EFFECTS OF PROTEIN SOURCE AND FAT LEVEL ON PERFORMANCE, GROWTH OF THE PANCREAS, STOMACH, AND SMALL INTESTINE, AND ON PANCREATIC ENZYME ACTIVITY AND GENE EXPRESSION IN EARLY-WEANED PIGS

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July, 2006

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ACKNOWLEDGEMENTS

I would not have accomplished this academic pursuit without the help of so many kind and selflessly giving souls….

My adviser and committee members who have been an inspiration to me, for what I have learned both in and out of the classroom with them, and the incredible moral support they have given… My adviser, Dr. Scott D. Carter, for accepting me into the OSU swine nutrition research program, for believing in me and totally supporting the research I wanted to pursue, for being ever patient and understanding of my family situation and personal dilemmas through the program, for the tireless guidance, for putting me in perspective and back in focus when needed especially with the writing of this manuscript, I can't thank him enough. To Dr. Robert G. Teeter for being generous with positive comments that I have always considered as rewards to hard work, to Dr. James E. Breazile for imparting and sharing so much knowledge and for being a great spiritual inspiration, and to Dr. Elizabeth A. Droke for the friendship and for taking the time to accommodate me after having left OSU… my heartfelt gratitude.

I would like to extend my deep gratitude to the Oklahoma Pork Council for awarding me the Bill Luce fellowship in 2004. The conduct of my research would not have been possible without the support of many including the professors who have generously accommodated me in their labs and allowing me use of their

equipment: Dr. Rod Geisart, Dr. Udaya de Silva, Dr. Jerry Ritchey, Dr. Stanley Gilliland, Dr. Glenn Zhang, Dr. Bob Wetteman, Dr. Leon Spicer, Dr. Elizabeth Droke, and Dr. Edralin Lucas. Many graduate students and researchers have extended help, advice, and positive inputs and helped me through the actual lab work especially Anthony Luyai, Jason Ross, Morgan Ashworth, Paloma, Saumnya Wickramasinghe, Asitha Vasudevan pillai, Xiaoping Guo, and special thanks to Charaka Fernando who helped me with the determination of gene expression.

The Swine Nutrition group all share this accomplishment… Jin Seong Park, Jason Schneider, Rodel Cueno, and Mariela Lachmann… I thank them for the camaraderie and helping me with the experimental part, I couldn't have handled the pigs without their help and the help and support of Kim Brock and Cecil Hooper of the OSU Swine Farm, as well as the students making up the farm crew who worked tirelessly with us. Diana Bateson and Carrianne often went out of their way to see to many important details to many of our activities… they are truly appreciated.

I am grateful for the resources made available by the Oklahoma State University Edmon Low Library, and especially the staff of the Interlibrary Services who helped me obtain much needed references; the Office of the International Students and Scholars, especially Regina Henry who answered all immigration concerns, and Linda Dunbar for help with tax concerns. I thank the faculty members of the Department of Animal Science who have contributed to my

learning experience, and the staff and graduate students who have shared special times and friendships that will be treasured. The Filipino community in Stillwater has become like family to me and I'll treasure the support they have extended as well as making special occasions real special and memorable. I also thank my friends and colleagues in the Philippine veterinary profession who have stayed in touch through all these years.

My family has been my rock, especially my parents, Pedro and Florecita, whose selfless support, love and prayers are invaluable; my children Anne, Benjie, and CJ, who have had to bear with me through all these and who have made me proud of their own accomplishments; my granddaughters Sam and Mikha who I have longed to hug since they were born… to them this work is lovingly dedicated. My special thanks to my husband Ralph for his love and support, as well as to his family who have become my second family.

Above all, I thank God for making all these possible and whose guiding hands I have always felt and relied on.

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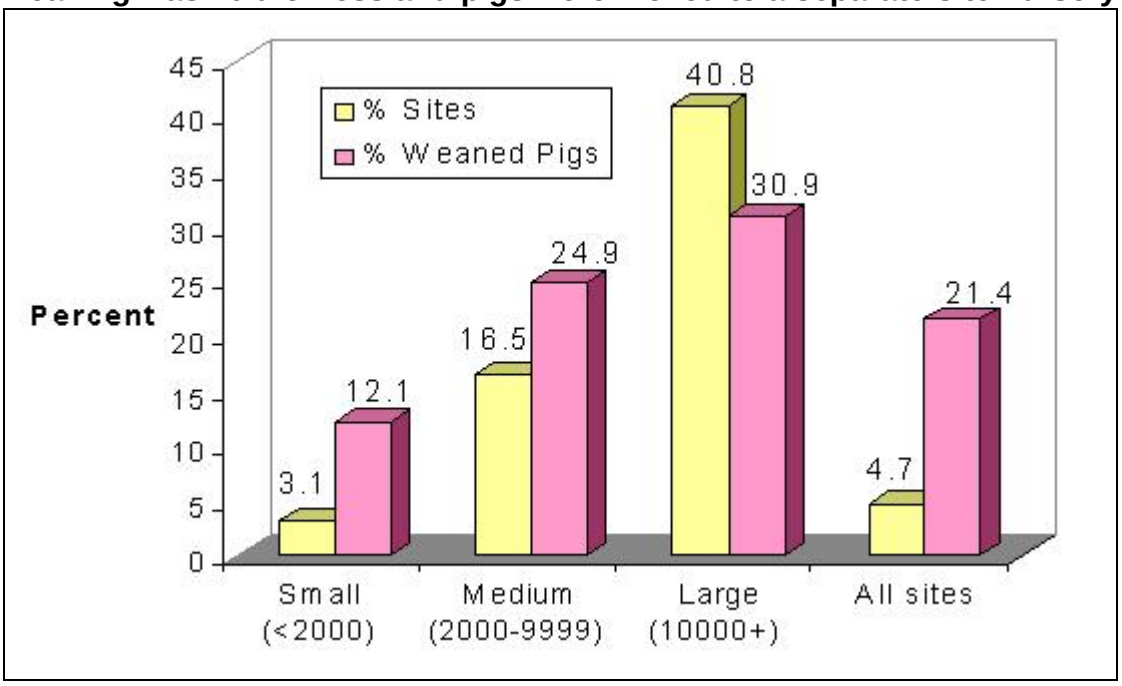
CHAPTER I

INTRODUCTION

Early weaning technology has been implemented in the swine industry since the 1980's. Piglets are weaned as early as 10 d after birth or up to 21 d, but usually average less than 21 d. The piglets are then moved to a nursery site away from the sow or other animals, a system known as Segregated Early Weaning (SEW). The USDA's National Animal Health Monitoring System (NAHMS) collected swine nursery management data from 2,499 swine production sites in 17 states from June 1, 2000 through July 14, 2000 (USDA, 2000). Data showed that the average age of weaning ranged from 18 or 19 d for medium (2,000 to 9,999 pigs in total inventory) or large (10,000 or more) to 26 d for small sites (less than 2,000). In the NAHMS report, SEW sites were those that weaned pigs at age 20 d or less and moved weaned pigs to a separate-site nursery. Data showed that only 21.4% of all weaned pigs and 30.9 % of pigs on large sites were raised using SEW (Figure 1.1). But survey data also showed that 84.1% of sites (accounting for 97.7% of all nursery pigs) raised nursery pigs in facilities that did not allow access to the outside. The major objective of segregated early weaning was to develop sanitary strategies to control or eradicate diseases for better production (Robert et al., 1999).

However, USDA's NAHMS reported no significant difference in nursery deaths between sites that used SEW and those that did not (USDA, 2000).

Figure 1.1. Percent sites and percent pigs where the maximum age of weaning was 20 d or less and pigs were moved to a separate-site nursery.



A study of piglet behavior showed that weaning piglets at 14 d or less may result in reduced performance and behavioral patterns causing or indicating reduced welfare (Worobec et al., 1999). Weaning is a very stressful period and brings about major changes in the weanling pig's life. Funderburke and Seerly (1990) enumerated three sources of stress including psychological (removal from sow, new surroundings, and new penmates), nutritional (change from liquid to dry diet and the rapid change in pancreatic enzyme production), and environmental (temperatures outside of the piglet's thermoneutral zone).

Worobec et al. (1999) described the effects of weaning age on piglet behavior and found marked differences in behavior with different weaning age. They grouped newly-weaned pig behavior into maintenance (lying, feeding, and drinking), oral-nasal activities (belly-nosing, nosing and chewing pen-mates or objects, and interaction with neighbors), and aggression and escape behavior. At weaning, mixing pigs from different litters result in aggressive behavior within the first few hours (Fraser, 1978; Friend et al., 1983). This would imply added cost of energy for aggressive activity and less time for eating and lying.

Although the newly-weaned pig consumes less, especially within the first 3 d post-weaning, its demand for energy is high. The relative weight of the small intestine increases by 25% at 3 to 7 d post-weaning and by 52% at 10 to 14 d (Le Dividich and Sève, 2000). This intestinal growth proceeds even though during the first wk post-weaning, metabolizable energy (ME) intake is only 60 to 70% of pre-weaning milk ME intake (Le Dividich and Sève, 2000). In addition to energy demands for aggressive activity and intestinal growth, there may be disease challenges that can impose additional energy cost. Energy is needed to mount an immune response and immunological stress accelerates fat tissue and muscle protein breakdown (Johnson, 1997).

Many studies have been conducted to better understand the effects of weaning and stress on the piglet digestive (Miller et al., 1986; Kelly et al., 1991; Madec et al., 1998; McCracken et al., 1999; Tang et al., 1999; Madec et al., 2000; Spreeuwenberg, et al., 2001) and endocrine physiology (White et al., 1991; Carroll et al, 1998; Le Dividich and Sève, 2000; Matteri et al., 2000; Hay et al.,

2001). Weaning stress can be minimized by ensuring that the piglet's environment is comfortable (i.e. optimum temperature, space allocation, humidity and airflow, etc.) with available water and properly formulated diets. Providing a comfortable environment cannot be underrated and it is not simple because of the psychological factors mixed in. However, formulating the diet for optimum nutrition is probably the most complex factor that has to be considered because of the variety of choices of available feed ingredients and additives that may be utilized. In addition, the interactions of the nutrients in the diet (e.g. fat and protein) have an impact on the early-weaned pig's digestive processes and consequently its growth and development.

One of the feed ingredients that has gained popularity in the last decade is spray-dried animal plasma (SDAP). It is a high quality protein ingredient, commonly of porcine origin (spray-dried porcine plasma or SDPP), that improves average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (gain to feed ratio) during the first two weeks post-weaning. Although expensive, it is considered an essential protein source in the early-weaned pig diet. In addition to improvements in growth performance, SDPP has been shown to reduce small intestinal growth (Jiang et al., 2000a). Considering the very rapid relative growth of the intestines in the first two wk post-weaning, reduced small intestinal growth in pigs fed SDPP may suggest a lower ME requirement for the intestine, thus increasing ME available for body growth (protein accretion). It is therefore hypothesized that reducing ME in newly-weaned pig diets

supplemented with SDPP might result in growth performance similar to pigs given a control diet with soy protein concentrate.

Objectives of the Study

In formulating diets, ME is easily increased or decreased with the addition or reduction of a fat source, such as soybean oil; thus, impacting the fat level in the diet. A study was initially performed to determine the effects of reducing the ME concentration of diets containing SDPP on weanling pig performance. This was followed up by two more experiments that were performed to determine the effects of two protein sources (soy protein concentrate vs. SDPP) and two ME levels (3,523 vs 3,323 kcal/kg) on growth performance of early-weaned pigs, growth of the pancreas, stomach, and small intestine, morphology of the small intestine, levels of immunoglobulins (IgG and IgA) in serum and small intestinal contents (chyme), pancreatic amylase and lipase activities in pancreatic tissue and intestinal chyme, and gene expression of pancreatic triglyceride lipase, α-amylase and trypsinogen.

CHAPTER II

REVIEW OF LITERATURE

At birth, the newborn pig's digestive system adapts to the sudden influx of liquid milk. However, colostrum in milk paves the way for a relatively smooth transition. The baby pig thrives on the highly digestible milk from the sow at a time when innate immunity is high and the baby pig is secure in the warmth of its mother. Physiologic and psychologic factors work in favor of the suckling pig. Weaning is a much more harsh transition and it would seem that the weaned pig is totally unprepared for such a change. The weanling pig diet is a drastic change from the liquid, high-fat sow milk (Perrin, 1954) to a dry diet that is mostly of plant origin. The body of the newly-weaned pig is subjected to changes in practically all aspects, from its digestive morphology and function, including changes in brushborder enzyme activity (Miller et al., 1986; Kelly et al., 1991a, b; Tang et al., 1999), to metabolism of nutrients and hormonal adjustments (Carroll et al, 1998; Le Dividich and Sève, 2000; Hay et al., 2001), and changes in the immune system (Bianchi et al., 1992; Pié et al., 2004; Stokes et al., 2004). Most of the adaptive changes taking place in the immediate post-weaning period (within the first 24 to 36 hr) are dictated, for the most part, by the level of oral nutrient intake, and by the composition of the diet. Feed intake during the first

wk post-weaning had a strong impact on the severity of digestive disorders in the first four wk post-weaning (Madec et al., 1998).

The stomach, intestines, pancreas and spleen comprise the portal-drained viscera (PDV) that, taken together, serve a complex web of functions in nutrient digestion, absorption, and metabolism, and for the intestines and spleen, in mounting an appropriate immune response. Due to advances in technology and laboratory techniques we have easier and more convenient ways of gathering data and performing laboratory analysis. Therefore we have more abundant information from research. Experiments also have been performed towards understanding the events in the immediate post-weaning period and the consequent growth and development of the early-weaned pig.

Pancreatic Development and Function

The pancreas plays a major role in the process of digestion by synthesizing and secreting the important digestive enzymes (i.e. amylase, lipase and several proteases). It also synthesizes and secretes hormones like insulin and glucagon that are key components in the absorption and metabolism of nutrients. It is well known that the secretion of enzymes and hormones by the pancreas is affected by changes in the diet. A very drastic change in diet is exemplified at weaning. The suckling pig diet is the sow's liquid high fat milk and at weaning, the piglet is subjected to the stress of separation from the sow and a shift to a dry diet of

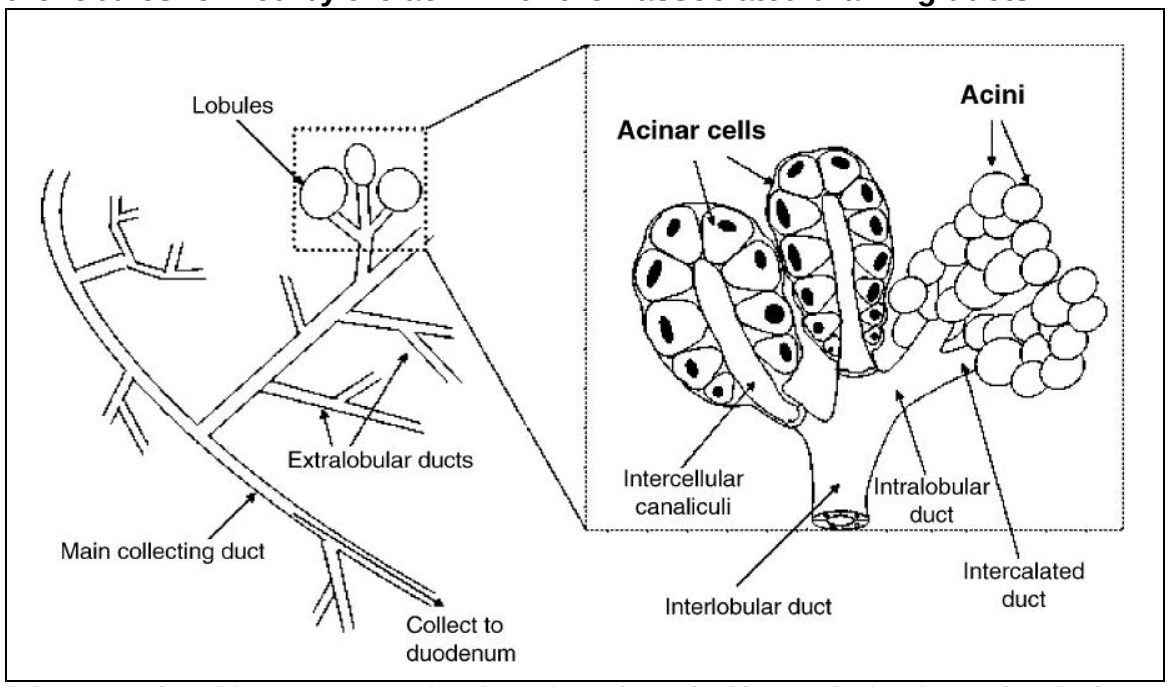
generally plant origin. Thus, major changes in pancreatic function and secretion take place in the weanling pig.

Regulation of Pancreatic Function

The pancreas is composed of about 90 to 95% exocrine tissue (acinar cells, centroacinar, and ductal cells) and only 2-3% endocrine tissue (islets of Langerhans) (Brannon, 1990) that produce digestive enzymes and hormones, respectively.

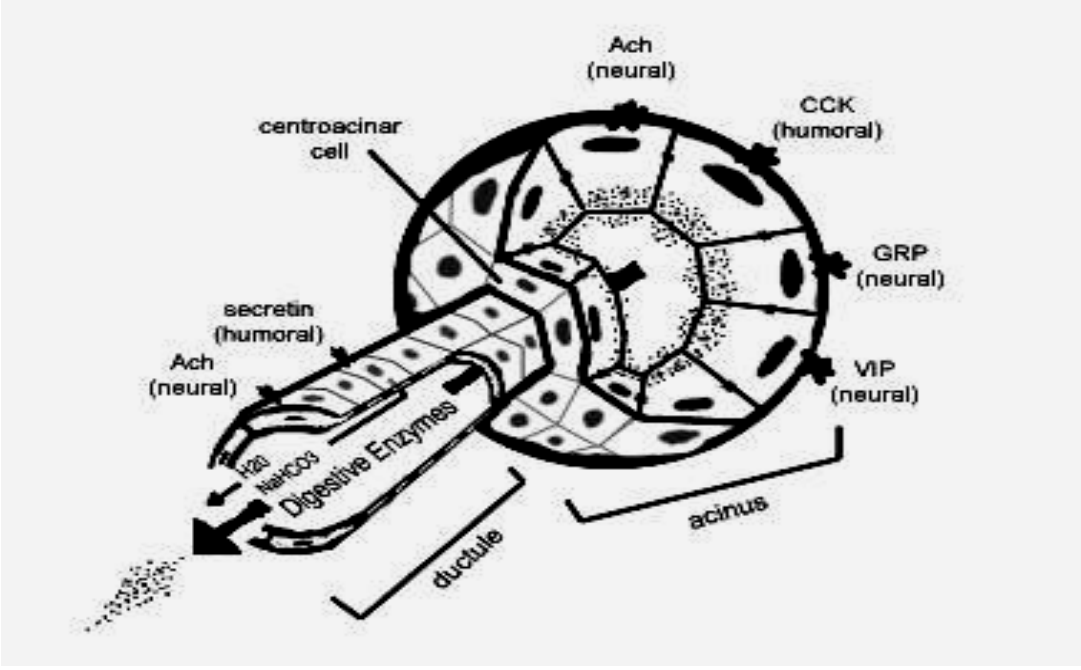
A schematic representation of the exocrine pancreas is shown in Figure 2.1 (Leung and Ip, 2006). The fine structure of the pancreas resembles a bunch of grapes with the pancreatic acinar cells forming the functional exocrine unit. Adjacent acinar cells form clusters of acini that surround a lumen into which they release the synthesized secretory vesicles or zymogen granules (Figure 2.2). Ductules or intercalated ducts that are lined by duct (ductal) cells drain the acini and these secrete water and HCO_3^-. Groups of acini form lobules; ductules join with other ductules to form intralobular ducts that drain a lobule into extralobular ducts that finally converge into the main pancreatic duct which empties into the duodenum (Bockman, 1986; Leung and Ip, 2006). The islets of Langerhans are scattered throughout the acini and secrete directly into the blood the hormones insulin, glucagon, somatostatin, pancreatic polypeptide (Bockman, 1986), and probably also neuropeptide Y (Konturek et al., 2003).

Figure 2.1. A schematic representation of the exocrine pancreas showing the lobules formed by the acini with their associated draining ducts.



(Figure reprinted from Leung and Ip (2006), and used with permission from Elsevier.)

Figure 2.2. The acinus and associated draining pancreatic ductule showing the receptors and mediators of a secretory response (Adapted from Konturek et al., 2003b).



The basolateral membrane of the acinar cell contains receptors that bind neurotransmitters or neuropeptides to stimulate secretion of zymogen granules (Figure 2.2 and 2.3). The synthesis and subsequent release of the pancreatic enzymes and hormones are under complex hormonal (Chey, 1986), neural (Holst, 1986), and neurohormonal (Singer, 1986; Niebergall-Roth and Singer, 2006) controls. These involve the interrelationships between the endocrine and exocrine pancreas described as the insulin-acinar relationship (Williams and Goldfine, 1986; Schweiger et al., 2005), between the gut and pancreatic islets, the enteroinsular axis (Creutzfeldt and Ebert, 1986), and the central control exerted by the brain via the brain-gut axis (Konturek et al., 2003a, b).

There is no pancreatic nerve but the pancreas has a rich supply of nerve fibers mostly to its periphery, originating from the celiac plexus, mainly from the vagus and splanchnic nerves (Holst, 1986). Pigs are very responsive to vagal (parasympathetic) stimulation that increases pancreatic outflow while sympathetic stimulation via the splanchnic nerve inhibits secretion (Holst, 1986). The intestinal hormone cholecystokinin (CCK) is a major stimulator of pancreatic growth and enzyme release (Rehfeld, 2004). Schweiger et al. (2005) recently reported finding CCK_A receptors exclusively in the glucagon-producing cells of the islets of porcine pancreas, and not in insulin- or somatostatin-producing cells as reported in the rat and mouse. Thus, in the pig, CCK may play a role in glucagon release and consequently, influence glucose homeostasis (Schweiger et al., 2005).

