Crypt depth has been considered to be an imperative factor to determine the crypt's capability to sustain the surrounding villi (Wang and Peng, 2008).

2.2.4.3 Microvilli

As previously mentioned, villi are covered in microvilli, which are cylindrical projections on the plasma membrane that are about 0.1 μm in diameter and about 1 μm in length (Kenny and Booth, 1978; Crawley et al., 2014b), and are structural features of polarized epithelial cells lining the gut, kidney proximal tubule, duct glands, and even airways of respiratory systems (Kenny and Booth, 1978). Microvilli, or the brush border, cover the enterocyte and function to increase the surface of the enterocyte to maximize luminal interaction (Stevens and Humes, 1995). A fully developed brush border on an enterocyte consists of densely packed microvilli in a hexagonal pattern (Crawley et al., 2014a) They also have the role of performing a variety of digestive and transport functions in the GIT (Brasitus et al., 1979). Microvilli are devoid of microtubules that are the principal feature in cilia; however, microvilli have a methodically organized infrastructure of parallel actin filaments that support the extension of the microvilli into the lumen of the gut (Crawley et al., 2014b; Kenny and Booth, 1978).

Work has been done to identify enzymes that are associated with the microvilli in the gut. Glycosidases, responsible for carbohydrate digestion, in particular have been associated with microvilli and their role in aiding in digestion in the gut along with peptidases (Kenny and Booth, 1978).

2.2.4.4 Cells Present on Villi in the Small Intestine

There are four major epithelial cell types that constitutes the epithelium that lines the small intestine: entero-endocrine cells, paneth cells, absorptive enterocyte cells, and goblet cells (Ensari and Marsh, 2018). Entero-endocrine cells export peptide hormones (Ensari and Marsh, 2018), and paneth cells secrete anti-microbial proteins (Hooper, 2015).

Enterocytes, or absorptive cells, begin as undifferentiated cells at the base of the crypt and migrate towards the tip of the villous as they mature (Stevens and Humes, 1995; Cheeke and Dierenfeld, 2010). As the enterocytes mature, their brush border membrane becomes more developed (Crawley et al., 2014b). Brush border development is depicted in Figure 2.5, showing that the microvilli elongate and have a higher density as maturation occurs.

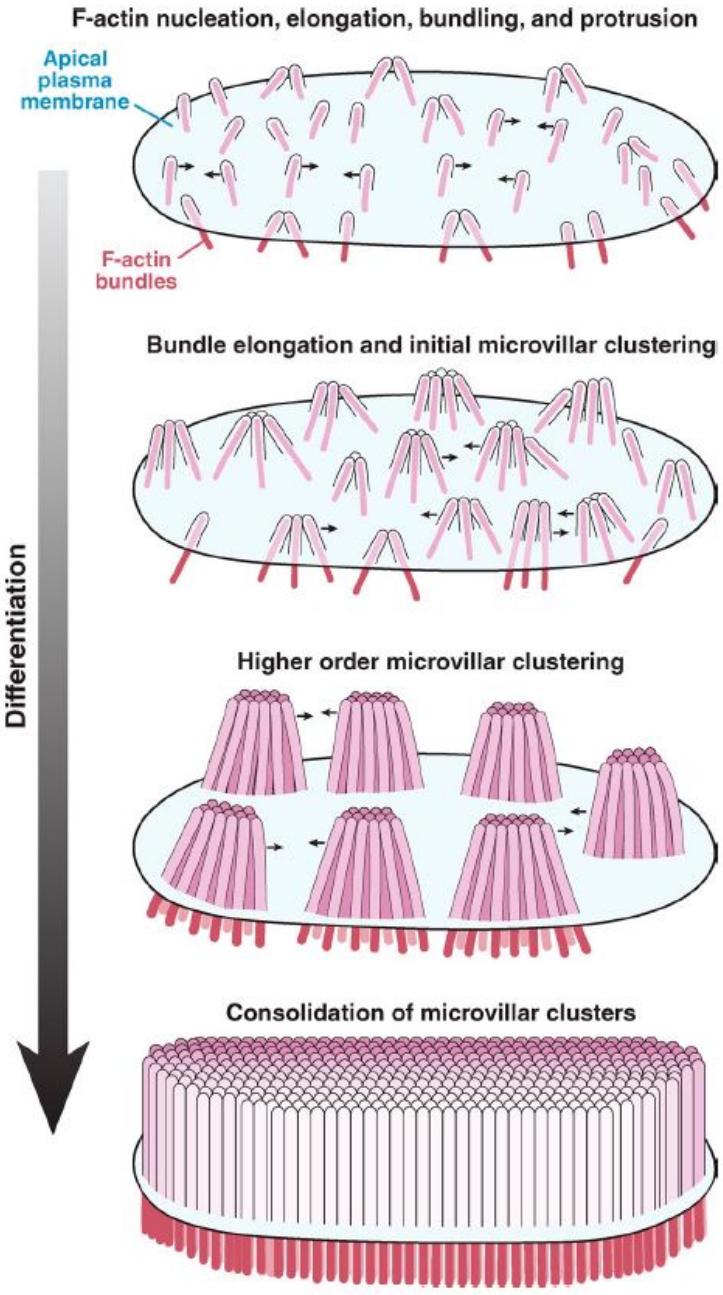

Figure 2.5 Development of the brush border in maturing enterocytes. Initially, microvilli appear as "buds" on the apical surface of the enterocyte. Being formed from polymerization and bundling of actin filaments, the microvilli projections begin forming clusters at their distal tips. As maturation occurs, the clusters grow in numbers and consolidate until a uniform surfaced is formed. Figure taken from Crawley et al. (2014b.)

As seen in Figure 2.4, intestinal cell maturation involves the development of the brush border membrane. Along with the changes in the phenotype of the cell as maturation occurs, the cytoskeleton in the villus cells are enriched for altered gene expression, with the majority of the expressed genes being up-regulated with maturation (Mariadason et al., 2005). This up-regulation of genes on the villus cells allows for better absorption of nutrients, particularly with the absorption of ingested fats (Mariadason et al., 2005).

Enterocytes comprise more than 95% of the cells on the villous (Fan et al., 2001) and, as previously mentioned, as the enterocytes mature, they develop distinguishing absorptive cell features such as microvilli (Skrzypek et al., 2009). In addition to the development of the brush border, as enterocytes mature, they express digestive enzymes (Cheeke and Dierenfeld, 2010). When the mature enterocyte reaches the top of the villi, they are sloughed off the villous and are considered endogenous loss (Skrzypek et al., 2009). Enterocytes secrete transmembrane mucins MUC1, MUC3, MUC4, MUC12, MUC13, and MUC17 (Pelaseyed et al., 2014). MUC3, MUC12, and MUC17 are the major components that can be found in the enterocyte glycocalyx (Pelaseyed et al., 2014).

Goblet cells are responsible for the production of mucins. These mucins form a gel-like substance, which is critical for maintaining proper health and barrier function (Smith et al., 2009). Goblet cells produce and secrete a variety of mucins (Ensari and Marsh, 2018), with the secretion of mucins occurring via secretory vesicles or exocytosis (Birchenough et al., 2016; Pelaseyed et al., 2014). Goblet cells can increase in both size and number throughout the gut (Ma et al., 2018). MUC2 is the major structural mucin that is secreted in the small intestine (Pelaseyed et al., 2014).

Paneth cells are located in the crypts of the small intestine and are also secretory cells (Hooper, 2015). However, the secretions that come from paneth cells are not mucins, but rather microbial proteins, resulting in the paneth cells being responsible for the majority of antimicrobial secretions in the small intestine (Hooper, 2015; Pelaseyed et al., 2014). In addition to being responsible for antimicrobial output, paneth cells play a role in regulating epithelial renewal by secreting sustaining factors for the surrounding stem cells in the crypt (Hooper, 2015).

Entero-endocrine cells compose approximately 1% of the cells found in the small intestine and are responsible for sensing luminal contents and secreting regulatory factors such as gastric inhibitory peptide and glucagon-like peptide, that regulate digestion and intestinal mobility (Hooper, 2015). This regulatory function of the entero-endocrine cells throughout the small intestine makes it one of the largest endocrine systems in the body (Hooper, 2015).

In addition to the previously mentioned cells, other cells that can be found in the GIT are tuft cells and M cells. Tuft cells are noted for the appearance of their microvilli to stand up taller than the microvilli around them (von Moltke, 2018), such as an appearance of a tuft of hair. The purpose of tuft cells in the small intestine are chemosensing (von Moltke, 2018). Their exact function remains poorly defined currently; however, tuft cells are thought to be responsible for a sensory role largely due to their long microvilli (von Moltke, 2018). A final cell that can be found in the small intestine are M cells. The function of M cells includes antigen sampling, transporting the antigens via transepithelial transport, to generate an immune response (Hooper, 2015).

2.2.4.5 Mucus

Mucosal surfaces, such as in the GIT, are coated in a gel that is formed by secreted mucins that are produced from goblet cells. These mucins prevent the passages of unwanted molecules, such as bacteria, from coming into direct contact with the epithelial layer (Turner, 2009). Therefore, mucus production helps to keep the epithelial lining on the small intestine from being exposed to dangerous pathogens in the lumen (Yamashita et al., 2018). In addition to mucus protecting the intestinal lining from unwanted molecules, mucus also helps to assist in the movement of digesta through the intestine (Cheeke and Dierenfeld, 2010).

Mucins are first translocated in the rough endoplasmic reticulum of the goblet cell (Pelaseyed et al., 2014). Once the mucin is correctly assembled it then passes to the Golgi apparatus where *O*-glycosylation occurs (Freeman, 1962; Pelaseyed et al., 2014). The mucin is then sorted into the secretory pathway, where it is organized and highly packed before secretion occurs (Pelaseyed et al., 2014). The organization of mucus is prompted by a low pH and a high calcium concentration (Pelaseyed et al., 2014). The secretion of mucus requires highly organized packing that allows for an expansion of >1,000-fold in volume, which is triggered by a rise in pH and the lowering of the calcium concentration by the removal of calcium ions (Pelaseyed et al., 2014).

The primary mucus that is secreted in the small intestine by goblet cells is MUC2 (Pelaseyed et al., 2014). In addition to secreting MUC2 in the small intestine, goblet cells are also known to secrete AGR2, which is an endoplasmic reticulum residential protein that is essential for the production of intestinal mucus (Park et al., 2009).

2.2.5 *Small Intestine Morphology in the Pig*

The ingestion of feedstuffs with nutrients is essential for growth and development of the intestinal epithelia of the pig. Zhang et al. (1997) reported that the most dramatic changes in the GIT structures occurred within in the first day postpartum and within the first hours of suckling. When the piglet is suckling from the sow, the intestinal villi appear to be long finger-like projections (Cera et al., 1988). Location in the small intestine, i.e. duodenum, jejunum, or ileum, affects the phenotype, such as smoothness and variation in shape, of the intestinal villi (Skrzypek et al., 2005). The shape of the villi is important to consider as the features present on the surface area of the villi can affect the nutrient absorption in the gut (Skrzypek et al., 2005). Understanding how the villi change throughout the maturation of the pig can also lead to a better understanding of gut function (Smith et al., 1977). The size of the intestinal villi increases during the first day of life, possibly due to the intake of colostrum (Kanka et al., 2016). Zabielski et al. (2008) reports that the increase in villi height occurs from blood being distributed to the intestinal mucosa. Villus height is said to increase around 33% on the first day of life (Xu et al., 1992).

At weaning, the gastrointestinal tract (GIT) of the pig needs to adapt to the diet change from highly digestible milk to a plant-based diet (Xu et al., 2000; Skrzypek et al., 2005). Intestinal villi help with the adaptation of the GIT, as they change phenotypically over time as the pig matures to adapt to the diet that the pig consumes, which keep gut function efficient (Skrzypek et al., 2005). One obvious change that occurs to the villi at weaning includes a shortening of the villi height and an increase in crypt depth (Pluske et al., 1997). The villi that are finger-like projections initially shift to more leaf- or tongue-like projections as the pig approaches weaning, and this shift in the structure typically is a

response to development or an exposure to a new food component (Skrzypek et al., 2005). The age that pig is weaned does affect the physiological stress that the pig will undergo (Lallès et al., 2004). The earlier that the pig is weaned, the more physiological stress that the GIT undergoes (Smith et al., 2009), and the later in life that the pig is weaned, the less stress that the GIT undergoes (Lallès et al., 2004).

The height of the villi is reported to have a relationship with feed intake (Pluske et al., 1997). It is very common at weaning for a pig to have a decrease in feed intake, and the villi height is stunted as a result of lack of new cell production and rapid loss of mature enterocytes (Pluske et al., 1997; Nabuurs et al., 1993). Villi growth is location dependent and it is reported that within the first week after weaning the proximal section of the small intestine had more growth than the mid-section (Vente-Spreeuwenberg et al., 2003). And as previously mentioned, the shape of the villi also changes around the time of weaning to be more leaf-like.

In addition to the shortening of the villi, there is a deepening of the crypts (Pluske et al., 1997). A deeper crypt is indicative of a fast cell turnover in the intestine to continue the renewal of cells on the villus, a mechanism done in attempt to try to compensate for the sloughing on villi potentially due to an inflammation from the presence of a new feedstuff (Laudadio et al., 2012). Since the crypts are responsible for cell renewal, a deeper crypt means an increase in cell proliferation, but a shorter villi means the cells are sloughed off quicker and lost as endogenous loss, this relationship between the villus height and the crypt depth during a stressful event shows a low villus height:crypt depth ratio (V:C ratio). In turn, a healthy pig would have a higher V:C ratio, showing a taller villous and a more shallow crypt which is indicative of a slower epithelium cell turnover rate and therefore a

lower maintenance requirement, which in turn can lead to a higher growth rate in the animal (Laudadio et al., 2012). A higher V:C ratio is also viewed as an indicator of the animal being in overall good health (Wang and Peng, 2008). When assessing the V:C ratio, many researchers highlight the importance of the shortening of the villi rather than the deepening of the crypt. However, the deepening of the crypt may be a more important parameter because it determines the villus-crypt junction, thus the length of the villi (da Cunha Ferreira et al., 1990).

2.3 Summary

As previously mentioned, there are a number of factors that affect the general growth of pigs. For example, selecting pigs for leanness so that there is minimal fat on the pig at market has become a common practice (Theil et al., 2012; Cliplef et al., 1993). The selected for leaner pig has less back-fat, but has also been known to have larger visceral organs, resulting in an overall lower dressing percentage of the carcass (Pond et al., 1988; Cliplef et al., 1993).

There have been reports that there is a difference in the weight of the organs among breeds (Cliplef et al., 1993), type of diet consumed (Anugwa et al., 1989), and feeding regimen of the diet (de Lange et al., 2003). For example, diets that are high in fiber result in larger VO (Jørgensen et al., 1985; Pond et al., 1988; Anugwa et al., 1989; de Lange et al., 2003). Leroch et al. (2003) reported that an increase of fiber in the diet increased the volume of the cecum and large intestine in swine. An example of fiber effecting the volume of the GIT, in addition to the age of the pig, is reported by Jha and Berrocoso (2015) who concluded that mature sows can digest dietary fibers at greater rates than growing pig,

which meant that growing pigs had higher retention times for dietary fibers and in turn this lead to greater GIT volume. Wiseman (2006) reported that lean tissue growth was affected among genetic lines and breed of pigs. In addition to lean tissue growth being affected, VO growth and body composition were affected in pigs from 20 kg to 75 kg by genetic line and sex (Wiseman, 2006).

The type of feed and the amount of feed intake can affect the morphology of the small intestine. When the pig is consuming highly digestible milk, intestinal villi are longer than when the pig's diet changes to a less-digestible plant-based diet (Skrypek et al., 2005). A study conducted by Deprez et al. (1987) fed equivalent diets in a pelleted form and slurry form and found that the pigs that consumed the slurry feed had longer villi. The slurry pigs having a greater villi height may be due to either a lack of abrasive feed being consumed or an increase in feed consumption. Beers-Schreurs et al. (1995) found that energy intake, independent of diet, effects the height of the villus after weaning. A number of other factors such as BW and maturity, health status (de Lange., 2003), sex, environment, and any interactions between listed factors also influence development of the pig (Wagner et al., 1999).

2.4 Research Objectives

Early stages of life are crucial, not just to pigs, but also for many species as this is a time when the offspring is rapidly growing. With documented changes that occur early in the pig's life, a more complete understanding of how the pig develops is established. This more complete understanding of the pig's development provides opportunities to identify critical times in the pig's life in which targets dietary or management interventions

might occur to improve the pig's performance. Expanding specifically on current knowledge body composition, tissue glycogen reserves, visceral organ growth and small intestine development will highlight key points in the pig's life that can be improved through dietary changes that could result in an increased feed efficiency for the pig and, thereby, an extension of limited resources for global food production. This study therefore aims to document the teleological and allometric changes that occur in the developing pig. Frequent sampling occurred from birth, through the suckling phase, at weaning, and after weaning till six weeks post-weaning, roughly 25 kg BW, to closely track subtle changes that may occur in the developing pig. Additional collection occurred at late gestation to obtain a general idea of the pig's body just before birth.

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CHAPTER 3. Developmental changes in the pig from birth through 42 d post-weaning

3.1 Introduction

With the constant challenge of improving the growth rate and efficiency of pigs, a detailed knowledge of pig anatomy and physiology is needed to understand where improvements can be made. For example, over recent years there has been a vast amount of work done to alter the carcass composition of the pig through genetic selection to meet consumer demands for leaner meat. In addition, pigs have been genetically selected for improved growth rates, resulting in pigs that reach market weight sooner (Correa et al., 2006).

The typical composition of adult mammals is approximately: 60% water, 16% protein, 20% fat, and 4% ash (Lloyd et al., 1978). Carbohydrates, another component of proximate analysis, are typically not reported in proximate analysis reports because there is less than one percent of carbohydrates in the body at a given moment, and those carbohydrates present are constantly being formed and broken down during metabolism for a multitude of vital functions in the body (Maynard and Loosli, 1962; Lloyd et al., 1978). Carbohydrates are stored in the body in the form of glycogen, which serves as an energy reservoir when needed by the animal. During the time immediately after birth, glycogen serves as a critical source of metabolic fuel for the neonate and may comprise a greater portion of the body (Mersmann et al., 1972).

Visceral organs (VO) are crucial for life in all animals due to their essential and critical role in all aspects of physiology including the metabolism of nutrients that are then used by the pig for growth and development. Although VO do not contribute to the final

value of the carcass, they are responsible for the conversion of dietary nutrients into pork (de Lange et al., 2003). Knowledge of VO growth can help shape concepts about the development of the pig.

The gastrointestinal tract (GIT), particularly the small intestine, is responsible for the absorption of nutrients that are needed to sustain life and support growth and development of the pig. The intestinal villi, finger-like projections, line the small intestine and increase the luminal surface area (Cera et al., 1988). Located on the villi are absorptive cells, called enterocytes. Enterocytes that are fully differentiated can be identified by a prominent brush border, comprised of densely packed microvilli (Palay and Karlin, 1959; Crawley et al., 2014a; Crawley et al., 2014b). As far as changes to microvilli and enterocytes related to major events of the pig's life (e.g., birth and weaning), there is very little information published as most of the concern over maturation in the swine intestine revolves around villi.

This study evaluated the above components at times that were surrounding stressful events in the young pig's life, i.e., birth and weaning, by frequent collection during these times in order to identify any subtle changes that might occur in the pig at these times.

3.2 Materials and Methods

The study was carried out at the University of Kentucky and was conducted under protocols approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

3.2.1 *Animals and diets*

A total of 96 crossbred pigs from 20 litters were randomly euthanized (n = 6, 3 barrows and 3 gilts) over 16 time points from late gestation through 42 d post-weaning. After weaning all pigs had ad libitum access to water and common diets (Table 3.1) that were fed in two phases. Phase 1 of the diet was fed from weaning through d 14 post-weaning. On d 14 post-weaning, Phase 2 of the common diet was fed through the end of the study. All diets (Table 3.1) met or exceeded all NRC (2012) requirements. Pigs were randomly selected over 16 time points for slaughter. Pigs were randomly selected by dividing the pigs into 3 weight groups (heavy, average, or light) and choosing a pig from each group, but making sure to not select the heaviest or lightest pig from each weight group. This process allows for pig selection of a variety of weights without creating a bi-modal curve for the weights of the pigs remaining in the pig pool. The times in which pigs were slaughtered included: birth (pre-suckle), days 1, 2, 3, 5, 7, 14 after birth, weaning (d 21), and days 1, 2, 3, 5, 7, 14, 28, and 42 post-weaning. The study was divided into two parts; from birth (pre-suckle) to weaning (d 21) was the suckling period of the study, and from weaning (d 21) through 42 d post-weaning was the post-weaning period of the study.

In addition, there were 6 collection time points (n = 6, 3 barrows and 3 gilts) on gestation days 108, 109, 110, 111, 112, and 113. However, the data collected from the fetuses were not statistically analyzed as the 6 fetuses collected per gestation day came from the only one sow. Because of this, the sow became the experimental unit and n = 1.

Table 3.1 Formulation and calculated nutrient composition of basal diets

	Basal Diet	
Ingredient, %	Phase 1	Phase 2
Corn	51.05	61.33
Soybean meal, 48%	29.59	34.00
Fish meal (Menhaden)	2.00	0.00
Spray-dried animal plasma	2.00	0.00
Whey dried	10.00	0.00
Grease	2.60	1.50
L-Lysine	0.23	0.28
DL-Methionine	0.17	0.18
L-Threonine	0.08	0.12
Dicalcium Phosphate	0.56	0.88
Limestone	1.08	1.07
Salt	0.50	0.50
TM premix[1]	0.06	0.06
Zinc oxide[2]	0.01	0.01
Iron sulfate[3]	0.02	0.02
Vitamin mix[4]	0.04	0.04
Santoquin[5]	0.02	0.02
Calculated Composition		
ME, kcal/kg	3401.00	3348.00
Ca, %	0.80	0.70
Total P, %	0.60	0.56
Available P, %	0.40	0.33
Total Amino Acid Basis, %		
Lysine	1.51	1.38
Threonine	1.00	0.92
Tryptophan	0.74	0.70
Methionine	0.52	0.51
Cysteine	0.40	0.35
Methionine + Cysteine	0.92	0.87
Isoleucine	0.95	0.90
Histidine	0.62	0.58
Valine	1.08	0.99
Arginine	1.40	1.40
Phenylalanine	1.09	1.06
Tyrosine	0.74	0.70
Phenylalanine + Tyrosine	1.83	1.76
Leucine	1.92	1.82
Standardized Ileal Digestible Amino Acid Basis, %		
Lysine	1.35	1.23

Table 3.1 (continued) Formulation and calculated nutrient composition of basal diets

Threonine	0.85	0.79
Tryptophan	0.25	0.23
Methionine	0.48	0.47
Methionine + Cysteine	0.81	0.77
Isoleucine	0.83	0.79
Histidine	0.54	0.51
Valine	0.92	0.85
Arginine	1.29	1.30
Phenylalanine	0.95	0.92
Tyrosine	0.64	0.60
Phenylalanine + Tyrosine	1.59	1.52
Leucine	1.68	1.60

[1]The trace mineral premix supplied the following per kilogram of diet: 27.5 mg of Mn as manganous oxide, 60.5 mg of Fe as ferrous sulfate monohydrate, 60.5 mg of Zn as zinc sulfate, 9.9 mg of Cu as copper sulfate, 0.39 mg of I as calcium iodate, and 0.17 mg of sodium selenite.
[2]The zinc oxide supplied 50 mg/kg Zn.
[3]The iron sulfate supplied 50 mg/kg Fe.
[4]The vitamin premix supplied the following per kilogram of diet: 9,359 IU of vitamin A, 2,341 IU of vitamin D_3, 62.3 IU of vitamin E, 6.9 mg of vitamin K, 0.03 mg of vitamin B_{12}, 7.32 mg of riboflavin, 20.84 mg of pantothenic acid, 41.46 mg of niacin, 1.72 mg of folic acid, 4.16 mg of vitamin B_6, 1.15 mg of thiamin, and 0.23 mg of biotin.
[5]Santoquin (Monsanto, St. Louis, MO) supplied 130 mg/kg ethoxyquin.

3.2.2 *Experimental Procedures*

3.2.2.1 Body composition and glycogen analysis

Body weight was recorded weekly as well as at the time of slaughter. At the time of slaughter, a portion of the left medial lobe of the liver (~10 g), and a section (~10 g) of the right gluteal muscle group of the pig was collected, both were immediately frozen in liquid nitrogen. Samples were stored at -80 ºC until further analysis could occur. The previous samples were used to assess glycogen concentrations in the pig. The whole body of the pig, minus the gastrointestinal tract, was collected and stored at -20 ºC until the whole body could be ground into a composite.

Whole body samples were analyzed for dry matter (DM), ash, ether extract (EE), nitrogen (N), calcium (Ca), phosphorus (P), and glycogen concentrations in the body. When analyzing for DM, ash, EE, N, Ca, and P, Association of Official Agricultural Chemists and/or Association of Official Analytical Chemists protocols were followed accordingly. Glycogen was analyzed using the EnzyChrom^{TM} Glycogen Assay Kit (Cat # E2GN-100, BioAssay Systems, Hayward, CA, US).

3.2.2.2 Visceral organs

At slaughter, all VO were removed and weighed, and intestinal length measured. The volume of the stomach and cecum were measured by filling the empty organ with phosphate buffered saline (PBS) until it was subjectively determined as "full." The intestines were measured for total length and sections at the predetermined length of the small and large intestine were taken (small intestine: 0%, 25%, 50%, 75%, and 100%; large intestine: 0%, 50%, and 100%) and measured to determine circumference. The recorded dimensions of the intestine, length and circumference, were used to determine the overall volume of the GIT with an equation for volume of a tapered cylinder. This was done with the assumption that the intestinal lumen changes evenly between the GIT sections.

3.2.2.3 Small intestine morphology

Once the length of the small intestine was recorded, 2 sections of the small intestine were collected at 25%, 50%, and 75% of the total length for further analysis by light microscopy (LM) and scanning electron microscopy (ScEM). All sections of the small intestine were gently rinsed to remove digesta using PBS (pH 7.4) and stored in 10%

neutral buffered formalin at room temperature and out of direct sunlight until further processing and analysis.

All samples were subjected to LM analysis, and selected samples from the 50% and 75% distances (gestation days 108, 112, 113, birth, day 1, day 7, weaning, post-weaning days 1, 2, 3, 5, 7, 14, 28, 42) were subjected to ScEM. Then LM was used to assess villi height, crypt depth, villus height:crypt depth ratio, and goblet cell count while ScEM was used to assess villi width and density, enterocyte width, perimeter, and area, and microvilli density, diameter, and length. In addition to the previous ScEM assessments, enterocyte width, microvilli density, microvilli diameter, and microvilli length were measured at the apical end, middle, and base of the villi.

3.2.3 *Laboratory Procedures*

3.2.3.1 Body composition and glycogen analysis

3.2.3.1.1 Carcass Grinding

All bodies, minus the GIT, were stored at -20 ºC after collection until further processing and analysis. Carcasses were partially thawed before being ground to facilitate fitting the carcass into the grinder. Carcasses that were less than or equal to 2.3 kg body weight (BW), were thoroughly ground in a grinder (TorRey model M22-R-2, Houston, TX) using an 82.6-mm kidney plate (TorRey model TOR 223KP, Houston, TX) prior to a 4.8-mm plate (TorRey model TOR 12P 3/16) twice each. The ground carcass was collected in a plastic tub, thoroughly mixed by hand before randomly subsampled. Subsamples (500

- 1200 g) were collected in two separate plastic containers and stored at -20° C until further analysis.