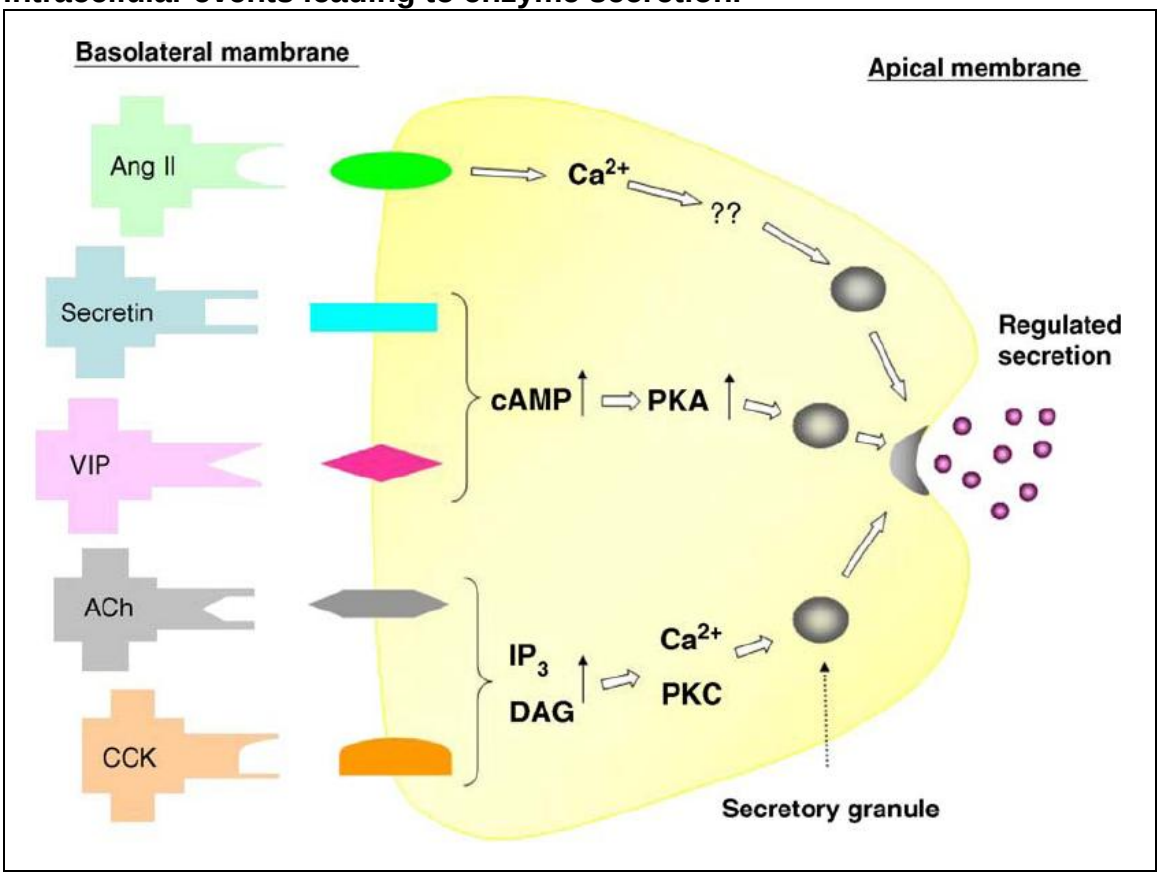
Several hormones, neurotransmitters, peptides or neuropeptides influence pancreatic secretion and a summary is given in Table 2.1 (Konturek et al., 2003a). These substances bind with receptors on the basolateral surface of the acinar cells as shown in Figure 2.3 (Leung and Ip, 2006) to initiate a cascade of events that lead to the secretion of zymogen granules that are transported to the small intestine via the main pancreatic duct.

Table 2.1. The hormones, neuropeptides or neurotransmitters that stimulate or inhibit pancreatic secretion (Adapted from Konturek et al., 2003b).

Stimulation	Inhibition
[ab]Cholecystokinin (CCK)	[ab] Pancreatic Polypeptide (PP)
[b] Secretin	[ab] Leptin
[a] Gastrin	[ab] Ghrelin
[ab] Gastrin-Releasing Peptide (GRP)	[b] Peptide YY
[ab] Insulin	[ab] Neuropeptide Y
[ab] Vasoactive Intestinal peptide (VIP)	[ab] Calcitonin Gene-Related Peptide
[ab] Cyclase-Activating peptide	[ab] Somatostatin
[ab] Substance P and other tachykinines	Glucagon
[a] Adenosine 5'-triphospate (ATP)	Glucagon-Like Peptide -1 (GLP-1)
Uridine triphosphate (UTP)	[ab] Thyrotropin-Releasing Hormone (TRH)
[ab] Histamine	[ab] Enkephalin (Met- or Leu-)
Panacreatic phospholipase A2	[ab] Nitric Oxide (NO)
[c] Acetylcholine (ACh)	[ab] Dopamine
[c] Angiotensin II (Ang II)	

[a] Present both in the brain and gut; [b]Acting through neural pathway; [c]From Leung and Ip, 2006.

Figure 2.3. The membrane receptors on the basolateral surface of an acinar cell bind hormones, neuropeptides, or neurotransmitters to initiate intracellular events leading to enzyme secretion.



(Figure reprinted from Leung and Ip (2006), and used with permission from Elsevier.)

Pancreatic Growth and Function in the Newly-Weaned Pig

The pancreas of piglets undergoes a very rapid growth and development during the 1st wk after birth (Corring et al., 1978) probably due to high amounts of growth factors present in sow milk and the high levels of glucocorticoids in the plasma of the newborn (Mubiru and Xu, 1998). Corring et al. (1978) reported a second phase of rapid pancreatic tissue growth between the 4th and 8th wk after birth, coinciding with the intake of creep feed by unweaned piglets. There was a linear increase in body weight and pancreas weight from birth to six wk with a

markedly greater slope after weaning at 4 wk (Lindemann et al., 1986). During the first 4 wk after birth, growth of the pancreas was due to the increase in number (hyperplasia) of pancreatic cells but after the 4th wk, growth was due to both hyperplasia and hypertrophy (increase in size) of pancreatic cells (Corring et al., 1978). Cera et al. (1990a) reported a linear increase in pancreas weights from d 2 to 35 in suckling pigs. There was a decline in pancreas weight at 3 d post-weaning (weaned at 21 d) but a consequent linear increase thereafter such that at 35 d, weaned pigs had greater relative pancreas weight compared to their suckling counterparts (Cera et al., 1990a). Pancreas weight was greater from the 2nd wk onward in pigs weaned at 7 d of age compared to suckling pigs the same age (Hartman et al., 1961).

The growth of the pancreas is affected by the source of dietary protein in weaned pigs (Makkink et al., 1994; Peiniau et al., 1996). Pigs weaned at 28 d and fed diets containing fish meal had greater pancreas weight (g/kg live weight) at d 3 post-weaning but was then lower on the 6th and 10th d post-weaning compared to pigs fed diets containing soybean meal (Makkink et al., 1994). Peiniau et al. (1996) reported that 35 d after weaning, the fresh weight and protein content of the pancreas was significantly lower in pigs fed diets containing soluble fish protein concentrate compared to those fed diets containing casein, soybean meal, or soyabean concentrate. Efird et al. (1982a) reported increased growth of pancreas in relation to body weight in pigs fed a soy protein diet compared to pigs fed diets with milk protein. They noted that growth inhibitors in soy protein may have caused the increase in the pancreas weight.

Development of pancreatic exocrine function at weaning. The exocrine pancreas of unsuckled newborn pigs has been shown to respond to administration of CCK and secretin although volume and enzyme content of secretion was very low (Peirzynowski et al., 1995). This response appears to increase with age and pigs at 3 to 4 wk of age showed significant stimulation compared to basal (before feeding) levels and it is surmised that pancreatic secretion might be dependent on CCK receptor maturation (Peirzynowski et al., 1995). The maturation of the exocrine pancreas function in terms of volume, protein content, and trypsin activity, was induced by weaning, and by the shift to solid feed at weaning either at 4 or 6 wk of age (Peirzynowski et al., 1993). A parallel weaning-dependent increase in post-prandial (after feeding) insulin secretion was observed which was not seen in suckling pigs even though glucose levels were increased after suckling of milk (Peirzynowski et al., 1993).

Rantzer et al. (1997) reported that the major changes in pancreatic exocrine secretion taking place during the immediate post-weaning period corresponded more to the disappearance of milk from the gut rather than to the consumption of solid feed so that milk may possibly play a role in suppressing pancreatic secretion. The weaning-dependent development of the endocrine pancreas may be related to its exocrine function (Peirzynowski et al., 1995) because of the insulin-acinar relationship described by Williams and Goldfine (1986). Insulin has been reported to influence pancreatic amylase mRNA levels in rats (Korc et al., 1981) and shown to decrease pancreatic lipase mRNA and lipase and colipase synthesis in rats (Duan et al., 1991).

Changes in pancreatic enzyme activity at weaning. The release of gut hormones such as CCK and secretin, is dictated for the most part by the type of nutrients present in the stomach and small intestine. These hormones play major roles in pancreatic secretion through the brain-gut axis (Konturek et al., 2003) or enteropancreatic reflexes (Niebergall-Roth and Singer, 2006); therefore, the type of nutrients present in the stomach and small intestine must affect the quality and quantity of pancreatic secretion. For example, protein and fat digestion products in the small intestine stimulate the release of CCK and increased levels of CCK act on the acinar cells to release zymogen granules (Konturek et al., 2003). Also, it has been demonstrated that administration of emulsified oleic acid in the intestinal lumen significantly increased CCK and secretin release (Schaffalitzky de Muckadell et al., 1986). Intravenous administration of secretin in rats increased lipase synthesis with only a slight change in amylase (Rausch et al., 1986). Thus, secretin may play a role in pancreatic adaptation to dietary fat (Wicker et al., 1988).

Adaptive changes in pancreatic enzyme secretion have been observed in rats within 24 hr of dietary change, reaching a peak after 7 to 10 d (Flores et al., 1988). Weaning is characterized by a drastic change in diet, therefore we expect major changes in pancreatic function in the immediate post-weaning period. Several authors have reported that weaning results in a general decrease in the activity of pancreatic enzymes (Hartman et al., 1961; Lindemann et al., 1986; Cera et al., 1990a; Jensen et al., 1997; Zabielski et al., 1999; Marion et al., 2003) regardless of weaning age. Marion et al. (2003) reported that circulating levels of

CCK and gastrin were markedly reduced at weaning and were positively correlated with feed intake, pancreatic enzymes, and mRNA.

Corring et al. (1978) reported that in suckling pigs from birth to 8 wk of age, there were two distinct phases in the development of total activities of pancreatic enzymes lipase, amylase, and the proteases trypsin and chymotrypsin. During the first phase, from birth to about 3 to 4 wk when creep feed intake started to increase, the total enzyme activities of all pancreatic enzymes steadily increased (by 2 to 18 units). In the second phase between the 4^{th} to the 8^{th} wk, the increase was much more rapid (lipase by 300, amylase by 240, chymotrypsin by 10, and trypsin by 23). This coincided with the increasing intake of creep feed. The consumption of milk protein and milk fat increased only very slightly from birth to 8 wk while the increase in consumption of creep feed carbohydrates was much greater (Corring et al., 1978). Lindemann et al. (1986) reported a similar trend in suckling pigs: from birth to four wk, the increase in total activity of lipase and amylase was much higher compared to the increase in total trypsin and chymotrypsin activities. The pigs were weaned at 4 wk resulting to a decrease in the activities of all pancreatic enzymes. Two wk after weaning, the total activity of lipase continued to decrease, the total activities of amylase and chymotrypsin increased to near pre-weaning levels, while that of trypsin increased to greater than pre-weaning levels (Lindemann et al., 1986). Efird et al. (1982b) compared enzyme activities on d 16 and 22 between suckling pigs and pigs weaned at 16 d. They reported a greater increase in trypsin and chymotrypsin activity in chyme of weaned pigs compared to suckling pigs.

While sow milk has very high fat content (around 40% on a dry matter basis), the weanling pig diet contains more complex carbohydrate and protein. Flores et al. (1988) weaned pigs at 4 wk and fed them either a high carbohydrate/low fat or low-carbohydrate/high fat diet for d 7 or 30. For both d 7 and 30, the high carbohydrate diet increased pancreatic amylase activity and the high fat diet increased pancreatic lipase activity while protease activity was not affected (Flores et al., 1988).

Effects of diet and feed intake. Dietary protein source and feed intake also affect pancreatic enzyme activity as reported by Makkink et al. (1994). Pigs were weaned at 28 d and fed diets containing either skimmed-milk powder (SMP), soya-bean-protein concentrate (SPC), soya-bean meal (SBM), or fish meal (FM). On d 3 post-weaning, SMP stimulated trypsin synthesis and secretion more than the other protein sources while on d 6, pigs fed SPC had higher pancreatic and jejunal trypsin activity; a higher feed intake resulted in higher pancreatic trypsin activity (Makkink et al., 1994). Efird et al. (1982a) reported higher levels of trypsin and chymotrypsin activity in chyme but lower levels in the pancreas of pigs fed soy protein compared to pigs fed milk protein. They noted that pigs fed soy protein had a higher rate of digesta passage through the stomach and that soy has a greater buffering effect. This may decrease proteolytic activity in the stomach resulting in more intact protein entering the intestine that may affect pancreatic secretion.

Changes in synthesis of proteases, amylase, and lipase parallel the changes in their mRNA levels in response to their respective substrates (Brannon, 1990). In pigs weaned at 7 d of age, trypsin mRNA levels decreased 3 d after weaning, but increased linearly in the 2-wk period post-weaning, while relative activity of trypsin decreased during the 3rd and 7th d post-weaning before increasing on the 14th d (Marion et al., 2003). Lipase mRNA also decreased 3 d after weaning, but increased linearly during the 2-wk period post-weaning, while relative lipase activity decreased linearly during the same period (Marion et al., 2003). The decrease in pancreatic enzyme gene expression was enhanced with low feed intake (Marion et al., 2003).

Growth, Morphology, and Function of the Gastrointestinal System

The gastrointestinal system develops functionally and grows rapidly after birth when enteral feeding commences. The ingestion of colostrum or milk stimulates protein synthesis in the intestines, other visceral tissues, and muscular tissue (Burrin et al., 1992), resulting in increased gastrointestinal protein accretion. The presence of nutrients in the gut is known to be stimulatory to gastrointestinal growth (Thomson and Keelan, 1986) and total parenteral or intravenous nutrition results in decreased intestinal mass (Goldstein et al., 1985; Morgan et al., 1987). Cranwell and Moughan (1989) summarized the relationship of the weight of the stomach, small intestine, and pancreas, with that of the body weight of suckling piglets from birth up to 38 d of age (Table 2.2).

Table 2.2. The weight (g) of stomach, small intestine, and pancreas per kg body weight of suckling pigs from birth to 38 d of age (Adapted from Cranwell and Moughan, 1989)

Age (days)	0	1-3	5-12	14-18	21-23	24-28	29-38
Stomach (g/kg BW)							
Widdowson et al. (1976)[1]	4.0	4.5	4.2	-[2]	-	-	-
Braude et al. (1981)[1]	4.5	5.5	5.1	4.8	-	5.1	-
Cranwell (1985a)[1]	-	-	5.9	4.7	4.7	5.2	5.2
Cranwell (1985b)[1]	-	5.3	5.3	4.3	4.3	4.7	4.7
Xu (1989)[1]	4.9	5.2	5.3	4.6	4.2	4.5	4.0
Small Intestine (g/kg BW)							
Widdowson et al. (1976)	23.0	33.0	29.0	-	-	-	-
Braude (1981)	24.0	27.0	26.0	27.0	-	26.0	-
Efird et al. (1982)[1]	-	34.0	33.0	33.0	26.0	-	-
Cera et al. (1988a)[1]	-	34.0	32.0	-	30.0	36.0	41.0
Pancreas (g/kg BW)							
Friend et al. (1970)[1]	-	-	-	-	1.1	-	1.1
Widdowson and Crabb (1976)[1]	1.0	1.6	1.4	-	-	-	-
Corring et al. (1978)[3]	1.0	-	1.5	1.4	1.4	1.6	1.7
Shields et al. (1980)	1.7	-	-	0.8	-	-	-
Efird et al. (1982)	-	1.0	1.2	1.1	1.0	-	-
Lindemann et al. (1986)[1]	0.8	-	1.4	1.1	1.2	1.1	-
Owsley et al. (1986)[4]	1.0	-	-	1.2	-	1.2	-

[1]No creep feed; [2]no observations at this age; [3]creep feed from 10 days of age; [4]creep feed from 14 days of age

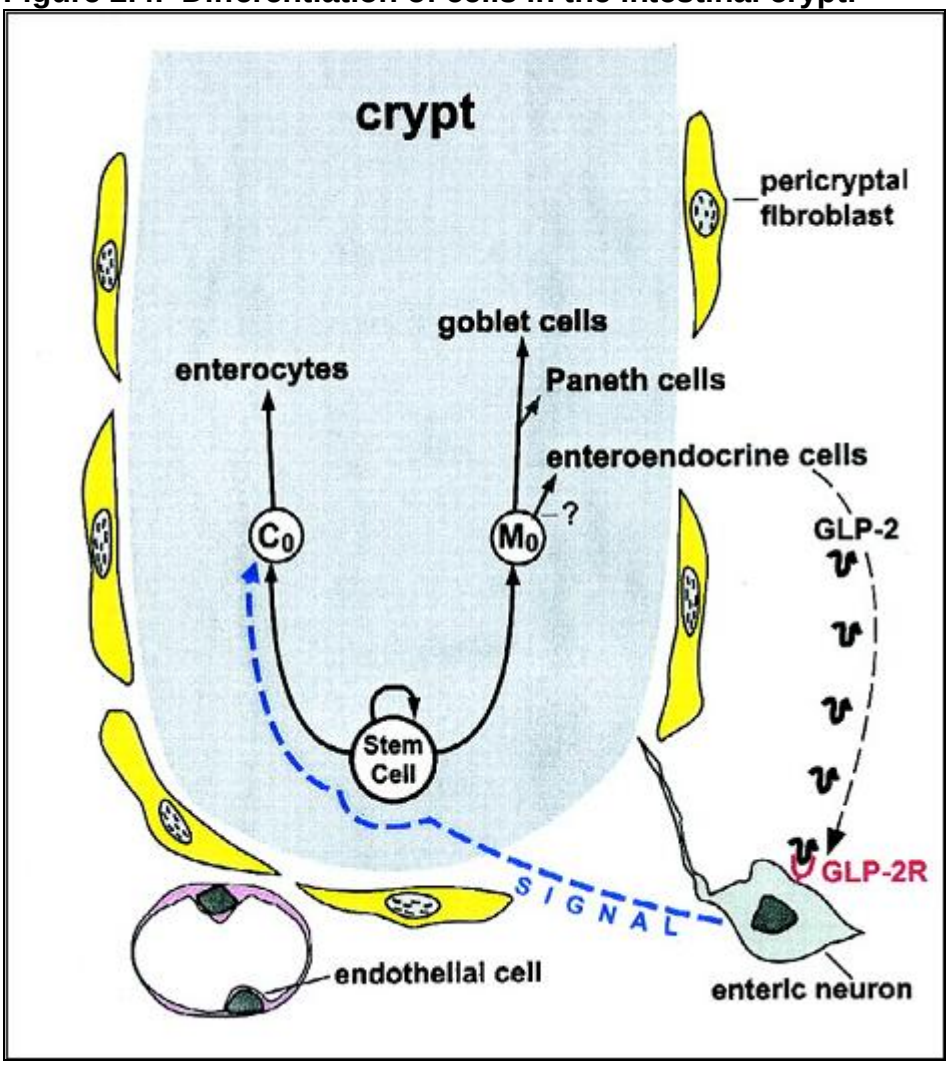
After weaning, the growth rate and weight of these organs in relation to body weight is much greater especially the small intestine (Cranwell and Moughan, 1989). Regardless of weaning age, the relative weight of the small intestine increases by 25% from 3 to 7 d post-weaning, and up to 52% at 10 to 14 d while the relative weight of the pancreas has a corresponding 17% and 30% increase, respectively (Le Dividich and Sève, 2000). This increase in gastrointestinal weight relative to body weight is attributed to the change in diet because growth is higher in pigs fed solid feed compared to those fed a liquid milk substitute (McCracken et al., 1995). The weaned pig apparently requires a larger digestive system (compared to the suckling pig counterpart) to properly digest and absorb the more complex post-weaning diet and the period needed to upgrade its digestive system is probably one of the limitations affecting post-weaning performance (Cranwell and Moughan, 1989).

Morphology and Function of the Small Intestinal Epithelium

The surface of the small intestine (the mucosa) is composed of fingerlike projections (villi) that protrude into the lumen, and invaginations of the epithelium around the villi called crypts of Lieberkuhn. The villi and crypts are lined with columnar epithelial cells (enterocytes) that arise from stem cells at the base of the crypts. The stem cells are continually dividing and differentiating into one of four cells (Figures 2.4 and 2.5): an enterocyte, enteroendocrine cell, goblet cell, or Paneth cell (Smith, 1985). The cells that become enterocytes continue to

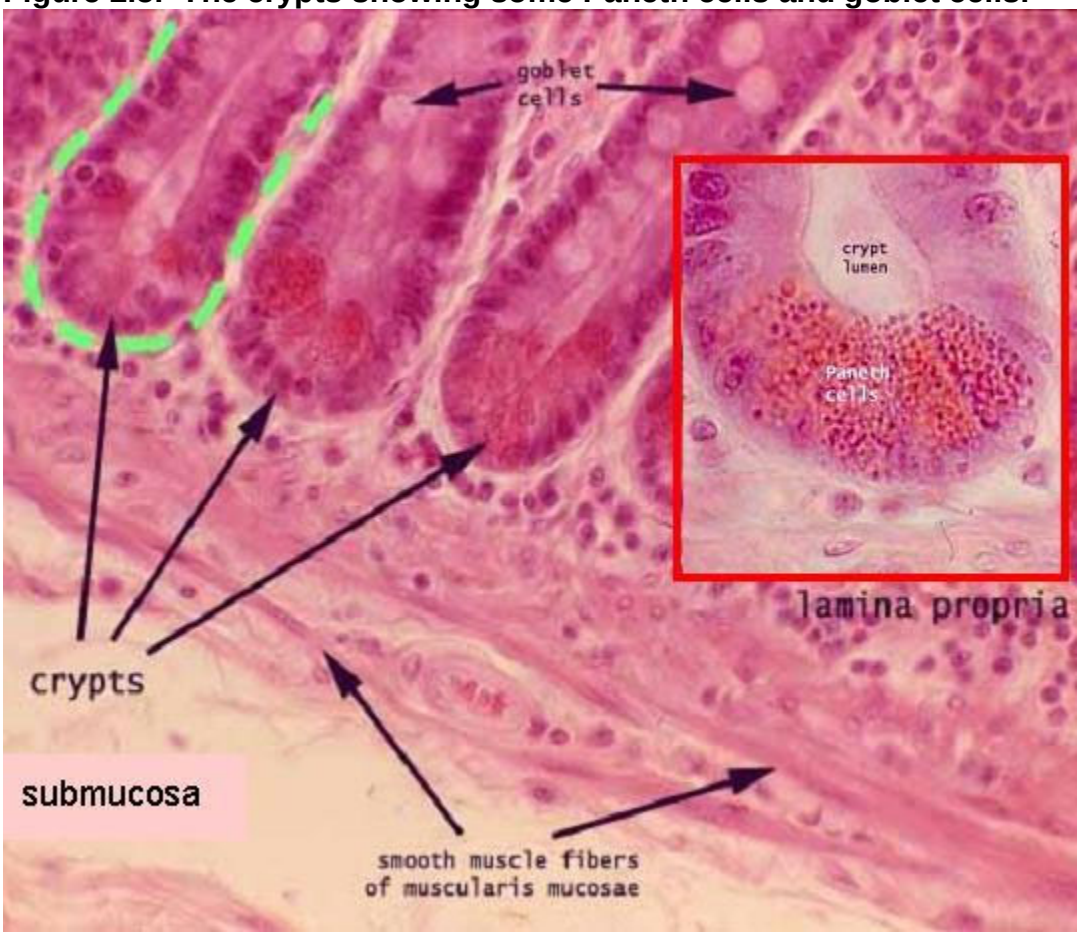
divide as they migrate up the crypts maturing into digestive and absorptive cells in the villous, and they finally slough off from the villous tip as their life cycle ends (Figure 2.6). Thus, the villi are lined with mature absorptive cells while the epithelium lining the crypts contains immature enterocytes. The luminal plasma membrane of the villous epithelium is lined with densely packed microvilli referred to as the brushborder that, in mature enterocytes, contain digestive enzymes and transport proteins essential for their digestive and absorptive functions. The synthesis of these enzymes and other microvillar proteins has been reviewed by Danielsen et al. (1984). Mucus-secreting goblet cells are interspersed along the epithelium. Enteroendocrine cells that secrete the hormones cholecystokinin and gastrin also form part of the epithelium.

Figure 2.4. Differentiation of cells in the intestinal crypt.



(Figure reprinted from Mills and Gordon (2001), used with permission from the National Academy of Sciences, USA.)

Figure 2.5. The crypts showing some Paneth cells and goblet cells.



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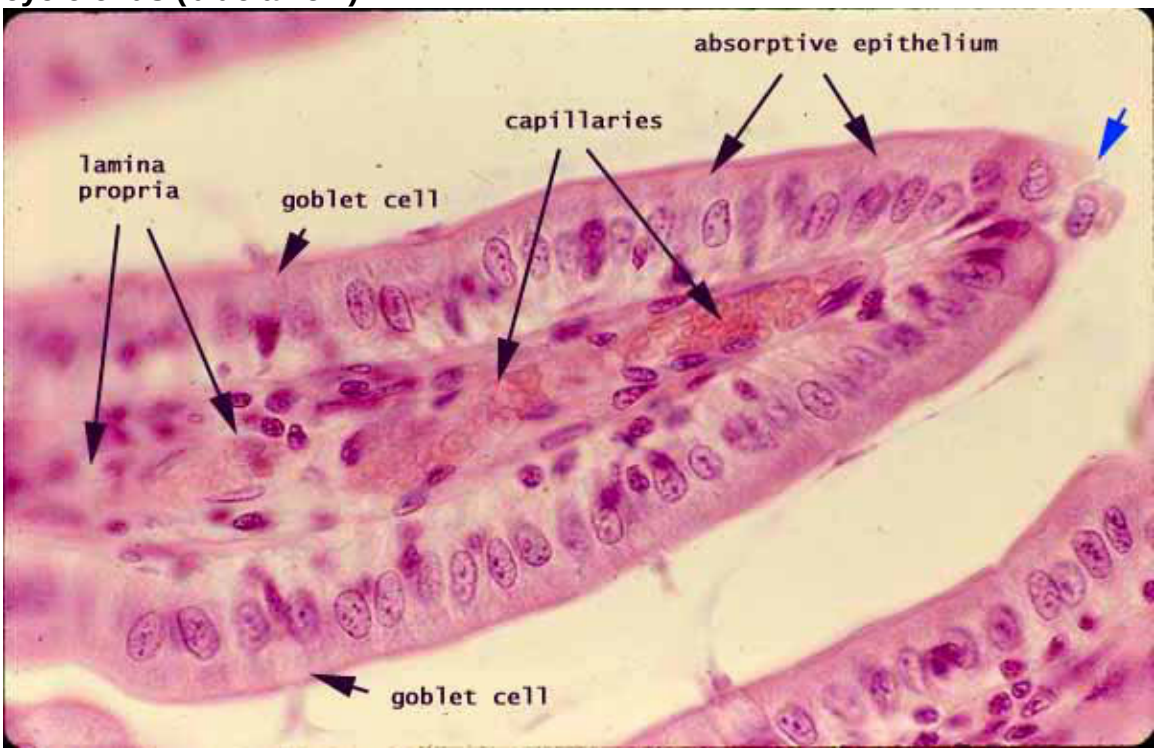
The lamina propria makes up the core of the intestinal villus and is separated from the epithelium by a basement membrane (Figure 2.6). Capillaries and lymphatic vessels are embedded in the lamina propria connective tissue along with smooth muscle, nerve fibers, variable white blood cells, mast cells, and others. The capacity of the brushborder to transport and absorb nutrients does not take place unless structural differentiation has ended (Smith, 1985). Nutrients are absorbed into the villous epithelium through the brushborder and undergo processing in the enterocyte. The nutrients enter the systemic circulation via the capillaries and lymph vessels in the lamina propria.

Figure 2.6. The intestinal villus with the lamina propria and mature enterocytes with a villus tip showing a mature cell sloughing off as its life cycle ends (blue arrow).



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Changes in Morphology at Weaning

The mucosa of the small intestine undergoes dramatic changes at weaning. The long slender villi are drastically reduced in height (30 to 63% reduction) and the crypt depth is increased by 76 to 180% (Hampson, 1986; Miller et al., 1986; Cranwell and Moughan, 1989; Kelly et al., 1991a, b; van Beers-Schreurs et al., 1998; Hedemann et al., 2003) with mitotic counts showing increased proliferative activity from the 3rd d post-weaning (Hedemann et al., 2003). There appears to be a good correlation between crypt depth and mitotic counts. Onset of crypt elongation and increased mitotic counts occur with increased amount of ingesta in the gastrointestinal tract (Hedemann et al., 2003).

Van Beers-Schreurs et al. (1998) attempted to determine whether villous atrophy was caused by separation of the pigs from the sow or by changes in the quantity and composition of the diet. They reported that the degree of villous atrophy was more closely associated with the level of feed intake rather than the composition of the diet (sow milk vs commercial weanling diet) and that villous atrophy was partly caused by separation from the sow. Marion et al. (2002) also reported that decreasing energy (feed) intake is the major factor responsible for villous atrophy at weaning. Kelly et al. (1991) intubated weaned pigs with a pelleted diet to insure uniform feed intake and they reported that villous atrophy was not prevented by relatively high nutrient supply to the gut. Dietary digestible carbohydrate composition (lactose vs. glucose vs. starch) did not prevent villous atrophy and an increase in crypt depth at weaning (Vente-Spreeuwenberg et al., 2003). However, between d 3 to 10, the increase in villous height was greater in

pigs fed diets with lactose (Vente-Spreeuwenberg et al., 2003). Zijlstra et al. (1996) reported that 7 d post-weaning, pigs weaned at 14 or 21 d to a milk replacer diet had 74% longer villi in the proximal small intestine compared to suckled pigs. Pigs weaned to a starter diet had a 28% shorter villi. Pluske et al. (1996c) reported that feeding whole cow's milk to weaned pigs for 5 d maintained villous height and crypt depth.

Pluske et al. (1997) performed an extensive review of factors that influence the intestinal structure and function in the newly-weaned pig and listed five major factors that contribute to the changes in gut structure and function at weaning including: 1) the presence of pathogenic bacteria, 2) maladaptation to weaning stressors, 3) withdrawal of sow's milk that contains high levels of epidermal growth factor, polyamines, insulin, insulin-like growth factors, and glutamine, 4) dietary change (causing decreased feed intake and possible exposure to anti-nutritional factors), and 5) cytokines and the role they play in epithelial cell growth. There is a great interplay of these factors and aside from level of feed intake, the health status of the gut also plays a major role in maintenance of mucosal structure. Recently, Mei and Xu (2005) reported a significant decrease in transforming growth factor-β1 (TGF-β1) at the apical membrane of the intestinal villi epithelium on d 4 post-weaning; this was transient, returning to the pre-weaning level by the 8^{th} d. This decrease in TGF-β1 was associated with atrophy of the villi and a marked reduction in digestive enzyme activities (Mei and Xu, 2005).

Digestive Function of the Brushborder

The microvillus has a very highly ordered structure of generating mature enterocytes possessing substantial integral membrane proteins necessary for their digestive and absorptive functions. An increase in enterocyte migration from crypt to villus and a lower proportion of mature enterocytes result in inadequate development of microvillar proteins such as digestive brushborder enzymes and transport proteins (Cera et al., 1988a). Some brushborder peptidases (Table 2.2) and carbohydrases (Table 2.3) have been detected in weanling pig intestines. Also, digestive brushborder enzyme activities (Kidder and Manners, 1980; Miller et al., 1986; Kelly et al., 1991a, b; Hedemann et al., 2003) and gene expression (Torp et al., 1993; Marion et al., 2005) have been quantified during the immediate post-weaning period. In general, the levels of activity and mRNA gene expression of brushborder enzymes are greatly affected by the integrity of the mucosal epithelium, by nutrients in the diet, and by proteases in the intestinal lumen.

Lactase. Lactase is the predominant enzyme during the suckling period and the activity level is high at birth, peaking at 3 wk of age, and declining rapidly during the weaning period (Hampson and Kidder, 1986; Miller at al., 1986; Kelly and King, 2001). Boudry et al. (2004) reported a nearly 90% decrease in lactase specific activity in the proximal jejunum and distal ileum between d 0 and 5 post-weaning. There is a significant decline in lactase activity in suckling pigs that consumed creep feed (Hampson and Kidder, 1986).

Table 2.3. The peptidases on the intestinal brushborder.

Common name (Other name(s))	E.C. #	Reaction	References
Aminopeptidase N (microsomal aminopeptidase; aminopeptidase M; membrane aminopeptidase I)	E.C. 3.4.11.2	Release of an N-terminal amino acid, Xaa┼Yaa- from a peptide, amide or arylamide. Xaa is preferably Ala, but may be most amino acids including Pro (slow action). When a terminal hydrophobic residue is followed by a prolyl residue, the two may be released as an intact Xaa-Pro dipeptide	Danielsen (1984); Torp et al. (1993); Hedemann et al. (2003); Marion et al. (2005)
Amino-peptidase A (glutamyl aminopeptidase; aspartate aminopeptidase; glutamyl peptidase; Ca^{2+}-activated glutamate aminopeptidase; membrane aminopeptidase II)	E.C. 3.4.11.7	Release of N-terminal glutamate (and to a lesser extent aspartate) from a peptide; Ca^{2+}-activated and generally membrane-bound	Danielsen et al. (1984); Marion et al. (2005)
Dipeptidylpeptidase IV (dipeptidyl aminopeptidase IV; glycylproline aminopeptidase; X-prolyl dipeptidyl aminopeptidase)	E.C. 3.4.14.5	Release of an N-terminal dipeptide, Xaa-Yaa┼Zaa-, from a polypeptide, preferentially when Yaa is Pro, provided Zaa is neither Pro nor hydroxyproline	Danielsen et al. (1984); Hedemann et al. (2003)
γ-glutamyl transpeptidase (glutamyl transpeptidase; α-glutamyl transpeptidase; γ-glutamyl peptidyltransferase; γ-glutamyl transpeptidase; L-γ-glutamyl transpeptidase; L-γ-glutamyltransferase)	E.C. 2.3.2.2	(5-L-glutamyl)-peptide + an amino acid = peptide + 5-L-glutamyl amino acid	Hedemann et al. (2003)

[1]**IUBMB Enzyme Nomenclature**

Table 2.4. The carbohydrases on the intestinal brushborder.

Common name (Other name(s))	E.C. #	Reaction	References
Maltase Ib (sucrase(invertase); sucrase-isomaltase; sucrose-α-glucosidase; sucrose-α-D-glucohydrolase; sucrose.alpha.-glucohydrolase; intestinal sucrase)	E.C. 3.2.1.48	Hydrolysis of sucrose and maltose by an α-D-glucosidase-type action	Kidder and Manners (1980); Danielsen et al. (1984); Kelly at al. (1991); Marion et al. (2005)
Maltase Ia (Isomaltase; isomaltase oligo-1,6-glucosidase; sucrase-isomaltase)	E.C. 3.2.1.10	Hydrolysis of 1,6-α-D-glucosidic linkages in some oligosaccharides produced from starch and glycogen by EC 3.2.1.1 (α-amylase), and in isomaltose	Kidder and Manners (1980)
Maltase II and Maltase III (glucoamylase; glucan 1, 4-α-glucosidase; 1,4-α-D-glucan glucohydrolase; amyloglucosidase; γ-amylase; acid maltase; exo-1,4-α-glucosidase; glucose amylase; γ-1,4-glucan glucohydrolase)	E.C. 3.2.1.3	Hydrolysis of terminal 1,4-linked α-D-glucose residues successively from non-reducing ends of the chains with release of β-D-glucose; most forms of the enzyme can rapidly hydrolyse 1,6-α-D-glucosidic bonds when the next bond in the sequence is 1,4, and some preparations of this enzyme hydrolyse 1,6- and 1,3-α-D-glucosidic bonds in other polysaccharides. This entry covers all such enzymes acting on polysaccharides more rapidly than on oligosaccharides.	Kidder and Manners (1980); Miller et al. (1986); Kelly at al. (1991);

[1]**IUBMB Enzyme Nomenclature**

Table 2.4 (Continued)

Common name (Other name(s))	E.C. #	Reaction	References
Maltase (α-glucosidase; glucoinvertase; glucosidosucrase; maltase-glucoamylase; α-glucopyranosidase; glucosidoinvertase; α-D-glucosidase; α-glucoside hydrolase; α-1,4-glucosidase)	E.C. 3.2.1.20	Hydrolysis of terminal, non-reducing 1,4-linked α-D-glucose residues with release of α-D-glucose; This single entry covers a group of enzymes whose specificity is directed mainly towards the exohydrolysis of 1,4-α-glucosidic linkages, and that hydrolyse oligosaccharides rapidly, relative to polysaccharide, which are hydrolysed relatively slowly, or not at all. The intestinal enzyme also hydrolyses polysaccharides, catalysing the reactions of EC 3.2.1.3 glucan 1,4-α-glucosidase.	Danielsen et al. (1984); Miller et al. (1986); Flores et al. (1988); Kelly at al. (1991a, b); Marion et al. (2005)
Trehalase (α,α-trehalase; α,α-trehalose glucohydrolase)	E.C. 3.2.1.28	α,α-trehalose + H_2O = 2 D-glucose	Kidder and Manners (1980)
Lactase (β-galactosidase; β-lactosidase; β-D-lactosidase; trilactase; β-D-galactanase)	E.C. 3.2.1.23	Hydrolysis of terminal non-reducing -D-galactose residues in β-D-galactosides; Some enzymes in this group hydrolyse α-L-arabinosides; some animal enzymes also hydrolyse β-D-fucosides and β-D-glucosides	Kidder and Manners (1980); Hampson and Kidder (1986); Miller et al. (1986); Flores et al. (1988); Kelly at al. (1991a, b); Marion et al. (2005)
Lactase-phlorizin hydrolase	E.C. 3.2.1.23-62		Danielsen et al. (1984); Sangild et al. (1995); Torp et al. (1993)

Lactase activity is higher in the intestines of piglets kept in a cleaner environment (Miller et al., 1986). There is low lactase activity in enterocytes emerging from the crypts, subsequently increasing as enterocytes migrate along the villus reaching a constant or beginning to fall slightly as enterocytes reach the tip of the villus (Miller et al., 1986). Absolute lactase activity differed with the age of the piglet and whether it has been weaned or not, but a reduction in villous height results in a decrease in total digestive surface area (Miller et al., 1986). Kelly et al. (1991) weaned piglets at 14 d to a cereal-based diet (containing fat-filled skim milk powder) that was either restricted or tube-fed continuously six or seven times daily. Pigs were slaughtered at weaning, then 3, 5, and 7 d post-weaning. They sampled five sites from the small intestine (10, 30, 50, 70, and 90% from pylorus to ileo-cecal valve) and reported a sharp increase in lactase activity from the 10% to the 30% site and then a steady decline with the last site having the lowest value (Kelly et al., 1991).

Torp et al. (1993) reported that there seems to be a close correlation between mRNA levels and protein synthesis along the small intestine with maximum mRNA expression in the proximal jejunum, intermediate in the proximal ileum and very low in the duodenum and terminal ileum.

Sucrase, maltase and other disaccharidases. Sucrase and maltase activities are very low at birth, increased during the first 3 wk, then rapidly increased from the 3rd wk onward (Kelly and King, 2001). Hampson and Kidder (1986) reported a reduction in sucrase activity in the immediate post-weaning

period. Miller et al. (1986) reported higher sucrase and isomaltase activity, but not maltase II and III, in piglets kept in a cleaner environment at weaning. As pig enterocytes migrate from crypt to the tip of the villus, the activity of maltase increases just like lactase activity (Miller et al., 1986) but age did not affect the levels and the decrease at weaning is less pronounced compared to lactase (Miller et al., 1986; Tang et al., 1999). Kelly et al. (1991) reported dramatic increases in maltase and glucoamylase by the 3rd d post-weaning suggesting the influence of nutrient level (cereal-based diets) on rapid substrate induction of the enzymes. Boudry et al. (2002) also concluded that switching from milk to a cereal diet increased the activities of some mucosal enzymes. Lactase and maltase II decreased significantly in the intestine of unweaned pigs when they reached six wk of age (Miller et al., 1986).

Peptidases. The activities of aminopeptidase N and dipeptidylpeptidase IV decreased from the day of weaning up to d 3 in all segments of the small intestine, but weaning only had minor effects on γ-glutamyl transpeptidase per gram of mucosa (Hedemann et al., 2003). The decline in peptidase activity in the proximal intestine is likely due to the observed villous atrophy and loss of mature enterocytes while starvation may be the cause of the reduction in activity in the distal intestine (Hedemann et al., 2003). Torp et al. (1993) reported a fairly constant aminopeptidase N mRNA expression along the length of the intestine that roughly correlated with the specific enzyme activity in the ileum. However, the duodenum and proximal jejunum had a much higher ratio of mRNA/specific

enzyme activity indicating rapid turnover that is most likely due to higher degradation by pancreatic enzymes (Torp et al., 1993).

Energy Demands and Growth of the Small Intestine

The small intestine serves a major function in the body that utilizes protein and energy sources directly from dietary nutrients in the intestinal lumen (first-pass metabolism), and from the blood (arterial mesenteric circulation). Recent studies by Burrin et al. (2001) quantified the nutrient requirements of gut tissues and they concluded that gut tissues consume a significant proportion of the whole body nutrient needs. This is mainly due to the high rates of protein synthesis and energy metabolism in the gastrointestinal tract. It is interesting to note that although the morphology and function of the intestinal epithelium show drastic changes, tissue accretion (growth) of the gut is relatively unaffected by dietary composition and nutrient intake (Reeds et al., 1993). When piglets were fed a high protein diet, portal drained viscera (PDV) utilized lysine from arterial blood supply (i.e. lysine in blood circulation), but when the diet was low in protein, the lysine requirement of PDV was supplied by both arterial and by first-pass metabolism (van Goudoever et al., 2000). Thus, the gut lysine requirement was met, but because lysine is generally the first limiting amino acid, this may limit the systemic lysine available for lean tissue growth.

Gastrointestinal vs. Muscular Tissue Energy Utilization

While the gastrointestinal tissues can source out nutrients from the lumen and from the arterial supply, the muscular system, on the other hand, can only take nutrients from arterial circulation for its growth and function. This bears a significant impact on how these two systems influence the utilization of dietary energy and protein. The metabolic activity levels of PDV and the muscular system are different during resting or in active states. In the resting state, the overall expenditure of energy is almost the same, about 25% of the whole body, even though PDV makes up only 3 to 6 g/kg BW while muscle tissue is 50 g/kg BW (Reeds et al., 1993). Also, the two systems have major functional differences. About 50% of the protein synthesized by muscle tissue is accumulated as new tissue and contributes to growth while only 10% of the protein synthesized by the gastrointestinal tissue is accrued as new tissue (Reeds et al., 1993). Due to the continual regeneration of the intestinal epithelium, most of the protein synthesized in the gut is toward production of new cells to replace those that are sloughed off and lost into the lumen. In addition, there is a very high fractional rate of mucosal protein (mucin) synthesis and brushborder enzymes (Danielsen et al., 1984) so that over 30% of the protein synthesized by the intestinal mucosa is lost (Reeds et al., 1993).

Intestinal Catabolism of Dietary Amino Acids

The utilization of dietary first-pass amino acids by intestinal mucosa has been described (Wu, 1998; Stoll et al., 1999a, b; Mariotti et al., 2000; van Goudoever et al., 2000; van der Schoor et al., 2001, 2002). Wu (1998) summarized that the main source of amino acids for intestinal mucosa is dietary and that amino acid metabolism in the mucosa is essential for the maintenance of intestinal mucosal mass and integrity. Goldstein et al. (1985) and Morgan et al. (1987) reported that total parenteral feeding decreases protein synthesis in the small intestine and results in intestinal atrophy.