Bodies that exceeded 2.3 kg of BW were ground and thoroughly mixed following a similar procedure as above, but using a larger grinder (AUTIO model 804GH50T, AUTIO Company, Astoria, OR), and grinding plates were stacked in the grinder in the following order: 160-mm kidney plate and 12-mm plate, so that the carcass passed through both plates at once. Partially frozen carcasses were run through the larger grinder once, which provided a ground composite. If large components of the carcass were still present after the first grind, the partially ground carcass was put back through the grinder once more. The entire ground carcass was thoroughly mixed by hand before being subsampled (500 - 1200 g) and stored in two plastic containers at -20° C until further analysis.

3.2.3.1.2 *Proximate Analysis*

3.2.3.1.2.1 Calculations:

The chemical composition (%), on a dry matter (DM) basis of the whole carcass was calculated by dividing the analyzed percentage of the nutrient by the fraction of the dry chemical component. All chemical components are reported on a DM basis.

3.2.3.1.2.2 Water/DM

Water content of the samples were obtained by placing 2.0 - 3.0 g of the sample in a ceramic crucible, then placing the crucible in an oven (Gravity convection oven Cat# 414005-110, VWR, Radnor, PA) at 110 °C for two days. After 48 h, samples were cooled, and then the final weight was recorded. All DM measures were conducted in duplicate with an acceptable coefficient of variance (CV) of 15%.

3.2.3.1.2.3 Ash

Inorganic material in the body of the pig was obtained by placing ~5 g of fresh sample in a dry and pre-weighed porcelain crucible and placing it in a furnace (Isotemp muffle furnace, Fisher Scientfic, Pittsburg, PA) at 600° C for 24 h. After 24 h, samples were removed from the furnace at 150 ^{o}C, cooled, and then weighed. Ash measures were conducted in duplicate with a coefficient of variance of 25% with a few exceptions for samples at 35% due to variability of bone chips within the sample during the time of analysis.

3.2.3.1.2.4 Nitrogen

Nitrogen was determined by placing ~ 40 g of sample into a drying oven (Tru Temp model 214300, Hotpack Corp., Philadelphia, PA.) at 50^{o} C for 6 d to ensure the sample was completely dry. After 3 d, samples were broken in their weigh boat to increase the surface area of the sample and safeguard that the middle of the sample was fully dried. Cooled samples were then ground using a coffee grinder (Model CBG100S, Black and Decker Inc., Shelpton, CT) and sent to the Experiment Station Chemical Laboratories at the University of Missouri for nitrogen analysis via LECO. Nitrogen measures were conducted in singles.

3.2.3.1.2.5 Lipid

Lipid content was analyzed by ether extraction (procedure for lipid analysis from AOAC, Official Methods of Analysis (1995), 16th Ed., Arlington, Virginia, USA). A 3 - 5 g composite sample of the whole body was weighed, mixed with a small amount of sand onto a 12.5-cm sheet of filter paper (P8, Fisher Scientific, Pittsburg PA), and dried for 1 hr in an oven before being placed in the solvent extractor. The solvent for extraction is 70 ml of petroleum ether at 40-60^{o} C with an evaporation residue not higher than 10 mg/ml.

Samples were immersed in boiling solvent for 60 minutes in a solvent extractor (Velp SER 148 Mfr # F30310242, VELP Scientifica, Bohemia, NY) and experienced a reflux washing for an additional 60 minutes. Extraction vessels were then dried in an oven at 110º C for 30 minutes, cooled in a desiccator, and then weighed. Lipid measures were conducted in duplicates with an acceptable CV of 20% apart from a few exceptions that had a higher CV.

3.2.3.1.2.6 Phosphorus

Phosphorus was determined using gravimetric quimociac technique explained in Shaver (2008). Subsamples of 3 - 5 g whole body composite were measured into a dry crucible and ashed overnight in a muffle furnace at 600° C. These were the same samples from which ash was determined. As previously mentioned, the samples were then cooled and weighed. A total of 40 ml of 3N HCl was then added to the ashed sample and the solution was heated to boiling for 15 minutes. The solution was quantitatively transferred to a volumetric flask and diluted with distilled, deionized water to 250 ml. The sample solution stood overnight to allow any heavy particles to settle, and this solution was used to analyze both phosphorus and calcium. A 10 ml aliquot of digested sample solution was then taken by volumetric pipette from this solution and placed into a 250-ml Erlenmeyer flask, heated, and mixed with 20 ml of Quimociac solution (Appendix I). A yellow precipitate was formed after boiling the sample for 2 minutes. This precipitate was gravimetrically filtered into a porcelain crucible and dried overnight at 110° C. The weight of the precipitate was taken and the percentage phosphorus in the sample was calculated as follows:

$$\text{Total P (\%)} = \left[\frac{(precipitate\ wt\ x\ 250\ ml)}{Aliquot, ml}\ x \frac{(0.013997\ x\ 100)}{sample\ wt}\right]$$

Phosphorus measures were conducted in duplicates with an acceptable CV of 25% apart from a few exceptions that had a higher CV once again due to variability of bone in subsample at time of ashing.

3.2.3.1.2.7 Calcium

From the diluted solution that was mentioned in the sample preparation for phosphorous, a 10 to 50 ml aliquot was taken from the flask and stored in a covered, plastic test tube. Samples were diluted with a 0.1% NaCl solution at a salt solution to sample solution ratio of 199:1. After dilution, samples were mixed by vortex and analyzed with an atomic absorption spectrometer at a wavelength of 422.7 nm (Solaar M-series). Calcium measures were conducted in duplicates with an acceptable CV of 25% apart from a few exceptions that had a higher CV once again due to variability of bone in subsample at time of ashing.

3.2.3.2 Glycogen Homogenization

When ready for analysis, 0.2 g liver and 0.4 g muscle were homogenized with 4 mL homogenization solution (25 mM citrate, pH 4.2, 2.5 g/L NaF). Samples were kept on ice for the entirety of the homogenization process. After homogenization, samples were centrifuged at 14,000 g for 5 minutes in a refrigerator. The supernatant was then removed and divided between three aliquots. The aliquots of the supernatant were stored at -80 ^{o}C until further analysis. EnzyChrom^{TM} Glycogen Assay Kit (Cat # E2GN-100, BioAssay Systems, Hayward, CA, US) was used to determine the concentration of glycogen. Standards of 0, 50, 100, 150, and 200 µg/mL were used to create a standard curve to calculate concentration, and a plate reader (Spectramax 250, Molecular Devices, San Jose, CA) was utilized at 570 nm to obtain optical density in each well in the plate. The principal

of the kit was to hydrolyze all glycogen into glucose, the more glycogen/glucose present, the higher the optical density. This process assumes that all detected glucose is from the breakdown of glycogen in the tissues. Glycogen analysis was conducted in duplicates with an acceptable CV of 10%.

3.2.3.3 Small intestine morphology

The intestine samples were collected, cleaned by gently rinsing the sample with PBS (pH 7.4) to remove digesta, and then fixed in 10% neutral buffered formalin solution before being processed through several steps for each microscopy procedure. For light microscopy (LM), samples of intestine were transferred to Histo-Scientific Research Laboratories (Mt Jackson, VA, US), who were employed for staining and mounting samples on microscope slides. Each sample underwent a total of 6 slices, producing 6 cross-sections of the intestine. Of the 6 cross sections produced, half the cross sections were stained with both *Hematoxylin and eosin* stain (H&E stain) for measurements of fully intact villi and crypts. The remaining cross sections were stained with alcian blue periodic acid Schiff (PAS). The PAS stain detects polysaccharides. Because polysaccharides comprise a large portion of goblet cell-produced mucins, this stain is used to identify goblet cells.

Ten fully structured villi and crypts of each sample were randomly selected to measure villi height, crypt depth, and goblet cell count per villi. Figure 3.1 and Figure 3.2 illustrate an example of villi and crypt measurements that were recorded. NDP.view2 Viewing Software (Hamamatsu Photonics, Naka-ku, Japan) was used to obtain measurements. The long black line represents villous height, the short black line represents

crypt depth, and the purple dots are representatives of goblet cells. The mean of the ten measurements for each response measure was used to represent each sample.

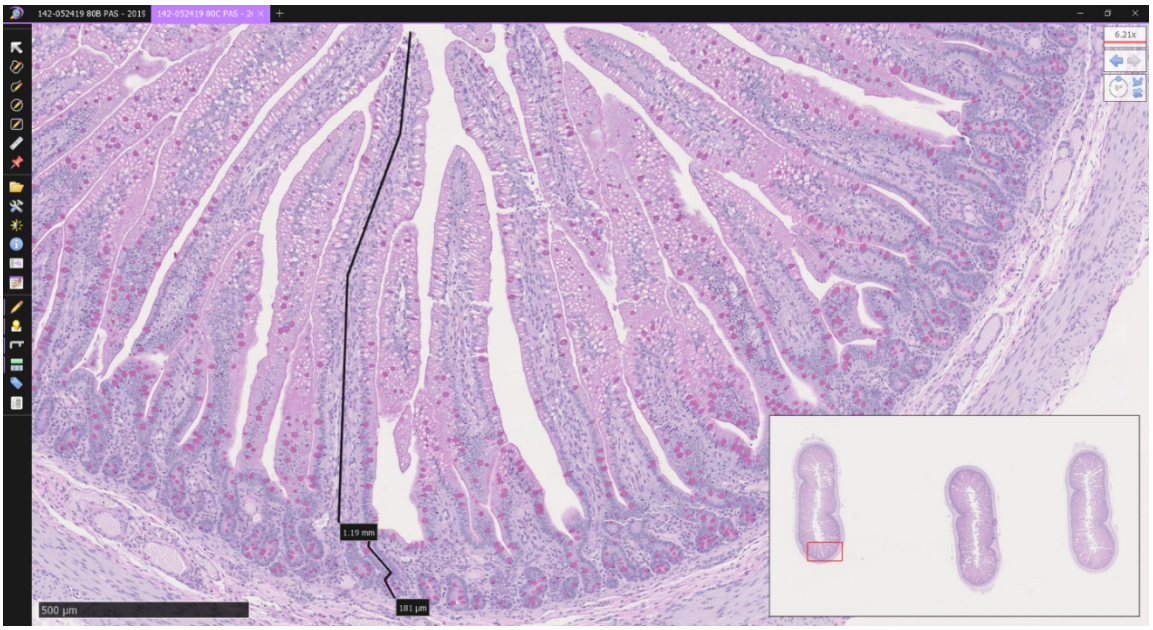

Figure 3.1. Example of villous height measurement

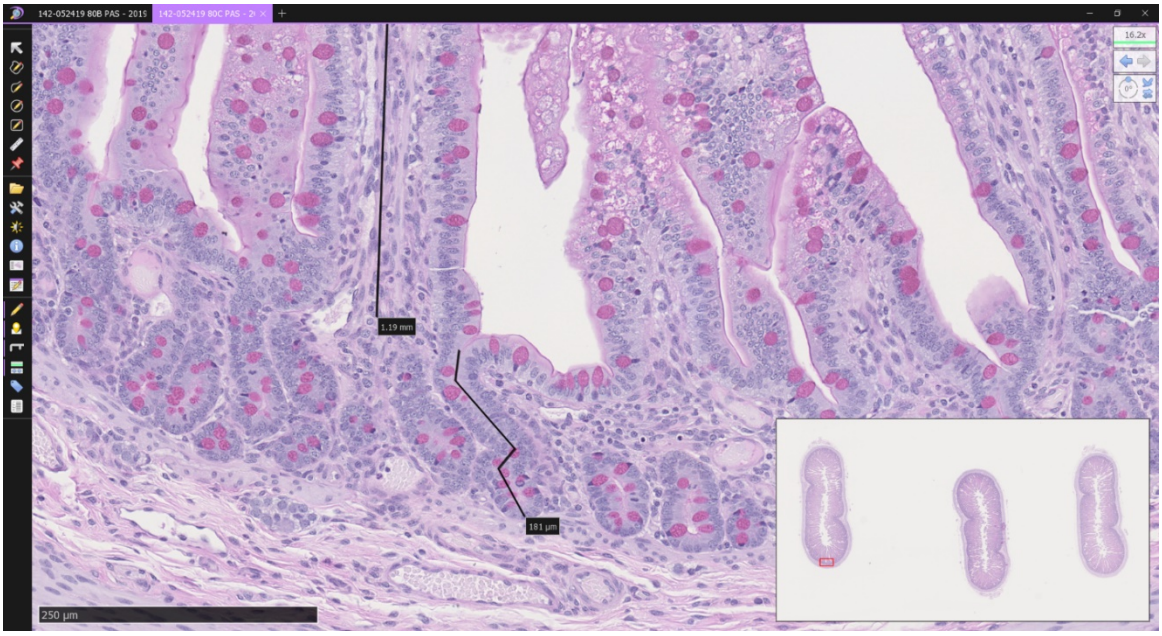

Figure 3.2. Example of crypt depth measurement

Samples for scanning electron microscopy (ScEM), underwent a dehydration process and were coated with platinum, details of the dehydration are in Appendix II. When assessing intestines using ScEM, Image J (NIH, 2018) was utilized to make measurements on the scanning electron micrographs. Of the villi present in the micrograph, 15 intact villi were randomly selected for width measurements. Three areas of 0.25 mm^2 with intact villi were counted for density. Two intact villi were then selected at random and further examined at their apical, middle, and base ends for enterocyte width, microvilli density, microvilli diameter, and microvilli length. At each position on the villi, apical, middle, and base, ten enterocytes were selected at random and their width was measured. Three areas of 0.25 μm^2 with intact microvilli were counted for density. Ten microvilli were randomly selected and measured for diameter. Microvilli length was not always available to be measured, and there were several instances where length measures were compromised by angles that appeared in the micrograph. The mean of the measurements for all response measures at their appropriate locations served as the representative for that response measure at that location. Figures 3.3 - 3.8 show an example of measurements previously mentioned indicated by red markings. A scale bar located in the bottom left corner of the micrograph was produced by the microscope and used to accurately make measurements. Some of the following examples are missing scales bars due to the image being "zoomed in" for precision when making measurements of structural features.

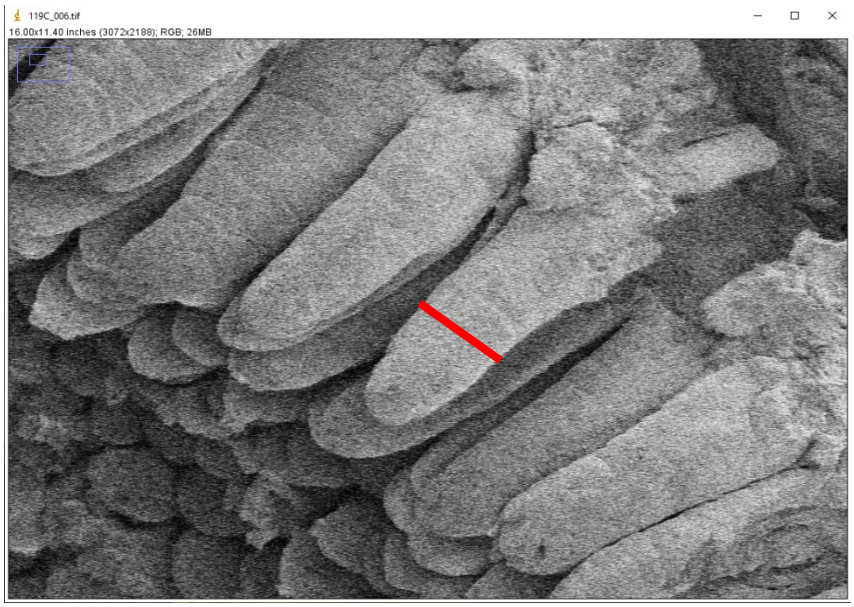

Figure 3.3. Example of villi width measurement on a scanning electron micrograph via ImageJ

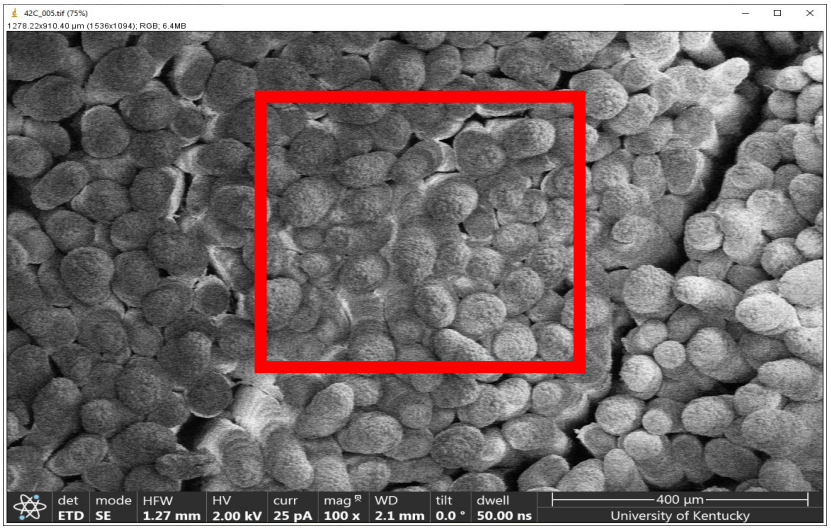

Figure 3.4. Example of measuring villi density in a scanning electron micrograph via ImageJ.

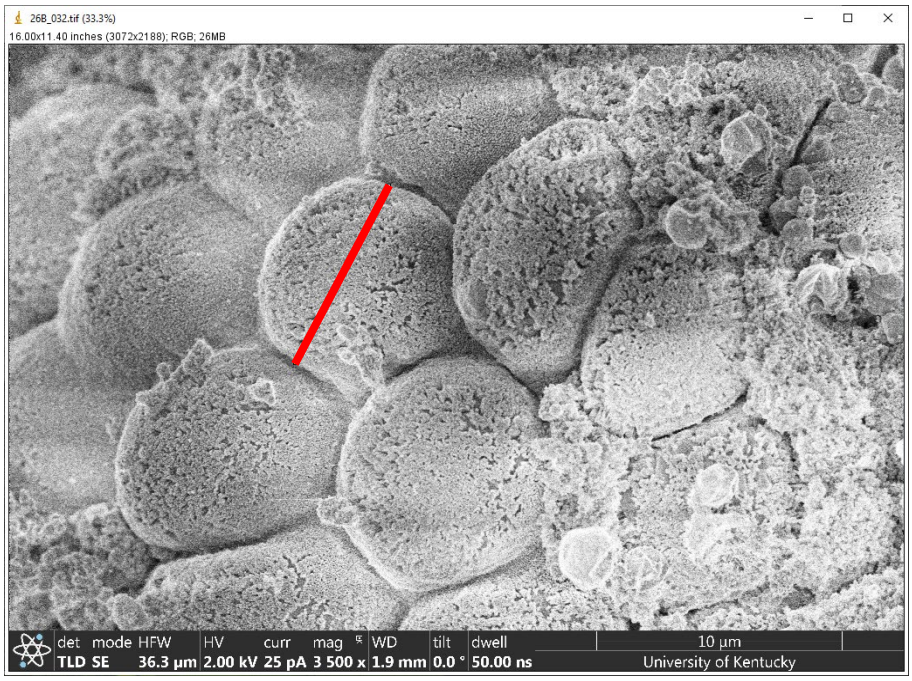

Figure 3.5. Example of enterocyte width measurement on scanning electron micrograph via ImageJ

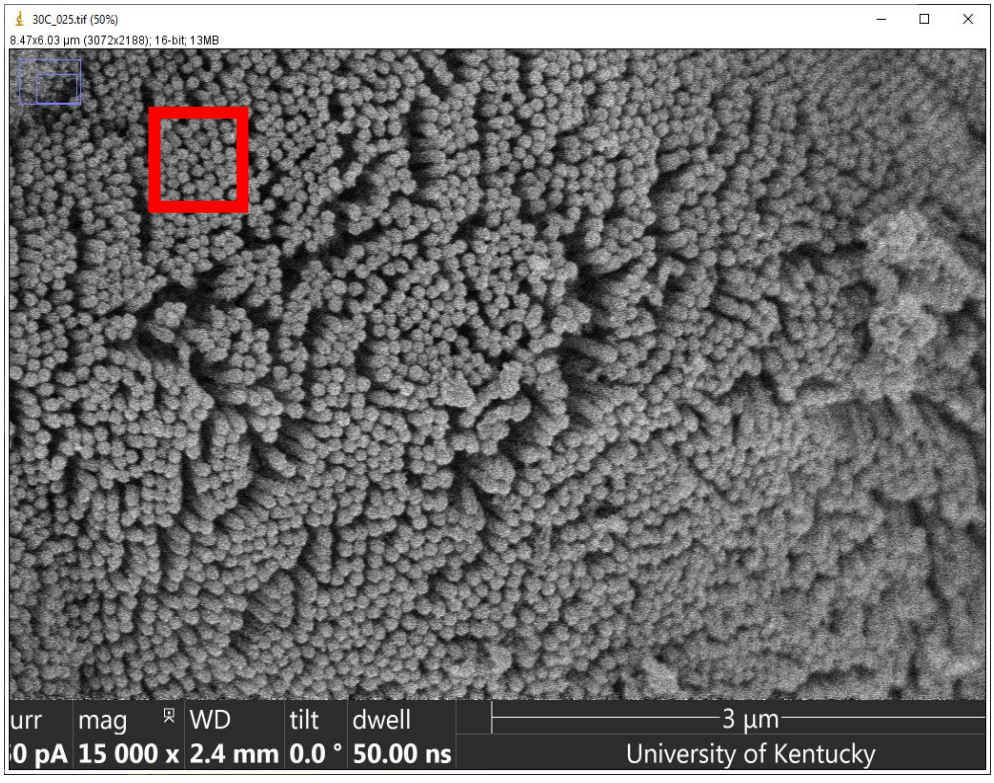

Figure 3.6. Example of microvilli density on a scanning electron micrograph via ImageJ

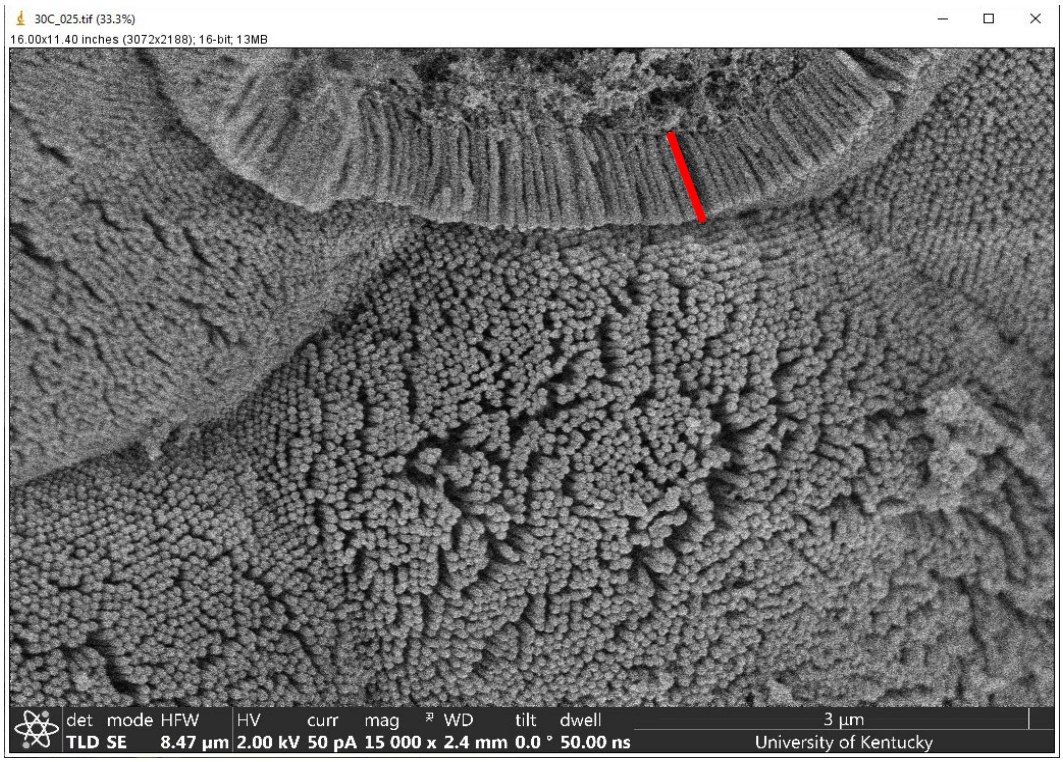

Figure 3.7. Example of microvilli length measurement on a scanning electron micrograph via ImageJ

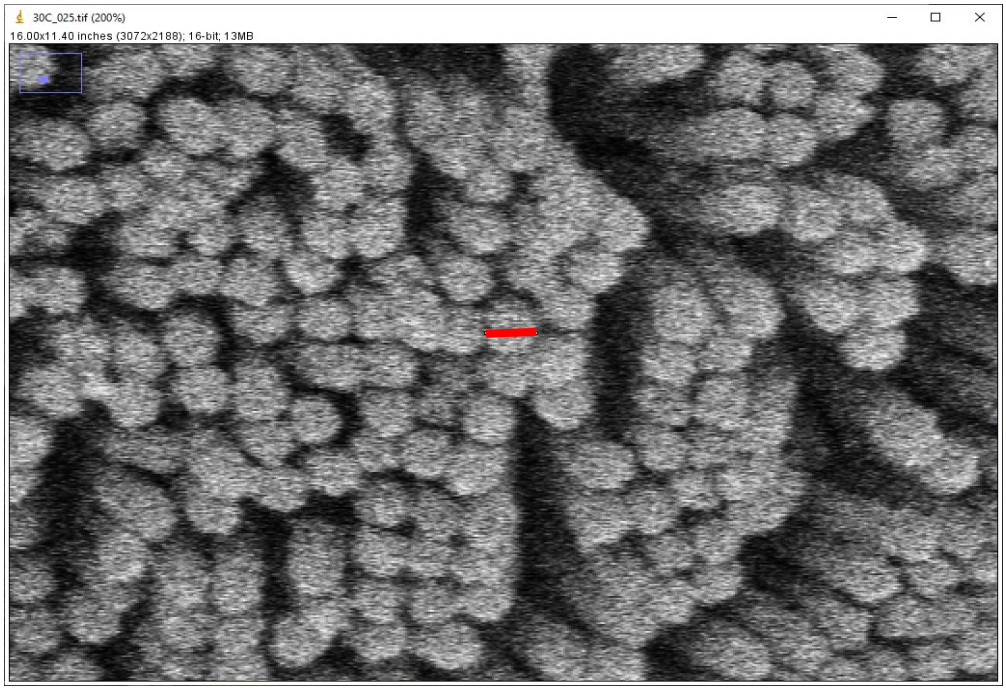

Figure 3.8. Example of microvilli diameter measurement on a scanning electron micrograph via ImageJ

3.3 Calculations and Statistical Analysis

3.3.1 *Body composition calculations and glycogen calculations*

All chemical components, ash, ether extract (EE), nitrogen (N), calcium (Ca), phosphorus (P), and glycogen concentrations, were reported on a DM basis for chemical composition of the pig. Crude protein (CP) was determined by multiplying N x 6.25. When glycogen is not reported with the chemical proximate composition of the pig, it is reported on a wet tissue basis to be consistent with published literature values.

The total amount of skeletal muscle in the body was calculated by multiplying live body weight by 40%. The 40% coefficient represents the literature values (de Lange et al., 2003) of ~54% muscle in the carcass and ~74% of BW being carcass weight. Then the total amounts (g) of glycogen in the liver and body muscle were added together and divided by BW to get percent glycogen in the total body.

The pig was the experimental unit and all data were subjected to analysis of variance using the PROC GLM procedure in SAS (SAS Inst. Inc., Gary, NC). Dependent variables evaluated were: live body weight (BW), DM, ash, EE, N, Ca, P, and glycogen concentrations in the body.