The major fuels for the small intestinal mucosa are dietary glutamine, glutamate, and aspartate, these being preferentially channeled towards mitochondrial oxidation compared to a much less complete oxidation of glucose to CO_2 (Burrin et al., 2001). Arginine, glutamine, and proline are needed to synthesize ornithine, a precursor for polyamines, essential for the normal growth, proliferation, migration, and repair of intestinal epithelium (Luk, 1990; McCormack and Johnson, 2001; McCormack et al., 2002).

Threonine and cysteine are both abundantly utilized by intestinal mucosa for the synthesis of mucin, the innate immune defense of the intestinal mucosa. In addition, cysteine is a component of glutathione, an essential antioxidant needed to maintain structural integrity and barrier function in the mucosa (Martensson et al., 1990). Cysteine is non-essential and is synthesized from methionine. Results from Shoveller et al. (2000) suggest that there is substantial

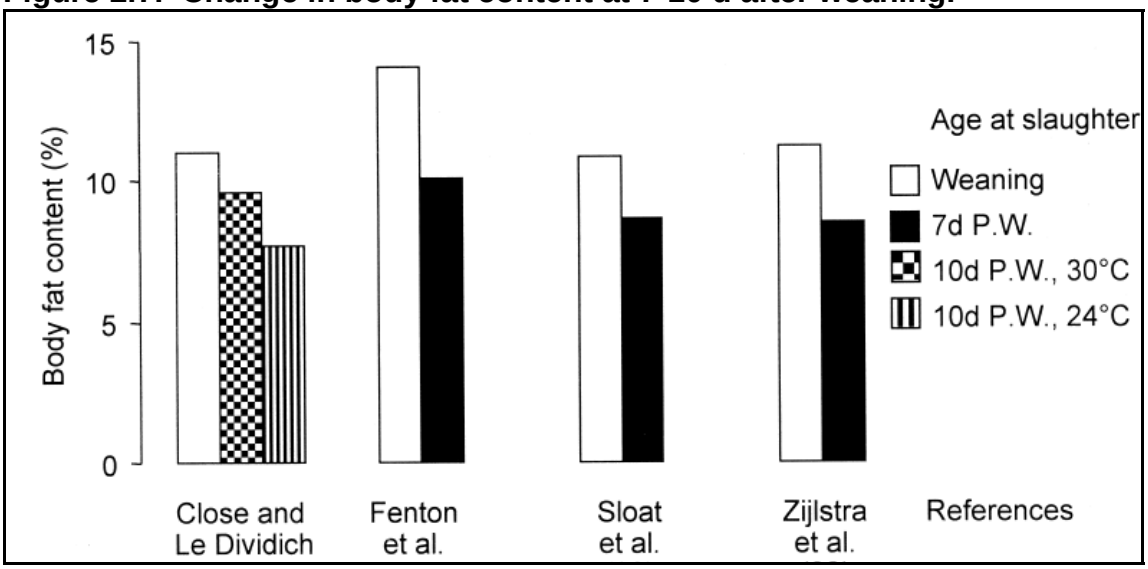
methionine utilization by the gut so methionine may be metabolized to cysteine. When gut utilization of methionine is high, this will have impact on the amount of methionine available for growth (Burrin et al., 2001).

The presence of pathogens can also impact utilization of amino acids by intestinal mucosa. Yu et al., (2000) reported increased leucine metabolism in the gastrointestinal tract of sheep with subclinical nematode infection, reducing leucine availability for other tissues.

Post-Weaning Energy, Protein, and Fat Metabolism

Underfeeding during the immediate post-weaning period results in decreased ME and nutrient intake and a negative energy balance (Le Dividich and Sève, 2000). The main source of energy for the newly-weaned pig is body fat. Le Dividich and Sève (2000) summarized the results of four studies that showed the change in body fat content after weaning (Figure 2.7). They also cited literature that reported increased body fat catabolism immediately post-weaning as shown by a decrease in backfat thickness, increased plasma free fatty acid levels, and decreased adipocyte size.

Figure 2.7. Change in body fat content at 7-10 d after weaning.



(Figure reprinted from Le Dividich and Sève (2002), used with permission from Elsevier.)

Funderburke and Seerley (1990) reported that symptoms of post-weaning growth lag included low plasma glucose, high free fatty acids, low plasma insulin, low liver glycogen, and high cortisol values. Weaning has marginal effects on blood urea nitrogen concentration (Pluske et al., 1997). Jiang et al. (2000a) reported increased lean tissue mass and fat free mass with lower plasma urea concentrations in weanling pigs fed porcine plasma protein compared to pigs fed extruded soy protein, thus demonstrating increased efficiency of dietary protein use for lean tissue growth.

Health and Gut Immunity of the Weanling Pig

There are two types of immune response to pathogens and antigens and these are generated by the innate and the acquired (adaptive) immune systems that work very closely together. The innate immune system response is immediate and is considered the first line of defense. It is generally non-specific, does not develop memory, and includes physical and chemical barriers, and cellular and soluble components. The physical barriers include the skin, gastrointestinal epithelial lining, respiratory tract, and reproductive tract epithelium, while an example of a chemical barrier is the acid in the stomach. Cellular components include many different cell types such as neutrophils, macrophages, dendritic cells, eosinophils, and mast cells. Acute phase proteins and cytokines are the soluble components. Cytokines are non-specific for any particular antigen but are produced by lymphocytes and monocytes, components of the adaptive immune system, therefore they are considered as a bridge between the innate and adaptive immune systems.

The adaptive immune system is a specific response to a pathogen or antigen, but is a slower memory generated response (Thacker, 2003) and involves a cellular component, the lymphocytes, and a soluble component, the immunoglobulins. The gastrointestinal immune system is capable of mounting both innate and adaptive immune responses. Considering the large total mucosal surface area of the gastrointestinal tract (e.g. the adult human gastrointestinal mucosal surface area totals 200-400 m^2), and the volume and array of food antigens and pathogens it has to deal with daily, this makes the

intestinal mucosa a major ‘immunological site’ (Stokes et al., 1996). Understanding the immune processes taking place in the young pig is important in the continuing search for alternatives to antibiotics and in order to take advantage of developing trends in disease prevention especially in the early-weaned pig.

Gastrointestinal Immune Function

The epithelium of the gastrointestinal tract is not only important in the digestion and absorption of nutrients but it also plays a major role in innate immunity serving as a very specialized physical and functional barrier to microbial and dietary antigens present in the gut lumen (Stokes et al., 2004; Bailey et al., 2005). The intestinal mucosa and, in some areas, including the submucosa, contains a rich array of immunologic components that perform a variety of complex functions geared towards recognizing non-harmful from harmful elements and developing tolerance or mounting an immune reaction (Baumgart and Dignass, 2002).

The mucus layer. The intestinal epithelium possesses an overlying layer of mucus gel that serves to protect, lubricate, and act as a transport medium between the contents of the gut lumen and the epithelium (Deplancke and Gaskins, 2001). Mucin is secreted by goblet cells, the specialized columnar cells in the gastrointestinal tract epithelial lining. The gene expression of mucin, and

the type and composition of mucus secretion varies with anatomical location, the microbial population present, and the age of the animal (Deplancke and Gaskins, 2001). Mucins in the stomach are predominantly neutral while acidic mucin predominates in the small and large intestines (Sheahan and Jervis, 1976). Changes in the type of mucin can influence the type of gut microflora and the presence of microbes (e.g. more neutral mucin in the intestines can favor growth of undesirable *E. coli*). Probiotics generally increase mucus secretion (Mack et al., 1999) and pathogens can enzymatically degrade mucins (Corfield et al., 1992).

Dunsford et al. (1991) reported that in piglets weaned at 21 d and regardless of diet, the goblet cell populations were markedly decreased 3 d post-weaning. They noted that the villi goblet cell populations tended to increase after 3 to 15 d post-weaning while those in the crypts remained low.

The epithelium and lamina propria. Under the mucus layer, the surface of the epithelial cells express diverse receptor systems (e.g. glycan receptors and toll-like receptors) that recognize bacteria then send signals to immune cells in the underlying lamina propria to activate signal transduction pathways that initiate adaptive immune responses (Stokes et al., 2004). The mucus coat serves to prevent adhesion of bacteria to epithelial cell surface receptors, but mucins also have the capacity to entrap bacteria and facilitate adhesion. However, adhesion alone does not correlate with disease. Francis et al. (1998) identified a mucin-type sialoglycoprotein receptor in porcine enterocyte

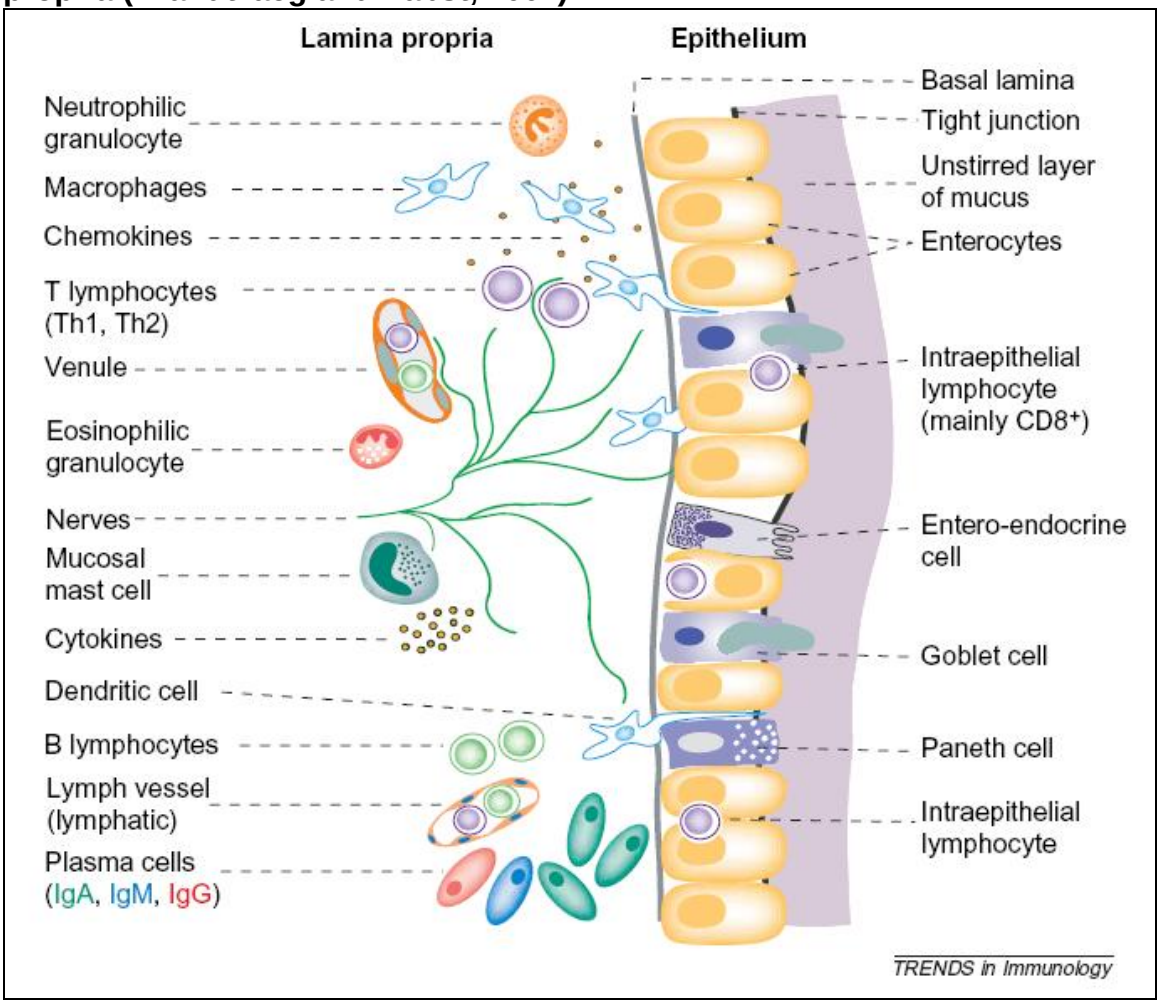
membranes that bind K88 fimbriae of enterotoxigenic *E. coli* and the expression of this receptor strongly correlated with the susceptibility of the piglet to enterotoxigenic *E. coli*, while adhesion without the receptor did not. Maintenance of the integrity of the gastrointestinal epithelium is critical in the prevention of infections (Stokes et al., 2004).

Figure 2.8 illustrates the cellular components within the lamina propria and epithelium of the gut mucosa. Shown are the surface mucus, the columnar epithelium with enterocytes and other cell types, the basal lamina (basement membrane) and the diffusely distributed cellular components (Brandtzaeg and Pabst, 2004). The lamina propria contains B and T lymphocytes, dendritic cells, mast cells, IgA and IgM plasma cells, IgG plasma cells, as well as mediators such as chemokines and cytokines (Brandtzaeg and Pabst, 2004). Immunoglobulin A-producing plasma cells are predominant and the different cell types can modulate the microenvironment by secreting cytokines and interacting with adjacent nerve fibers (Brandtzaeg and Pabst, 2004). Dendritic cells can penetrate the basal lamina and epithelial tight junctions to actively sample antigens from the mucosal surface while maintaining barrier integrity by expressing tight junction proteins (Brandtzaeg and Pabst, 2004).

The lymphocyte subsets in the small intestine compartments of piglets have been described (Bianchi et al., 1992; Pabst and Rothkötter, 1999). There were no differences in the proportion of T lymphocytes in the blood of piglets from birth to weaning compared to adult pigs (McCauley and Hartmann, 1984). The number of T lymphocytes was very low at birth but increased thereafter,

being significantly higher at 12 d post-weaning compared to that in adults (McCauley and Hartmann, 1984). The presence of microorganisms in the intestinal lumen has great impact on the lymphocyte composition (Pabst and Rothkötter, 1999).

Figure 2.8. The cellular components of the gut epithelium and lamina propria (Brandtzaeg and Pabst, 2004).



(Figure reprinted from Brandtzaeg and Pabst (2004), and used with permission from Elsevier.)

Intestinal Peyer's patches. Peyer's patches (PP) are aggregates of lymphoid tissue that are found in the small and large intestines. The Peyer's patches make up most of the gut-associated lymphoid tissue (GALT, also includes the appendix in humans and other isolated lymphoid follicles), that is a part of the mucosa-associated lymphoid tissue, or MALT, that includes lymphoid tissue and associated lymph nodes in mucosa of the gut and respiratory tract (Brandtzaeg and Pabst, 2004). The pig has three types of Peyer's patches, named according to their location: jejunal Peyer's patches (JPP), ileal Peyer's patches (IPP), and the spiral colon Peyer's patches (Andersen et al., 1999).

The development and distribution of the porcine discrete (jejunal) Peyer's patches were investigated by Makala et al. (2000) using immuno-histology and image analysis. They summarized the morphological characteristics (Table 2.5) and the development of PP compartments from birth to 35 d of age (Table 2.6). It was noted that the JPP was not morphologically differentiated until d 10 and it wasn't until d 21 to 35 when the PP reached their adult appearance (Makala et al., 2000). The results of Makala et al. (2000) also suggested that T cell compartment of the JPP reaches its adult structure earlier than the B cell compartment while Boecker et al. (1999) demonstrated that there are more T cells than B cells in the JPP than in IPP.

Table 2.5. Morphological characteristics of discrete (jejunal) Peyer's patches (Adapted from Makala et al., 2000).

Compartments	Sub-compartment	Main cell type lymphoid	Non-lymphoid
Follicle	corona medulla cortex	T and B cells Small T and B cells Lymphoblasts	Follicular dendritic cells, macrophages
Inter-follicular area		T lymphocytes	IDC, macrophages
Dome		Plasma cells B cells T cells	Macrophages IDC monocytes
Lympho-epithelium			M cells, enterocytes

IDC: interdigitating dendritic cells

Table 2.6. Development of the compartments of discrete (jejunal) Peyer's patches (Adapted from Makala et al., 2000).

Days	0	1-8	8-12	12-14	14-18	18-28	28-35
Separated T and B cell areas	(-)	+	+	+	+	+	+
Dome region	(-)	+/-	+	+	+	+	+
Primary follicle	(-)	+/-	+/-	+/-	+/-	+	+
Secondary follicle	(-)	(-)	(-)	(-)	(-)	+	+

+: Seen in all PP; +/-: occasionally seen; (-): not seen

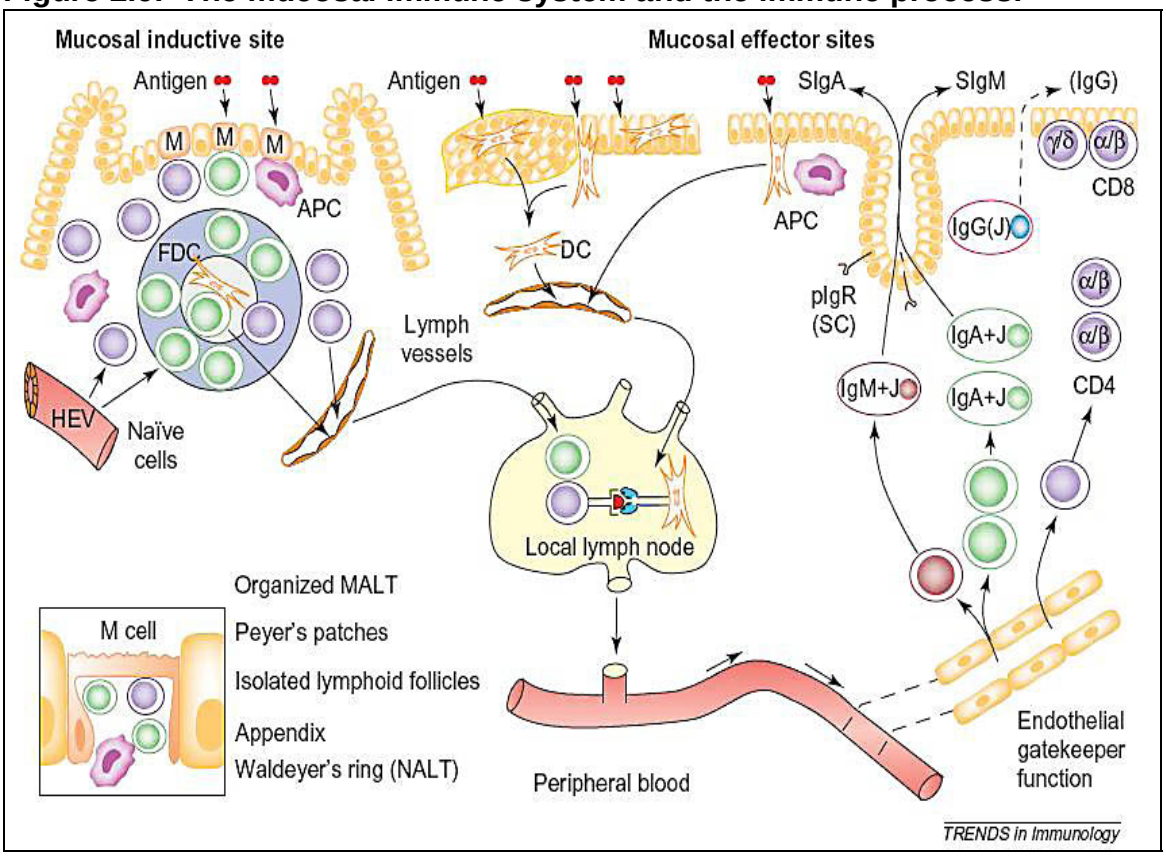
Andersen et al. (1999) characterized the porcine ileal Peyer's patches and reported that more than 90% of the IPP cells are B cells that are phenotypically distinct from mature circulating or lymph node B cells and they noted extensive apoptosis taking place in porcine IPP. Earlier studies in sheep have indicated that sheep IPP is a primary site for B cell production contributing to the systemic B cell pool and Andersen et al. (1999) demonstrated that porcine IPP shared anatomical, histological, and functional similarities with sheep IPP concluding that the pig IPP is a primary lymphoid organ that generates B lymphocytes independent of the presence of antigen.

The Peyer's patches, as all other MALT, lack a lymphatic supply (afferent) because they actively and directly sample antigens from the mucosal lining through the M cells ('microfold' or 'membranous') present in the follicle-associated epithelium (FAE) (Brandtzaeg and Pabst, 2004). M cells are epithelial cells exclusive to the MALT that possesses the capability of transcytotic transport of antigens (Gebert et al., 1996). The basolateral membrane of M cells usually forms a 'pocket' that contains B and T lymphocytes, macrophages, and sometimes plasma cells (Gebert et al., 1996).

Figure 2.9 illustrates the mucosal immune system (Brandtzaeg and Pabst, 2004). Shown are the inductive sites (the mucosa-associated lymphoid tissue or MALT) and effector sites (the epithelium and lamina propria). The MALT M cell (M)-containing follicle-associated epithelium actively transport exogenous antigens that reach antigen-presenting cells (APCs) such as dendritic cells (DCs), macrophages, B cells and follicular dendritic cells (FDCs) (Brandtzaeg

and Pabst, 2004). Naive B and T cells from circulation enter MALT (and lymph nodes) via high endothelial venules (HEVs) then become primed to become memory–effector B and T cells that migrate from MALT and lymph nodes to the peripheral blood for subsequent release into the mucosal effector site (Brandtzaeg and Pabst, 2004). The mechanism of lymphocyte transendothelial migration in mucosa-associated lymphoid tissue has been reviewed (Azalli, 2003).

Figure 2.9. The mucosal immune system and the immune process.



(Figure reprinted from Brandtzaeg and Pabst (2004) and used with permission from Elsevier.)