The effect of time/age was assessed using the following model:

$$Y_{ij} = \mu + \alpha_i + b_j + (ab)_{ij} + \varepsilon_{ij};$$

In this equation, the components represent:

Y_{ij} = the response variables (BW, DM, ash, EE, CP, Ca, P, and glycogen)

μ = a constant common to all observations

α_i = the time/age effects

b_j = the sex effects

$(ab)_{ij}$ = the interaction between time/age and sex

ε_{ij} = the error term of the model

Orthogonal polynomial contrasts were also used to determine the linear, quadratic, and/or cubic effect throughout the study as a response to age. PROC IML was used to obtain values to be used in orthogonal polynomial contrast statements, only through cubic contrasts, for the suckling and post-weaning period. Effects were considered significant if $P < 0.05$.

Data were subjected to outlier analysis prior to final statistical analyses. The process involved identifying potential outliers that were 3 standard deviations from the mean of the response measure within a collection day when the sample was not included in the day mean and then comparing that value to other values within the day, as well as reviewing study notes, before a final decision was made to exclude that value.

3.3.2 *Visceral organ*

Relative weights of the VO were determined by taking the absolute weight of the VO divided by the BW of the pig. Relative volumes of the GIT were determined by dividing the volume of a section of the GIT by total GIT volume. To determine the volume of the intestines, the circumference and the length were used in a tapered cylinder formula:

$$V = \frac{1}{3}\,\pi\,(r_1^2 + r_1 r_2 + r_2^2)\,h$$

In this equation, the components represent:

V = the volume

r_1 = the first end of the cylinder

r_2 = the second end of the cylinder

h = the length between each recorded circumference

Each circumference measurement was determined to be the end of the "cylinder" (0% and 25%, 25% and 50%, 50% and 75%, and 75% and 100% for the small intestine and 0% and 50%, and 50% and 100% for the large intestine). In all, four volume measurements were determined for the small intestine and two volume measurements were determined for the large intestine. Total volume is determined by the sum of the volume of the stomach, small intestine, cecum, and large intestine. To obtain the relative length of VO, the length of either the small or large intestine was divided by the sum of the small and large intestine length. Total length is the sum of the small and large intestine length

All data were subjected to regression analysis in the PROC GLM using contrast coefficients obtained from PROC IML in SAS (SAS Inst., Inc., Cary, NC) with the pig as the experimental unit. All results were reported as least square means. Dependent variables include heart, lungs, liver, kidneys, spleen, pancreas, stomach, cecum, volume of the stomach, small intestine, cecum, and large intestine, and length of the small and large intestine. Relative quantities of the VO were determined by taking absolute weight, volume, or length of organ and dividing it by the BW, total volume, or total length, respectively.

The effect of time/age was assessed using the following model:

$$Y_{ij} = \mu + \alpha_i + b_j + (ab)_{ij} + \varepsilon_{ij};$$

In this equation, the components represent:

Y_{ij} = the response variables (heart, lungs, liver, kidneys, spleen, pancreas, stomach, cecum, volume of the stomach, small intestine, cecum, and large intestine, length of the small and large intestine and their relative values)

μ = a constant common to all observations

α_i = the time/age effects

b_j = the sex effects

$(ab)_{ij}$ = the interaction between time/age and sex

ε_{ij} = the error term of the model

Orthogonal polynomial contrasts were also used to determine the linear, quadratic, and/or cubic effect throughout the study as a response to age. PROC IML was used to obtain values to be used in orthogonal polynomial contrast statements, only through cubic contrasts, for the suckling and post-weaning period. Effects were considered significant if $P < 0.05$.

Data were subjected to outlier analysis prior to final statistical analyses. The process involved identifying potential outliers that were 3 standard deviations from the mean of the response measure within a collection day when the sample was not included in the day mean and then comparing that value to other values within the day, as well as reviewing study notes, before a final decision was made to exclude that value.

3.3.3 *Small intestine morphology*

Analysis of the small intestine morphology was done via light microscopy to evaluate villi height, crypt depth, villi height:crypt depth ratio (V:C ratio), and goblet cell count and via scanning electron microscopy to evaluate villi width, villi density, enterocyte

width, microvilli diameter, and microvilli diameter. Enterocyte width was determined by the following information:

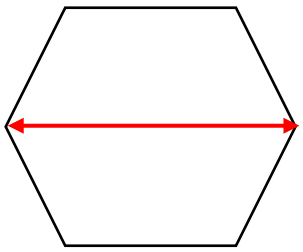

Figure 3.9. Example of measurement of enterocyte width

The above figure represents an enterocyte, as they are known for the hexagonal shape (Crawley et al., 2014a). The red line represents the measurement that was taken for "width" as it was the most accessible area of the enterocyte to measure consistently.

Small intestine surface area (SA) was determined using the formula for an "open cylinder":

$$SA = 2\pi \; X \; r \; X \; h$$

Where r = the radius of the small intestine, and h = the length of the intestine. This equation represents the small intestine if it were a "simple", flat tube. The calculation was also made using measurements at 25 - 50% and 75% - 100% measurements of the small intestine to calculate SA and to best utilize measurements obtained using both LM and ScEM. However, the equation above does not take into consideration the projections of villi and microvilli. To take these projections into consideration, the surface area of the villi and microvilli are calculated using a "half opened cylinder" SA equation:

$$SA = \; (2\pi \; X \; r \; X \; h) + \; (\pi \; X \; r^2)$$

Where r = the radius of the villi or microvilli, and h = the length of the villi or microvilli. Since there were complications obtaining the length of the microvilli, a literature value of 1.0 μm (Crawley et al., 2014b) was used in the calculation. When determining the amount of absorptive area the villi contribute to the small intestine, the surface area of the small intestine was multiplied by the density of the villi to get the total number of villi in the small intestine. This was done using measurements obtained at 50% and 75% to best utilize the data previously collected. Once the number of villi was determined, the number of villi was multiplied by the surface area of the villi to obtain the absorptive area of the villi in the small intestine. The amount that the villi increased the absorptive area of the small intestine was determined by dividing the absorptive area of the villi by the absorptive area of the assumed "flat" small intestine. The same calculation was done for microvilli with the exception that the absorptive area of the villi was multiplied by the density of the microvilli to take into consideration the already increased surface area due to villi.

All data were subjected to ANOVA with the PROC GLM procedure in SAS (SAS Inst. Inc., Gary, NC) with the pig being the experimental unit. Dependent variables that were evaluated are the following: body weight (BW), villi height, crypt depth, villus height:crypt depth ratio, goblet cell count, villi diameter, villi density, enterocyte width, microvilli density, microvilli diameter, microvilli length, small intestinal surface area, small intestine surface area due to villi, and small intestine surface area due to microvilli at the time of collection. Villi height, crypt depth, villus height:crypt depth ratio, goblet cell count, villi diameter, villi density, enterocyte width, microvilli density, microvilli diameter, microvilli length, small intestinal surface area, small intestine surface area due

to villi, and small intestine surface area due to microvilli were assessed based on their location in the small intestine. Furthermore, enterocyte width, microvilli density, microvilli diameter, and microvilli length were observed based on the position on the villi in addition to location in the small intestine.

The effect of time/age on BW, villi height, crypt depth, villus height:crypt depth ratio, goblet cell count, villi diameter, villi density, enterocyte width, microvilli density, microvilli diameter, microvilli length, and small intestinal surface areas was assessed using the following model:

$$Y_{ij} = \mu + \alpha_i + b_j + (ab)_{ij} + \varepsilon_{ij};$$

In this equation, the components represent:

Y_{ij} = the response variables (BW, villi height, crypt depth, villus height:crypt depth ratio, goblet cell count, villi diameter, villi density, enterocyte width, microvilli density, microvilli diameter, microvilli length, and small intestinal surface areas)

μ = a constant common to all observations

α_i = the time/age effect

b_j = the sex effects

$(ab)_{ij}$ = the interaction between time/age and sex

ε_{ij} = the error term of the model

The effect of time/age on BW, villi height, crypt depth, goblet cell count, villi diameter, villi density, enterocyte width, microvilli density, microvilli diameter, microvilli length when location in the small intestine is taken into consideration was assessed using the following model:

$$Y_{ij} = \mu + \alpha_i + b_j + (ab)_{ij} + L_{k(i)} + \varepsilon_{ijk};$$

In this equation, the components represent:

Y_{ij} = the response variables (BW, villi height, crypt depth, goblet cell count, villi diameter, villi density, enterocyte width, microvilli density, microvilli diameter, and microvilli length)

μ = a constant common to all observations

α_i = the time/age effect

b_j = the sex effects

$(ab)_{ij}$ = the interaction between time/age and sex

$L_{k(i)}$ = the location of the small intestine nested inside the effect of time/age

ε_{ijk} = the error term of the model

Orthogonal polynomial contrasts were also used to determine the linear, quadratic, and/or cubic effect throughout the study as a response to age. PROC IML was used to obtain values to be used in orthogonal polynomial contrast statements, only through cubic contrasts, for the suckling and post-weaning period. Effects were considered significant if $P < 0.05$.

Data were subjected to outlier analysis prior to final statistical analyses. The process involved identifying potential outliers that had a coefficient of variance over 20% within a collection day and then comparing that value to other values within the day, as well as reviewing study notes, before a decision was made to exclude that value. When n=3, the mean of the remaining values within a collection day were used to replace missing values. This was done because too many values were not included within a day to obtain orthogonal polynomial contrast during statistical analysis.

3.4 Results

Results tables are presented as LSMeans for data from late gestation through 42 d post-weaning to facilitate continuity of the entire data sets. Whereas data sets are presented in their entirety, the statistical evaluations were conducted, and are presented, for the subsets of the data. Although the gestational values are for 6 pigs at each time point, they are from one sow and are therefore one experimental unit and were not a part of statistical analysis; they are presented for reference only. The remaining data are divided into the suckling and post-weaning (PW) periods (with the weaning day included in each period) and are presented accordingly.

Tables are presented in the following order:

1) Body composition: Table 3.2.a, Table 3.2.b, and Figure 3.10
2) Glycogen; Table 3.3 and Figure 3.11
3) Visceral organ measures; Table 3.4.a, Table 3.4.b, Table 3.5.a, Table 3.5.b, Table 3.6, Figure 3.12, Figure 3.13, and Figure 3.14
4) Small intestine light microscopy: Table 3.7 Table 3.8, Table 3.9, and Figure 3.15, Figure 3.16, Figure 3.17, and Figure 3.18
5) Small intestine scanning electron microscopy: Table 3.10.a, Table 3.10.b, Table 3.11.a, Table 3.11.b, Figure 3.19, Figure 3.20, Figure 3.21, Figure 3.22, Figure 3.23, and Figure 3.24
6) Small intestine absorptive surface area: Table 3.12 and Table 3.13

Sex effect, location, age by sex, and/or age by location interactions will not be shown in the tables, but will be listed textually in the Results section when they occur.

3.4.1 *Body Composition*

The body composition of the pig over the course of the study are shown in Table 3.2a. and Figure 3.10 There were no observed sex effect or age by sex interactions for the chemical components of the body in the suckling period.

The responses ($P < 0.05$) to increasing age in the suckling period are the following:

DM: linear, quadratic, and cubic

Ash: linear and cubic

Crude Protein (CP; nitrogen x 6.25): linear and cubic

EE: linear and quadratic

Ca: linear

DM decreased immediately after birth from 20% to 18%, but then increased to 27.67% through the remainder of the suckling period. Ash was around 20% in the suckling period from birth to d 7 postpartum, until a decrease occurred at d 14 postpartum to 9.47%. CP increased till d 2 postpartum, when it then decreased from 61.45% to 49.85% through the rest of the suckling period. EE decreased d 1 post-partum, but then increased from d 2 post-partum at 5.12% through weaning at 19.62%. Total body glycogen in the suckling period was the greatest at birth (12.44%) and then continuously decreased till weaning (0.38%).

In the post-weaning period (Table 3.2.a), there was an age by sex interaction ($P = 0.02$) for EE in which there were multiple points of interaction but no clear pattern; at the end of the post-weaning period the barrows had more fat than the gilts. Post-weaning, BW of the pig increased (linear and cubic, $P < 0.05$) with age.

The responses ($P < 0.05$) to increasing age in the PW period are the following:

Ash: linear

CP: quadratic and cubic

Ca: linear

CP increased d 2 post-weaning, immediately decreased, increased at d 5 post-weaning, and then decreased through the rest of the post-weaning period. Total body glycogen had a relatively low inclusion rate in the body during the post-weaning period (0.38 - 2.27%) when compared to the suckling period (12.44 – 0.38%).

The total unaccounted for percent of the body composition (Table 3.2.b) was calculated by subtracting the sum of ash, CP, EE, and total body glycogen percentages from 100. The result from this calculation should be essentially zero, which would show that the entirety of the pig was accounted for in the laboratory analysis. Unexpectedly, the total unaccounted for percent of the body composition is above zero (between 2 - 22%) in both the suckling and PW periods of the study, showing that there was inaccuracy in the laboratory analysis.

In the suckling period, the unaccounted for fraction is consistently under 20%, with the days from birth till 5 days postpartum being between 2 - 12%. Post-weaning, the unaccounted for fraction was around 20% with the exception of day 5 PW, in which the unaccounted for fraction was 8.44%. However, overall the unaccounted for fraction in the PW period was greater than that in the suckling period.

Table 3.2a. Proximate analysis (DM basis) of pigs from birth through 42 d PW[1]

Age, days	BW, kg[2,5,6]	DM, %[2,3,4]	%, on DM basis				
			Ash[2,4,5]	Crude Protein[2,4,6,7]	EE[2,3]	Ca[2,5]	P
Gest. 108	1.15	19.57[8]	15.97	53.84	3.22	7.19[8]	4.17
Gest. 109	1.42	19.51	19.49[8]	61.63[8]	2.62	6.33[8]	3.83[8]
Gest. 110	1.50	17.94	22.34[8]	58.45	2.36	7.30	3.92
Gest. 111	1.51	19.53[8]	19.27[8]	59.44[8]	2.36	5.40[8]	3.56[8]
Gest. 112	1.24	19.63[8]	21.78	55.41	3.41	6.54	4.04
Gest. 113	1.36	21.28	23.24[8]	56.36	2.48	7.55	4.15
0 (Birth)	1.56	20.08[8]	15.62[8]	54.75	5.75	6.00	3.29
1	1.57	18.24[8]	20.06[8]	58.63	3.90[8]	5.86[8]	3.38[8]
2	1.74	19.97[8]	21.01[8]	61.45[8]	5.12	5.47[8]	2.96[8]
3	1.67	24.77[8]	16.10	59.45	8.61	4.79[8]	2.55[8]
5	2.47	25.23	20.53	57.61[8]	8.48	6.59	3.50
7	2.67	25.49[8]	17.16[8]	53.36[8]	15.21	5.73[8]	3.07[8]
14	5.26	27.46	9.47[8]	51.40	18.34	2.94[8]	1.66[8]
21 (W)	6.40	27.67[8]	13.32	49.85	19.62	4.79	2.80
22 (PW1)	5.62	28.45	9.55[8]	53.95[8]	19.35	2.96[8]	1.62[8]
23 (PW2)	6.27	26.70[8]	12.06[8]	57.35[8]	20.90	3.68	2.06
24 (PW3)	5.77	26.79	9.78[8]	56.20	18.97	2.78[8]	1.69[8]
26 (PW5)	6.34	26.32[8]	14.48	58.04[8]	17.74	4.46	2.52
28 (PW7)	6.69	25.56	10.43	57.07[8]	18.50	3.52[8]	1.76
35 (PW14)	9.55	27.56[8]	9.32	55.018	19.70	2.47[8]	1.29[8]
49 (PW28)	18.55	26.82[8]	8.88[8]	55.88[8]	18.15	3.06	1.89
63 (PW42)	28.12	27.48	7.10	54.28[8]	18.24[8]	2.18	1.38
SEM[9]	0.40/ 0.94	0.67/ 0.79	2.54/ 1.83	1.42/1.45	1.97/ 3.67	0.83/ 0.69	0.46/ 0.39

[1]Proximate analysis was conducted on the whole body, except the gastrointestinal tract, of the pig; PW=post-weaning, crude protein=nitrogen X 6.25, EE=ether extract, Ca=calcium, P=phosphorous, Gest.=gestation day, W=weaning; gestation day means are reported but not included in the statistical analysis, statistical analysis was assessed from birth to weaning (the suckling period) and from weaning to 42 d PW (the post-weaning period); n=6 per mean unless otherwise noted.

[2,3,4]Linear, quadratic, or cubic response, respectively, to age in the suckling period.

[5,6,7]Linear, quadratic, or cubic response, respectively, to age in the PW period.

[8]n=5.

[9]SEM values are presented as suckling period SEM / PW period SEM.

Table 3.3b. Unaccounted percent for body composition of pigs from birth through 42 d PW[1]

Age, days	BW, kg[2,5,6]	%, on DM Basis				Total unaccounted for, DM%
		Ash[2,4,5]	Crude Protein[2,4,6,7]	EE[2,3]	Total body glycogen	
Gest.108	1.15	15.97	53.84	3.22	12.94[8]	13.76
Gest.109	1.42	19.49[8]	61.63[8]	2.62	9.78[8]	4.85
Gest.110	1.50	22.34[8]	58.45	2.36	12.49[8]	6.71
Gest.111	1.51	19.27[8]	59.44[8]	2.36	19.06[8]	0.04
Gest.112	1.24	21.78	55.41	3.41	13.07	6.97
Gest.113	1.36	23.24[8]	56.36	2.48	16.39[8]	3.71
0 (Birth)	1.56	15.62[8]	54.75	5.75	12.44[8]	10.84
1	1.57	20.06[8]	58.63	3.90[8]	6.85[8]	9.72
2	1.74	21.01[8]	61.45[8]	5.12	4.33[8]	2.03
3	1.67	16.10	59.45	8.61	3.77	11.82
5	2.47	20.53	57.61[8]	8.48	2.01	7.51
7	2.67	17.16[8]	53.36[8]	15.21	1.36	18.86
14	5.26	9.47[8]	51.40	18.34	0.65[8]	17.81
21 (W)	6.40	13.32	49.85	19.62	0.38[8]	18.50
22 (PW1)	5.62	9.55[8]	53.95[8]	19.35	0.65[8]	20.74
23 (PW2)	6.27	12.06[8]	57.35[8]	20.90	0.70[8]	15.86
24 (PW3)	5.77	9.78[8]	56.20	18.97	1.34	18.67
26 (PW5)	6.34	14.48	58.04[8]	17.74	1.93[8]	8.44
28 (PW7)	6.69	10.43	57.07[8]	18.50	2.27	16.44
35 (PW14)	9.55	9.32	55.018	19.70	1.51	14.42
49 (PW28)	18.55	8.88[8]	55.88[8]	18.15	0.69[8]	21.01
63 (PW42)	28.12	7.10	54.28[8]	18.24[8]	0.26	17.01
SEM[9]	0.72	2.54/ 1.83	1.42/1.45	1.97/ 3.67	1.26/0.37	8.86/8.81

[1] Proximate analysis was conducted on the whole body, except the gastrointestinal tract, of the pig; Total unaccounted for determined by subtracting the sum of ash, protein, ether extract, and glycogen, all on a DM basis, from 100; PW=post-weaning, Gest.=gestation day, W=weaning; gestation day means reported but not included in statistical analysis, n=6 per mean unless otherwise noted.

[2,3,4]Linear, quadratic, or cubic response ($P < 0.05$) to age in the suckling period.

[5,6,7]Linear, quadratic, or cubic response ($P < 0.05$) to age in the PW period.

[8]n=5.

[9]SEM values are presented as suckling period SEM / PW period SEM.

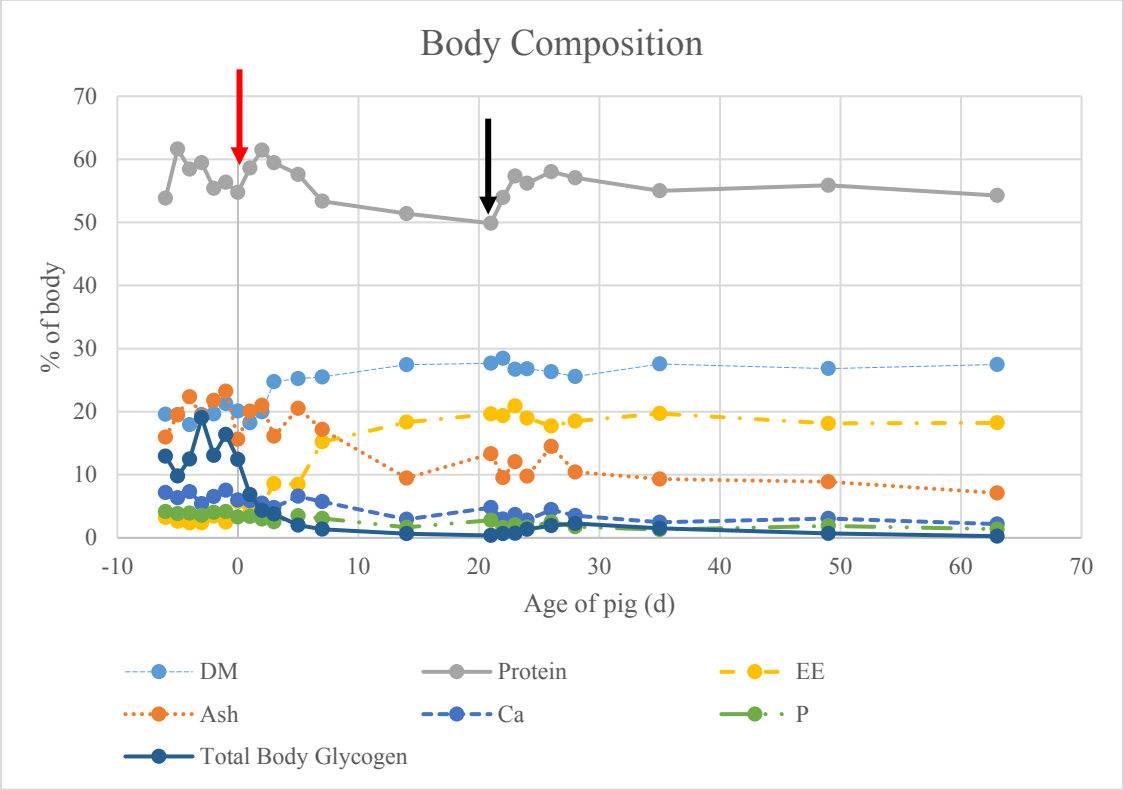

Figure 3.10 Body composition from birth through 42 d post-weaning. Birth is indicated by a red arrow, and weaning is indicated by a black arrow.

3.4.2 *Glycogen*

The effect of age on glycogen response measures (as is basis) is reported in Table 3.3 and Figure 3.11. There were no sex or age by sex interaction effects for any of the glycogen measures in either the suckling or PW period.

The responses ($P < 0.05$) to increasing age in the suckling period are the following:

Concentration liver glycogen: linear, quadratic, and cubic

Concentration muscle glycogen: linear, quadratic, and cubic

Amount (g) liver glycogen: linear and cubic

Amount (g) muscle glycogen: linear, quadratic, and cubic

Total body glycogen (%): linear, quadratic, and cubic

In the suckling period, the concentration of liver glycogen decreased immediately following birth from 76 mg/g to roughly 14 mg/g. On d 3 post-partum, liver glycogen increased until d 5 post-partum. Following d 5 in the suckling period, liver glycogen decreased from 42 mg/g to roughly 23 mg/g. The concentration of muscle glycogen decreased from 62.81 mg/g at birth to roughly 22 mg/g on d 2 postpartum. Muscle glycogen concentration then continued to decrease after d 3 postpartum from roughly 22 mg/g to 1.61 mg/g.

The responses ($P < 0.05$) to increasing age in the PW period are the following:

Concentration liver glycogen: linear, quadratic, and cubic

Concentration muscle glycogen: linear, quadratic, and cubic

Amount (g) liver glycogen: linear and quadratic

Amount (g) muscle glycogen: linear, quadratic, and cubic

Total body glycogen (%): linear, quadratic, and cubic

In the post-weaning period, there is an immediate decrease in the liver glycogen concentration from 23.36 mg/g to 9.61 mg/g on d 1 post-weaning. This is followed by an increase in the liver glycogen concentration to 37.68 mg/g on d 3 PW. Liver glycogen concentration then decreased once more to 8.69 mg/g on d 42 PW. Muscle glycogen concentration increased from 1.61 mg/g at weaning to 9.73 mg/g on d 5 PW, then it decreased to 1.97 mg/g on d 42 PW.

Table 3.4 Glycogen concentration, on an as is basis, of pigs from birth through 42 d PW[1]

Age, days	BW, kg[2,5,6]	Concentration, mg/g		Glycogen in tissue, g		Total glycogen in body, %[2,3,4,5,6,7]
		Liver[2,3,4,5,6,7]	Muscle[2,3,4,5,6,7]	Liver[2,4,5,6]	Muscle[2,3,4,5,6,7]	
Gest.108	1.15	35.95	60.00[8]	1.14	29.08[8]	2.50[5]
Gest.109	1.42	40.48[8]	49.87	1.58[8]	28.53	2.15[5]
Gest.110	1.50	35.26	53.89[8]	1.52	31.98[8]	2.26[5]
Gest.111	1.51	47.24	65.93[8]	2.67	40.06[8]	2.79[5]
Gest.112	1.24	61.28	61.67	2.90	30.55	2.70
Gest.113	1.36	66.23[8]	68.47	3.39[8]	36.80	2.92[5]
0 (Birth)	1.56	76.00	62.81[8]	3.69	36.08[8]	2.73[5]
1	1.57	13.37	29.15[8]	0.63	16.07[8]	1.19[5]
2	1.74	14.24[8]	22.57	0.91[8]	16.65	0.91[5]
3	1.67	24.39	21.42	1.69	14.00	0.96
5	2.47	42.65	11.13	4.39	10.69	0.62
7	2.67	24.49	6.35	2.48	6.85	0.35
14	5.26	25.70[8]	2.55	4.56[8]	4.92	0.16[5]
21 (W)	6.40	23.36	1.61[8]	3.89	4.26[8]	0.12[5]
22 (PW1)	5.62	9.61	4.26[8]	1.19	10.08[8]	0.19[5]
23 (PW2)	6.27	22.28[8]	2.71[8]	3.47[8]	6.46[8]	0.13[5]
24 (PW3)	5.77	37.68	7.27	5.77	17.30	0.39
26 (PW5)	6.34	36.16	9.73[8]	6.73	23.27[8]	0.49[5]
28 (PW7)	6.69	23.78	9.51	4.27	25.04	0.45
35 (PW14)	9.55	26.21	9.54	7.67	37.47	0.46
49 (PW28)	18.55	13.03[8]	2.99	8.06[8]	22.23	0.17[5]
63 (PW42)	28.12	8.69	1.97	6.86	21.71	0.10
SEM[9]	0.40/ 0.94	4.33/ 3.43	2.91/ 1.03	0.58/ 1.23	3.42/5.15	0.12/0.06

[1]Glycogen analysis on the left medial lobe in the liver and right gluteal muscle group of the pig; PW=post-weaning, Gest.=gestation day, W=weaning; gestation day means are reported but not included in the statistical analysis, statistical analysis was assessed from birth to weaning (the suckling period) and from weaning to 42 d post-weaning (the post-weaning period); n=6 per mean unless otherwise noted.

[2,3,4]Linear, quadratic, or cubic response ($P < 0.05$), respectively, to age in the suckling period.

[5,6,7]Linear, quadratic, or cubic response ($P < 0.05$), respectively, to age in the PW period.

[8]n=5.

[9]SEM values are presented as suckling period SEM / PW period SEM.