Immunoglobulins in Swine

The three major isotypes of immunoglobulins in swine are immunoglobulin M (IgM), immunoglobulin A (IgA), and immunoglobulin G (IgG) (Butler and Brown, 1994; Butler et al., 2006). The concentration of immunoglobulins in porcine serum, milk, and colostrum is summarized in Table 2.6 below. A wide range in the levels is sometimes seen between and within herds that may be attributed to age, environment, stage of lactation, or individual differences (Klobasa and Butler, 1987). It may also be due to the differences in methods used in measuring immunoglobulin levels (Bokhout et al., 1986). Sow parity influences not only the immunoglobulin levels in serum and milk of sow but also immunoglobulin synthesis by their piglets (Klobasa et al., 1986). None of the immunoglobulins cross the placenta. Curtis and Bourne (1971) measured the levels of IgG, IgA, and IgM in piglet serum and reported that at 16 wk, the pig had not attained a mature serum immunoglobulin profile.

Table 2.7. Concentration of porcine immunoglobulins (mg/mL) in serum, colostrum, and milk (Adapted from Curtis and Bourne, 1971).

	IgG	IgM	IgA
Pork pig serum	18.31 ± 0.67	3.15 ± 0.19	1.44 ± 0.12
Sow serum	24.33 ± 0.94	2.92 ± 0.18	2.37 ± 0.20
Colostrum	61.80 ± 2.44	3.19 ± 0.21	9.66 ± 0.59
Milk, 1-2 d	8.0 – 11.8	1.8 ± 0.40	2.7 – 3.8
Milk, 3-35 d	1.4 – 1.9	0.9 – 1.2	3.0 – 3.4

Immunoglobulin G is the predominant immunoglobulin in the serum and in colostrum. All of the IgG in colostrum, most of the IgM, and about 40% of IgA comes from the serum of the sow (Curtis and Bourne, 1971; Bourne and Curtis, 1973). Immunoglobulin G is rapidly absorbed by the intestinal epithelium during the first 12 hr after birth and until 'gut closure' while IgA and IgM tend to accumulate in the crypts of week-old piglets (Butler et al., 1981).

Most of IgA in the body is found in epithelial secretions such as in the lumen of the gut where it forms the first line of defense against food-borne pathogens (Underdown and Schiff, 1986; Fagarasan and Honjo, 2003; Monteiro et al., 2003). This IgA is synthesized in dimer form by plasma cells in the lamina propria while serum IgA is synthesized in the bone marrow mostly in monomeric form (Kerr, 1990). Dimeric IgA is associated with a J chain protein. Immunoglobulin M is either in pentameric or hexameric form but only the pentameric form has the J chain (Randall et al., 1992).

Immunoglobulin A and M in the lamina propria cross the epithelial lining and enter the gut lumen (Allen et al., 1976). Transport of immunoglobulins takes place via the transmembrane protein, polymeric Ig receptor (pIgR), which is cleaved thus releasing the secretory component bound with the immunoglobulin into the lumen (Brandtzaeg and Pabst, 2004). The J chain is important for affinity with the secretory component (Hanson and Brandtzaeg, 1993), so pIgR transports only the dimeric IgA and the pentameric IgM (Figure 2.9). Immunoglobulin G is not associated with the J chain and does not form a binding

site for pIgR but some local and serum-derived IgG enter the gut lumen via paracellular leakage as shown in Figure 2.9 (Brandtzaeg and Pabst, 2004).

Immunoglobulins in the intestinal mucosa. Allen and Porter (1973, 1977) reported the distribution of immunoglobulin-bearing cells in the intestinal mucosa of suckling and weaned pigs. They noted that IgA- and IgM-bearing cells were predominant in the lamina propria of suckling pigs up to 4 wk of age and that the duodenum consistently contained more immunoglobulin-containing cells. The proportion of IgM-containing cells gradually declined after weaning with IgA-containing cells increasing in proportion, reaching 90% by 12 wk of age (Allen and Porter, 1977). Thus, weaning seems to influence the relative population of immunoglobulin-containing cells (Allen and Porter, 1977). Antigenic challenge is also a factor that may influence the extent of plasma cell (immunoglobulin-bearing cell) proliferation because very few of these cells occurred in gnotobiotic animals compared to those given oral *E. coli* antigen (Porter et al., 1974).

Secreted immunoglobulins in the gut lumen serve as a first line of defense against antigens and pathogens (Fagarasan and Honjo, 2003). McClelland at al. (1972) demonstrated that secreted IgA agglutinated a wide range of organisms in the intestinal tract. Secretory IgA is considered a more efficient immunologic agent compared to serum IgG but this might be due to the abundance of IgA, being the major secretory immunoglobulin in mucosal surfaces (Blum et al., 1981). Immunoglobulin G may be as effective if available in sufficient amounts

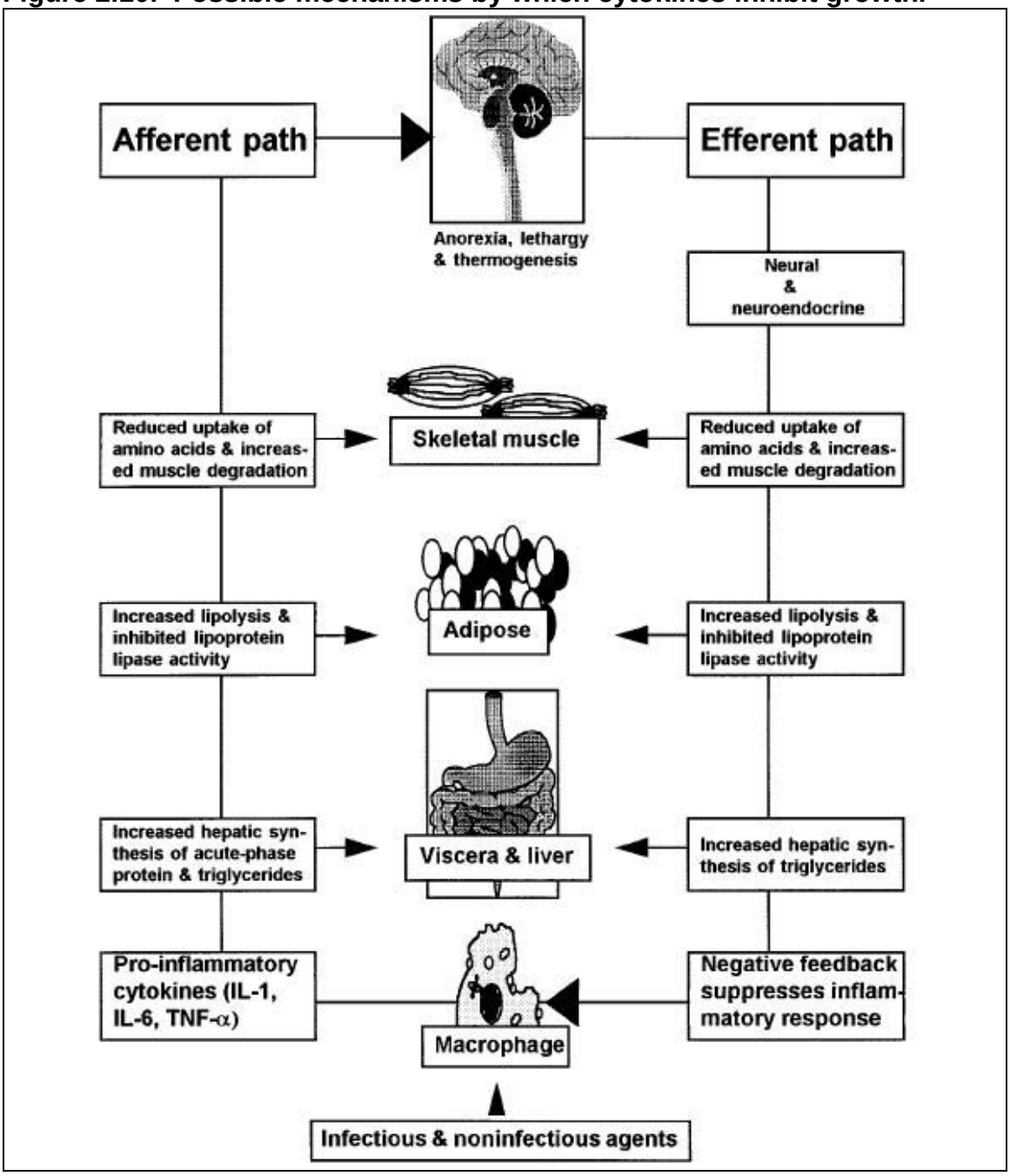
(Blum et al., 1981). Svanborg-Eden et al. (1978) have shown that both IgG and secretory IgA antibodies were effective in blocking adhesion of *E. coli* to human urinary tract epithelial cells in vitro.

Cytokines

Cytokines are pro-inflammatory peptides that are important in the development of an immune reaction and they play a major role in the immunological stress process that results in inhibition of growth (Johnson, 1997). Cytokines are mostly secreted by lymphocytes and macrophages, but these are also be produced by epithelial cells, endothelial cells, and fibroblasts (Pié et al., 2004).

Johnson (1997) provided an integrated view of how cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and interleukin 6 (IL-6) act on the brain and other major target organs to alter metabolism and inhibit growth. The possible mechanisms of cytokine effects are summarized in Figure 2.10 (Johnson, 1997). The presence of cytokines in the brain causes a reduction in appetite, while it increases lipolysis in adipose tissue and increases protein muscle degradation. It is interesting to note that there is an associated upregulation of inflammatory cytokine expression in the intestine of piglets at weaning (Pié et al., 2004). There is a transient intestinal inflammation with upregulation of IL-1β, IL-6, and TNF-α mRNA during the first 2 d post-weaning (Pié et al., 2004).

Figure 2.10. Possible mechanisms by which cytokines inhibit growth.



(Figure reprinted from Johnson (1997) and used with permission from the American Society of Animal Science.)

Immune challenges at weaning

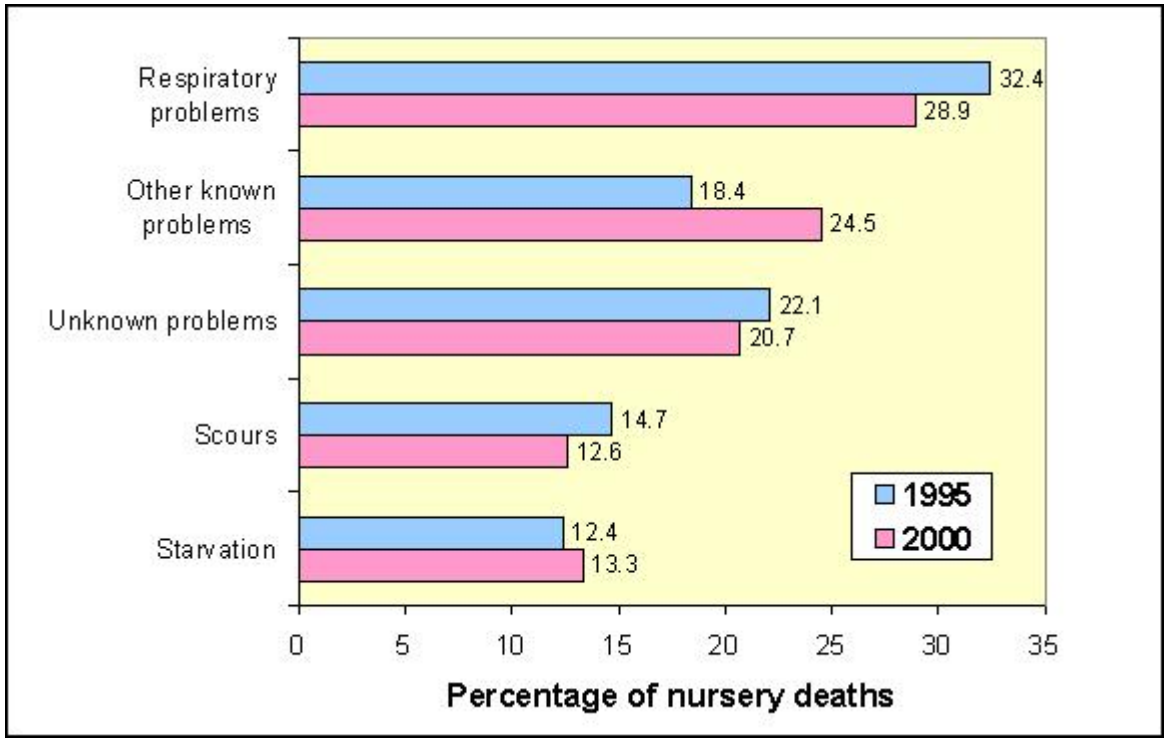
Segregated early weaning takes advantage of the reduced exposure to infectious disease and colostral immunity: piglets are “clean” at birth, then colostrum intake provides them maternal immunity, and piglets are weaned while their maternal immunity is still relatively high (Leiting, 1998; Robert et al., 1999). However, Leiting (1998) cited that earlier SEW techniques weaned pigs before 14 to 16 days while today, SEW age at weaning can be up to 24 d of age, a time when piglet immune status has greatly diminished. Robert et al. (1999) noted that age of weaning would vary from less than 10 d of age to control *Mycoplasma hyopneumoniae*, to 21 d of age to control transmissible gastroenteritis virus.

The use of antibiotics has also become part of SEW program to target such pathogens as *Actinobacillus pleuropneumonia*, *Pasteurella multocida*, and *Bordetella bronchiseptica* (Leiting, 1998). The USDA NAHMS reported that the top six disease problems present in sites with nursery pigs included meningitis (*Streptococcus suis*), greasy pig disease (*Staphylococcus hyicus*), *E. coli* diarrhea, *Mycoplasma pneumonia*, roundworms, and PRRS (USDA, 2000). Table 2.8 gives a breakdown of these diseases in the different sites where, except for roundworms, they were more common in medium or large sites (with over 2000 pigs) compared to sites with less than 2000 pigs. The NAHMS report in 2000 also showed that respiratory problems were the leading cause of nursery deaths, followed by other known (such as *Streptococcus suis*, fighting, hernias, etc.), or unknown problems, then digestive problems as scours and starvation (Figure 2.11).

Table 2.8. Most common disease problems in nursery pigs in 2000 (Adapted from USDA, 2000).

Disease	Small (<2,000)	Medium (2,000-9,999)	Large (>10,000)	All sites
Streptococcus suis (meningitis)	24.0	64.9	76.7	31.6
Greasy pig disease	21.3	43.9	34.2	25.3
E. coli diarrhea	22.1	32.1	40.7	24.0
Mycoplasma pneumonia	14.6	41.5	52.7	19.6
Roundworms	20.9	4.5	6.9	18.0
PRRS	13.4	33.8	58.0	17.5

Figure 2.11. Percent nursery deaths from Dec 1999-May 2000 and Dec 1994- May 1995, by producer-identified cause (Adapted from USDA, 2000).



Impact of Diet on the Immune System

The quantity and quality of feed entering the stomach and intestines has a marked impact on the gastrointestinal epithelium and the consequent immune reaction mounted by the immune cells along the epithelial lining, the lamina propria, and the gut-associated lymphoid tissue. Different dietary factors have been shown to increase or decrease the immune reaction along the gut mucosa to improve growth performance of early-weaned pigs, but no single specific strategy has been totally effective. The more popular approaches used at present are inclusion of antibiotics in feed (Gaskins et al., 2002) and use of spray-dried animal plasma. Other strategies explored were acidification of the diet, supplementation of feed with probiotics (Mack et al., 1999; Reid et al., 2003; Fairbrother et al., 2005), egg yolk antibodies (Owusu-Asiedu et al., 2002; Owusu-Asiedu et al., 2003a,b; Fairbrother et al., 2005), zinc (van Heugten et al., 2003; Davis et al., 2004a; Fairbrother et al., 2005; Broom et al., 2006), glutamine supplementation (Yi et al., 2005; Domeneghini et al., 2006), mannan oligosaccharides (White et al., 2002; Davis et al., 2002; LeMieux et al., 2003; Davis et al., 2004a, b), or manipulation of the type and level of fat (van Heugten et al., 1996; Liu, et al., 2003) and protein (van Heugten et al., 1994; Gu and Li, 2004; Nyachoti et al., 2006) levels in the diet. There are also non-diet related strategies that vary from simple manipulation of duration of lighting (Salak-Johnson et al., 2004) to more complex genetic selection for disease resistance (Wilkie and Mallard, 1999; Fairbrother et al., 2005).

Spray-Dried Animal Plasma in Weanling Pig Diets

Spray-dried animal plasma (SDAP) has become a popular protein source in post-weaning pig diets. Its use is limited to the first 10 to 14 days post weaning when the improvements in average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (gain to feed ratio) are significant. It has also been shown to increase villous height (Spencer et al., 1997; Torrallardona et al., 2003) and reduce small intestinal growth (Jiang et al., 2000b). Torrallardona et al. (2002, 2003) have even suggested that spray-dried animal plasma might be used as an alternative to antibiotics in the early-weaned pig diet.

Source, Processing, and Safety of Spray-Dried Animal Plasma

Spray-dried animal plasma is mostly of porcine origin (spray-dried porcine plasma or SDPP), but it may also be of bovine origin (SDBP), or a mixture of both. Commercial preparations come in powder form with neutral to rather distinct odor and it may have a uniform off white to beige-color, or be light tan to medium brown in color. Spray-dried animal plasma is processed from whole blood coming from animals identified as fit for slaughter for human consumption, in federally-inspected, commercial slaughter facilities (Polo et al., 2005). Because of questions regarding the potential role of SDAP in spreading diseases, companies involved in the collection and processing of spray-dried blood and plasma have formed the North American Spray-Dried Blood and Plasma Producers Association that have established Good Manufacturing

Practices detailing the proper sourcing, collection, and processing of animal blood and blood products to maintain safety.

Two experiments were conducted to determine the survival of viral contamination during the spray-drying process (Polo et al., 2005). Bovine plasma samples were inoculated with either pseudorabies virus or porcine reproductive and respiratory syndrome (PRRS) virus and the presence of the virus was confirmed for each before spray-drying. After spray-drying the investigators were unable to detect any live virus demonstrating that the spray-drying process used in the study is capable of eliminating viable pseudorabies and PRRS viruses from bovine plasma (Polo et al., 2005).

Nutrient Value and Digestibility

Spray-dried animal plasma is a high quality protein ingredient with at least 75% crude protein (CP). It is relatively high in lysine and threonine but low in methionine (Chae et al., 1999). Hansen et al. (1993) used spray-dried animal products to replace milk products in diets for early-weaned pigs and noted that the experimental diet containing porcine plasma had a calculated methionine content of 0.3%, a value lower than the NRC interpolated methionine requirement of 0.37%. Chae et al. (1999) compared the ileal digestibilities of nutrients in different protein sources, and the comparative feeding value of these protein sources for early- (14 d old) or conventionally- (26 d old) weaned pigs. These authors noted that, except for His, Lys, and Arg, the digestibility of most

essential amino acids were lower in SDPP- than in dried skim milk (DSM)-based diet for early weaned pigs. In conventionally-weaned pigs, the digestibility of valine was lower in SDPP than in DSM diet. Van Dijk et al. (2001a) compared the amino acid composition and apparent ileal digestibility of spray-dried animal plasma preparations with casein and soybean meal. These authors noted that the protein content and apparent ileal digestibility of amino acids were lower in SDAP compared to casein but that the essential amino acid content of SDAP is superior to soybean protein. Van Dijk et al. (2001a) further noted that, except for methionine, SDAP provision of essential amino acids is in agreement with the NRC (1998) requirements for piglets.

Effects on Growth Performance of Weanling Pigs

Spray-dried animal plasma improves average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (gain to feed ratio) during the first two weeks post-weaning. The improvement in weanling pig growth performance was significant even with as low as a 2% inclusion rate (Kats et al., 1994a, b). Coffey and Cromwell (2001) performed an extensive literature review of 79 experiments conducted with spray-dried animal plasma from 1987 to 2001. They reported that 70 experiments showed a positive response from plasma with respect to both growth rate and feed intake while 42 experiments showed a positive response in feed efficiency. They further computed a 25% average improvement in growth rate, 21% improvement in feed intake, and 4% improvement in feed efficiency. Van Dijk et al. (2001a) summarized published

studies done from 1990 to 1997 and reported similar results during the first 2 wk post weaning. The overall mean improvement in ADG was 26.8%, ADFI improvement was 24.5% while the SDAP-induced change in FCR was -3.2%. They concluded that the improvements in ADG and ADFI were generally consistent when up to 6% of dietary spray-dried animal plasma was used while SDAP improved FCR at levels below 6%. They noted that responses to higher inclusion levels were more variable. Coffey and Cromwell (2001) enumerated such factors as age at weaning, level of environmental stress, and density and complexity of the diet, to be important determinants of the optimal inclusion rate of SDAP in weanling pig diets.

Factors Affecting Growth Response to Spray-Dried Animal Plasma

Van Dijk et al. (2001a) noted that the response to SDAP is dependent on several factors such as the diet composition of the control group, the origin of SDAP (porcine vs. bovine), and feed processing (pellet vs. meal form). The health status and degree of pathogen exposure of the weanling pigs is another determinant of the level of response to SDAP (Coffey and Cromwell, 1995; Bergstrom et al., 1997; Van Dijk et al., 2001a, 2002b).

Protein source used to compare response. The type of protein used in the control diet (whether from plant or animal source) appears important in quantifying improvements in growth performance of weanling pigs fed diets

containing SDAP. However, SDAP-induced improvements have been observed regardless of protein source in the control diet, even when compared with milk proteins (Gatnau and Zimmermannn, 1990; Hansen et al., 1993). Hansen et al. (1993) used porcine plasma in combination with lactose or starch, with or without whey, and this was compared with a control diet containing either dried skim milk without lactose, skim milk with whey, or skim milk with whey and casein. Spray-dried porcine plasma (regardless of combination) was better than dried skim milk and the highest ADG and ADFI were obtained when SDPP was used in combination with dried whey and lactose. Hansen et al. (1993) also compared the effects of spray-dried porcine plasma (SDPP) with different supplemental animal protein sources namely, porcine blood, meat extract and bovine plasma. For the first two weeks post-weaning, pigs fed diets supplemented with SDPP had higher ADG compared to those fed the diets with either skim milk, meat extract, or bovine plasma, but did not differ from those fed porcine blood. In the same period, pigs fed diets supplemented with SDPP had higher ADFI compared to all other treatments. The gain:feed ratio of pigs fed diets supplemented with SDPP was higher compared to pigs fed meat extract, but did not differ from those fed diets supplemented with either skim milk, porcine blood, or bovine plasma. Angulo and Cubiló (1998) reported that pigs fed SDPP had higher ADFI and G:F compared to pigs fed modified soy protein. Van Dijk et al. (2002b) used SDPP at 3%, replacing portions of both fishmeal and dried skim milk or fishmeal alone, and they concluded that SDPP is superior to fishmeal. Van Dijk et al. (2001a) noted that from the 15 published studies they reviewed, the mean percentage

SDAP-induced improvement in growth performance was greater when soy protein was used in the control diet compared to when milk protein was used.