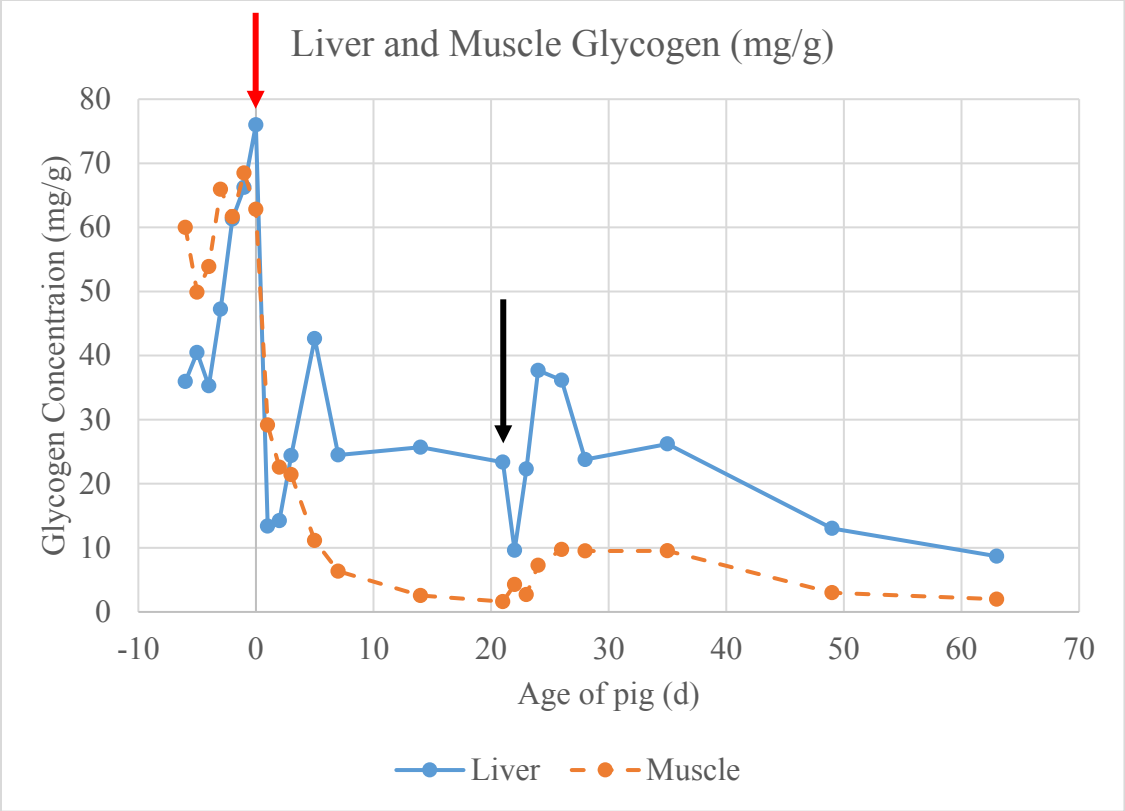

Figure 3.11. Liver and muscle glycogen concentrations from birth to 42 d post-weaning. Birth is represented by the red arrow, and weaning is represented by the black arrow.

3.4.3 *Visceral organs*

All visceral organ (VO) absolute weight increased with age (Table 3.4a and Table 3.4.b, and Figure 3.12). In the suckling period of the study, there were no observed sex effect or age by sex interaction.

The responses ($P < 0.05$) for absolute and relative weight, as a percent of body weight, of the VO to increasing age in the suckling period are the following:

Liver absolute weight: linear and quadratic

Liver relative weight: linear, quadratic, and cubic

Kidneys absolute weight: linear

Kidneys relative weight: linear, quadratic, and cubic

Spleen absolute weight: linear

Spleen relative weight: linear, quadratic, and cubic

Pancreas absolute weight: linear

Pancreas relative weight: linear

Heart absolute weight: linear

Heart relative weight: linear, quadratic, and cubic

Lungs absolute weight: linear, quadratic, and cubic

Lungs relative weight: linear and cubic

Stomach absolute weight: linear

Stomach relative weight: linear, quadratic, and cubic

Cecum absolute weight: linear

Cecum relative weight: linear and cubic

In the suckling period, all absolute weights increased with increasing age. The relative weight of the liver increased from 2.99% at birth to 4.20% at d 5 postpartum. After d 5 postpartum, relative liver weight decreased to 2.73% at weaning. The relative weight of the pancreas increased from birth to d 3 postpartum, 0.14% to 0.21%, and then decreased from 0.21% to 0.12% at the end of the suckling period. The relative weight of the spleen and the cecum increased through the entire suckling period from 0.09% to roughly 0.23% and 0.09% to 0.13%, respectively.

After weaning occurred, all absolute weights of the VO increased (linear, $P < 0.05$). There was a sex effect for the absolute weight of the spleen ($P = 0.03$) where the barrows had heavier spleens than the gilts. The responses ($P < 0.05$) for absolute and relative weight of the VO to increasing age in the PW period are the following:

Liver absolute weight: linear and cubic

Liver relative weight: linear and quadratic

Kidneys absolute weight: linear

Spleen absolute weight: linear and quadratic

Spleen relative weight: linear and quadratic

Pancreas absolute weight: linear, quadratic, and cubic

Pancreas relative weight: linear and quadratic

Heart absolute weight: linear and quadratic

Heart relative weight: linear

Lungs absolute weight: linear and quadratic

Lungs relative weight: linear

Stomach absolute weight: linear

Stomach relative weight: linear, quadratic, and cubic

Cecum absolute weight: linear

Cecum relative weight: linear, quadratic, and cubic

In the post-weaning period, the relative weight of the liver had a drop from 2.73% to 2.31% immediately following weaning, but then increased through the rest of the PW period until d 42 PW, were there was a decrease from 3.15% to 2.86%. The relative weight of the pancreas increased from weaning at 0.12% to d 28 PW at 0.26%. The relative weight of the spleen increased from 0.23% at weaning to 0.25% on d 3 PW. The relative weight of the spleen then decreased to 0.17% on d 28 PW, and then increased to 0.20% on d 42 PW. Similar to the suckling period, the relative weight of the cecum increased from 0.13% to 0.25% in the PW period.

The effect of age on the volume of the gastrointestinal organs can be found in Table 3.5a., Table 3.5b., and Figure 3.13. During the suckling period, there was no sex effect or age by sex interaction. The responses ($P < 0.05$) for absolute and relative volume of the VO to increasing age in the suckling period are the following:

Total volume: linear, quadratic, and cubic

Stomach absolute volume: linear and quadratic

Stomach relative volume: linear, quadratic, and cubic

Small intestine absolute volume: linear and quadratic

Small intestine relative volume: quadratic

Cecum absolute volume: linear

Cecum relative volume: linear

Large intestine absolute volume: linear and cubic

Large intestine relative volume: linear

All absolute volumes increased in both the suckling and PW period with an increase in age. The relative volume of the stomach increased from birth to d 3 postpartum, from 15.08% to 22.38%, from there it then decreased to 8.14% at weaning. The relative volume of the small intestine decreased from birth to d 7 postpartum, from 73.88% to 62.38%. Relative volume of the small intestine then increased till weaning at 6.25%. The relative volume of the cecum increased from 1.07% to 4.91% at weaning. The relative volume of the large intestine increased immediately after birth from 9.97% to 19.45% d 1 postpartum. Relative volume of the large intestine then decreased till d 5 postpartum at 12.66%. Large intestine relative volume then increased to roughly 20% at the time of weaning.

Post-weaning, there was an age by sex interaction for the relative volume of the small intestine ($P = 0.01$) with two points of interaction between day 5 and 14 PW and the gilts had greater relative small intestine volumes at the end of the PW period, and there was a sex effect for the relative large intestine volume ($P = 0.02$) where the barrows had greater relative large intestinal volumes than the gilts. The responses ($P < 0.05$) for absolute and relative volume of the VO to increasing age in the PW period are the following:

Stomach absolute volume: linear

Stomach relative volume: linear and cubic

Small intestine absolute volume: linear, quadratic, and cubic

Small intestine relative volume: linear, quadratic, and cubic

Cecum absolute volume: linear and cubic

Cecum relative volume: cubic

Large intestine absolute volume: linear and quadratic

Large intestine relative volume: linear, quadratic, and cubic

In the post-weaning period of the study, the relative volume of the stomach decreased immediately post-weaning from 8.14% to 6.37%. Stomach relative volume then increased to 16.70% on d 3 PW. Stomach relative volume then decreased to 6.58% on d 42 PW. The relative volume of the small intestine decreased from weaning to d 5 PW, from 67.25% to 44.87%. The relative volume of the small intestine then increased to 54.51% on d 14 PW and then decreased to 50.28% on d 42 PW. The relative volume of the cecum increased from 4.91% at weaning to 6.28% on d 5 PW. Relative volume of the cecum then decreased to 4.34% on d 14 PW. It then increased to 8.65% and decreased to 5.05% on d 28 PW and d 42 PW, respectively. The relative volume of the large intestine increased from weaning to d 5 PW, from 19.70% to 36.13%. The relative volume of the large intestine then decreased to 38.08% on d 42 PW.

The effect of an increasing age on the length of the small and large intestine can be found in Table 3.6 and Figure 3.14. There were no sex effect or age by sex interactions in either the suckling or PW period. The responses ($P < 0.05$) for absolute and relative length of the VO to increasing age in the suckling period are the following:

Total length: linear and quadratic

Small intestine absolute length: linear and quadratic

Large intestine absolute length: linear and quadratic

The responses ($P < 0.05$) for absolute and relative volume of the VO to increasing age in the PW period are the following:

Total length: linear

Small intestine absolute length: linear and cubic

Small intestine relative length: linear and quadratic

Large intestine absolute length: linear and cubic

Large intestine relative length: linear and quadratic

In the post-weaning period of the study, the relative length of the small intestine decreased from 85.46% to 81.30% between weaning and d 7 PW. The relative length of the small intestine then increased to 82.26% on d 42 PW. The relative length of the large intestine increased from 14.54% at weaning to 18.70% on d 7 PW. Then the relative length of the large intestine decreased to roughly 17.50% at the end of the PW period.

Table 3.5a. Absolute and relative weights of selected visceral organs from birth to 42 d PW[1]

Age, days	BW, kg[2,6,7]	Organ weight, absolute g and as (% BW)			
		Liver[2,3,4,5,6,8,9]	Kidneys[2,4,5,6]	Spleen[2,4,5,6,7,9]	Pancreas[2,4,6,7,8,9]
Gest.108	1.15	31.20 (2.67)	8.37 (0.73)	1.38 (0.12)	1.37 (0.12)[10]
Gest.109	1.42	38.29 (2.67)	8.84 (0.62)	1.69 (0.12)	1.66 (0.12)
Gest.110	1.50	42.80 (2.86)	10.30 (0.69)	1.88 (0.13)	1.63 (0.11)
Gest.111	1.51	54.18 (3.58)	8.97 (0.61)	1.68 (0.11)	1.61 (0.11)[10]
Gest.112	1.24	45.81 (3.69)	8.93 (0.72)	1.34 (0.11)	1.35 (0.11)
Gest.113	1.36	50.99 (3.73)	9.16 (0.67)	1.44 (0.10)	1.66 (0.12)
0 (Birth)	1.55	47.28 (2.99)	8.78 (0.57)	1.47 (0.09)	2.38 (0.14)
1	1.57	41.73 (2.55)	11.88 (0.78)	1.95 (0.12)	2.32 (0.14)
2	1.74	61.88 (3.54)	13.22 (0.76)	2.28 (0.13)	2.94 (0.17)[10]
3	1.67	68.08 (4.09)	13.53 (0.82)	2.97 (0.18)	3.52 (0.21)
5	2.47	103.00 (4.20)	19.38 (0.79)	5.52 (0.22)	4.20 (0.17)
7	2.67	98.72 (3.71)	21.37 (0.80)	7.23 (0.27)	4.35 (0.16)
14	5.26	166.25 (3.16)	30.70 (0.59)	12.63 (0.24)	6.98 (0.13)
21 (W)	6.40	176.17 (2.73)	35.93 (0.55)	14.58 (0.23)	7.63 (0.12)
22 (PW1)	5.62	130.07 (2.31)	31.10 (0.56)	12.25 (0.22)	7.53 (0.13)
23 (PW2)	6.27	159.55 (2.54)	32.00 (0.51)	13.13 (0.21)	7.53 (0.12)
24 (PW3)	5.77	159.13 (2.76)	29.17 (0.50)	14.47 (0.25)	7.40 (0.13)
26 (PW5)	6.34	182.65 (2.83)	33.60 (0.53)	12.23 (0.20)	9.62 (0.16)
28 (PW7)	6.69	195.17 (2.91)	39.53 (0.58)	14.72 (0.22)	13.98 (0.21)
35 (PW14)	9.55	302.57 (3.17)	57.32 (0.59)	16.42 (0.17)	20.72 (0.22)
49 (PW28)	18.55	586.25 (3.15)	94.42 (0.51)	30.93 (0.17)	47.5 (0.26)
63 (PW42)	28.12	800.68 (2.86)	137.38 (0.49)	54.75 (0.20)	67.77 (0.24)
SEM[11]	0.40/ 0.94	13.47/28.42 (0.18/0.08)	2.67/5.47 (0.04/0.02)	1.02/2.23 (0.01/0.01)	0.75/1.62 (0.02/0.01)

[1]Values in the parentheses represent the relative weight to bodyweight; PW=post-weaning, Gest.=gestation day, W=weaning; gestation day means are reported but not included in the statistical analysis, statistical analysis was assessed from birth to weaning (the suckling period) and from weaning to 42 d PW (the post-weaning period); n=6 per mean unless otherwise noted.

[2,3]Linear or quadratic response ($P < 0.05$) to age for absolute weight, respectively, suckling.

[4]Linear and cubic response ($P < 0.05$) to age for relative weight suckling.

[5]Quadratic response ($P < 0.05$) to age for relative weight suckling.

[6,7,8]Linear, quadratic, or cubic response ($P < 0.05$) to age for absolute weight, respectively, PW.

[9]Linear and quadratic response ($P < 0.05$) to age for relative weight PW.

[10]n=5.

[11]SEM values are presented as absolute weights suckling period SEM /PW period SEM (relative weights suckling period SEM / PW period SEM).

Table 3.6b. Absolute and relative weights of selected visceral organs from birth to 42 d PW[1]

Age, days	BW, kg[2,7,8]	Organ weight, absolute g and as (% BW)			
		Heart[2,5,6,7,8,9,10]	Lungs[2,3,4,5,7,8,9,10]	Stomach[2,5,6,7,8,11,12,13]	Cecum[2,5,7,8,11,12,13]
Gest.108	1.15	7.77 (0.67)	30.83 (2.67)	6.22 (0.52)[14]	0.67 (0.06)[14]
Gest.109	1.42	11.76 (0.82)	36.93 (2.59)	9.00 (0.63)	0.9 (0.06)
Gest.110	1.50	11.95 (0.80)	36.70 (2.43)	8.50 (0.57)	0.97 (0.06)
Gest.111	1.51	9.90 (0.66)	47.05 (3.10)	9.42 (0.62)	0.82 (0.06)
Gest.112	1.24	8.81 (0.71)	29.09 (2.36)	7.20 (0.58)	0.68 (0.05)
Gest.113	1.36	9.25 (0.68)	34.46 (2.53)	7.74 (0.57)	0.64 (0.05)
0 (Birth)	1.55	11.08 (0.72)	27.67 (1.86)	7.18 (0.56)[15]	1.25 (0.09)
1	1.57	10.80 (0.69)	26.72 (1.77)	8.95 (0.59)	1.52 (0.10)
2	1.74	12.48 (0.73)	32.00 (1.88)	12.10 (0.67)[14]	1.55 (0.09)
3	1.67	13.55 (0.81)	33.63 (2.01)	11.17 (0.67)	1.70 (0.10)
5	2.47	18.28 (0.74)	42.75 (1.73)	15.48 (0.62)	2.12 (0.09)
7	2.67	17.30 (0.66)	45.78 (1.74)	16.60 (0.62)	2.85 (0.11)
14	5.26	28.47 (0.54)	88.88 (1.65)	24.62 (0.47)	6.38 (0.12)
21 (W)	6.40	32.32 (0.51)	74.75 (1.17)	30.45 (0.49)	8.20 (0.13)
22 (PW1)	5.62	29.07 (0.52)	74.25 (1.34)	28.68 (0.51)	7.07 (0.13)
23 (PW2)	6.27	34.70 (0.56)	81.80 (1.30)	33.03 (0.54)	9.20 (0.15)
24 (PW3)	5.77	31.42 (0.54)	67.32 (1.17)	35.25 (0.61)	7.92 (0.14)
26 (PW5)	6.34	33.78 (0.53)	72.18 (1.17)	42.70 (0.69)	14.2 (0.24)
28 (PW7)	6.69	35.83 (0.53)	74.80 (1.13)	54.90 (0.83)	14.2 (0.21)
35 (PW14)	9.55	47.48 (0.50)	106.47 (1.12)	78.30 (0.82)	25.42 (0.28)
49 (PW28)	18.55	87.03 (0.47)	164.88 (0.90)	145.65 (0.79)	52.82 (0.28)
63 (PW42)	28.12	130.07 (0.46)	242.47 (0.87)	197.48 (0.71)	69.27 (0.25)
SEM[16]	0.40/ 0.94	2.08/3.98 (0.04/0.02)	7.36/8.51 (0.17/0.06)	2.19/6,92 (0.05/0.03)	0.63/2.63 (0.01/0.02)

[1]Values in the parentheses represent the relative weight to bodyweight; PW=post-weaning, Gest.=gestation day, W=weaning; gestation day means are reported but not included in the statistical analysis, statistical analysis was assessed from birth to weaning (the suckling period) and from weaning to 42 d PW (the post-weaning period); n=6 per mean unless otherwise noted.

[2,3,4,5,6,7]Linear, quadratic, or cubic response ($P < 0.05$) for absolute weight and linear, quadratic, or cubic response ($P < 0.05$) for relative weight, respectively, to age in the suckling period.

[8,9,10,11,12,13]Linear, quadratic, or cubic response ($P < 0.05$) for absolute weight and linear, quadratic, or cubic response ($P < 0.05$) for relative weight, respectively, to age in the PW period.

[14,15]n=5, n=4, respectively.

[16]SEM values are presented as absolute weights suckling period SEM /PW period SEM (relative weights suckling period SEM / PW period SEM).

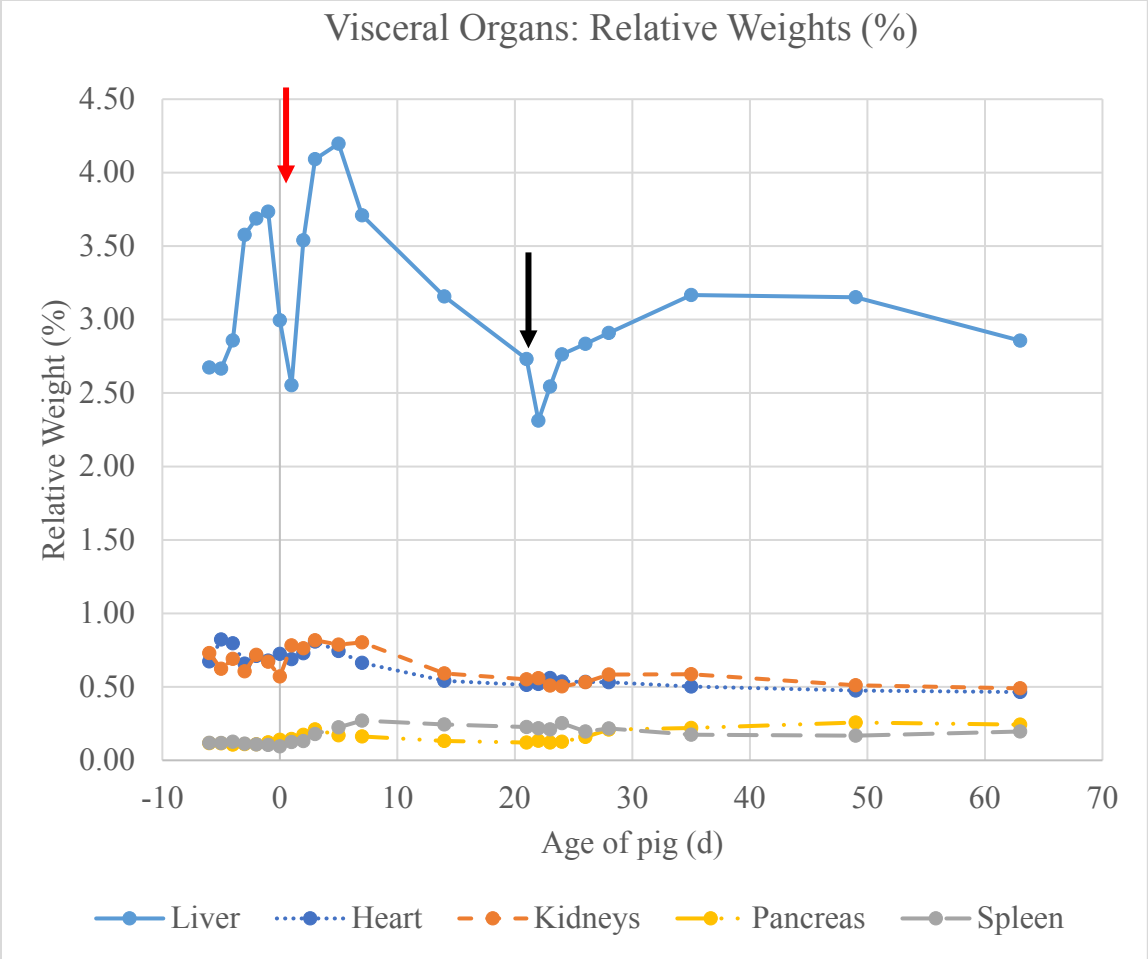

Figure 3.12 Relative weights of selected organs from birth through 42 d post-weaning. Birth is represented by the red arrow, and the black arrow indicates weaning.

Table 3.7a. Absolute and relative volume of selected visceral organs from birth to 42 d PW[1]

Age, days	BW, kg[2,8,9]	Organ volume, absolute ml and as (% Total Volume)		
		Total Volume[2,3,4,8,9]	Stomach[2,3,5,6,7,8,11,13]	Small Intestine[2,4,6,8,9,10,11,12,13]
Gest.108	1.15	30.18	6.09 (22.73)	17.82 (57.02)
Gest.109	1.42	42.65	10.05 (23.85)	24.58 (57.34)
Gest.110	1.50	40.36	5.75 (14.59)	25.26 (62.01)
Gest.111	1.51	50.45	12.63 (26.25)	29.76 (57.86)
Gest.112	1.24	42.48	9.77 (23.01)	25.75 (60.54)
Gest.113	1.36	47.21	10.58 (22.38)	28.1 (59.76)
0 (Birth)	1.55	74.32	11.28 (15.08)[14]	56.02 (73.88)
1	1.57	86.15	10.17 (12.41)	57.35 (66.13)
2	1.74	116.65	23.68 (20.50)	75.83 (65.01)
3	1.67	105.91	23.84 (22.38)	66.99 (63.44)
5	2.47	158.17	34.82 (21.99)	99.96 (63.36)
7	2.67	200.30	34.74 (17.61)	126.83 (62.38)
14	5.26	404.00	39.33 (9.83)	260.47 (65.00)
21 (W)	6.40	419.15	33.20 (8.14)	278.09 (67.25)
22 (PW1)	5.62	332.28	19.27 (6.37)	206.4 (65.28)
23 (PW2)	6.27	486.74	68.09 (14.05)	267.79 (55.00)
24 (PW3)	5.77	450.52	74.55 (16.70)	204.79 (45.97)
26 (PW5)	6.34	649.34	82.35 (12.71)[14]	264. (44.87)
28 (PW7)	6.69	788.64	91.08 (12.56)	391.06 (47.27)
35 (PW14)	9.55	1334.18	125.86 (9.07)	728. (54.51)
49 (PW28)	18.55	3187.75	228.75 (7.08)	1663.45 (52.77)
63 (PW42)	28.12	4796.57	314.54 (6.58)	2477.08 (50.28)
SEM[15]	0.40/ 0.94	27.83/172.74	3.94/16.30 (2.67/1.31)	14.76/82.95 (3.38/2.53)

[1]Values in the parentheses represent the relative volume to total volume, total volume= sum of all individual segment volumes; PW=post-weaning, Gest.=gestation day, W=weaning; gestation day means are reported but not included in the statistical analysis, statistical analysis was assessed from birth to weaning (the suckling period) and from weaning to 42 d PW (the post-weaning period); n=6 per mean unless otherwise noted.

[2,3,4,5,6,7]Linear, quadratic, or cubic response ($P < 0.05$) for absolute volume and linear, quadratic, or cubic response ($P < 0.05$) for relative volume, respectively, to age in the suckling period.

[8,9,10,11,12,13]Linear, quadratic, or cubic response ($P < 0.05$) for absolute volume and linear, quadratic, or cubic response ($P < 0.05$) for relative volume, respectively, to age in the PW period.

[14]n=5.

[15]SEM values are presented as absolute weights suckling period SEM /PW period SEM (relative weights suckling period SEM / PW period SEM).

Table 3.8b. Absolute and relative volume of selected visceral organs from birth to 42 d PW[1]

Age, days	BW, kg[2,6,7]	Organ volume, absolute ml and as (% Total Volume)		
		Total Volume[2,3,4,6,7]	Cecum[2,5,6,8,11]	Large Intestine[2,4,5,6,7,9,10,11]
Gest.108	1.15	30.18	0.27 (0.97)[12]	5.78 (19.28)
Gest.109	1.42	42.65	0.49 (1.12)	7.53 (17.69)
Gest.110	1.50	40.36	0.49 (1.20)	8.87 (22.20)
Gest.111	1.51	50.45	0.69 (1.32)	7.37 (14.58)
Gest.112	1.24	42.48	0.61 (1.49)	6.35 (14.96)
Gest.113	1.36	47.21	0.39 (0.83)	8.14 (17.03)
0 (Birth)	1.55	74.32	0.71 (1.07)	9.48 (9.97)
1	1.57	86.15	1.66 (2.00)	16.97 (19.45)
2	1.74	116.65	2.01 (1.87)	15.13 (12.62)
3	1.67	105.91	2.13 (1.90)	12.96 (12.28)
5	2.47	158.17	3.09 (2.00)	20.3 (12.66)
7	2.67	200.30	6.63 (3.47)	32.1 (16.55)
14	5.26	404.00	16.77 (4.17)	87.43 (21.00)
21 (W)	6.40	419.15	20.82 (4.91)	87.04 (19.70)
22 (PW1)	5.62	332.28	15.17 (4.66)	73.68 (23.69)
23 (PW2)	6.27	486.74	32.32 (6.52)[12]	118.54 (24.42)
24 (PW3)	5.77	450.52	23.71 (5.42)	147.46 (31.91)
26 (PW5)	6.34	649.34	36.47 (6.28)	215.41 (36.13)
28 (PW7)	6.69	788.64	33.48 (4.63)	273.02 (35.54)
35 (PW14)	9.55	1334.18	56.11 (4.34)	424.22 (32.09)
49 (PW28)	18.55	3187.75	276.67 (8.65)	1018.89 (31.50)
63 (PW42)	28.12	4796.57	252.47 (5.05)	1824.19 (38.08)[12]
SEM[13]	0.40/ 0.94	27.83/172.74	2.01/17.72 (0.66/1.07)	9.47/74.47 (2.53/2.09)

[1]Values in the parentheses represent the relative volume to total volume, total volume= sum of all individual segment volumes; PW=post-weaning, Gest.=gestation day, W=weaning; gestation day means are reported but not included in the statistical analysis, statistical analysis was assessed from birth to weaning (the suckling period) and from weaning to 42 d PW (the post-weaning period); n=6 per mean unless otherwise noted.

[2,3,4,5]Linear, quadratic, or cubic response ($P < 0.05$) for absolute volume and linear response ($P < 0.05$) for relative volume, respectively, to age in the suckling period.

[6,7,8,9,10,11]Linear, quadratic, or cubic response ($P < 0.05$) for absolute volume and linear, quadratic, or cubic response ($P < 0.05$) for relative volume, respectively, to age in the PW period.

[12]n=5.

[13]SEM values are presented as absolute weights suckling period SEM /PW period SEM (relative weights suckling period SEM / PW period SEM).

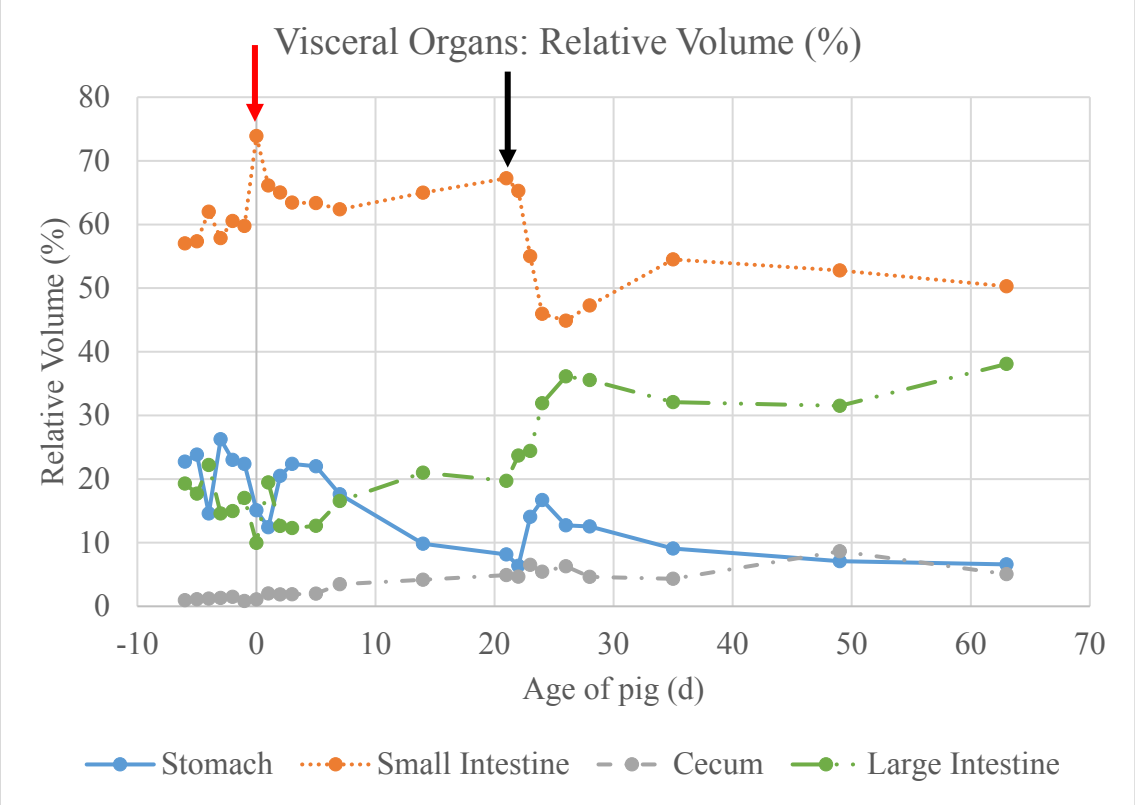

Figure 3.13 Relative volume of the GIT from birth through 42 d post-weaning. Birth is indicated by the red arrow, and weaning is indicated by the black arrow.

Table 3.9. Absolute and relative length of selected visceral organs from birth to 42 d PW[1]

Age, days	BW, kg[2,4,5]	Organ length, absolute m and as (% Total Length)		
		Total Length[2,3,4]	Small Intestine[2,3,4,5,6,7]	Large Intestine[2,3,4,5,6,7]
Gest.108	1.15	3.30	2.64 (79.93)	0.66 (20.07)
Gest.109	1.42	4.00	3.24 (80.88)	0.76 (19.12)
Gest.110	1.50	4.14	3.35 (80.77)	0.79 (19.23)
Gest.111	1.51	4.24	3.48 (81.93)	0.76 (18.07)
Gest.112	1.24	4.12	3.41 (82.71)	0.71 (17.29)
Gest.113	1.36	4.21	3.49 (82.66)	0.73 (17.34)
0 (Birth)	1.55	4.44	3.72 (84.19)	0.72 (15.81)
1	1.57	5.31	4.44 (83.35)	0.88 (16.65)
2	1.74	5.45	4.52 (83.03)	0.93 (16.97)
3	1.67	5.41	4.57 (84.42)	0.84 (15.58)
5	2.47	6.43	5.40 (83.92)	1.03 (16.08)
7	2.67	7.09	5.94 (83.61)	1.15 (16.39)
14	5.26	9.70	8.26 (85.23)	1.43 (14.77)
21 (W)	6.40	9.98	8.52 (85.46)	1.46 (14.54)
22 (PW1)	5.62	9.47	8.02 (84.70)	1.45 (15.30)
23 (PW2)	6.27	9.66	8.18 (84.58)	1.49 (15.42)
24 (PW3)	5.77	10.07	8.41 (83.49)	1.66 (16.51)
26 (PW5)	6.34	10.45	8.75 (83.64)	1.70 (16.36)
28 (PW7)	6.69	10.72	8.72 (81.30)	2.00 (18.70)
35 (PW14)	9.55	12.80	10.51 (82.08)	2.29 (17.92)
49 (PW28)	18.55	16.86	13.98 (82.88)	2.89 (17.12)
63 (PW42)	28.12	19.70	16.20 (82.26)	3.50 (17.74)
SEM	0.40/ 0.94	0.36/ 0.47	0.30/0.41 (1.14/82.26)	0.08/0.11 (1.14/0.63)

[1]Values in the parentheses represent the relative length to total length, total length = sum of small and large intestine length; PW=post-weaning, Gest.=gestation day, W=weaning; gestation day means are reported but not included in the statistical analysis, statistical analysis was assessed from birth to weaning (the suckling period) and from weaning to 42 d PW (the post-weaning period); n=6 per mean unless otherwise noted.

[2,3]Linear or quadratic response ($P < 0.05$) to age for absolute length in the suckling period.

[4,5,6,7]Linear or cubic response ($P < 0.05$) for absolute length and linear or quadratic response ($P < 0.05$) for relative length to age in the PW period.

[8]SEM values are presented as absolute weights suckling period SEM /PW period SEM (relative weights suckling period SEM / PW period SEM).

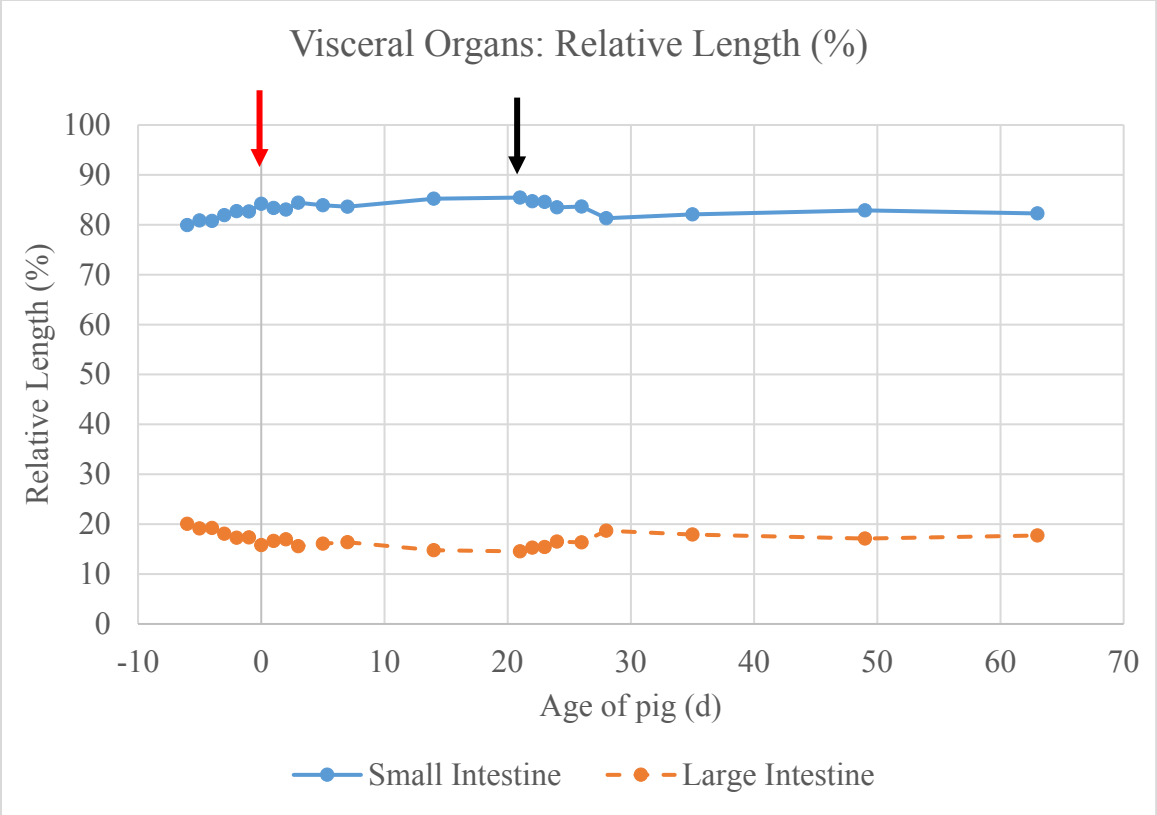

Figure 3.14 Relative length of the GIT. Birth is indicated by the red arrow, and weaning is indicated by the black arrow.

3.4.4 *Small intestine morphology*

The effect that age had on various parameters of the small intestine (SI) morphology can be found in Tables 3.7, 3.8, 3.9, 3.10, 3.11, and 3.12 (light microscopy data can be found in Tables 3.7, 3.8 and 3.9, scanning electron microscopy data can be found in Tables 3.10.a, 3.10.b, 3.11.a, and 3.11.b, and absorptive surface area measures can be found in Tables 3.12 and 3.13) and additional light microscope histology measurements can be seen in Figure 3.15, Figure 3.16. Figure 3.17, and Figure 3.18 and scanning electron micrographs can be seen in Figure 3.19, Figure 3.20, Figure 3.21, Figure 3.22, Figure 3.23, and Figure 3.24. When location effect occurs, it means that the location, 25%, 50%, or 75% or the total length, of the SI in which response measures where measured were significantly different in each location from the other location(s).

Assessing the effect of age and location on the SI in the suckling period, there was an age by location interaction for: villi height ($P = 0.04$; Figure 3.15), V:C ratio ($P = 0.04$; Figure 3.17). There was an age by sex interaction for the average apical, and middle enterocyte widths on the villous ($P < 0.03$), and villi density ($P < 0.05$), in which there are many points of interactions but, in general, the gilts have greater values at the end of the suckling period for the enterocyte widths, and the barrows had greater densities than the gilts. There was a location effect for the base microvilli diameter (distal portion of the small intestine had greater diameter values than the middle portion of the small intestine; $P < 0.01$), average, middle, and base microvilli density (the middle portion of the small intestine had greater densities than the distal portion of the small intestine; $P < 0.03$), and

a sex effect for the apical microvilli diameter (gilts had greater diameters than barrows) and density (barrows had a greater apical microvilli density than gilts; $P < 0.05$).

Evaluating the PW period, there is an age by sex interaction for crypt depth ($P < 0.01$) where gilts had greater crypt depth values than barrows, and an age by location interaction for: villi height ($P < 0.001$; Figure 3.15), V:C ratio ($P < 0.001$; Figure 3.17), all enterocyte widths ($P < 0.02$; points of interaction between days PW 7 and PW 14, and PW 14 and PW 28 with the distal location of the small intestine having greater values than the middle section), apical microvilli diameter ($P < 0.01$; with multiple points of interaction immediately following weaning but with the distal location of the small intestine having greater values than the middle section), all microvilli densities ($P < 0.01$, with points of interactions between days PW 7 and PW 14, PW 14 and PW 28, and PW 28 and PW 42 with the distal location of the small intestine having greater values than the middle section), villi density ($P < 0.001$; with a point of interaction occurring between day PW 2 and PW 3 with the distal location of the small intestine having greater values than the middle section). Also, there was a location effect for all enterocyte widths (the middle portion of the small intestine had greater values than the distal portion of the small intestine; $P < 0.001$), average, apical, and base microvilli diameter (the middle portion of the small intestine had greater values than the distal portion of the small intestine; $P < 0.03$), and all microvilli densities (the distal portion of the small intestine had greater density values than the middle portion of the small intestine $P < 0.01$).

During the suckling period of the study in the proximal section of the small intestine, or 25% of the total length of the small intestine (Table 3.7), villous height (Figure

3.15), crypt depth (Figure 3.16), V:C ratio (Figure 3.17), and goblet cell count (Figure 3.18) responses were the following:

Villus height: linear and cubic

Crypt depth: linear and cubic

V:C ratio: linear

Goblet cell count: linear

The response ($P < 0.05$) for parameters in the proximal section of the small intestine in the PW period is the following:

Villus height: quadratic and cubic

Crypt depth: linear, quadratic, and cubic

V:C ratio: linear, quadratic, and cubic

Goblet cell count: cubic

During the suckling period of the study in the middle section of the small intestine, or 50% of the total length of the small intestine (Table 3.8), villous height (Figure 3.15), crypt depth (Figure 3.16), V:C ratio (Figure 3.17), and goblet cell count (Figure 3.18) responses were the following:

Villus height: quadratic

Crypt depth: linear and quadratic

V:C ratio: linear and quadratic

Goblet cell count: linear

The response ($P < 0.05$) for parameters in the middle section of the small intestine in the PW period is the following:

Villus height: cubic

Crypt depth: linear, quadratic, and cubic

V:C ratio: linear, quadratic, and cubic

Goblet cell count: linear and cubic

During the suckling period of the study in the distal section of the small intestine, or 75% of the total length of the small intestine (Table 3.9), villous height (Figure 3.11), crypt depth (Figure 3.12), V:C ratio (Figure 3.17), and goblet cell count (Figure 3.18) responses were the following:

Villus height: quadratic

Crypt depth: linear

V:C ratio: quadratic

Goblet cell count: linear

The response ($P < 0.05$) for parameters in the distal section of the small intestine in the PW period is the following:

Villus height: linear, quadratic, and cubic

Crypt depth: linear, quadratic, and cubic

V:C ratio: linear, quadratic, and cubic

Goblet cell count: quadratic and cubic

During the suckling period of the study, the villus height increased immediately after birth at d 1 postpartum. In the proximal section of the small intestine, there is a decrease in the villi height on d 7 postpartum. The middle and distal sections of the small intestine also decrease prior to weaning on d 14 of the suckling period. The crypt depths increased immediately after birth, and then continued to increase through the rest of the suckling period. V:C ratio increased postpartum and then decreased on d 7 postpartum.

Goblet cell count decreased postpartum, but then increased on d 5 of the suckling period and continued to increase through weaning.

In the PW period of the study, the villi height decreased immediately post-weaning, increased by d 5 PW, and then plateaued through the remainder of the PW period. The crypt depth decreased first thing PW, but then increased by d 3 PW, and continued to increase through the PW period. V:C ratio decreased from weaning and through the post-weaning period. Goblet cell count decreased following weaning, but by d 5 PW, goblet cell count increased through d 28 PW. After d 28 PW, the distal section of the small intestine had a decrease in its number of goblet cells on the villus, but the proximal and middle section of the small intestine appeared to plateau.

When assessing the response measures recorded from scanning electron microscopy (ScEM) in the suckling period, responses ($P < 0.05$) for the middle section of the SI, or 50% of total length(Table 3.10a and 3.10b, and Figures 3.20, 3.21, 3.22, 3.23, and 3.24), were the following:

Villi density: linear

Average enterocyte width: linear

Average microvilli density: linear

Average microvilli diameter: linear

Apical enterocyte width: linear and quadratic

Apical microvilli density: linear

Apical microvilli diameter: linear

Middle enterocyte width: linear

Middle microvilli density: linear

Base enterocyte width: linear

Base microvilli density: linear

Base microvilli diameter: linear

In the PW period responses ($P < 0.05$) for the middle section, 50% of total length, of the SI (Table 3.10a and Table 3.10b, and Figures 3.20, 3.21, 3.22, 3.23, and 3.24) were the following:

Villi density: linear and quadratic

Villi width: quadratic and cubic

Average microvilli density: linear

Average microvilli diameter: cubic

Apical microvilli density: linear and quadratic

Apical microvilli diameter: quadratic

Middle microvilli density: linear

Middle microvilli diameter: quadratic

Base microvilli density: linear and cubic

When assessing the response measures recorded from ScEM in the suckling period, responses ($P < 0.05$) for the distal section of the SI, or 75% of total length (Table 3.11a and Table 3.11b, and Figures 3.20, 3.21, 3.22, 3.23, and 3.24), were the following:

Villi density: linear and cubic

Villi width: linear and cubic

Average enterocyte width: cubic

Average microvilli density: linear

Average microvilli diameter: linear

Apical microvilli density: linear

Apical microvilli diameter: linear

Middle enterocyte width: cubic

Middle microvilli density: linear, quadratic, and cubic

Middle microvilli diameter: linear

Base enterocyte width: cubic

Base microvilli density: linear

In the PW period, responses ($P < 0.05$) for the distal section of the SI, or 75% of total length (Table 3.11a and Table 3.11b, and Figures 3.20, 3.21, 3.22, 3.23, and 3.24), were the following:

Villi density: linear and cubic

Villi width: linear, quadratic, and cubic

Average enterocyte width: linear, quadratic, and cubic

Average microvilli density: linear and quadratic

Apical enterocyte width: linear and quadratic

Apical microvilli density: linear and quadratic

Apical microvilli diameter: linear and quadratic

Middle enterocyte width: linear, quadratic, and cubic

Middle microvilli density: linear and quadratic

Middle microvilli diameter: quadratic

Base microvilli density: linear

Base microvilli diameter: cubic

During the suckling period of the study, villi density in middle section of the small intestine was relatively static around 21 villi per 0.25 square millimeter , while there is a decrease in the villi density following birth in the distal section of the small intestine from 26 to 20 villi per 0.25 square millimeter. Villi width in both the middle and distal section of the small intestine increased from roughly 80 μm to 110 μm. Average microvilli density decreased in the middle section of the small intestine from roughly 32 to 29 microvilli per 0.25 square micrometer, and decreased from roughly 32 to 28 microvilli per square micrometer in the distal section of the small intestine. Average microvilli diameter increased in both sections of the small intestine from 0.075 μm to roughly 0.080 μm.

During the PW period, villi density increased to d 2 PW in the middle section of the small intestine and d 3 PW in the distal section, from 21 to 27.5 and 17.47 to 26.08 villi per square millimeter, respectively. Then villi density decreased to 9.48 and 10.17 villi per square millimeter in the middle and distal sections, respectively. Villi width in the middle section of the small intestine decreased from 110 μm to 87 μm on day 3 PW, but then increased to roughly 196 μm by the end of the PW period. In the distal section of the small intestine, villi width decreased from 119 μm to roughly 84 μm on d 3 PW. It then increased to 212 μm on d 42 PW. Average microvilli density in the middle section of the small intestine increased from 28.6 to 30.31 microvilli per square micrometer between weaning and d 5 PW, then density decreased to 23.41 microvilli per square micrometer. In the distal section of the small intestine, average microvilli density decreased from roughly 28 to 25 microvilli per square micrometer from d 3 PW to d 42 PW. Average microvilli diameter in the middle section of the small intestine, stayed around 0.080 μm, but increased to 0.083 μm on d 7 and d 14 PW. In the distal section of the small intestine, average microvilli

diameter increased from 0.083 μm to 0.087 μm between weaning and d 3 PW, microvilli diameter then immediately decreased back to 0.080 μm and then increased to 0.088 μm on d 3 and d 5 PW.

When combining some of the response measures from the light microscopy measures and the ScEM to obtain the surface area of the SI with villi and microvilli taken into consideration, in response to age, villi and microvilli increase the surface area of the SI. Suckling, in the middle section, 25 - 50% of the total length of the SI (Table 3.12), villi increased surface area in a quadratic and cubic ($P < 0.05$) manner, while microvilli increased surface area cubically ($P < 0.05$). In the distal portion, 75 - 100% of the total length of the SI (Table 3.13), the villi increased the surface area cubically ($P < 0.05$).

During the suckling period of the study, the surface area of the middle section of the small intestine increased roughly 900,000 square millimeters due to villi from birth to d 1 postpartum and increased roughly 800,000 square millimeters due to villi in the distal section of the small intestine during the same time. Microvilli increased the surface area in both the middle and distal section of the small intestine by roughly 20,000,000 square millimeters between birth and d 1 postpartum.

Post-weaning, both the villi increased surface area cubically ($P < 0.05$) in the middle section of the SI (Table 3.12). Additionally, the microvilli increased surface area linearly and cubically ($P < 0.05$) in the middle section of the SI. In the distal portion of the SI (Table 3.13), both villi and microvilli increased surface area quadratically and cubically ($P < 0.05$).

The surface area of the middle and distal section of the small intestine approximately doubled between d 14 and d 28 PW for surface area contributed from villi

and microvilli. Greatest surface area for both sections of the small intestine and for both surface area from villi and microvilli occurs on d 28 and d 42 PW.

Table 3.10. Light microscopy histology measurements at 25% of the total length of the small intestine for pigs from birth through 42 d PW[1]

Age, days	BW, kg[2,5,6]	Villous Height, µm[2,4,6,7]	Crypt Depth, µm[2,4,5,6,7]	V:C Ratio[2,5,6,7]	Goblet Cell Count, per villous[2,7]
Gest.108	1.15	667.5	99.8	6.67	12.5
Gest.109	1.42	701.8	105.2	6.67	14.2
Gest.110	1.50	651.0	93.4	6.98	13.3
Gest.111	1.51	637.1	91.5	6.96	12.3
Gest.112	1.24	644.1	98.4	6.55	14.4
Gest.113	1.36	700.9	107.2	6.55	14.8
0 (Birth)	1.55	839.7	110.6	7.58	15.4
1	1.57	1012.9	139.6	7.25	14.8[8]
2	1.74	1188.5	144.7	8.28	15.0
3	1.67	1140.0	143.9	7.95	15.2
5	2.47	1159.4	146.6	7.94	18.0[8]
7	2.67	905.6	139.1	6.56	18.7
14	5.26	917.0[8]	150.9[8]	6.26[8]	17.3[9]
21 (W)	6.40	818.2	156.9	5.20	20.2[8]
22 (PW1)	5.62	557.2	161.9	3.47	19.3
23 (PW2)	6.27	629.9	152.2	4.16	20.1
24 (PW3)	5.77	576.8	175.1	3.32	14.9
26 (PW5)	6.34	580.1	204.4	2.84	14.4
28 (PW7)	6.69	621.5	210.9	2.96	15.0
35 (PW14)	9.55	580.5	252.2	2.31	17.9
49 (PW28)	18.55	652.4	251.0	2.61	19.5
63 (PW42)	28.12	646.9	301.7	2.18	19.9
SEM[10]	0.40/0.94	69.69/ 31.48	5.50/7.30	0.57/0.22	1.26/1.41

[1]PW=post-weaning, Gest.=gestation day, W=weaning; gestation day means are reported but not included in the statistical analysis, statistical analysis was assessed from birth to weaning (the suckling period) and from weaning to 42 d PW (the post-weaning period); n=6 per mean unless otherwise noted.

[2,3,4]Linear, quadratic, or cubic response ($P < 0.05$) to age in the suckling period.

[5,6,7]Linear, quadratic, or cubic response ($P < 0.05$) to in the PW period.

[8, 9]n=5, n=4, respectively.

[10]SEM values are presented as suckling period SEM / PW period SEM.

Table 3.11. Light microscopy histology measurements at 50% of the total length of the small intestine for pigs from birth through 42 d PW[1]

Age, days	BW, kg[2,5,6]	Villous Height, µm[3,7]	Crypt Depth, µm[2,3,5,6,7]	V:C Ratio[2,3,5,6,7]	Goblet Cell Count, per villous[2,5,7]
Gest.108	1.15	625.9	97.4	6.43	14.0
Gest.109	1.42	666.8	98.1	6.83	15.0
Gest.110	1.50	692.3	89.0	7.81	15.0
Gest.111	1.51	661.6	87.1	7.55	15.1[8]
Gest.112	1.24	624.1	92.5	6.75	14.9
Gest.113	1.36	802.0	92.2	8.66	16.7
0 (Birth)	1.55	779.0	95.7	8.08	17.3
1	1.57	1086.5	116.0	9.35	16.3[8]
2	1.74	1159.5	128.2	9.08	16.7
3	1.67	1079.6	127.9	8.46	15.5
5	2.47	1161.5	120.8	9.76	20.2
7	2.67	1156.5	129.7	8.89	19.6
14	5.26	1174.2[8]	139.8[8]	8.44[8]	21.7[8]
21 (W)	6.40	779.6	143.3	5.46	22.1
22 (PW1)	5.62	511.6	136.4	3.83	21.8
23 (PW2)	6.27	574.4	136.4	4.23	19.7
24 (PW3)	5.77	499.8	160.3	3.14	15.4
26 (PW5)	6.34	487.9	186.6	2.64	15.5
28 (PW7)	6.69	528.2	215.6	2.46	15.6
35 (PW14)	9.55	636.0	250.8	2.54	20.4
49 (PW28)	18.55	671.0[8]	261.9	2.49[8]	22.3
63 (PW42)	28.12	576.7	319.9	1.83	22.5
SEM[9]	0.40/ 0.94	69.69/34.72	5.50/7.30	0.57/0.24	1.13/1.28

[1]PW=post-weaning, Gest.=gestation day, W=weaning; gestation day means are reported but not included in the statistical analysis, statistical analysis was assessed from birth to weaning (the suckling period) and from weaning to 42 d PW (the post-weaning period); n=6 per mean unless otherwise noted.

[2,3,4]Linear, quadratic, or cubic response ($P < 0.05$) to age in the suckling period.

[5,6,7]Linear, quadratic, or cubic response ($P < 0.05$) to age in the PW period.

[8]n=5.

[9]SEM values are presented as suckling period SEM / PW period SEM.

Table 3.12. Light microscopy histology measurements at 75% of the total length of the small intestine for pigs from birth through 42 days PW[1]

Age, days	BW, kg[2,5,6]	Villous Height, µm[3,5,6,7]	Crypt Depth, µm[2,5,6,7]	V:C Ratio[3,5,6,7]	Goblet Cell Count, per villous[2,6,7]
Gest.108	1.15	698.8	98.5	7.14	16.2
Gest.109	1.42	665.1	94.1	7.11	16.4
Gest.110	1.50	683.4	88.9	7.71	16.5
Gest.111	1.51	627.6	89.0	7.04	14.9
Gest.112	1.24	650.6	91.0	7.19	16.7
Gest.113	1.36	705.5	91.9	7.66	17.5
0 (Birth)	1.55	762.2	101.3	7.51	18.5
1	1.57	1135.6[8]	114.1	9.64[8]	16.1
2	1.74	1076.5	131.0	8.27	17.8
3	1.67	1087.2	129.9	8.40	17.3
5	2.47	1224.3	120.1	10.23	20.9
7	2.67	1102.4	122.6	8.91	20.7
14	5.26	1193.7[8]	131.2[8]	9.41[8]	22.4[8]
21 (W)	6.40	1010.9[8]	136.2	7.36[8]	24.9[8]
22 (PW1)	5.62	765.9	130.5	5.92	22.8
23 (PW2)	6.27	493.2	134.3	3.74	16.7
24 (PW3)	5.77	528.2	165.2	3.22	13.9
26 (PW5)	6.34	510.6	192.0	2.67	15.6
28 (PW7)	6.69	542.2	210.7	2.59	16.0
35 (PW14)	9.55	562.5	239.4	2.35	17.3[8]
49 (PW28)	18.55	595.4[9]	239.2[9]	2.49[9]	22.4[10]
63 (PW42)[8]	28.12	587.3[10]	294.9[10]	2.00[10]	18.6[10]
SEM[11]	0.40/0.94	69.62/49.77	5.50/11.54	0.57/0.35	1.13/2.02

[1]PW=post-weaning, Gest.=gestation day, W=weaning; gestation day means are reported but not included in the statistical analysis, statistical analysis was assessed from birth to weaning (the suckling period) and from weaning to 42 d PW (the post-weaning period); n=6 per mean unless otherwise noted.