Health status of weanling pigs and sanitation. The health status and degree of pathogen exposure of the weanling pig is probably the major determinant of the level of response to SDAP, and this may be an important clue to the mode of action of SDAP. Coffey and Cromwell (1995) reported that the response to SDPP was greater in pigs reared in a conventional, on-farm nursery setting compared to those in a cleaner, off-site nursery. Segregated early-weaning (SEW) has been used as a means to improve herd health so that weanling pigs raised under this off-site nursery system are considered to have high health status. Bergström et al. (1997) evaluated both SDAP and select Menhaden fishmeal (SMFM) in diets for pigs weaned at 12 to 14 d and reared in different production systems. They compared the response from pigs weaned to an off-site nursery with a high health status to those of lower health status reared in an on-site nursery and found that SEW pigs responded less to both SDAP and SMFM. Van Dijk et al. (2002b) evaluated the effects of SDPP on weanling pig growth performance and health status under typical Northern European conditions. The magnitude of improvement in growth performance due to SDPP was not as high compared to most experiments performed in the U.S. as reported by Van Dijk et al. (2001b). The authors noted that the high hygiene status in their research station might be a reason. In addition, they concluded

that low inclusion levels (up to 3%) have positive effects on weanling pig performance under Northern European conditions.

Other factors. Three of the studies reviewed by Van Dijk et al. (2001a) made a direct comparison between spray-dried plasma from porcine (SDPP) vs. that of bovine origin (SDBP). Results showed that although both improved growth performance, the response to SDPP was greater compared to that of SDBP. However, Pierce et al. (2005) reported that the immunoglobulin G (IgG)-rich fraction of both spray-dried porcine plasma (SDPP) and spray-dried bovine plasma (SDBP) was responsible for improvement in growth performance of early-weaned pigs and it didn't matter whether the IgG was porcine or bovine.

Van Dijk et al. (2001a) also noted that feed processing, whether meal or pellet form, has an effect on the response to SDAP. They reported that although both meal and pelleted feed containing SDAP improved growth performance of weanling pigs, there was a much greater ADFI response to SDAP in meal form thus lowering feed efficiency; therefore, feed efficiency appeared to be better in pigs fed pelleted feed.

Effect on Gut Morphology

The inclusion of SDPP in weanling pig diet has been shown to increase villous height (Spencer et al., 1997; Torrallardona et al., 2003) and reduce small intestinal growth (Jiang et al., 2000b). Jiang et al. (2000b) did not observe

effects on villous height, crypt depth or cell proliferation index but they reported a significantly lower small intestinal mass (g/kg BW) in pigs fed diets with SDPP compared to those fed diets with extruded soy protein by d 16 post-weaning. The pigs fed diets with SDPP had lower jejunal protein masses, lower jejunal and ileal DNA masses, and lower intravillous lamina propria cell density with decreased numbers of macrophages (Jiang et al., 2000b). Van Dijk et al. (2001b, 2002c) used SDPP vs. casein and reported no significant effect on villous length, but observed less mitotic activity in SDPP-fed pigs on d 4 and 7 post-weaning (Van Dijk et al., 2001b). Compared to casein, SDPP had no effect on crypt depth, enterocyte mitotic activity, and intestinal brushborder disaccharidases in piglets kept under low infection pressure (Van Dijk et al., 2002c).

Effect on the Immune System

Spray-dried animal plasma (SDAP), whether porcine (SDPP) or bovine (SDBP) origin, can be separated into three fractions: the immunoglobulin G (IgG)-rich fraction, the albumin-rich fraction, and the low molecular weight (LMW) fraction. The results of several experiments have shown that the IgG-rich fraction is responsible for the improvements in the early-weaned pig growth performance (Owen et al., 1995; Pierce et al., 1995; Weaver et al., 1995; Pierce et al., 2005) regardless of origin (Pierce et al., 2005). However, Owasu-Aseidu et al. (2002) reported that spray-dried animal plasma (mostly bovine in origin) contains less anti-*E. coli* antibody (anti-K88 and F18) compared to SDPP and

that these antibodies might be responsible for the positive effects of SDPP on growth of early-weaned pigs especially when challenged with enterotoxigenic *E. coli*.

Nollet et al. (1999) weaned pigs at 4 wk onto a commercial weaner diet (control) or a diet supplemented with porcine plasma powder. The pigs were orally infected with an *E. coli* strain two d after weaning and the fecal consistency and fecal excretion of total coliforms were not different in the two groups. However, the fecal excretion of the challenge strain was significantly lower from d 6 to 16 (except d 10 and 11) in pigs given 45 g plasma powder compared to the control group (Nollet et al., 1999). Van Dijk et al. (2002a) showed that when SDPP was added to the diet at an economically feasible percentage (8%), it did not prevent losses when pigs were challenged with pathogenic *E. coli*, although they reported improvements in ADG, ADFI, and fecal and condition scores. However, others have reported positive effects of SDPP on growth and immune status of pigs weaned at 21-24 d and challenged with *E. coli* (Torrallardona et al., 2003; Bosi et al., 2004). In the absence of *E. coli* challenge, pigs fed an unmedicated diet supplemented with spray-dried animal plasma (SDAP) had similar growth performance as pigs fed a medicated diet supplemented with fishmeal suggesting that SDAP can be used as an alternative to antibiotics (Torrallardona et al., 2002).

Torrallardona et al. (2003) reported that the antimicrobial colistin sulfate (from Andrés Pintaluba, S. A., Reus, Spain) dramatically reduced the number of *E. coli* colony-forming units in the contents of the ileum and cecum of early-

weaned pigs challenged with *E. coli* K99. Spray-dried animal plasma did not significantly reduce *E. coli*, but increased the lactobacilli population so that the immunoglobulin fraction of SDAP may have acted against *E. coli* K99 (Torrallardona et al., 2003). Torrallardona et al. (2003) also reported some interactions between colistin and SDAP in contrast to earlier reports that had suggested that the effects of SDPP and antibiotics are independent and non-additive (Coffey and Cromwell, 1995; Torrallardona et al., 2002). However, Coffey and Cromwell (1995) and Torrallardona et al. (2002) did not challenge the newly-weaned pigs with *E. coli*. Torrallardona et al. (2003) concluded that SDAP may be a good alternative to antibiotics since the magnitude of the response to SDAP was similar to that observed with colistin.

Bosi et al. (2004) also reported that spray-dried plasma (SDP) may be used as an alternative to antibiotics. Spray-dried plasma was effective in improving growth, and reducing *E. coli* infection via reduced intestinal mucosal damage and *E. coli* shedding in early-weaned pigs challenged with *E. coli* K88 (Bosi et al., 2004). And compared to antibiotics, SDP is even more efficient in reducing the expression of pro-inflammatory cytokines IL-8, TNF-α in the intestines; expression of these cytokines is markedly increased in inflamed gut mucosa (Bosi et al., 2004).

Jiang et al. (2000b) earlier reported that pigs fed diets with SDPP had lower intravillous lamina propria cell density with decreased numbers of macrophages indicative of reduced inflammation. Touchette et al. (2002) reported a reduction in cytokine TNF-α and IL-1β expression in the adrenal

gland, spleen, hypothalamus, pituitary gland, and liver in early-weaned pigs fed diets containing 7% SDP. Carroll et al. (2002) also reported lower mRNA levels of hypothalamic corticotropin-releasing hormone (CRH), in pituitary gland CRH receptor, and adrenal gland adrenocorticotropin-releasing hormone receptor in these same pigs. Touchette et al. (2002) and Carroll et al. (2002) then challenged the pigs with intraperitonial injection of lipopolysaccharide resulting in a dramatic increased activation of the pituitary-adrenal axis (Carroll et al., 2002) and increased serum TNF-α and interferon-γ (IFN-γ) with major villous atrophy (Touchette et al., 2002) in pigs fed diets containing SDP. These results indicate that early-weaned pigs fed diets supplemented with SDAP are 'naïve' pigs with lower immune system activation making them susceptible to major immunological challenges as shown by how they appear to over-respond to the LPS challenge.

Fat in Weanling Pig Diets

The value of adding fat to weanling pig diets has been debated and research data, particularly during the first 2 wk post-weaning, have been inconclusive due to inconsistent results. Several issues have been raised to explain the differences in the results such as the type or source of oil used in the trial and its digestibility, the differences in the level of nutrients between treatment groups (such as the lysine to energy ratio), and the activity of pancreatic lipase.

Digestibility of Different Sources of Fat

Different fat sources have different profiles of fatty acid content that differ in length and saturation, factors that can impact digestibility. Fats from vegetable sources differ in digestibility from those of animal origin (Cera et al., 1988a; Cera et al., 1989; Cera et al., 1990b). Conflicting reports regarding digestibility of different fat sources may be due to different analytical methods used (Cera et al., 1988b). Using corn oil, lard, or tallow, Cera et al. (1988b) reported a consistently higher apparent fat digestibility in corn oil-supplemented diets compared to the animal fat sources with the difference being greater during the immediate post-weaning period and narrowing by the 3rd wk. The addition of coconut oil has resulted in higher digestibility and growth rate compared to other vegetable oils (Cera et al., 1989; Cera et al., 1990b) and this has been attributed to the higher proportion of shorter fatty acid chain length in coconut oil, with consequent higher absorption directly into the blood stream. Soybean oil contains more unsaturated, long-chain fatty acids (Li et al., 1990). The apparent fat digestibility was greater in diets containing medium-chain triglyceride (MCT) or coconut oil compared to soybean oil or roasted soybean diets during the first 2 wk post-weaning (Cera et al., 1990b). Fish oil showed similar digestibility to that of coconut oil but 10% fish oil in the diet increased both the volume of pancreatic juice and output of pancreatic enzymes including lipase while coconut oil did not stimulate lipase secretion (Hedemann et al., 2001).

In general, the apparent digestibility of fat increased with age, reaching a plateau between 3 to 4 wk post-weaning (Cera et al., 1988a, b). Frobish et al.

(1970, 1971) also reported a higher apparent fat digestibility 28 d after weaning compared to 10 d post-weaning. This increase in digestibility coincides with the increase in pancreatic lipase activity after 3 to 4 wk post-weaning.

The addition of fat to weanling pig diets during the first wk post-weaning decreased ileal DM digestibility (Cera et al., 1988a; Li et al., 1990), nitrogen retention and increased serum urea concentration (Cera et al., 1988a), increased carcass fat (Endres et al., 1988) and decreased protein gain (Leibbrandt et al., 1975). Fat supplementation has also been shown to decrease jejunal villi and reduce absorptive area (Cera et al., 1988b). Li et al. (1990) also demonstrated shorter and more slender villi in pigs fed diets supplemented with coconut oil or soybean oil alone.

Effects on Growth and Immunity of the Early-weaned Pig

Several authors have described different methods of adding fat to weanling pig diets: either fat replaced a carbohydrate source such as cornstarch on equal weight basis but maintaining the levels of all other nutrients constant (Frobish et al., 1970, 1971); or adding fat but increasing lysine and other nutrient levels to maintain a constant ratio of the nutrients with energy (i.e. constant lysine:energy) (Lawrence and Maxwell, 1983; Li et al., 1990; Mahan, 1991; Tokach et al., 1995); or formulating isocaloric diets with equal protein levels among treatments (Cline et al., 1977). The experimental results varied depending on how diets were formulated.

Allee et al. (1971b) noted that if fat replaced an equivalent amount of carbohydrate, increased caloric density of the diet, and decreased feed intake, this might result in deficient amino acid intake. Thus, this may be the cause of the depression of growth rather than fat addition. Allee et al. (1971a) reported an increase in gain and gain per unit energy consumed in pigs fed diets containing corn oil or tallow. However, they had used older pigs (weaned at 5 wk) and reported data over a 21 d period. The inclusion of oil 2 wk after weaning has generally been beneficial to the weaned pig.

Frobish et al. (1970, 1971) reported reduction in weight gain and higher energy required per unit of weight gain when fat was added to the weanling pig diet. Cline et al. (1977) concluded that the young pig can utilize fat efficiently when all other nutrients are provided or when the protein:calorie ratio is kept constant; however, all the diets used in their experiments contained fat and they just showed no differences in the growth of pigs fed diets containing low fat (43% of non-protein calories) compared to those fed diets with higher fat level (74% of non-protein calories). Also, Lawrence and Maxwell (1983) reported a linear decrease in weight gain and feed consumption as fat level increased, during the first 2 wk post-weaning. However, soybean oil in weanling pig diets at 0, 3, 6, or 9% did not affect growth performance during the first 2 wk post-weaning (Tokach et al., 1995). And the addition of either soybean oil, coconut oil, or choice white grease had no effect on the ADG or F:G during the first 2 wk post weaning (Li et al., 1990).

Li et al. (1990) supplemented weanling pig diets with soybean oil, coconut oil, or a combination (50:50) of the two and reported that pigs fed the 50% soybean oil:50% coconut oil combination had longer villi that coincided with greater net ileal disappearance of unsaturated fatty acids compared to those supplemented with soybean oil or coconut oil alone. Pigs fed this combination also had better growth performance compared to pigs fed diets with coconut oil alone (Li et al., 1990).

As described earlier, pancreatic lipase activity drops at weaning and does not return to pre-weaning levels until around the 3rd wk. Fat digestibility appears to be relatively low during the first 2 wk post-weaning and there is a marked increase in apparent fat absorption from weaning (at 21 d) to the 3rd wk post-weaning (Cera et al., 1988b). In addition, intestinal morphology is compromised especially during the immediate post-weaning period. Therefore, the supplementation of oil during this phase is probably not needed because it does not improve weanling pig performance. Positive response to fat supplementation does not occur until after 2 to 3 wk post-weaning and this improvement in growth was observed even though fat was added only on the 3rd wk post-weaning (no adaptation) (Howard et al., 1990) and had no effect on the subsequent grow-finish stages (Tokach et al., 1995). However, Tokach et al. (1995) points out the importance of fat in the pelleting process especially for diets containing high levels of milk products to reduce dustiness and improve pellet quality.

CHAPTER III

EXPERIMENT 1

EFFECTS OF REDUCING METABOLIZABLE ENERGY CONCENTRATION IN DIETS CONTAINING SPRAY-DRIED PORCINE PLASMA ON WEANLING PIG PERFORMANCE

ABSTRACT: An experiment was conducted to determine the effects of reducing the ME concentration of diets containing spray-dried porcine plasma (SDPP) on weanling pig performance. A total of 232 pigs (avg BW = 5.8 kg) were weaned at approximately 21 d and housed (6-7 pigs/pen) in a temperature-controlled nursery for 18 d. Pigs were blocked by weight and randomly allotted to four dietary treatments (9 pens/trt). Diet 1 (3,471 kcal ME/kg) was composed primarily of corn, soybean meal, dried whey, lactose, soy protein concentrate (SPC), fishmeal, and soybean oil. Diet 2 (3,471 kcal/kg) was similar to Diet 1 with the exception that SDPP replaced SPC. Diets 3 and 4 were similar to Diet 2 except that soybean oil was decreased to provide 3,371 and 3,271 kcal ME/kg, respectively. All diets contained 1.35% digestible Lys. Pigs and feeders were weighed on d 0, 7, 14, and 18 to determine ADG, ADFI, and gain:feed (G:F) ratio. On d 0, 7, and 14, blood was collected from two pigs per pen for determination of blood urea nitrogen, triglycerides, and glucose. Pigs fed SDPP had greater ($P < 0.01$) ADG, ADFI, and G:F than pigs fed SPC from d 0 to 18.

Decreasing the ME in SDPP diets had no effect ($P > 0.10$) on growth performance, but it increased (linear, $P < 0.01$) gain/100 kcal ME intake. Blood urea nitrogen was lower ($P < 0.03$) in pigs fed SDPP than those fed SPC. On d 7, blood glucose was higher ($P < 0.03$) while triglyceride levels were lower ($P < 0.03$) in pigs fed SDPP compared to pigs fed SPC. The results suggest that lowering ME in SDPP diets does not affect growth performance of weanling pigs.

Key Words: Weanling Pig, Porcine Plasma, Metabolizable Energy

Introduction

Weaning is a stressful event in the pig's life and most of the adaptive changes taking place within the first 24 to 36 hr post-weaning are, for the most part, dictated by the level of nutrient intake. In fact, feed intake during the first wk post-weaning was found to have a strong impact on the severity of digestive disorders during the first four wk post-weaning (Madec et al., 1998). Spray-dried porcine plasma (SDPP) has become a popular protein source in post-weaning pig diets. Weanling pigs showed preference for diets containing SDPP compared to diets containing dried skim milk resulting in increased feed consumption (Ermer et al., 1994). In fact, Van Dijk et al. (2001a), in a review on the effects of spray dried animal plasma, concluded that dietary spray-dried animal plasma levels up to 6% improved average daily gain (ADG) and average daily feed intake (ADFI) the first two wk post-weaning. In addition, up to 6% SDPP improved feed:gain ratio.

The gastrointestinal system develops functionally and grows rapidly after birth when enteral feeding commences. After weaning, the growth rate and weight of these organs in relation to body weight is much greater especially the small intestine (Cranwell and Moughan, 1989). This increase in gastrointestinal weight relative to body weight is attributed to the change in diet because growth is higher in pigs fed solid feed compared to those fed a liquid milk substitute (McCracken et al., 1995).

Hypothesis and Objective

The inclusion of SDPP in weanling pig diets has been shown to reduce small intestinal growth (Jiang et al., 2000b). Le Dividich and Sève (2000) noted that metabolizable energy (ME) intake at the end of the first wk post-weaning is only 60 to 70% of the pre-weaning milk ME intake. Yet, despite the reduced intake, the relative weight of the small intestine increases by 25% at 3 to 7 d post weaning and by 52% at 10 to 14 d. Reduced intestinal growth in pigs fed SDPP may suggest a lower ME requirement for the intestine, thus increasing ME available for body growth.

This experiment was performed to determine the effects of reducing the ME concentration of diets containing SDPP on weanling pig performance.

Materials and Methods

The effect of reducing the ME concentration in weanling pig diets supplemented with SDPP was investigated using a randomized complete block design.

Animals, Treatments, and Diets

A total of 232 crossbred pigs with an average BW of 5.8 kg were weaned at approximately 21 d. The pigs were housed (6-7 pigs/pen) in an environmentally-regulated nursery with pens measuring 1.14 x 1.5 m on raised woven wire floor. The temperature of the nursery was maintained at 31 to 32°C throughout the experimental period. Pigs were blocked by weight and randomly allotted to four dietary treatments (9 pens/trt). The composition of the diets is shown in Table 3.1 and was as follows: 1) Control diet containing soy protein concentrate (SPC) with ME = 3,471 kcal/kg, 2) SDPP replaced SPC, ME = 3,471 kcal/kg, 3) SDPP diet with ME level reduced by 100 kcal/kg, and 4) SDPP diet with ME level reduced by 200 kcal/kg. Substitutions were made on an equal lysine basis. To lower the ME concentration, soybean oil was replaced by cornstarch and in part by corn grain. All diets were pelleted and formulated to contain 1.35% digestible lysine, 0.90 % Ca, and 0.75% P. Feed and water were provided on an ad libitum basis using nipple waterers and a common feeder per pen. All procedures were approved by the OSU Institutional Animal Care and Use Committee (IACUC).

Table 3.1. Composition of the diets, Exp 1.

Treatment	1	2	3	4
Protein source	SPC	SDPP	SDPP	SDPP
ME concentration, kcal/kg	3,471	3,471	3,371	3,271
Ingredients:				
Corn	27.44	30.04	30.04	30.04
Soybean meal (48% CP)	20.00	20.00	20.00	20.00
Whey, dried	20.00	20.00	20.00	20.00
Lactose	10.00	10.00	10.00	10.00
Fish meal, Menhaden	5.55	5.55	5.55	5.55
Soy protein concentrate [a]	9.45	0.00	0.00	0.00
Plasma, spray dried [a]	0.00	6.00	6.00	6.00
L-lysine·HCl	0.05	0.05	0.05	0.05
DL-methionine	0.15	0.27	0.27	0.27
L-threonine	0.03	0.03	0.03	0.03
Soybean oil [a]	4.26	5.00	2.75	0.50
Cornstarch [a]	0.00	0.02	2.27	4.52
Dicalcium phosphate	0.51	0.27	0.27	0.27
Limestone, ground	0.66	0.87	0.87	0.87
Salt	0.50	0.50	0.50	0.50
Antibiotic[b]	1.00	1.00	1.00	1.00
Vitamin/TM premix[c]	0.40	0.40	0.40	0.40
Composition of the diet:				
Crude protein, %	23.82	22.66	22.70	22.70
Ca	0.90	0.90	0.90	0.90
P(a)	0.43	0.50	0.48	0.48
Total Lys, %	1.56	1.58	1.58	1.58
Apparent ileal digestible AA, %				
Lysine	1.35	1.35	1.35	1.35
Met + Cys	0.80	0.80	0.80	0.80
Threonine	0.85	0.85	0.85	0.85
Total lysine: ME, g/Mcal	4.49	4.55	4.69	4.83

[a] ME values, kcal/kg: Soybean oil: 8,400; Cornstarch: 3,985.

[b] Neo-Terramycin® provided 50 mg oxytetracycline hydrochloride and 35 mg neomycin sulfate per kg of complete feed.

[c] Provided the following per kg diet: 5,506 IU vitamin A, 551 IU vitamin D, 33 IU vitamin E, 3.6 g vitamin K (as menadione), 221 µg biotin, 137 mg choline, 33.04 mg niacin, 24.78 mg pantothenic acid (as d-pantothenate), 5.51 mg riboflavin, 27.55 µg vitamin B_{12}, 1.66 mg folacin, 100 mg Zn, 2 mg Mn, 100 mg Fe, 10 mg Cu, 0.30 mg I, and 0.30 Se.