[2,3,4]Linear, quadratic, or cubic response ($P < 0.05$) to age in the suckling period.

[5,6,7]Linear, quadratic, or cubic response ($P < 0.05$) to age in the PW period.

[8,9,10]n=5, n=4, n=3, respectively.

[11]SEM values are presented as suckling period SEM / PW period SEM.

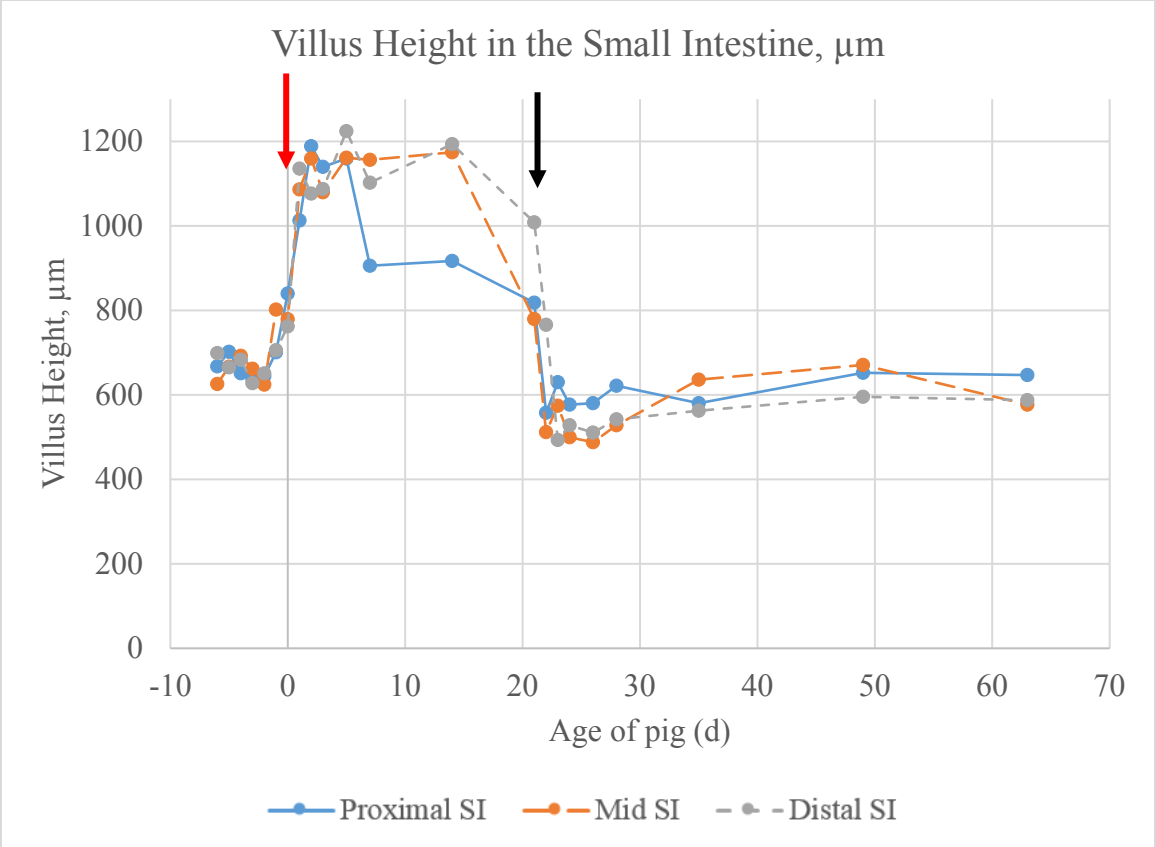

Figure 3.15. Villus height measurement in the proximal, middle, and distal portion of the small intestine (SI). Birth is indicated by the red arrow, and weaning is indicated by the black arrow.

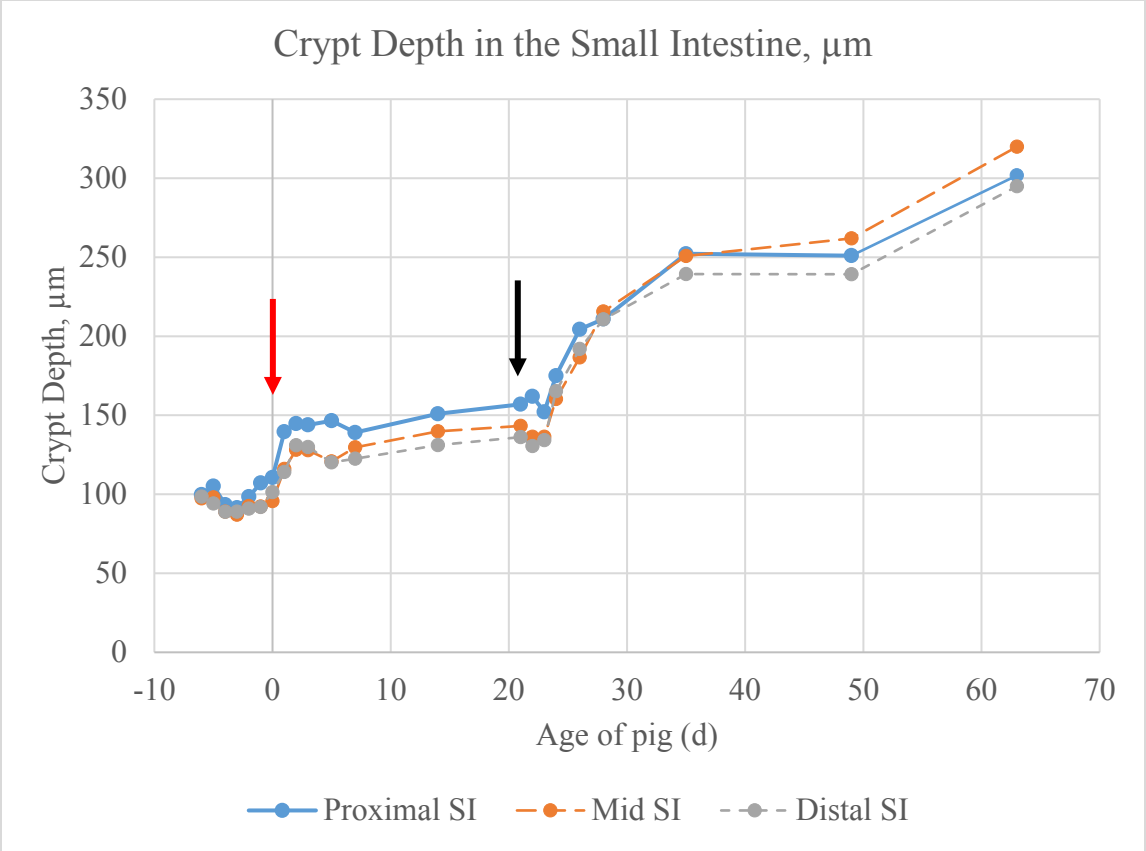

Figure 3.16. Crypt depth measurement in the proximal, middle, and distal portion of the small intestine (SI). Birth is indicated by the red arrow, and weaning is indicated by the black arrow.

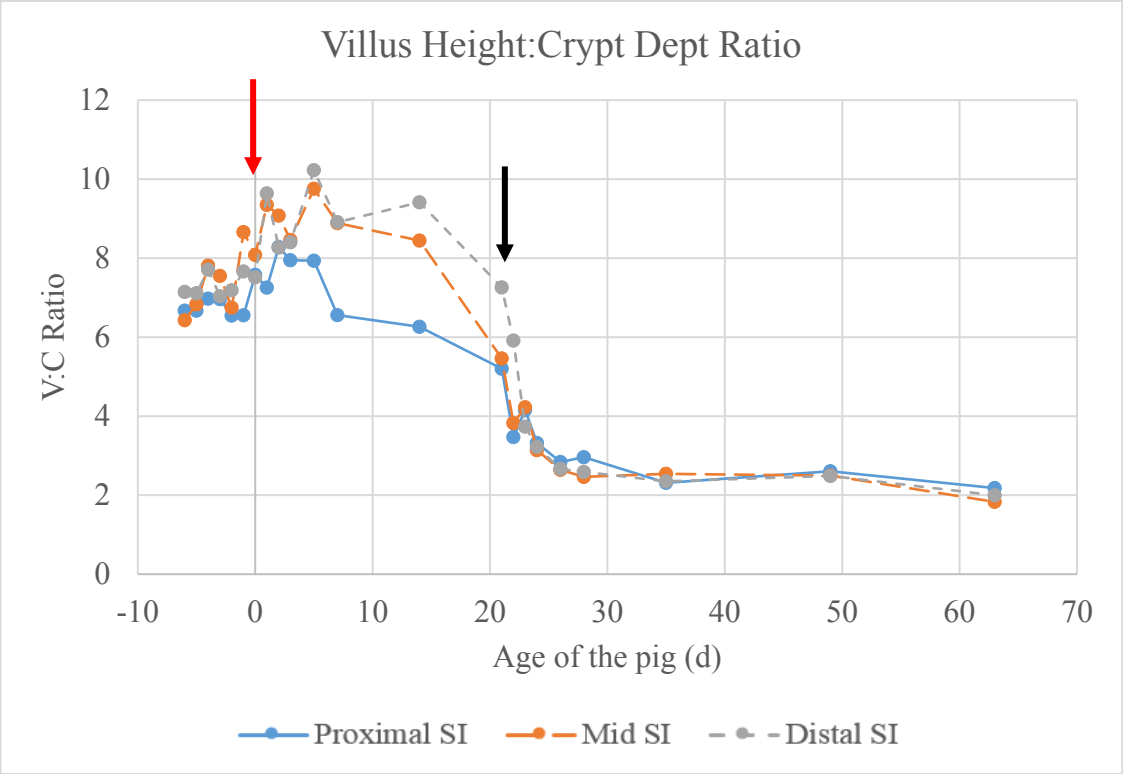

Figure 3.17. Villus height:crypt depth ratio measurement in the proximal, middle, and distal portion of the small intestine (SI). Birth is indicated by the red arrow, and weaning is indicated by the black arrow.

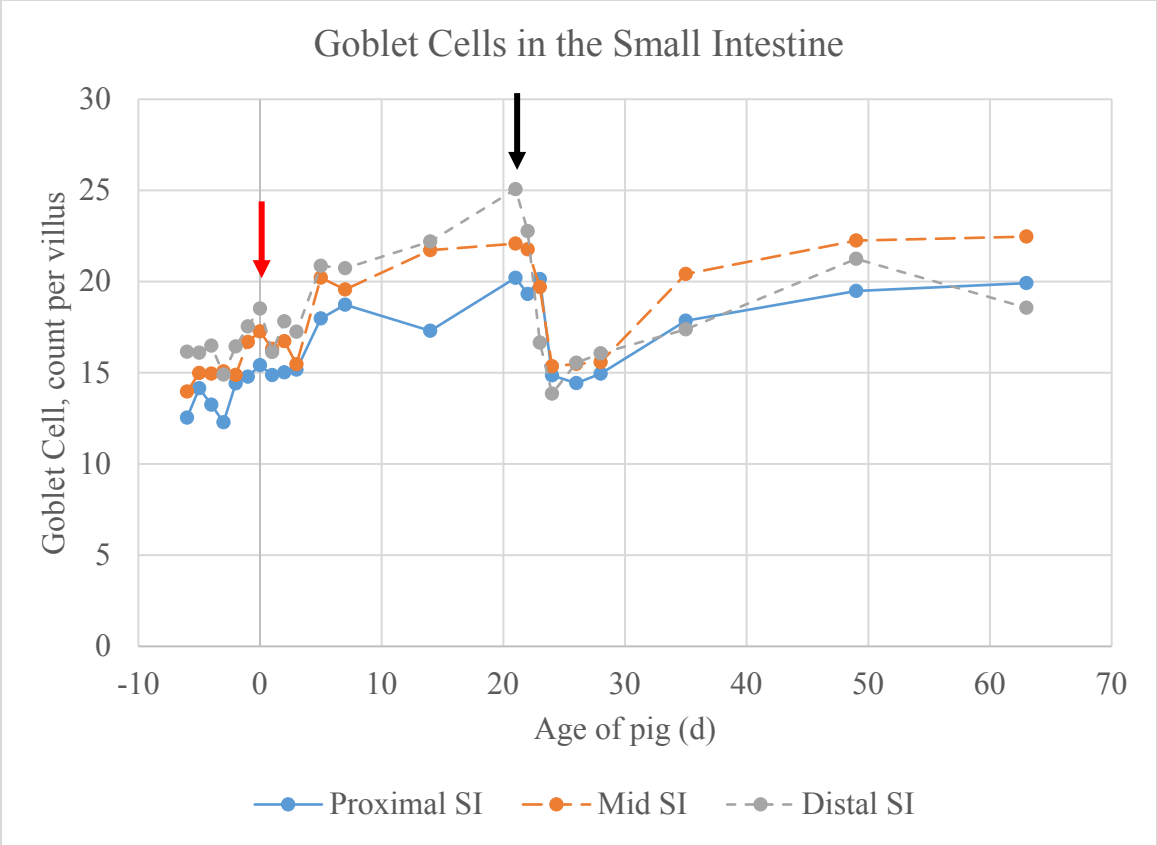

Figure 3.18. Goblet cell count, per villus, in the proximal, middle, and distal portion of the small intestine (SI). Birth is indicated by the red arrow, and weaning is indicated by the black arrow.

Table 3.13a. ScEM measurements at 50% of the total length of the small intestine of pigs from birth through 42 d PW[1]

				Average per villous			
		Villi		Enterocyte	Microvilli		
Age, days	BW, kg[2,5,6]	Density, /0.25sq mm [2,5,6]	Width, µm[6,7]	Width, µm^2	Density, /0.25sq µm[2,5]	Diameter, µm[2,7]	Length, µm
Gest.108	1.15	25.72	80.53	7.41	28.56	0.085	-
Gest.112	1.24	22.26	93.75	8.14	29.59	0.084	0.72
Gest.113	1.36	21.34[8]	85.86	7.94	31.90	0.079	0.69
0 (Birth)	1.55	22.56	88.45	7.81	31.98	0.075	0.49
1	1.57	23.02[8]	103.94	7.96	32.98	0.072	0.69
7	2.67	20.64	97.29	7.22	31.09	0.077	0.59
21 (W)	6.40	21.00	110.36	6.33	28.60	0.080	0.69
22 (PW1)	5.62	23.78	92.85	6.07	29.78	0.080	0.54
23 (PW2)	6.27	27.50	87.29	6.30	29.02	0.082	0.57
24 (PW3)	5.77	23.31	87.74	6.32	29.36	0.080	0.77
26 (PW5)	6.34	20.36	93.89	5.93	30.31	0.080	0.80
28 (PW7)	6.69	17.00	110.47	6.07	28.72	0.083	0.60
35 (PW14)	9.55	12.50	137.52	6.31	24.81	0.083	0.77
49 (PW28)	18.55	11.29[8]	197.71	6.08	25.32	0.080	0.73
63 (PW42)	28.12	9.48	195.47	6.16	23.41	0.082	0.72
SEM[9]	0.40/0.94	1.56/1.04	6.92/8.04	032/0.20	0.72/0.60	0.002/0.002	0.07/0.25

[1]PW=post-weaning, Gest.=gestation day, W=weaning; gestation day means are reported but not included in the statistical analysis, statistical analysis was assessed from birth to weaning (the suckling period) and from weaning to 42 d PW (the post-weaning period); n=6 per mean unless otherwise noted; - = missing values.

[2,3,4]Linear, quadratic, or cubic response ($P < 0.05$) to age in the suckling period.

[5,6,7]Linear, quadratic, or cubic response ($P < 0.05$) to age in the PW period.

[8]n=5.

[9]SEM values are presented as suckling period SEM /PW period SEM.

Table 3.14b. ScEM measurements at 50% of the total length of the small intestine of pigs from birth through 42 d PW[1]

		Apical section of the villous			Middle section of the villous			Base section of the villous		
		Enterocyte	Microvilli		Enterocyte	Microvilli		Enterocyte	Microvilli	
Age, days	BW, kg[2,5,6]	Width, μm[2,3]	Density, /0.25sq μm[2,5,6]	Diameter, μm[2,6]	Width, μm[2]	Density, /0.25sq μm[2,5]	Diameter, μm[6]	Width, μm[2]	Density, /0.25sq μm[2,5,7]	Diameter, μm[2]
Gest.108	1.15	7.99	27.45	0.085	7.42	27.42	0.083	6.83	30.81	0.083
Gest.112	1.24	8.52	28.54	0.082	8.44	28.47	0.084	7.46	31.76	0.080
Gest.113	1.36	8.39	29.94	0.078	8.00	31.76	0.078	7.42	33.28	0.081
0 (Birth)	1.55	8.50	29.50	0.077	8.01	32.95	0.073	6.91	33.50	0.073
1	1.57	8.66	31.61	0.073	8.00	32.47	0.072	7.23	34.97	0.070
7	2.67	7.36	30.00	0.082	7.83	30.61	0.077	6.46	32.67	0.075
21 (W)	6.40	6.37	27.17	0.083	6.72	28.83	0.078	5.89	29.83	0.080
22 (PW1)	5.62	6.44	29.81	0.085	6.18	28.97	0.080	5.60	30.61	0.075
23 (PW2)	6.27	7.12	27.42	0.090	6.36	28.89	0.080	5.43	30.81	0.080
24 (PW3)	5.77	6.53	27.75	0.085	6.37	29.42	0.080	6.05	30.92	0.077
26 (PW5)	6.34	6.77	28.67	0.083	5.85	30.86	0.078	5.17	31.39	0.078
28 (PW7)	6.69	6.56	27.42	0.092	6.27	28.25	0.083	5.38	30.53	0.078
35 (PW14)	9.55	6.67	21.64	0.090	6.50	25.69	0.087	5.77	27.11	0.075
49 (PW28)	18.55	6.34	25.05	0.085	6.02	25.83	0.082	5.89	25.06	0.080
63 (PW42)	28.12	6.13	22.92	0.082	6.25	23.58	0.082	6.12	25.03	0.078
SEM[8]	0.40/ 0.94	0.40/ 0.32	0.95/ 0.72	0.002/ 0.002	0.41/ 0.29	0.98/ 0.72	0.002/ 0.002	0.40/ 0.25	0.95/ 0.73	0.002/ 0.002

[1]PW=post-weaning, Gest.=gestation day, W=weaning; gestation day means are reported but not included in the statistical analysis, statistical analysis was assessed from birth to weaning (the suckling period) and from weaning to 42 d PW (the post-weaning period); n=6 per mean unless otherwise noted.

[2,3,4]Linear, quadratic, or cubic response ($P < 0.05$) to age in the suckling period.

[5,6,7]Linear, quadratic, or cubic response ($P < 0.05$) to age in the PW period.

[8]SEM values are presented as suckling period SEM /PW period SEM.

Table 3.15a. ScEM measurements at 75% of the total length of the small intestine of pigs from birth through 42 d PW[1]

		Villi		Average per villous			
				Enterocyte	Microvilli		
Age, days	BW, kg[2,5,6]	Density, /0.25sq mm[2,4,5,7]	Width, µm[2,4,5,6,7]	Width, µm[4,5,6,7]	Density, /0.25sq µm[2,5,6]	Diameter, µm[2]	Length, µm
Gest.108	1.15	25.14	78.83	6.75	32.02	0.072	-
Gest.112	1.24	25.81	84.77	7.36	32.27	0.074	0.52
Gest.113	1.36	25.08	80.34	8.04	31.67	0.074	-
0 (Birth)	1.55	26.78	83.15	6.85	31.87	0.075	0.51
1	1.57	19.75	115.42	8.13	29.69	0.077	0.49
7	2.67	20.19	106.35	7.48	29.27	0.078	-
21 (W)	6.40	17.47	119.41	7.89	27.70	0.083	-
22 (PW1)	5.62	17.73	110.70	7.53	28.06	0.080	0.78
23 (PW2)	6.27	25.61	97.95	6.78	26.68	0.087	-
24 (PW3)	5.77	26.08	83.85	6.66	27.93	0.080	0.61
26 (PW5)	6.34	25.25	95.19	6.59	26.08	0.088	0.71
28 (PW7)	6.69	21.25	95.79	6.68	26.87	0.083	1.05
35 (PW14)	9.55	14.25	121.45	6.10[8]	26.28[8]	0.086[8]	-
49 (PW28)	18.55	11.89	180.63	6.23[8]	24.69[8]	0.087[8]	0.89
63 (PW42)	28.12	10.17	212.13	6.65[9]	25.59[9]	0.083[9]	1.08
SEM[10]	0.40/0.94	1.50/0.88	6.64/6.90	0.30/0.24	0.65/0.52	0.002/0.002	0.06/0.10

[1]PW=post-weaning, Gest.=gestation day, W=weaning; gestation day means are reported but not included in the statistical analysis, statistical analysis was assessed from birth to weaning (the suckling period) and from weaning to 42 d PW (the post-weaning period); n=6 per mean unless otherwise noted; - = missing values.

[2,3,4]Linear, quadratic, or cubic response ($P < 0.05$) to age in the suckling period.

[5,6,7]Linear, quadratic, or cubic response ($P < 0.05$) to age in the PW period.

[8,9]n=5, n=3, respectively.

[10]SEM values are presented as suckling period SEM /PW period SEM.

Table 3.16b. ScEM measurements of the 75% of the total length of the small intestine of pigs from birth through 42 d PW[1]

		Apical section of the villous			Middle section of the villous			Base section of the villous		
		Enterocyte	Microvilli		Enterocyte	Microvilli		Enterocyte	Microvilli	
Age, days	BW, kg[2,5,6]	Width, µm[5,6]	Density, /0.25sq µm[2,5,6]	Diameter, µm[2,5,6]	Width, µm[4,5,6,7]	Density, /0.25sq µm[2,3,4,5,6]	Diameter, µm[2,6]	Width, µm[4]	Density, /0.25sq µm[2,5]	Diameter, µm[7]
Gest.108	1.15	7.18	30.09	0.077	6.94	33.06	0.073	6.14	33.67	0.072
Gest.112	1.24	8.11	31.83	0.070	7.42	32.81	0.074	6.57	32.19	0.080
Gest.113	1.36	8.79	30.04	0.078	8.20	32.71	0.073	7.12	32.27	0.078
0 (Birth)	1.55	7.83	30.50	0.077	6.70	32.39	0.073	6.01	32.72	0.078
1	1.57	8.76	29.44	0.075	8.22	29.36	0.078	7.41	30.28	0.077
7	2.67	7.65	28.31	0.078	8.10	28.81	0.078	6.69	30.70	0.078
21 (W)	6.40	8.39	26.58	0.088	8.07	27.92	0.082	7.20	28.86	0.083
22 (PW1)	5.62	7.93	27.67	0.082	8.26	27.78	0.082	6.40	28.75	0.080
23 (PW2)	6.27	7.56	26.04[8]	0.088	6.80	26.97	0.085	5.98	26.92	0.087
24 (PW3)	5.77	7.74	26.78	0.083	6.66	27.64	0.080	5.59	29.39	0.080
26 (PW5)	6.34	6.78	25.36	0.092	6.69	26.44	0.088	6.31	26.44	0.087
28 (PW7)	6.69	7.09	24.89	0.088	6.40	27.67	0.078	6.54	28.06	0.082
35 (PW14)	9.55	6.51[8]	25.10[8]	0.088[8]	5.74[8]	26.43[8]	0.085[8]	6.04[8]	27.32[8]	0.086[8]
49 (PW28)	18.55	6.12[8]	23.06[8]	0.098[8]	6.07[8]	24.14[8]	0.088[8]	6.39[8]	26.31[8]	0.078[8]
63 (PW42)	28.12	6.85[9]	23.89[9]	0.090[9]	6.83[9]	25.95[9]	0.080[9]	6.27[9]	26.95[9]	0.083[9]
SEM[10]	0.40/ 0.94	0.36/ 0.35	0.87/0.60	0.002/ 0.002	0.36/ 0.34	0.62/0.63	0.002/ 0.002	0.43/ 0.29	1.05/0.67	0.002/ 0.002

[1]PW=post-weaning, Gest.=gestation day, W=weaning; gestation day means are reported but not included in the statistical analysis, statistical analysis was assessed from birth to weaning (the suckling period) and from weaning to 42 d PW (the post-weaning period); n=6 per mean unless otherwise noted.

[2,3,4]Linear, quadratic, or cubic response ($P < 0.05$) to age in the suckling period.

[5,6,7]Linear, quadratic, or cubic response ($P < 0.05$) to age in the PW period.

[8,9]n=5, n=3, respectively.

[10]SEM values are presented as suckling period SEM /PW period SEM.

Table 3.17. Estimated increases in surface area of the small intestine from 25% - 50% of the total length with villi and microvilli projections in pigs from birth through 42 d PW[1]

Age, days	BW, kg[2,5,6]	SA of the SI, sq mm[2,5,7]	SA of the SI with villi, sq mm[2,3,5,7]	Approximate magnitude of SA increased with villi (mm^2/mm^2) [3,4,7]	SA of the SI with microvilli, sq mm[2,3,5,7]	Approximate magnitude of SA increased with microvilli (mm^2/mm^2) [4,5,7]
Gest.108	1.15	245,70	426,868	17.1	13,110,840	525.4
Gest.112	1.24	332,17	550,729	16.6	16,738,455	507.0
Gest.113	1.36	356,08	764,006[8]	22.7[8]	25,291,759[8]	753.0[8]
0 (Birth)	1.55	514,90	1,089,605	20.0	32,606,109	606.0
1	1.57	586,04	1,948,079[8]	30.7[8]	59,278,149[8]	936.0[8]
7	2.67	100,430	3,082,955	29.8	96,537,299	926.1
21 (W)	6.40	155,847	3,592,354	22.9	102,914,117	662.3
22 (PW1)	5.62	132,900	2,015,314	14.7	62,508,811	456.1
23 (PW2)	6.27	158,613	2,898,282	17.9	89,238,600	548.5
24 (PW3)	5.77	135,329	1,814,559	13.3	55,598,838	409.4
26 (PW5)	6.34	151,414	1,828,680	12.1	56,200,629	374.0
28 (PW7)	6.69	188,827	2,549,062	13.3	79,681,693	415.5
35 (PW14)	9.55	298,652	4,430,356	14.4	117,826,931	382.3
49 (PW28)	18.55	507,855	10,244,029[8]	20.7[8]	277,045,446[8]	555.9[8]
63 (PW42)	28.12	667,800	9,726,376	14.4	235,122,510	347.4
SEM[9]	0.40/0.94	8,476/23,176	389,832/681,213	2.77/1.22	1,1380,273/19,403,613	83.91/36.80

[1]SA= surface area, SI=small intestine, PW=post-weaning, Gest.=gestation day, W=weaning; gestation day means are reported but not included in the statistical analysis, statistical analysis was assessed from birth to weaning (the suckling period) and from weaning to 42 d PW (the post-weaning period); n=6 per mean unless otherwise noted.

[2,3,4] Linear, quadratic, or cubic response ($P < 0.05$), respectively, to age in the suckling period.

[5,6,7] Linear, quadratic, or cubic response ($P < 0.05$), respectively, to age in the PW period.

[8]n=5.

[9]SEM values are presented as suckling period SEM /PW period SEM.

Table 3.18. Estimated increases in surface area of the small intestine at 75% - 100% of the total length with villi and microvilli projections in pigs from birth through 42 d PW[1]

Age, days	BW, kg[2,5,6]	SA of the SI, sq mm[2,5,7]	SA of the SI with Villi, sq mm[2,5,6,7]	Approximate magnitude of SA with villi (mm^2/mm^2)[4,6,7]	SA of the SI with microvilli, sq mm[2,5,6,7]	Approximate magnitude of SA with microvilli (mm^2/mm^2)[6,7]
Gest.108	1.15	23,436	419,407	17.8	12,683,767	537.9
Gest.112	1.24	32,162	589,503	18.4	18,237,998	569.4
Gest.113	1.36	34,486	627,074	18.2	19,156,467	555.5
0 (Birth)	1.55	48,666	1,086,267	21.7	33,815,555	671.7
1	1.57	54,576	1,824,456[8]	32.0[8]	52,492,409[8]	922.2[8]
7	2.67	96,047	2,857,631	30.4	84,584,648	904.2
21 (W)	6.40	199,143	5,378,315[8]	26.8[8]	161,723,901[8]	804.6[8]
22 (PW1)	5.62	162,057	3,163,110	19.6	92,839,571	574.6
23 (PW2)	6.27	180,475	2,898,989	16.1	85,132,645	475.0
24 (PW3)	5.77	173,874	2,586,271	15.0	75,260,910	435.9
26 (PW5)	6.34	203,562	3,265,042	16.0	97,065,709	478.4
28 (PW7)	6.69	230,437	3,398,155	14.4	96,021,983	409.6
35 (PW14)	9.55	338,080	4,320,892[8]	12.8[8]	128,669,632[8]	364.5[8]
49 (PW28)	18.55	608,048	10,047,179[9]	17.1[9]	271,823,850[9]	463.4[9]
63 (PW42)	28.12	805,735	16,747,382[10]	20.6[10]	460,100,305[10]	565.5[10]
SEM[11]	0.40/0.94	8,658/17,155	312,625/722,272	2.75/1.38	9,918,576/20,314,939	89.45/43.90

[1]SA= surface area, SI=small intestine, PW=post-weaning, Gest.=gestation day, W=weaning; gestation day means are reported but not included in the statistical analysis, statistical analysis was assessed from birth to weaning (the suckling period) and from weaning to 42 d PW (the post-weaning period); n=6 per mean unless otherwise noted.

[2,3,4] Linear, quadratic, or cubic response ($P < 0.05$), respectively, to age in the suckling period.

[5,6,7] Linear, quadratic), or cubic response ($P < 0.05$), respectively, to age in the PW period.

[8,9,10]n=5, n=4, n=3, respectively.

[11]SEM values are presented as suckling period SEM /PW period SEM.

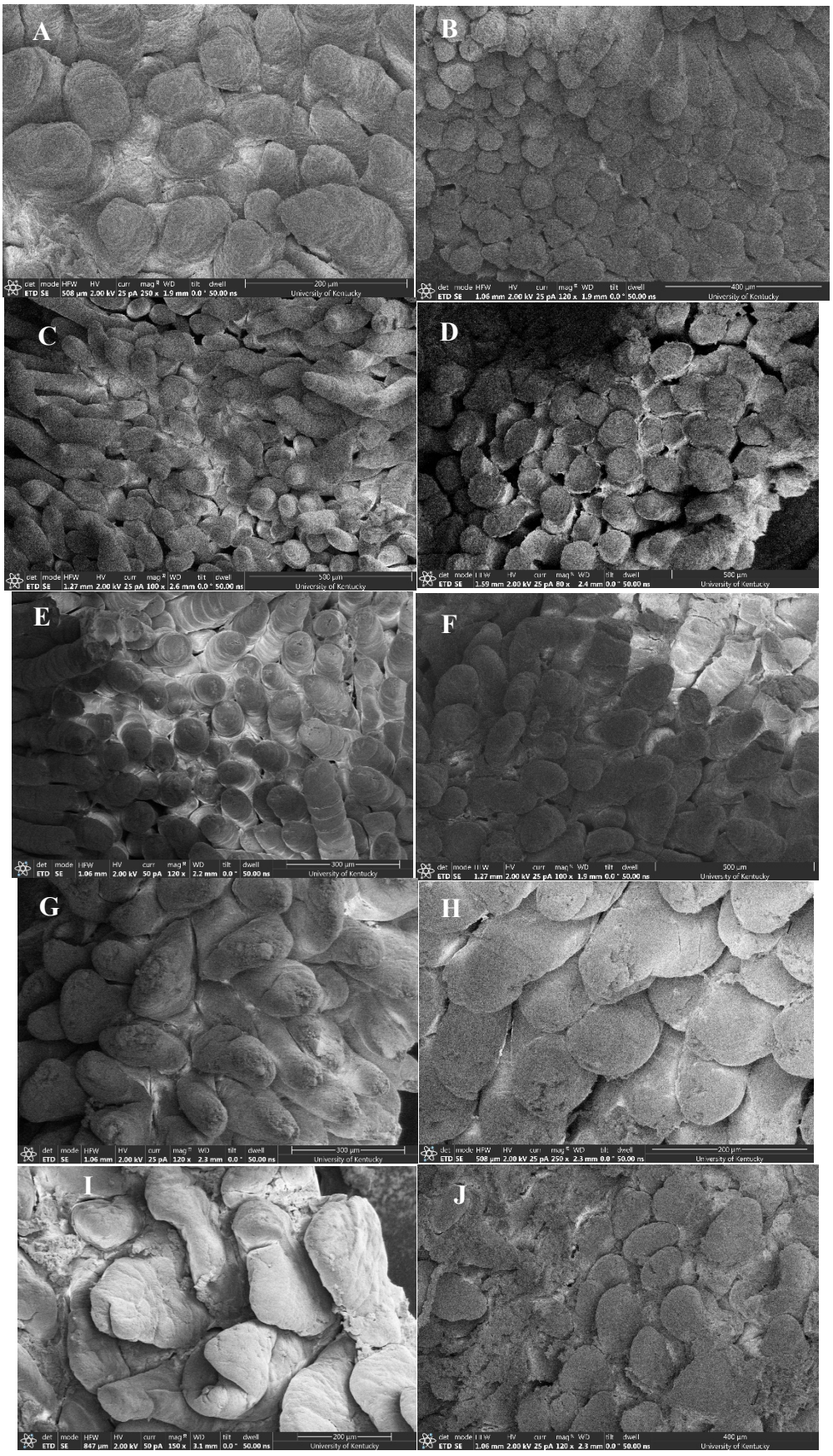
A
B
C
D
E
F
G
H
I
J

Figure 3.19. The above collage of images show the morphological changes in villi over the course of the study. On the left side of the collage are micrographs from the middle portion of the small intestine and the right side of the collage are micrographs from the distal portion of the small intestine. Each row of images are taken from the same age pig for comparison purposes. The first row (A and B) images are from fetal pigs on gestation day 108. Images C and D are 1 day after birth, E and F are from weaning, G and H are from day 7 post-weaning, and I and J are from day 42 post-weaning.

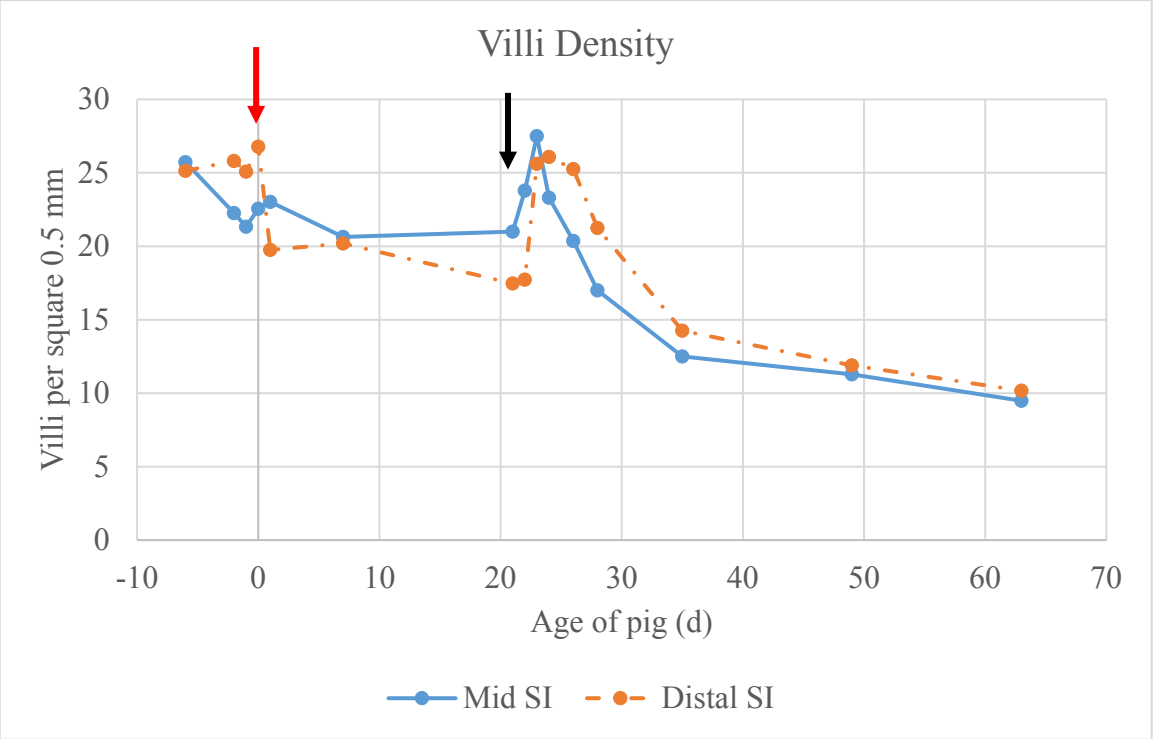

Figure 3.20 Villi density from birth through 42 d post-weaning. The red arrow indicates birth, and the black arrow indicates weaning.

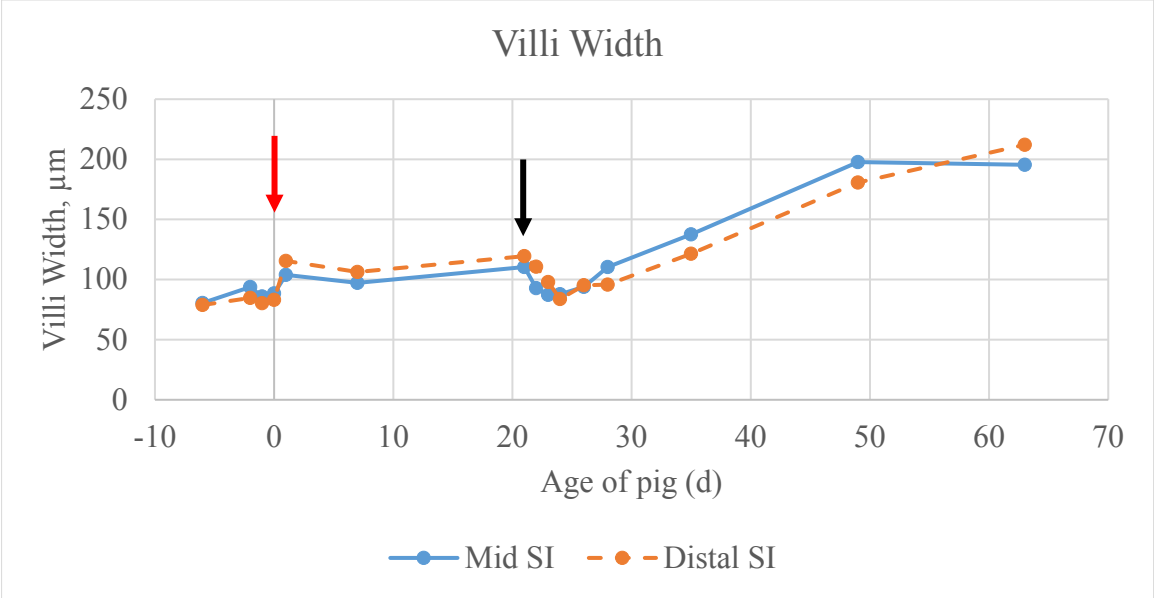

Figure 3.21 Villi width from birth through 42 d post-weaning. Birth is indicated by the red arrow, and weaning is indicated by the black arrow.

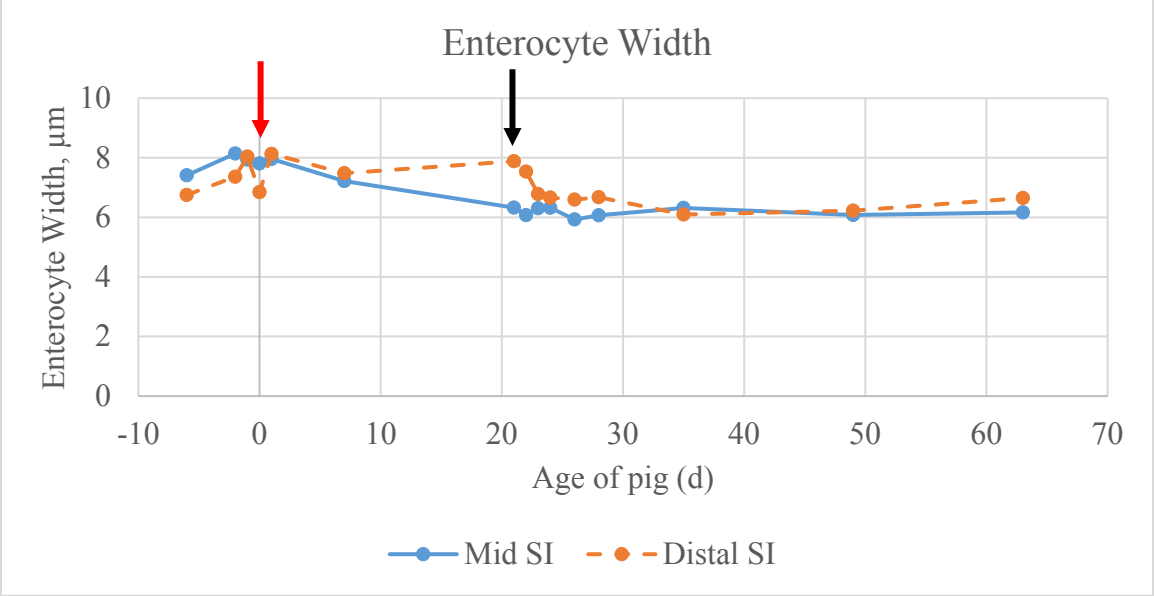

Figure 3.22 Enterocyte width from birth through 42 d post-weaning. Birth is indicated by the red arrow, and weaning is indicated by the black arrow.

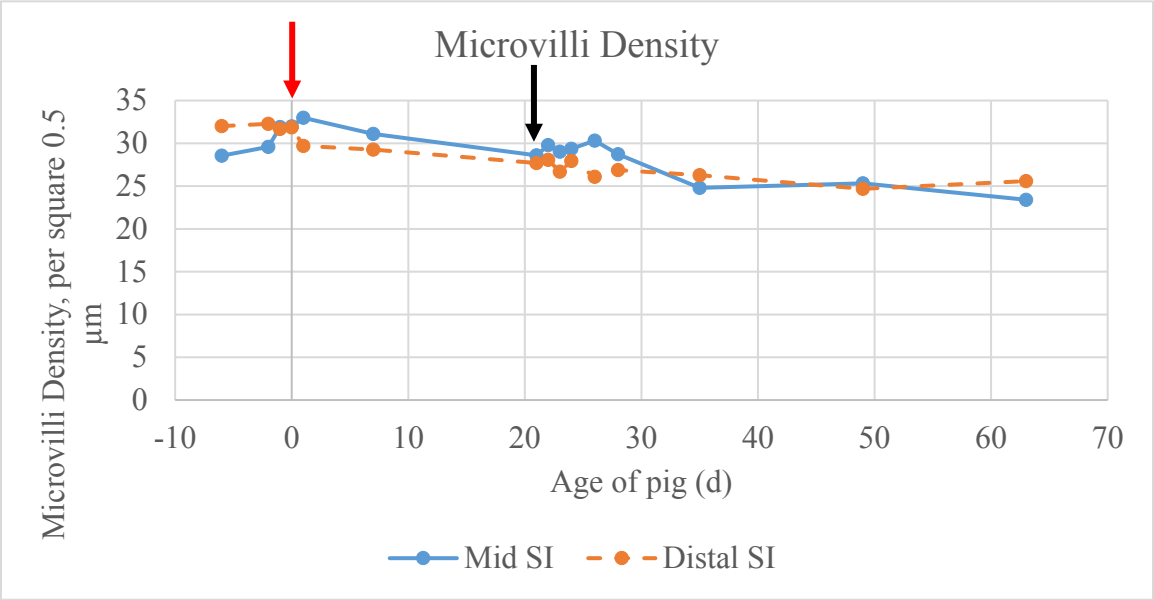

Figure 3.23 Microvilli density from birth through 42 d post-weaning. Birth is indicated by the red arrow, and weaning is indicated by the black arrow.

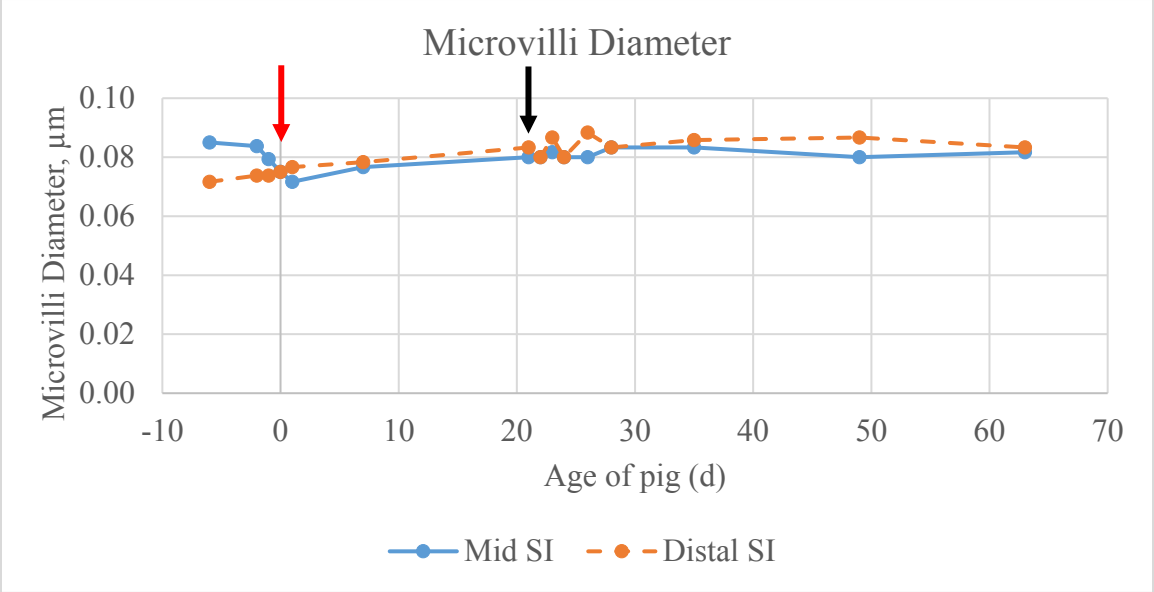

Figure 3.24 Microvilli density from birth through 42 d post-weaning. Birth is indicated by the red arrow, and weaning is indicated by the black arrow.

3.5 Discussion

This study was developed to establish an in-depth biological understanding of the young developing pig. Although there have been a number of studies that evaluate the changes that the pig undergoes either at birth or at weaning, there is limited data available over both events, let alone with frequent sampling around these physiologically stressful times/events for the pig to establish the magnitude of any changes observed and when those changes due to the stress could be minimized. General patterns can be observed in this study, but unlike blood data that is serially sampled, the measurements herein involved slaughter of different pigs at each age, which may contribute to some data variation.

3.5.1 *Body Composition*

In this study, the body composition of pigs from birth through 42 d PW was determined. Body composition is commonly assessed in pigs that are 25 kg body weight (BW) or greater as seen in Fisher et al., 2003, Wiseman, 2006, and Lu et al., 2018. Most commonly the body composition of the pig is reported with the carcass composition of the pig as well. The two are reported together mainly to assess how live chemical composition of the body relates to pork production, which can be used to assess the value of the pig's carcass at the time of harvest.

Overall, de Lange et al. (2003) has summarized the chemical composition of the pig after birth at 7 and 25 kg BW illustrating that the amount of water in the pig decreased and lipids increase, resulting in an inverse relationship for these two chemical components, which was similarly seen in Shields et al. (1983). The current study agreed with the inverse relationship between water and fat that both studies previously stated. As seen in Tables

3.2.a the amount of water decreased in the carcass, as shown by the increase in dry matter from 20% to nearly 30% from birth through 42 d PW, the amount of fat in the carcass increased from 2% to nearly 20% from birth to 42 d PW also. Throughout the study, water and protein quantitively comprised the majority of the chemical composition of the pig, which is consistent with findings reported by Shields et al. (1983).

Crude protein in the body herein was consistently around 50-60%, or 8-10% nitrogen given that protein was calculated from the analyzed nitrogen (Table 3.2a), which matches deposition trends found in Shields et al. (1983) although values are reported on a different basis, empty body versus whole carcass, explaining the differences in values. Ash values from this experiment decreased in response to age. The general decrease in ash over time was expected as the relative amount of bone in the pig decreased with the increasing proportion of muscle and fat. Calcium and phosphorus reported in the present study followed a very similar trend as the ash, which was expected, because ash is primarily calcium and phosphorus and in the body calcium and phosphorous is found primarily in the skeletal system of the animal. Additionally, the amount of calcium and phosphorus stayed in a rough 2:1 ratio throughout the course of the study, which was again expected as it is reported in a number of places (Lloyd et al., 1978; Pettey, 2004) that calcium and phosphorus exist in a 2:1 ratio in the body.

A previous study by Ma et al. (2011) observed the body composition (DM, ash, EE, and CP) of the neonate pig from birth to weaning. Note that carbohydrates were not analyzed in Ma et al. (2011), because it was anticipated as it has been previously mentioned that carbohydrates contribute a very small amount of the total body composition. After the proximate analysis of the pig was completed in Ma et al. (2011), the analyzed components

were added together with the expectation that the sum of the components would be 100%, representing that the entire pig had been accounted for. It was found that at birth 13.3% of the body composition of the pig was unaccounted for by proximate analysis, but by day 7 postpartum and through weaning the unaccounted fraction of the pig was essentially zero. In the study herein, at birth total body glycogen was observed to be around 12% (Table 3.2b). While total body glycogen quickly decreased in the body within the first week of life, the high amount of total body glycogen at birth observed in the present study along with the high unaccounted for percent of body composition observed in Ma et al (2011) shows that at birth carbohydrates need to be taken into consideration to obtain the entirety of the body composition of the neonate pig.

It should be noted in the present study that the total fraction of the pig that is unaccounted for by proximate analysis on a DM basis is between 2 – 20% in both the suckling and PW period (Table 3.2.b). This means that one or more of the analyses did not yield accurate results. In particular, the amount of fat in the carcass appeared to plateau at 20% EE at day 14 postpartum and through the rest of the study, which was unexpected as fat was predicted to continue to increase as age increased. For example, Ma et al., (2011) reported that by 7 d postpartum, EE was at roughly 34%. While the protocol for the AOAC Official Method was followed for the fat extraction from meat and meat products, the plateau at 20% fat in the whole body when a continuous increase was expected, means that there may be a different protocol that would be more appropriate to analyze a carcass sample and yield more accurate results.

For example, the protocol that was followed called for an hour long reflux of the sample, while there are other protocols, such as Bostian et al. (1985), that call for a longer

reflux and a different sample preparation before the fat is extracted. The longer reflux may result in more fat being extracted from the sample, which may get rid of the plateau that is seen in this experiment. Also, after the carcass was passed through the grinder, another preparatory step that could have been taken before further analysis occurred is that the carcass could have been dried and further ground to obtain a more uniform composite. Having a more uniform composite would help to avoid any large pieces of tissue or chips of bone in subsamples, which can skew results. There was also higher than desired coefficient of variance for all the samples in the chemical composition analysis of the pig. Although the data for body composition in this experiment may reflect to inaccurate results because of laboratory error, the results that were produced can be used to deduce relative trends of chemical composition of the young pig's body.

Carbohydrates, as previously mentioned are not typically reported in the chemical composition of the pig due to their low concentration (< 1%) in the body (Maynard and Loosli, 1962; Lloyd et al., 1978). However, as seen in the present study and with the data from Ma et al. (2011), at the time of birth, there is a considerable amount of carbohydrates in the body. After birth the amount of carbohydrates in the body, in the form of glycogen, is around 1%, which agrees with literature. The very low amount of carbohydrates in the body that have been previously reported, and reported in this experiment (Table 3.2.b), can give justification to not including carbohydrates as a main constituent with water, protein, fat, and ash in the chemical composition report after a BW of approximately 1.5 kg or one day postpartum, unless energy stores are an additional interest for the researcher.

3.5.2 *Glycogen*

Glycogen in the body, both liver and muscle, has been reported to be highest in the pig at the time of birth (Mersmann et al., 1974; Elliot and Lodge, 1977; Boyd et al., 1978); which this study agrees with as the highest concentration of glycogen is found at birth pre-suckle with a liver glycogen concentration of 76 mg/g, or 7.6% of wet tissue and a muscle glycogen concentration of 63 mg/g, or 6.3 % of wet tissue (Table 3.3 and Figure 3.10). Assuming that there is 25% dry matter in the liver (Ma et al., 2011) and 25% dry matter in the muscle (Lloyd et al., 1978), then the liver is 30% glycogen on a dry matter basis and the muscle is 25% glycogen on a dry matter basis. At birth, total body glycogen on a DM basis (Table 3.2b) is roughly 12%. This high amount of glycogen that is present in the neonate pig illuminates the importance of this form of energy reserve to the pig. The commonly observed trend for glycogen in the neonate's body is that there is a rapid depletion of the reserves immediately following birth. This was also observed in this study as the highest glycogen concentration was detected at birth (pre-suckle), followed by the glycogen levels dropping 82% and 53% in liver and muscle, respectively, 24 hours after birth, which are both roughly 10% higher than the percent glycogen declined reported by Elliot and Lodge (1977), but were near the values of 90% and 60% mobilization of glycogen in the liver and muscle, respectively, that were reported by Mellor and Cockburn (1986). Theil et al. (2014) reported that colostrum and milk consumption restores glycogen reserves in the pig after birth. This appeared to hold true in this study as well given that 24 hours after birth the glycogen levels in the pig began to rise again in the liver until weaning occurred while muscle glycogen continued to decrease gradually. Liver glycogen was depleted at a faster rate than the muscle glycogen, which was consistent with previous

studies (Elliot and Lodge, 1977; Boyd et al., 1978). Ezekwe and Martin (1978) stated that metabolism of glycogen in the muscle is slower than that in the liver, which explains why there is a more drastic drop in the liver glycogen. Also as mentioned before, when muscle mobilizes glycogen, it is for the purpose of using that mobilized glucose within itself as ATP, whereas liver glycogen redistributes its mobilized glucose throughout the body. This could be an explanation for the higher decrease in liver glycogen than muscle glycogen.

In this study, there was a second decrease in liver glycogen that was observed for the pig at the time of weaning. This decrease in glycogen could be associated with weaning. At the time of weaning, it is common that the pig will not consume solid feed for a short period of time due to either stress and/or the transition and adaptation to solid feed. During times of fasting, blood glucose in the body needs to be maintained, and this is typically done through hydrolysis of liver glycogen (Xie et al., 2016), which explains the decrease in tissue glycogen seen at weaning in this study. While there is not much work done assessing glycogen at the time of weaning, Xie et al. (2016) reported that weaning a pig early resulted in a significantly larger decrease in the glycogen reserves at d 21 of life.

Muscle glycogen at the time of weaning does not decrease as the liver glycogen does, but rather increased slightly. Unlike at the time of birth, at weaning the pig does not have as much of a challenge to maintain body temperature, there is more fat present in the pig's body, and the pig does not undergo as much physical exertion. The previous could explain the lack of a decrease in muscle glycogen at the time of weaning.