Data Collection

Pigs and feeders were weighed on d 0, 7, 14, and 18 to determine average daily gain (ADG), average daily feed intake (ADFI), and gain:feed (G:F) ratio. The average daily intake of energy (kcal ME) and lysine (g) as well as the weight gain (g)/100 kcal ME intake and weight gain (g)/lysine intake (g) were computed.

Blood was collected from two randomly selected pigs (one male and one female) per pen. Blood samples were taken via the vena cava on d 0, 7 and 18 using vacutainer tubes with anticoagulant. Collected blood was centrifuged and plasma was frozen until blood chemistry determination. Plasma urea nitrogen, triglycerides, and glucose were determined via colorimetric procedures using a Cobas Mira Clinical Analyzer.

Statistical Analysis

All data were analyzed as a randomized complete block design using procedures described by Steel et al. (1997). The model included the effects of replication (block), treatment, and replication x treatment (error). Orthogonal contrasts were used to compare treatment means for 1) SPC vs SDPP, 2) linear effects within pigs fed SDPP with decreasing ME levels, and 3) quadratic effects within pigs fed SDPP. The pen served as experimental unit.

Results and Discussion

Growth Performance

The inclusion of spray-dried plasma protein (SDPP) in the diet, regardless of ME level, improved ADG ($P < 0.01$), ADFI ($P < 0.01$), and G:F ratio ($P < 0.01$) on d 0 to 7 and overall, from d 0 to 18 (Table 3.2). In addition, the computed average daily ME ($P < 0.02$) and lysine intakes ($P < 0.01$) were greater for pigs fed SDPP compared to pigs fed SPC. The weight gain/100 kcal ME and gain/lysine intake were also higher ($P < 0.01$) in pigs fed SDPP as shown in Figure 3.1.

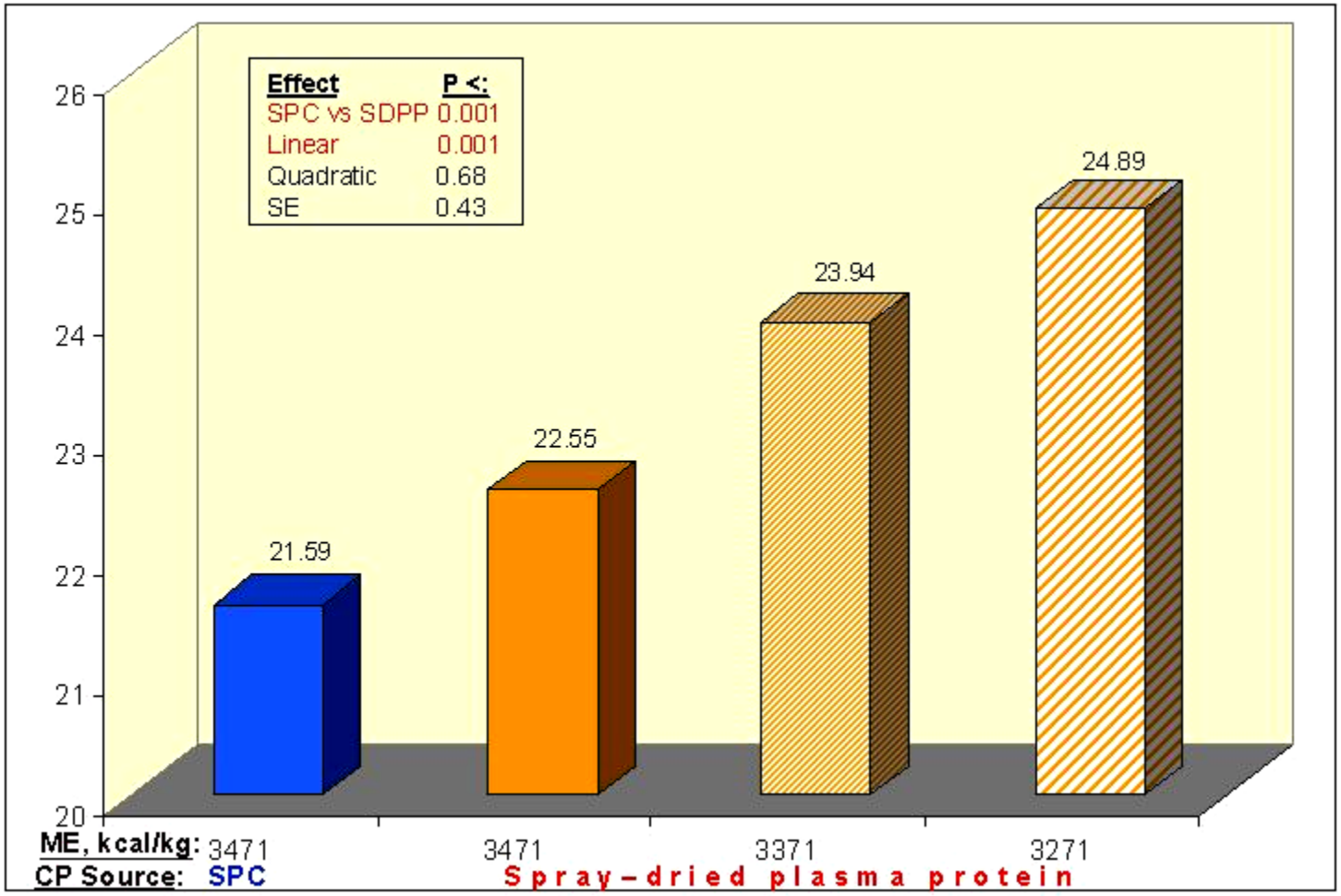
Reducing ME level in diets containing SDPP had no significant effect ($P > 0.10$) on the ADG, ADFI, and G:F on all days although decreasing ME level in SDPP diets numerically increased overall G:F (d 0 to 18) and final weight of weanling pigs on d 18 post-weaning. In addition, results suggest that pigs fed SDPP utilized the lower ME levels more efficiently as shown by the higher gain/100 kcal ME when ME level was decreased by 100 or 200 kcal as shown in Figure 3.1. There was a linear improvement ($P < 0.01$) in gain/100 kcal ME as the level of ME decreased. The weight gain/lysine intake numerically increased with decreasing ME levels in pigs fed SDPP but the differences were not significant ($P > 0.10$).

Table 3.2. Growth performance of weanling pigs[a], Exp 1.

Treatment	1	2	3	4		Probability, P <:		
Protein source	SPC	SDPP	SDPP	SDPP	SE	SPC vs SDPP	Linear	Quad
ME concentration, kcal/kg	3,471	3,471	3,371	3,271				
Initial weight, kg	5.81	5.82	5.80	5.82	0.02	1.00	1.00	0.53
Final weight, kg	15.90	16.37	16.55	16.83	0.20	0.01	0.12	0.85
d 0-7								
ADG, g	57.51	104.85	100.18	93.87	9.46	0.01	0.42	0.94
ADFI, g	106.57	139.22	128.07	128.40	7.44	0.01	0.31	0.53
G:F	0.51	0.71	0.78	0.71	0.05	0.01	0.98	0.31
d 7-14								
ADG, g	212.46	230.23	228.49	240.48	12.29	0.16	0.56	0.65
ADFI, g	244.44	263.98	271.76	276.46	11.39	0.06	0.45	0.91
G:F	0.87	0.86	0.84	0.87	0.03	0.83	0.86	0.41
d 0-18								
ADG, g	174.66	205.60	215.80	216.46	8.68	0.01	0.39	0.66
ADFI, g	232.38	261.84	266.86	265.35	8.88	0.01	0.78	0.77
G:F	0.75	0.78	0.80	0.81	0.01	0.01	0.15	0.59
Avg daily ME intake, kcal	806.42	879.12	892.27	857.66	29.29	0.04	0.61	0.51
Avg daily lysine intake, g	3.48	3.93	4.00	3.98	0.13	0.01	0.78	0.76
Wt gain (g)/lysine intake (g)	50.01	52.22	53.85	54.34	0.99	0.01	0.14	0.64

[a]Least squares means for 9 pens (6-7 pigs/pen) per treatment.

Figure 3.1. Weight gain (g)/100 kcal ME intake of weaned pigs fed diets supplemented with SDPP with decreasing ME levels.



The positive effect of the inclusion of SDPP on ADG and ADFI of weanling pigs is significant in the first 2 wk post-weaning (Grinstead et al., 2000; Van Dijk et al., 2001a). Pigs fed SDPP consistently had higher ADG and ADFI during the first wk (d 0 to 7). Although ADG was numerically higher in pigs fed diets containing SDPP from d 7 to 14, the difference was not significant ($P > 0.10$). Pigs fed SDPP tended to have higher ($P < 0.06$) ADFI from d 7 to14 compared to pigs fed SPC. Ermer et al. (1994) reported that weanling pigs showed preference for diets containing SDPP compared to diets with dried skim milk resulting in increased feed consumption. The G:F ratios were not different from d 7 to 14 but the improvement from d 0 to 7 in pigs fed SDPP was great enough to show an overall G:F improvement (d 0-18). De Rodas et al. (1995) and Kats et al. (1994) did not report any improvement in F:G although Van Dijk et al. (2001a) concluded, based on 15 published studies, that up to 6% SDAP improves F:G.

The process of weaning is associated with a variety of stressors resulting in growth reduction. The drastic decrease in feed intake immediately post weaning seems to be the major factor causing metabolic and endocrine adjustments (Le Dividich and Sève, 2000) and histological and biochemical changes in the small intestine of the weanling pig (Pluske et al., 1997). The inclusion of spray-dried animal plasma has become essential to weanling pig diets because of the consistent improvement in gain and feed intake.

The computed ratio of total lysine:ME (g/Mcal) in the formulated diets containing SDPP showed an increasing trend (4.55, 4.69, and 4.83 g/Mcal) as ME levels decreased from 3471 to 3271 kcal/kg. On the other hand, the ratio for

the SPC diet was numerically lower at 4.49 g total lysine/Mcal ME. Although the feed intake was higher in pigs fed SDPP compared to pigs fed SPC, there were no differences in feed intake among pigs fed the SDPP diets with decreasing ME levels. A greater feed intake in pigs fed SDPP increased lysine intake. Diets are generally formulated on a total, apparent, or true digestible lysine basis therefore increased lysine intake will necessarily mean increased intake of other amino acids. This is important because the gastrointestinal tissues utilize a significant amount of dietary amino acids.

The small intestine serves a major function in the body that utilizes protein and energy sources directly from dietary nutrients in the intestinal lumen (first-pass metabolism), and from the blood (arterial mesenteric circulation). Recent studies by Burrin et al. (2001) quantified the nutrient requirements of gut tissues and concluded that gut tissues consume a significant proportion of the whole body nutrient needs. This is mainly due to the high rates of protein synthesis and energy metabolism in the gastrointestinal tract.

The utilization of dietary first-pass amino acids by intestinal mucosa has been described (Wu, 1998; Stoll et al., 1999a, b; van Goudoever et al., 2000; van der Schoor et al., 2002). Wu (1998) summarized that the main source of amino acids for intestinal mucosa is dietary and that amino acid metabolism in the mucosa is essential for the maintenance of intestinal mucosal mass and integrity. The major fuels for the small intestinal mucosa are dietary glutamine, glutamate, and aspartate, these being preferentially channeled towards mitochondrial oxidation compared to a much less complete oxidation of glucose to CO_2 (Burrin

et al., 2001). Arginine, glutamine, and proline are needed to synthesize ornithine, a precursor for polyamines, essential for the normal growth, proliferation, migration, and repair of intestinal epithelium (Luk, 1990; McCormack and Johnson, 2001; McCormack et al., 2002).

Threonine and cysteine are both abundantly utilized by intestinal mucosa for the synthesis of mucin, the innate immune defense of the intestinal mucosa. In addition, cysteine is a component of glutathione, an essential antioxidant needed to maintain structural integrity and barrier function in the mucosa (Martensson et al., 1990). Cysteine is non-essential and is synthesized from methionine. Results from Shoveller et al. (2000) suggest that there is substantial methionine utilization by the gut suggesting that methionine may be metabolized to cysteine.

While the gastrointestinal tissues can source out nutrients from the lumen and from the arterial supply, the muscular system, on the other hand, can only take nutrients from arterial circulation for its growth and function. Thus, when the requirement of the gastrointestinal tissues is high, such as during weaning when growth is rapid, there may be greater sequestration of amino acids to these tissues at the expense of lean tissue deposition. Therefore, a greater feed intake with increased intake of amino acids may be beneficial to the newly-weaned pig. This may explain the higher weight gain and weight gain/lysine intake in pigs fed SDPP.

The reduction in feed intake at weaning is variable but regardless of weaning age, the ME intake of piglets during the first wk post-weaning is only 60 to 70% of the pre-weaning milk ME intake (Le Dividich and Sève, 2000). Yet, both gastric and intestinal mucosal weights dramatically increase even with the growth check immediately post-weaning. Thus, much of the nutrient intake of the newly-weaned piglet is probably channeled towards gastrointestinal and other organ growth and less toward lean tissue deposition. Energy is also spent on the mobilization of the immune system especially along the intestinal lining where major changes are taking place. This may imply that a healthy gastrointestinal tract with reduced inflammatory reaction would have a lower ME requirement for maintenance. Jiang et al. (2000b) had reported that pigs fed diets with SDPP had lower intravillous lamina propria cell density with decreased numbers of macrophages indicative of reduced inflammation. The increase in gain/100 kcal with decreasing ME levels in pigs fed SDPP was therefore not expected. And we do not know whether this increase was due to SDPP as the protein source or due to the decrease in ME level.

Blood Chemistry

Plasma urea nitrogen. Plasma urea nitrogen levels were lower before weaning compared to post-weaning levels. Levels were lower ($P < 0.03$) in pigs fed SDPP on d 7, with the differences being highly significant ($P < 0.001$) on d 18, compared to the levels in pigs fed SPC (Table 3.3). Jiang et al. (2000b) reported significantly lower plasma urea concentrations in weanling pigs fed porcine plasma protein compared to pigs fed extruded soy protein. Spray dried porcine and bovine plasma (vs extruded soy protein) increased efficiency of dietary protein use for lean tissue growth in weanling pigs, expressed as the ratio of weight gain to protein intake (Jiang et al., 2000b). However, it was not determined whether this change in amino acid metabolism is taking place in the gut or in other tissues such as the liver (Jiang et al., 2000b). In this experiment, SDPP seemed to increase efficiency of lysine use by the greater gain per gram of lysine intake and lower plasma urea nitrogen levels.

Plasma urea nitrogen levels increased post-weaning and were numerically higher 7 d post-weaning compared to the levels on d 18 for all pigs, and was numerically higher in pigs fed diets with 3471 kcal/kg ME concentration in both SPC and SDPP compared to that of pigs fed diets with 3271 kcal/kg ME concentration. Weaning has marginal effects on blood urea nitrogen concentration (Pluske et al., 1997). However, Cera et al. (1990b) observed that serum urea concentration was higher on d 7 post weaning compared to subsequent weekly periods and tended to be highest when fat was added to the

diets. Increased energy density or level of fat in the diet (increased blood triglyceride levels) may decrease utilization of amino acids thus increasing plasma urea nitrogen (Cera et al., 1990).

Triglycerides. Plasma triglyceride levels were higher on d 0 compared to post-weaning levels. This may be due to the high fat content of sow milk and greater absorption of fatty acids, thus, increasing fatty acids in plasma.

On d 7, triglyceride levels were lower ($P < 0.03$) in pigs fed SDPP. There were no differences ($P > 0.10$) in triglyceride levels on d 18 in pigs fed SDPP or SPC. Underfeeding during the immediate post-weaning period results in decreased ME and nutrient intake and a negative energy balance (Le Dividich and Sève, 2000). The main source of energy for the newly-weaned pig is body fat. Le Dividich and Sève (2000) cited literature that reported increased body fat catabolism immediately post-weaning as shown by a decrease in backfat thickness, increased plasma free fatty acid levels, and decreased adipocyte size. Results show numerically higher plasma triglycerides 7 d post-weaning compared to 18 d post-weaning. The greater plasma triglyceride levels in pigs fed SPC compared to pigs fed SDPP on d 7 may be due to the higher feed intake in pigs fed SDPP (with or without added fat) that may have decreased body fat catabolism thus decreasing plasma triglycerides, or this may signify better utilization of the fat absorbed, but the mechanism remains unclear.

Table 3.3. Blood chemistry of weanling pigs[a], Exp 1.

Treatment	1	2	3	4		Probability, P <:		
Protein source	SPC	SDPP	SDPP	SDPP	SE	SPC vs SDPP	Linear	Quad
ME concentration, kcal/kg	3,471	3,471	3,371	3,271				
Plasma urea nitrogen, mg/dL								
d 0	7.72	6.93	8.11	7.16	0.54	0.61	0.76	0.12
d 7	10.91	9.33	9.13	8.22	0.74	0.03	0.32	0.61
d 18	10.43	8.05	8.57	7.69	0.45	0.001	0.56	0.33
Triglycerides, mg/dL								
d 0	56.56	60.36	54.11	55.17	4.29	0.10	0.40	0.49
d 7	50.14	43.19	39.55	38.06	3.61	0.03	0.33	0.81
d 18	41.22	38.72	38.38	35.06	3.54	0.36	0.48	0.74
Glucose, mg/dL								
d 0	163.86	149.72	141.81	145.25	5.68	0.01	0.58	0.42
d 7	106.60	118.50	113.50	119.10	3.51	0.02	0.88	0.27
d 18	113.50	122.90	121.50	122.40	4.74	0.12	0.99	0.92

[a]Least squares means for 9 pens (2 pigs/pen) per treatment.

Decreasing ME level in diets containing SDPP had no effect (P > 0.10) on triglyceride levels but pigs fed the higher fat level (3471 kcal/kg ME with 4.26 or 5.0% soybean oil) had numerically higher triglyceride levels compared to those pigs fed diets containing very little fat (0.05% soybean oil). Jones et al. (1992) had reported that the addition of fat to diets increased serum triglycerides.

Glucose. Blood glucose levels were higher before weaning (d 0) compared to post-weaning levels (Table 3.3). On d 7, blood glucose levels were higher (P < 0.03) in pigs fed SDPP compared to pigs fed SPC. On d 18, blood glucose levels were numerically higher in pigs fed SDPP but the differences from those fed SPC were not significant (P > 0.10). Decreasing ME level in diets containing SDPP had no effect (P > 0.10) on glucose levels.

Blood glucose levels decreased after weaning and were numerically lower 7 d post-weaning compared to the levels 18 d post-weaning for all the pigs. Funderburke and Seerley (1990) reported that symptoms of post-weaning growth lag included low plasma glucose, high free fatty acids, low plasma insulin, low liver glycogen, and high cortisol values. If low plasma glucose is a symptom of post-weaning growth lag, the higher plasma glucose in pigs fed SDPP compared to those fed SPC may indicate that SDPP might have alleviated this growth lag and this may also be a consequence of the increased feed intake in pigs fed SDPP. However, de Rodas et al. (1995) did not observe any differences in plasma glucose concentrations in pigs fed either soybean meal or SDPP.

Implications

Reduced intestinal growth in pigs fed spray dried porcine plasma may lower ME requirement for maintenance in the gut therefore increasing ME available for lean tissue deposition. However, reducing ME concentration in SDPP diets did not affect growth performance of weanling pigs in this experiment, but linearly increased weight gain/ME intake. In the course of reducing the ME level of the diet, the added fat in the form of soybean oil was reduced. The apparent digestibility of fat is generally low at weaning and the use of fat in weanling pig diets is controversial. In addition, the digestibility coefficients for fat were reportedly higher for diets containing casein compared to soybean protein diets, implying that protein source may influence fat utilization. Therefore further investigation is needed to determine if SDPP, as the dietary protein source, and not just the low fat level, may have a role in the linear increase in weight gain/ME intake.

CHAPTER IV

EXPERIMENT 2

EFFECTS OF REDUCING METABOLIZABLE ENERGY CONCENTRATION IN DIETS CONTAINING EITHER SPRAY-DRIED PORCINE PLASMA OR SOY PROTEIN CONCENTRATE ON WEANLING PIG PERFORMANCE

ABSTRACT: An experiment was conducted to determine the effects of reducing the metabolizable energy (ME) concentration of diets containing either soy protein concentrate (SPC) or spray-dried porcine plasma (SDPP) on weanling pig performance. A total of 168 pigs (avg BW = 5.8 kg) were weaned at approximately 21 d and allotted to four dietary treatments (7 pens/trt) in a 2 x 2 factorial design with two crude protein (CP) sources (SPC vs SDPP) and two ME levels (3,523 vs 3,323). All diets contained 1.35% digestible lysine and were composed primarily of corn, soybean meal, dried whey, lactose, SPC or SDPP, fish meal, and soybean oil or cornstarch. All pigs were housed (6-7 pigs/pen) in a temperature-controlled nursery for 18 d. Pigs and feeders were weighed on d 0, 7, 14, and 18 to determine ADG, ADFI, and gain:feed (G:F) ratio. Pigs fed SDPP had greater ($P < 0.005$) ADG and ADFI and tended to have greater ($P < 0.09$) G:F and gain/100 kcal ME intake than pigs fed SPC from d 0 to 18. Reducing ME had no effect ($P > 0.10$) on growth performance, but it increased ($P < 0.01$) gain/100 kcal ME intake. The improvement in weight gain/ME intake

associated with reducing ME of the diet tended to be greater for pigs fed SPC than for pigs fed SDPP (CP source x ME level, P < 0.10). In addition, for pigs fed SPC, reduced ME tended to increase G:F ratio from d 7 to 14 and overall (d 0 to 18), as well as weight gain/lysine intake but there was no effect for pigs fed SDPP (CP source x ME level, P < 0.08). These results suggest that the source of dietary protein may affect energy (fat) utilization but further investigation is needed to elucidate underlying mechanisms.