For the liver, both after birth and weaning, there is a drop in glycogen stores followed by a rise in the reserves, which is expected. However, after the rise in reserves, there is another drop in glycogen stores that could be described as a gradual decrease into

an assumed plateau. This could be due to the body overcompensating the amount of glycogen in its reserves after having used glycogen during a stressful situation, and the small decrease which follows the increase in reserve is the body returning its glycogen reserves back to normal levels. At the end of the study, there is a steady decline in the concentration of glycogen present; however, the amount of glycogen in grams remains constant, showing that there is an increase in tissue mass while total glycogen content remains static.

3.5.3 *Visceral Organs*

Absolute visceral organ growth increased with age (Table 3.4.a and 3.4.b). The absolute and relative weights of the VO at 42 d PW (25 kg BW) were similar to the absolute and relative VO weights that were reported by Lu et al. (2018) at 30 kg BW. The absolute weight of the liver, heart, spleen, and kidneys are in agreement with those reported by Doornenbal and Tong (1981) at 10 kg BW. Absolute weights (g) of the stomach, liver, heart, spleen, as well as small intestine length (m) are also comparable to those reported by Craig et al. (2019) at birth and 29 days of age. The cubic response to age that is observed at the time of weaning could be explained by the apparent weight loss of the pig and also may be an artifact of pig selection.

Relative volume decreased for the stomach and small intestine, decreasing from roughly 20% to 10% and from 70% to 50%, respectively. In contrast, the relative volume of the cecum and large intestine increased with age from roughly 2% to 6% and 10% to 40%, respectively. Duke et al. (1970) reported that for a mature pig the volume of the GIT in relative percentages is the following, 8% stomach, 30% small intestine, 16% cecum, and 46% large intestine. Lu et al. (2018) reported the relative volumes of the gastrointestinal

tract of a 30 kg BW pig as 15% stomach, 36% small intestine, 16% cecum, and 33% large intestine. At the end of this study (25 kg BW), the reported relative volumes were: 7% stomach, 50% small intestine, 5% cecum, and 38% large intestine (Tables 3.5.a and 3.5.b). Not only are there age and body weight differences in the pigs between studies, another explanation for the difference in relative volumes between pigs in the present study and the other studies, is the way that volume was measured. For example, in this present study, the stomach and cecum were filled until they were subjectively determined as "full", not filled to a maximum capacity as it was in Lu et al. (2018), which can explain the wide variation between the two studies.

It was previously stated that the absolute length of the small intestine increased 24% in the first week of life (Skrzypek et al., 2010). This study does not agree with that as the small intestine increased from 3.72 m to 5.94 m, which is roughly 62%, within the first week of life. The large intestine also increased roughly 62% as well as the absolute length increased from 0.72 m to 1.15. Relative lengths of the intestine were rather static throughout the present study with the small intestine staying at 80% of the total length and the large intestine constituting 20% of the total length (Table 3.6). The relative lengths of the intestines are in agreement with G. Colins (1871) and Lu et al. (2018). The agreement of the presented data to the literature for isolated time points would suggest that the intermediate values generated herein are truly descriptive of the pig.

The absolute weights of the visceral organs increase with body weight and age over time. Overall this leads to the obvious, and unsurprising, conclusion that the VO weight of the pig is dependent on the BW of the pig. The continuous increase in absolute volume of the gastrointestinal tract may provide important information about VO capacity. In

addition, the increasing capacities of the gastrointestinal tract that were determined through volume and length, as well as continuous increase in weight of VO show that the young pig is rapidly developing from the time of birth through 25 kg BW. Future studies to assess the growth of the VO beyond 25 kg BW can evaluate the nutrient needs and digestive and absorptive capability of the VO across different growth stages of market pigs. More research will need to be done to assess growth differences across different breeds, genetics, management practice, etc. For example, feeding high fiber diets has been reported to increase the volume of the large intestine (Leroch et al., 2003). Additionally, a further step could be taken to calculate the growth rates of the VO and the deposition rates of the chemical components to determine any trends between the two and for further insight about relationships that VO have with the chemical composition of the pig.

3.5.4 *Small intestine development*

Villus height in the small intestine of the neonate pig increased roughly 20% within the first day of life across sections of the small intestine (Tables 3.7, 3.8, 3.9 and Figure 3.11). Villus height before weaning was roughly 300 μm taller than previously reported heights of suckling pigs (approximately 1100 μm compared to 800 μm in Cera et al. (1988) and Skrzypek et al. (2010)). However, the villus heights at the proximal and middle section of the small intestine are similar to those villus heights of suckling pigs reported on the third day of life by Skrzypek et al. (2018). The decrease in villous height prior to and at weaning was also observed by Skryzpek et al. (2010). It was reported by Skryzpek et al. (2005), that villus height reaches its maximum height at 2 weeks postpartum, which is true for the villi located in the middle section of the small intestine in the present study. Villi located in the proximal section of the present study reached maximum height 2 d

postpartum, and villi in the distal section reached their maximum height 5 d postpartum, although it should be noted that 2 weeks postpartum is the second greatest villi height recorded for the distal section. Immediately following weaning, there is a decrease in the villous height, which was expected due to the physiological stress that is caused by weaning (Pluske et al., 1997). Villous height will increase after the atrophy that occurs because of weaning, but villi do not reach the height that they were prior to weaning. The decrease in the villous height prior to weaning could have been a result of the piglet getting into the sow's feed as it was not restricted in this study. This could be a result of the diet that the pig is ingesting, a course plant-based diet versus a highly digestible milk diet, as demonstrated by Deprez et al. (1987) who fed diets that were equal in nutrient content but one was in a pelleted form and the other was in a slurry form. The study found that the pigs that consumed the slurry feed had longer villi, and this may be because a lack of abrasive feed being consumed or as a result of consuming more feed.

Villus width increased through the study, and the increase in villous width found in this study from birth to 7 days postpartum was about 20 μm (roughly 80 – 100 μm) in the middle portion of the SI, which is a similar increase as reported by Skrzypek et al. (2010), although the initial width was different (roughly 60 μm). Villi height decreased at the time of weaning, 100 μm in the proximal section of the small intestine and 300 μm in the middle and distal ends of the small intestine, which is consistent with values reported and compared by Pluske et al. (1997).

Crypt depths increased roughly 25% - 30% in the suckling period across the measured sections of the small intestine (Tables 3.7, 3.8, 3.9 and Figure 3.12). In the proximal portion of the small intestine, crypts deepened from 110 μm to 157 μm, in the

middle portion of the small intestine the crypts deepened from 95 μm to 143 μm, and finally in the distal portion of the small intestine the crypts deepened from 101 μm to 136 μm. Throughout the study, the crypt continues to deepen as the pig ages, which is seen in previous studies as well (Skrzypek et al., 2010; Skrzypek et al., 2018). At the time of weaning, there is not a drastic increase in crypt depth, although the crypt depth still increased through weaning, but the drastic loss of villous height, which accounts for the smaller villous height:crypt depth ratio (V:C ratio). The greatest increase in crypt depth occurs at both 3 and 5 days PW. In the post weaning period, the crypts depths increase from roughly 150 μm to 300 μm across the different locations in the small intestine.

Prior to weaning, V:C ratio is around 6 in the proximal section of the small intestine and around 9 in the middle and distal section of the small intestine, meaning that there is a much longer villous height than crypt depth (Tables 3.7, 3.8, 3.9 and Figure 3.13). A higher V:C ratio is associated with a taller villous and a shallow crypt, which is reflective of a slower epithelium cell turnover rate and in turn a lower maintenance requirement, this typically leads to a higher growth rate in the animal (Laudadio et al., 2012). Because there is a high V:C ratio in the suckling portion of this study, it is concluded that there is a low demand for tissue synthesis for epithelial cell regeneration during the suckling period. At weaning, and following weaning, there is an anticipated decrease in the V:C ratio. After weaning, V:C ratio decreased from 5 - 6 at weaning in all sections of the small intestine to roughly 3.5 1 d PW, showing that the pig is going through physiological stress. The smaller ratio results from both a decrease in villous height and an increase of crypt depth. This means that there is atrophy occurring for the villous and the crypt is increasing its depth to generate more epithelial cells to compensate for rapid sloughing (Wang and Peng, 2018).

With advancing age, villi density decreased which, with the increase in villi width, was anticipated (Table 3.10.a, 3.10.b, 3.11a, and 3.11.b). The decrease in villi density is consistent with reports made by Skrzypek et al. (2010) and Skrzypek et al. (2018). The most obvious morphological change over time occurred for the villi vs. all other observed structural features. Using the previous measurements from light microscopy and scanning electron microscopy to calculate the absorptive area increase due to villi, there is on average a roughly 18 fold increase in the absorptive area due to villi, which is comparable to Skrzypek et al. (2010). Using observed measurements to calculate surface area of the small intestine, microvilli increase the absorptive area of the small intestine around 900 times prior to weaning, and after weaning, microvilli increased the absorptive area of the small intestine on average by roughly 400 times.

In the suckling period, there is a large increase in the surface area for both the middle and distal section of the small intestine, as well as for the surface area from both villi and microvilli, between birth and d 1 postpartum. The surface area of the middle section of the small intestine increased approximately 900,000 square millimeters due to villi from birth to d 1 postpartum, and increased approximately 800,000 square millimeters due to villi in the distal section of the small intestine during the same time. Microvilli increased the surface area in both the middle and distal section of the small intestine by approximately 20,000,000 square millimeters. In both the 25 – 50% and 75 - 100% sections of the small intestine, the greatest calculated surface area was observed on days 28 and 42 post-weaning.

3.6 Conclusions and Implications

The present study provided insight into many aspects of the development of the young pig. The body composition that was reported in this study confirmed an inverse relationship between water and fat and showed a decreasing amount of ash towards the stated 3% ash in the mature pig as reported in Lloyd et al. (1978). The chemical composition report in this study with frequent collection around birth and weaning showed the progression of increasing dry matter, fat and protein, and a decreasing ash in the young pig. Also, this study confirmed that the amount of carbohydrates in the body, apart from birth, is low and not influential in the chemical composition of the body. However, the fact that the proximate components did not equal essentially 100% reveals that one or more of the assays were not correct.

Glycogen energy reserves are critical to the neonate, and that was seen in the present study by the drastic decrease in both the liver and muscle glycogen at the time of birth. While the data reported for liver and muscle glycogen reserves at the time of birth were similar to previous studies, the data that was collected after a week of life and through the end of the study was new and enlightening, showing what appears to be an over correction in the liver concentrations when glycogen reserves are replenishing after they had been used. Additionally, after weaning, there was a decrease in the liver glycogen as expected, but usage of the glycogen stores was not nearly as large as at the time of birth. Also at weaning, muscle glycogen increased, showing that weaning is not nearly as energy demanding as birth is for the pig, or that there may have been enough blood glucose in circulation for the muscle to extract, thus resulting in less glycogenolysis in the muscle.

To further increase the understanding of glycogen metabolism around the time of birth, fetal collection as well as even more frequent collections postpartum would need to be done. In addition, when looking into the glycogen stores of the fetal and neonate, assessing sow glucose levels during gestation may give knowledge to deposition of glycogen in the fetal pig since it was reported by Ezekwe and Martin (1978) that a higher blood glucose level in diabetic sow leads to higher liver glycogen stores in the fetal pig. An evaluation of similar insulin injections as from Ezekwe and Martin (1978), but at varying doses and times may influence the amount of glucose that is transferred to the fetal pig. Finding ways to increase the glycogen stores in the fetal pig in the neonate pig can increase piglet survival rates.

Visceral organs both suckling and PW increased, as was expected in young animals. The relative values of the organ weights, volume, and length allows for comparison amongst the organs across different pigs and ages. Volume of the visceral organs was measured in this study, but it was quickly found that there was not a truly accurate way to measure volume due to the elasticity of the organs, particularly the stomach and cecum. Finding a way to determine the capacity of the organs within the body cavity will improve those measurements. Also, the measurements for the volume of the intestine were calculated rather than measured which may add some degree of inaccuracy. In reality, none of the organs in the GIT exist at a maximum volume capacity, so determining a volume for the stomach, small intestine, cecum, and large intestine when they are determined "full" by the living pig would be needed for even more accurate volume measurements of the GIT.

A next step to further create a database on the growth of VO would be to assess the differences of VO growth among different breeds at common ages. For example, Len et

al. (2008) conducted a study to compare the VO of a Mong cai pig to a Yorkshire X Landrace pig. The Mong cai is a fatty breed of pig local to northern Vietnam and performs well on fibrous diets. It was concluded by Len et al. (2008) that the relative weights of the heart, stomach, and cecum were heavier in the Mong cai pig at 63 days of age, although there were no relative weight differences in the liver and kidney. It was also found that the Mong cai had a longer cecum and colon + rectum relative length than the Yorkshire X Landrace at 63 days of age. Assessments of different breeds at common ages and on a variety of diets can give knowledge to differences in VO growth rate among breeds, and the difference in nutrient handling capabilities.

The morphology of the small intestine yields information about the ostensible surface area of the small intestine epithelium, an aspect of the nutrient absorptive capability of the young pig. This study showed that there was rapid growth of the villi prior to weaning, although just as weaning is approaching there is a decrease in height. This decrease could be a result of the piglet getting into the sow's feed, although the piglets were not monitored in the present so this may not be true. Additionally, piglets fed creep feed would more than likely have different small intestine morphology than pigs that did not consume any solid, plant-based feed prior to weaning. Scanning electron microscopy showed the density of the villi decreased but was compensated with an increase in the villi width. Overall, even with the changes in the morphology of the villi and microvilli in both the suckling period and post-weaning period, absorptive surface area increased as a result from both the villi and microvilli as the pig matured. Finding a technique to accurately measure the microvilli length would help to contribute to the accuracy of the surface area that is contributed from the microvilli. Additionally, to improve the information that was

gathered from this study, the colon of the pig should have been examined using the same light microscopy techniques as the small intestine, particularly the examination of the goblet cells of the large intestine since there is more mucus secretion than nutrient absorption there. Staining for Paneth cells, responsible for antimicrobial secretions, in the small intestine could also help assess the gut health of the pig. Also, additional sections of the small intestine could be examined to create an even more complete database of the morphological changes that occur in the small intestine. For example, a section from the duodenum should be examined so that all three regions of the small intestine have been examined across the same time periods. Finally, mucosal folds in the small intestine could also be analyzed to improve the accuracy of the absorptive surface area.

The early stages of development are momentous as it is the time that all species undergo development that has the potential to affect the animal later in life. This is particularly true for the pig, which has an extremely fast relative growth rate (i.e. increasing birth weight 4-fold by weaning at 21 days and increasing weaning weight another 4-fold in the next 42 days). Having an in-depth knowledge of the general pig development during the early stages of life can give insight to times in which improvement to pig health and performance could occur or at least when particular care of management or diet change should be exercised. This is necessary as there have been many changes to the pig over the years through genetic selection. In all, this study provided an encompassing picture of the general growth and development of the pig, highlighted glycogen energy reservoir usage, as well as the morphological changes in the small intestine that effects nutrient absorption, all of which can be used to better understand the young pig and potentially improve its health and performance.

3.7 References

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APPENDIX I: QUIMOCIAC REAGENT PREPARATION

The following steps should be followed to prepare 1 L of reagent:

1. Dissolve 70 g sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$) into 150 ml deionized, distilled (DD) water.
2. Dissolve 63.8 g citric acid dihydrate [$HOCCOOH(CH_2COOH)_2 \cdot H_20$] into 150 ml DD water, add 85 ml concentrated nitric acid (HNO_3) and allow to cool.
3. Add the molybdate solution to the citric-nitric solution while stirring.
4. Add 5 ml synthetic quinoline (C_6H_4N:CHCH:CH) to a mixture of 100 ml DD water and 35 ml concentrated nitric acid.
5. Slowly add the quinoline mixture to the molybdate-citric-nitric solution, while stirring.
6. Let solution stand overnight.
7. Filter solution through No. 2 Whatman filter.
8. Add 280 ml C.P. acetone (CH_3COCH_3) and dilute to 1 l with DD water.

APPEDENIX II: SCANNING ELECTRON MICROSCOPE (ScEM) ANALYSIS, DEHYDRATION PROCESS

A small portion of the intestine sample was transferred to a Glutaraldehyde 3% in Sorenson Phosphate Buffer (pH 7.2; from Electron Microscope Sciences cat. #16539-50, Hatfield, PA, US) solution. Figure A.2.1 illustrates how the portion of the intestine was taken. Samples were then submerged in Glutaraldehyde where they remained in the solution for at least 24 hours. Samples then underwent an ethanol de-hydration process. Table A.2.1 lists solutions and times for the dehydration process. After the samples were dehydrated with ethanol, samples were furthered dehydrated using a Critical Point Dryer (CPD; Model Leica EM CPD300; Leica Microsystems, Buffalo Groove, IL, US). The CPD is used rather than an oven to prevent any damage to the surface of the sample. CPD replaces transitional liquids, in this case the transitional liquid is ethanol as any water was previously removed in the dehydration process. Liquid CO_2 is used in the CPD so that the surface tension on a sample is lowered and the sample can undergo air drying without damage to the surface of the sample. If water was still present in the sample and air drying occurred, the high surface tension that exists because of water causes surface damage to the sample. The CPD transfers CO_2 to a supercritical state in which, at an increasing temperature and pressure, the CO_2 will be converted into its gaseous phase without having to go through the phase change of liquid to gas. Figure A.2.2 below shows how the process occurs in a CPD. Samples underwent 30 slow exchanges in the CPD, resulting in the drying time lasting ~4 hr. After the sample is dried, it is then coated with of platinum at a current of 15 milliamps for 2.5 minutes using a coating machine (Model EM5550X, Serial No. 550X-065; Emitech

Ltd, Ashford, Kent, England). After being coated with platinum, samples are analyzed in a ScEM (FEI Helios Nanolab 660 Features, ThermoFisher Scientific Pittsburg, PA, US).

Table A.2.3.19. Intestine dehydration process

Step in dehydration process	Solution	Time (hr)	Temperature (°C)
1	3% Glutaraldehyde	24 – 48 hr	4
2	50%	At least 1hr	Room
3	70%	At least 1hr	Room
4	80%	At least 1 hr	Room
5	90%	At least 1hr	Room
6	100%	At least 1 hr, up to a couple weeks	Room

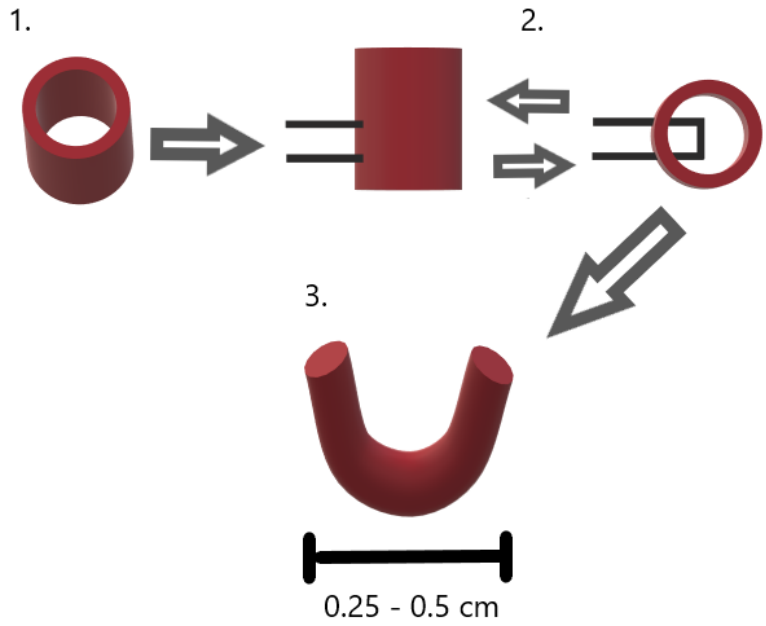

Figure A.2.1. Demonstration of how a section of the intestine was taken for ScEM. In the figure above, step 1 shows the intestine before any cuts are made. In step 2 black lines indicate where cuts on the intestine should be made. Step 3 demonstrates what the final piece of intestine will look before further preparation steps are taken.

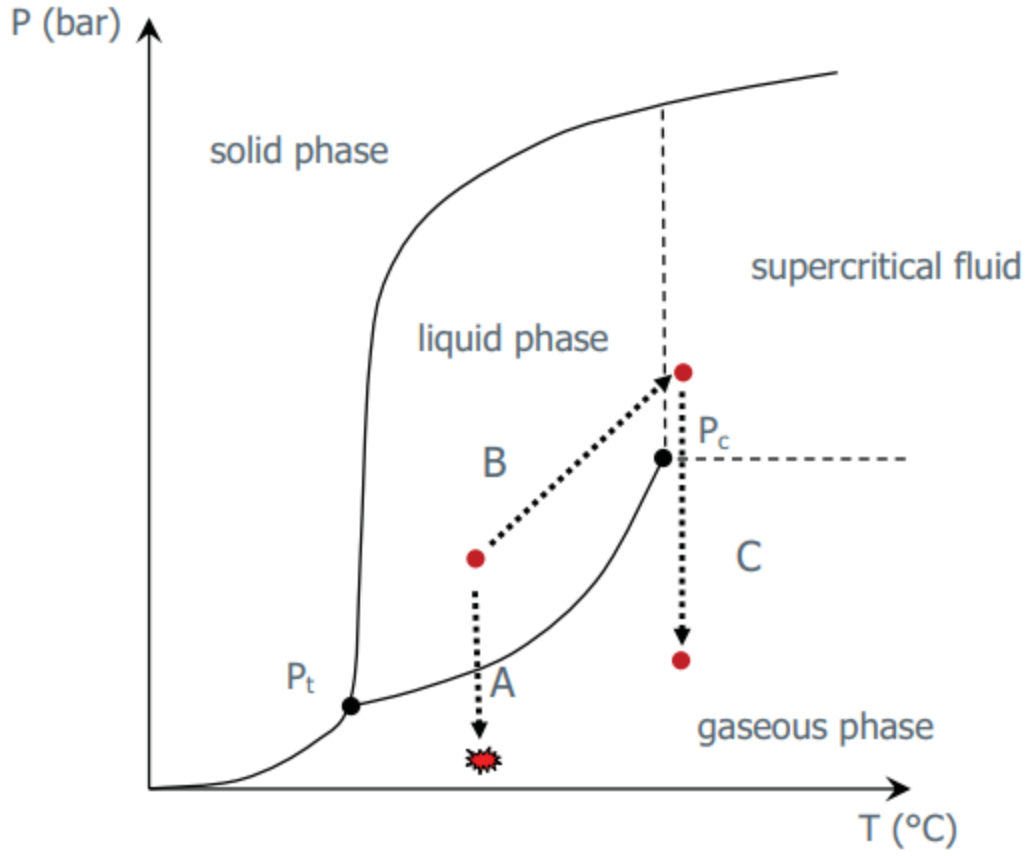

Figure A.2.2. Phase chart of the CPD. Figure from Leica Microsystems. 2012. Leica EM CPD300 Operating Manual, 167180032 Version 06/2012. Leica Mikrosysteme GmbH, Vienna, Austria. The phase chart shows how the drying process for sample bypasses the phase boundary crossing by utilizing the Critical Point (i.e. sample travels path from B to C, not B to A where the drying process would be crossing a phase boundary).

Appendix III: OUTLIER TABLES

Table A.3.20. Outliers (n) listed for chemical analysis of the pig's body[1]

Age, d	DM	Ash	N	EE	Ca	P
-6	1	-	-	-	1	-
-5	-	1	1	-	1	1
-4	-	1	-	-	-	-
-3	1	1	1	-	1	1
-2	1	-	-	-	-	-
-1	-	1	-	-	-	-
0	1	1	-	-	-	-
1	1	1	-	1	1	1
2	1	1	1	-	1	1
3	1	-	-	-	1	1
5	-	-	1	-	-	-
7	1	1	1	-	1	1
14	-	1	-	-	1	1
21	1	-	-	-	-	-
22	-	1	1	-	1	1
23	1	1	1	-	-	-
24	-	1	-	-	1	1
26	1	-	1	-	-	-
28	-	-	1	-	1	-
35	1	-	1	-	1	1
49	1	1	1	-	-	-
63	-	-	1	1	-	-
Total pigs removed	13	13	12	2	12	10

[1]Observations were identified as outliers if the sample was 3 standard deviations from the mean of the group when the sample was not included in the group mean; N=nitrogen, EE=ether extract, Ca=calcium, P=phosphorous.

Table A.3.21. Outliers (n) listed for liver and muscle glycogen concentration[1]

Age, d	Liver	Muscle
-6	-	1
-5	1	-
-4	-	1
-3	-	1
-2	-	-
-1	1	-
0	-	1
1	-	1
2	1	-
3	-	-
5	-	-
7	-	-
14	1	-
21	-	1
22	-	1
23	1	1
24	-	-
26	-	1
28	-	-
35	-	-
49	1	-
63	-	-
Total pigs removed	6	9

[1]Observations were identified as outliers if the sample was 3 standard deviations from the mean of the group when the sample was not included in the group mean.

Table A.3.22. Outliers (n) and damaged samples listed for 25% of the total length of the small intestine in light microscopy histology measurements[1]

	25% length of the total length of the small intestine			50% length of the total length of the small intestine			75% length of the total length of the small intestine		
Age, d	V	C	G	V	C	G	V	C	G
-6	-	-	-	-	-	-	-	-	-
-5	-	-	-	-	-	-	-	-	-
-4	-	-	-	-	-	-	-	-	-
-3	-	-	-	-	-	1	-	-	-
-2	-	-	-	-	-	-	-	-	-
-1	-	-	-	-	-	-	-	-	-
0	-	-	-	-	-	-	-	-	-
1	-	-	1	-	-	1	1	-	-
2	-	-	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-	-	-
5	-	-	1	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-
14	1	1	2	1	1	1	1	1	1
21	-	-	1	-	-	-	1	-	1
22	-	-	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-	-	-
28	-	-	-	-	-	-	-	-	-
35	-	-	-	-	-	-	-	-	1
49	-	-	-	1	-	-	2	2	3
63	-	-	-	-	-	-	3	3	3
Total pigs removed	1	1	5	2	1	3	8	6	9

[1]Observations were identified as outliers if an age mean had a relative standard deviation over 25%; V= Villi Height; C=Crypt Depth; G= Goblet cell count per villous

Table A.3.23. Outliers (n) and damaged samples listed for scanning electron microscopy[1]

Age, d	n	Location in the small intestine[2]	Response measure
-6	-		
-5	-		
-4	-		
-3	-		
-2	-		
-1	1	B	Villi density
0	-		
1	1	B	Villi density
1	-		
2	-		
3	-		
5	-		
7	-		
14	-		
21	-		
22	-		
23	1	C	Apical microvilli diameter and apical microvilli density
24	-		
26	-		
28	-		
35	1	C	All response measures except villous width and density
49	1	B; C	Villi density; All response measures except villous width and density
63	3	C[3]	All response measures except villous width and density
Total pigs removed	8		

[1]Outliers were identified if an age mean had a coefficient of variance over 25%.
[2]Locations labeled "B" mean the sample was taken at 50% of the total length of the small intestine; locations labeled "C" mean the sample was taken at 75% of the total length of the small intestine.

Table A.3.24. Outliers (n) and damaged samples listed for small intestine surface area[1]

	Location in the small intestine	
Age, d	50% of total length	75% of total length
-6	-	-
-5	-	-
-4	-	-
-3	-	-
-2	-	-
-1	1	-
0	-	-
1	-	1
2	-	-
3	-	-
5	-	-
7	-	-
14	-	-
21	-	1
22	-	-
23	-	-
24	-	-
26	-	-
28	-	-
35	-	1
49	1	2
63	-	3
Total pigs removed	3	8

[1]Outliers were identified from previous outlier analysis for light microscopy and scanning electron microscopy, any missing data for villi and microvilli height, width, and density from light microscopy and/or scanning electron microscopy resulted in the sample being removed from the surface area data.

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