Key Words: Weanling Pig, Porcine Plasma, Metabolizable Energy

Introduction

Spray-dried animal plasma (SDAP), usually of porcine origin (spray-dried porcine plasma or SDPP), improves average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F) during the first two wk post-weaning. Although expensive, it is considered an essential protein source in the early-weaned pig diet. In addition to improvements in growth performance, SDPP has been shown to reduce small intestinal growth (Jiang et al., 2000a). Considering the very rapid relative growth of the intestines in the first 2 wk post-weaning, reduced small intestinal growth in pigs fed SDPP may suggest a lower ME requirement for the intestine, thus increasing ME available for body growth (protein accretion). However, in a previous experiment, decreasing the metabolizable energy (ME) or fat level in diets containing SDPP for weanling pigs did not affect ADG, ADFI, and G:F, but resulted in a linear increase in body

weight gain/100 kcal ME intake. Decreasing ME level also numerically increased the final weight of weanling pigs on d 18 post-weaning as well as overall G:F (d 0 to 18) and weight gain/lysine intake.

To reduce the ME level of the diet, the added fat in the form of soybean oil was replaced with cornstarch. The use of fat in starter diets is controversial and results have not been consistent. Pigs fed a control diet gained more than pigs consuming diets with added fat, and fat in the diet increased the energy required per unit of weight gain (Frobish et al., 1970, 1971; Jones et al., 1992). Some authors have reported benefits from the addition of fat to weanling pig diets, although most of these benefits were seen at 3 to 5 wk post-weaning. Therefore, the value of adding fat to weanling pig diets, particularly during the first 2 wk post-weaning period, has been debated. The apparent digestibility of fat is generally low at weaning but increases with age, reaching a plateau between 3 to 4 wk post-weaning (Cera et al., 1988a, b). Frobish et al. (1970) noted that digestibility coefficients for fat were higher for diets containing casein compared to soybean protein diets, implying that protein source may influence fat utilization.

Objective

To determine the effects of reducing the metabolizable energy (ME) concentration (fat level) of diets containing either soy protein concentrate (SPC) or spray-dried porcine plasma (SDPP) on weanling pig performance.

Materials and Methods

The effect of reducing the ME concentration in weanling pig diets supplemented with either soy protein concentrate (SPC) or SDPP was investigated using a 2 x 2 factorial in a randomized complete block design.

Animals, Treatments, and Diets

A total of 168 crossbred pigs (avg BW = 5.8 kg) were weaned at approximately 21 d and allotted to four dietary treatments (7 pens/trt) in a 2 x 2 factorial with two CP sources (SPC vs SDPP) and two ME levels (3,523 vs 3,323). Weight, sex, and breed were equalized across treatment groups. The composition of the diets is shown in Table 4.1 and was as follows: 1) diet containing SPC with ME = 3,525 kcal/kg, 2) SPC diet with ME = 3,325 kcal/kg, 3) SDPP diet with ME = 3,525 kcal/kg, and 4) SDPP diet with ME = 3,325 kcal/kg. Substitutions were made on an equal lysine basis. To lower the ME concentration, soybean oil was replaced by cornstarch and in part by corn grain. All diets were pelleted and formulated to contain 1.35% digestible lysine, 0.90 % Ca, and 0.75% P. Feed and water were provided on an ad libitum basis using nipple waterers and a common feeder per pen. The pigs were housed in an environmentally-regulated nursery with pens measuring 1.14 x 1.5 m on a raised woven wire floor. The temperature of the nursery was maintained at 31 to 32°C throughout the experimental period. All procedures were approved by the OSU Institutional Animal Care and Use Committee (IACUC).

Table 4.1. Composition of the diets, Exp 2.

Protein source	SPC	SPC	SDPP	SDPP
ME concentration, kcal/kg	3,523	3,323	3,523	3,323
Ingredients:				
Corn	27.12	27.12	29.73	29.73
Soybean meal (48% CP)	20.00	20.00	20.00	20.00
Whey, dried	20.00	20.00	20.00	20.00
Lactose	10.00	10.00	10.00	10.00
Fish meal, Menhaden	5.55	5.55	5.55	5.55
Soy protein concentrate [a]	9.47	9.47	0.00	0.00
Plasma, spray dried [a]	0.00	0.00	6.00	6.00
L-lysine·HCl	0.05	0.05	0.05	0.05
DL-methionine	0.15	0.15	0.27	0.27
L-threonine	0.03	0.03	0.03	0.03
Soybean oil [a]	5.00	0.47	5.74	1.21
Cornstarch [a]	0.00	4.53	0.00	4.53
Dicalcium phosphate	0.51	0.51	0.33	0.33
Limestone, ground	0.66	0.66	0.84	0.84
Salt	0.50	0.50	0.50	0.50
Antibiotic [b]	0.25	0.25	0.25	0.25
ZnO	0.28	0.28	0.28	0.28
Ethoxyquin	0.03	0.03	0.03	0.03
OSU Vitamin mix[c]	0.25	0.25	0.25	0.25
OSU Trace mineral mix[d]	0.15	0.15	0.15	0.15
Composition of the diet:				
Crude protein, %	23.80	23.80	22.65	22.65
Ca	0.90	0.90	0.90	0.90
P(a)	0.44	0.44	0.50	0.50
Total Lys, %	1.56	1.56	1.58	1.58
Apparent ileal digestible AA, %				
Lysine	1.35	1.35	1.35	1.35
Met + Cys	0.80	0.80	0.80	0.80
Threonine	0.85	0.85	0.85	0.85
Total lysine: ME, g/Mcal	4.42	4.96	4.48	5.03

[a] ME values, kcal/kg: Soybean oil: 8,400; Cornstarch: 3,985.

[b] Mecadox® provided 50 mg of carbadox per kg of complete feed.

[c] Provided the following per kg diet: 11,013 IU vitamin A; 1,652 IU vitamin D3; 44 IU vitamin E; 4.4 mg vitamin K (menadione activity); 55 mg niacin; 10 mg riboflavin; 33 mg pantothenic acid (D-Ca pantothenate); and 44 μg vitamin B_{12}.

[d] Provided the following per kg diet: 16.5 mg $CuSO_4$; 165 mg $FeSO_4$; 0.30 mg I ($Ca(IO_3)_2$); 40 mg MnO_2; 0.30 mg Se (Na selenite); and 165 mg ZnO.

Data Collection

Pigs and feeders were weighed on d 0, 7, 14, and 18 to determine average daily gain (ADG), average daily feed intake (ADFI) and feed:gain (F:G) ratio. The average daily intake of energy (kcal ME) and lysine (g) as well as the weight gain (g)/100 kcal ME intake and weight gain (g)/lysine intake (g) were computed.

Statistical Analysis

Data were analyzed as a 2 x 2 factorial in a randomized complete block design using procedures described by Steel et al. (1997). Main effects for CP source and ME levels and CP x ME interaction were tested. The pen served as the experimental unit.

Results and Discussion

Effect of Protein Source (SPC vs SDPP)

The inclusion of SDPP in the diet increased ($P < 0.005$) both ADG and ADFI from d 0 to 7 and overall (d 0 to 18) as shown in Table 4.2. It also increased ($P < 0.01$) G:F from d 0 to 7. From d 7 to 14, ADG was greater ($P < 0.02$) while ADFI and G:F tended to be greater ($P < 0.10$) for pigs fed SDPP compared to pigs fed SPC. Overall, G:F tended to be greater ($P < 0.10$) in pigs fed SDPP.

The average daily ME and lysine intakes were greater ($P < 0.01$) for pigs fed SDPP due to greater ADFI. Spray-dried plasma protein inclusion also tended to increase ($P < 0.10$) weight gain/Lys intake. The improvements in growth, feed intake, and feed efficiency in pigs fed SDPP are consistent with the results of Exp. 1 except that the differences in ADG and ADFI between pigs fed SDPP and SPC are greater during the first wk post-weaning.

Van Dijk et al. (2001a) noted that the response to spray-dried animal plasma (SDAP) is dependent on several factors such as the diet composition of the control group, the origin of SDAP (porcine vs. bovine), and feed processing (pellet vs. meal form). The health status and degree of pathogen exposure of the weanling pig is another determinant of the level of response to SDAP (Coffey and Cromwell, 1995; Bergstrom et al., 1997; Van Dijk et al., 2001a, 2002b). The pigs were housed in the same environmentally-controlled nursery used in Exp. 1 under the same management procedures. In addition, the pigs used in this experiment had the same average initial and final body weights as the pigs used in Exp. 1. Thus, the greater differences in ADG and ADFI between pigs fed SDPP and those fed SPC, especially during the first wk post-weaning cannot be clearly explained. The health status and degree of pathogen exposure of the weanling pig is probably the major determinant of the level of response to SDPP. It is possible that the pigs used in Exp. 1 may have had a better health status compared to the pigs used in this experiment. However, this cannot be ascertained because health condition of the pigs such as incidence of diarrhea was not recorded.

Table 4.2. Growth performance of weanling pigs[a], Exp 2.

Protein source	SPC	SPC	SDPP	SDPP		Probability, P <:		
ME concentration, kcal/kg	3,523	3,323	3,523	3,323	SE	SPC vs SDPP	Fat Level	Interaction
Fat inclusion, %	5.0	0.47	5.74	1.21				
Initial weight, kg	5.81	5.82	5.81	5.81	0.01	0.82	0.78	0.89
Final weight, kg	15.90	15.79	16.53	16.18	0.27	0.14	0.25	0.91
d 0-7								
ADG, g	84.17	80.86	117.19	124.61	9.62	0.001	0.83	0.58
ADFI, g	133.86	124.15	166.20	161.72	8.56	0.001	0.42	0.76
G:F	0.61	0.64	0.69	0.75	0.03	0.01	0.24	0.59
d 7-14								
ADG, g	267.53	283.70	296.60	313.23	11.88	0.02	0.18	0.98
ADFI, g	306.34	296.54	311.84	328.85	9.96	0.07	0.72	0.19
G:F	0.88	0.96	0.96	0.96	0.03	0.10	0.09	0.08
d 0-18								
ADG, g	219.77	233.65	255.52	262.21	9.55	0.003	0.30	0.71
ADFI, g	278.22	280.78	304.98	315.34	9.49	0.005	0.50	0.69
G:F	0.79	0.83	0.84	0.83	0.02	0.07	0.16	0.07
Avg daily ME intake, kcal	980.00	932.90	1,074.20	1,047.70	32.48	0.01	0.27	0.76
Avg daily lysine intake, g	4.17	4.21	4.57	4.73	0.14	0.01	0.50	0.69
Wt gain (g)/lysine intake, g	52.54	55.34	55.68	55.32	0.86	0.09	0.17	0.08

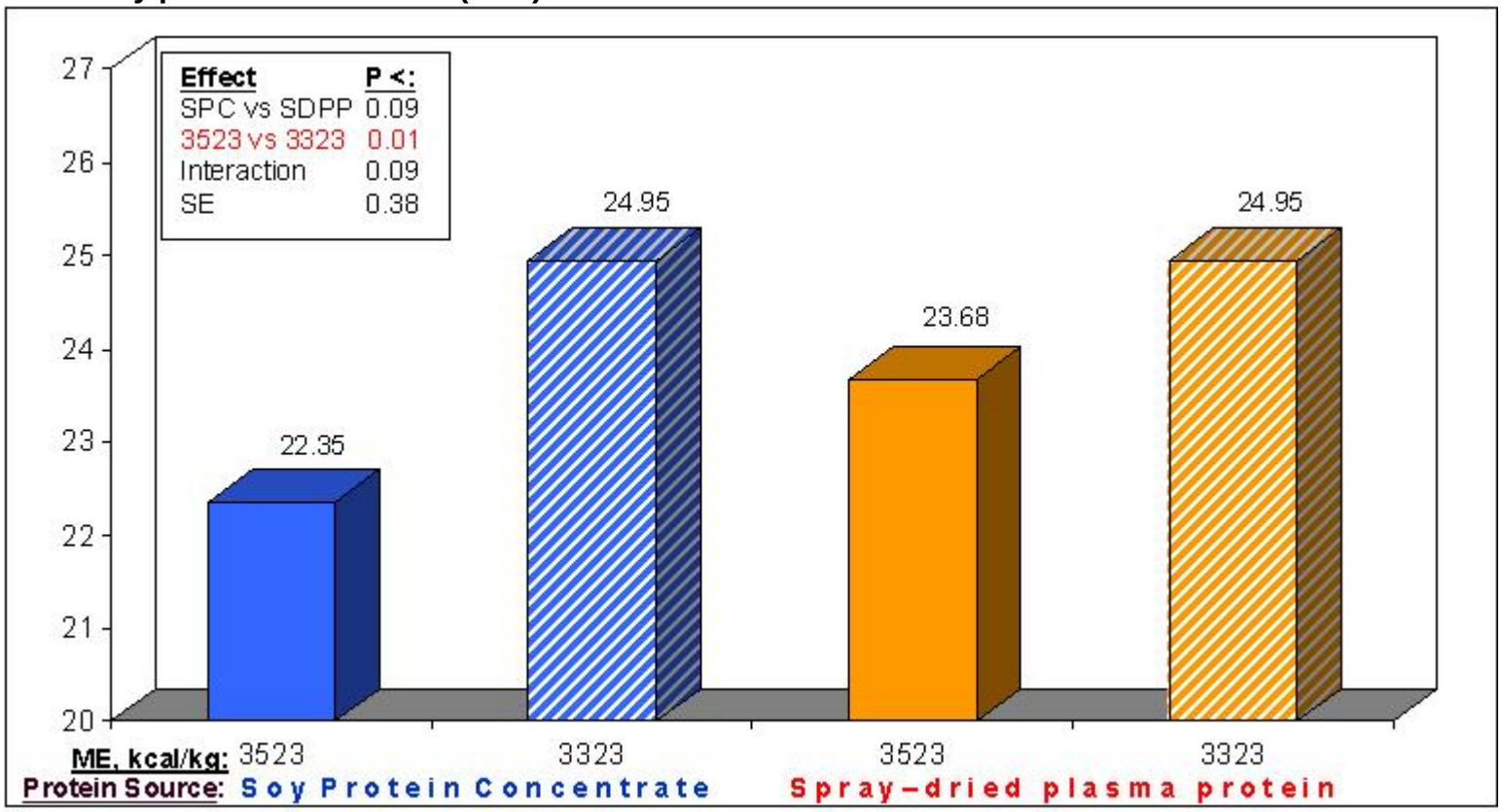
[a]Least squares means for 7 pens (6 to 7 pigs/pen)/trt.

Effect of Decreasing ME Level

Decreasing ME level did not affect growth performance but increased ($P < 0.01$) weight gain/100 kcal ME intake (Figure 4.1). For pigs fed SPC, reduced ME tended to increase G:F ratio (from d 7 to 14 and d 0 to 18) and weight gain/Lys intake while there was no effect for pigs fed SDPP (CP source x ME level, $P < 0.08$). In addition, improved weight gain/100 kcal ME intake associated with low ME levels was more pronounced in pigs fed SPC (CP source x ME level, $P < 0.09$).

The value of adding fat to weanling pig diets has been controversial due to inconsistent results. Lawrence and Maxwell (1983) reported a linear decrease in weight gain and feed consumption as fat level increased during the first two wk post-weaning. However, soybean oil in weanling pig diets at 0, 3, 6, or 9% did not affect growth performance during the first two wk post-weaning (Tokach et al., 1995). Also, the addition of either soybean oil, coconut oil, or choice white grease had no effect on ADG or F:G during the first two wk post-weaning (Li et al., 1990). Issues related to the inconsistency have been raised to explain the differences such as the type or source of oil used in the trial and its digestibility, the differences in the level of nutrients between treatment groups (such as lysine to energy ratio), and the activity of pancreatic lipase.

Figure 4.1. Weight gain (g)/100 kcal ME intake of weaned pigs fed diets supplemented with either soy protein concentrate (SPC) or SDPP.



Frobish et al. (1970, 1971) reported reduction in weight gain and higher energy required per unit of weight gain when fat was added to the weanling pig diet. They added fat in place of cornstarch on equal weight basis while maintaining the levels of all other nutrients constant, thus probably limiting other nutrients when fat was added. Cline et al. (1977) concluded that the young pig can utilize fat efficiently when all other nutrients are provided or when the protein:calorie ratio is kept constant; however, all the diets used in their experiments contained fat and they showed no differences in the growth of pigs fed diets containing low fat (43% of non-protein calories) compared to those fed diets with higher fat level (74% of non-protein calories).

In this experiment, fat level was reduced by replacing soybean oil with cornstarch to reduce ME by 200 kcal/kg while maintaining total lysine (%) constant. All other published literature have added fat to the experimental diets while maintaining or increasing caloric density and maintaining or increasing the levels of other nutrients such as amino acids.

The ability of the newly-weaned pig to digest fat has been questioned and most of the reported benefits from the addition of fat to weanling pig diets were seen at three to five wk post-weaning. Fat digestibility seems to be generally low during the first two wk post-weaning and there is a marked increase in apparent fat absorption from weaning (at 21 d) to the third wk post-weaning (Cera et al., 1988b). The addition of fat to weanling pig diets during the first two wk post-weaning also decreased ileal DM digestibility (Cera et al., 1988a; Li et al., 1990). The major factor seen as a reason for the low fat digestibility is the low lipase

activity in the weanling pig. Total lipase activity increased quadratically from birth to 4 wk then dramatically decreased immediately post-weaning (Lindemann et al., 1986).

Makkink et al. (1994) reported that dietary protein source and feed intake affect pancreatic enzyme activity. In their study, pigs were weaned at 28 d and fed diets containing either skimmed-milk powder (SMP), soya-bean-protein concentrate (SPC), soya-bean meal (SBM), or fish meal (FM). On d 3 post-weaning, SMP stimulated trypsin synthesis and secretion more than the other protein sources while on d 6, pigs fed SPC had higher pancreatic and jejunal trypsin activity and higher feed intake resulted in higher pancreatic trypsin activity. The source of dietary protein also affects growth of the pancreas in weaned pigs (Makkink et al., 1994; Peiniau et al., 1996); therefore, possibly affecting synthesis, secretion, and activity of pancreatic enzymes including pancreatic lipase.

Protein source may also influence digestibility coefficients of fat. Frobish et al. (1970) noted that digestibility coefficients for fat were higher for diets containing casein compared to soybean protein diets. Thus, protein source may influence fat utilization and explain why the improved G:F ratio and weight gain/100 kcal ME associated with reduced ME levels tended to be less pronounced for pigs fed SDPP.

Implications

Reducing ME had no effect on growth performance of weanling pigs, but it increased weight gain/ME intake. The improvement in weight gain/ME intake associated with reducing the ME of the diet tended to be greater for pigs fed SPC than for pigs fed SDPP. Also, lowering ME in SPC diets tended to improve G:F ratio and weight gain/Lys intake(d 0 to 18) while lowering ME in SDPP diets did not affect G:F ratio and weight gain/Lys intake in weanling pigs. These results suggest that the source of dietary protein may affect energy (fat) utilization. In addition, the results imply that fat supplementation may have no benefit on weanling pig growth during the first 2 wk post weaning. However, further investigation is needed to clarify the mechanisms by which low ME (low fat) diets improve ME utilization in weanling pigs and how dietary protein affects fat utilization.

CHAPTER V

EXPERIMENT 3

EFFECTS OF PROTEIN SOURCE AND FAT LEVEL ON GROWTH OF THE PANCREAS, STOMACH, AND SMALL INTESTINE, AND MORPHOLOGY OF THE SMALL INTESTINE IN EARLY-WEANED PIGS

ABSTRACT: Previous work from our lab suggests that CP source may affect ME utilization in early-weaned pigs. Therefore, as part of a larger study to examine the effects of CP source (soy protein concentrate, SPC vs spray-dried porcine plasma, SDPP) and ME level on organ and pancreatic enzyme development, an experiment utilizing 80 pigs (avg BW = 5.2 kg; avg age = 18 d) was conducted to determine the effects of these factors on the growth of the pancreas, stomach, and small intestine, and morphology of the small intestine in weanling pigs. Pigs were allotted to four dietary treatments (4 pigs/pen) in a 2 x 2 factorial design with two CP sources (SPC vs SDPP) and two ME levels (3,523 vs 3,323 kcal/kg). All diets (1.35% dig. Lys) contained corn, soybean meal, dried whey, lactose, and fishmeal. Spray-dried porcine plasma replaced SPC on an equivalent digestible lysine basis and the dietary ME level was adjusted using soybean oil. On d 0, 3, 7, and 14, one pig was removed from each pen, weighed, and euthanized.

The pancreas, stomach, and small intestine (SI) were excised carefully, emptied and washed as needed, and weighed. Samples of intestinal sections

were taken for morphological exam. Organ weights were computed in g per kg body weight (g/kg BW). Overall, the growth of the pancreas, stomach, and SI were highly correlated ($r > 0.85$, $P < 0.01$) with BW. Furthermore, the weights of these organs were associated with increasing BW and age ($R^2 > 0.70$, $P < 0.01$). There were no day by treatment interactions; and ME level did not affect ($P > 0.10$) the growth of the organs on any day. However, CP source affected the growth of the organs such that on d 7, SI weight was lower ($P < 0.05$) in pigs fed SDPP compared to pigs fed SPC. On d 14, both the individual and combined weights of the stomach and SI were lower ($P < 0.05$) in pigs fed SDPP compared to those fed SPC. Histologically, villi were longer ($P < 0.02$) in pigs fed SDPP on d 14, while villous height:crypt depth ratios were greater ($P < 0.01$) in pigs fed SDPP. Crypt depth was lower ($P < 0.04$) in pigs fed SDPP and tended to be lower in pigs fed the lower ME level. Villous width was lower ($P < 0.01$) in pigs fed SDPP and in pigs fed a lower ME level on d 7, but no differences ($P > 0.10$) were noted on d 3 and 14. There was no CP source by fat level interaction ($P > 0.10$). These results suggest that CP source (SPC vs SDPP), but not ME level, can dramatically affect the growth of the stomach and SI, while both CP source and ME level can affect villous morphology. Also, it appears that BW may be used to predict the weight of the pancreas, stomach, and small intestine in early-weaned pigs.

Key words: Pigs, protein, organ weights

Introduction

After weaning, the growth rate and weight (in relation to body weight) of the gastrointestinal organs and pancreas is much greater especially the small intestine (Cranwell and Moughan, 1989). This is attributed to the change in diet because growth is higher in pigs fed solid feed compared to those fed a liquid milk substitute (McCracken et al., 1995). Le Dividich and Sève (2000) noted that the relative weight of the small intestine increases by 25% at 3 to 7 d post weaning and by 52% at 10 to 14 d.

The mucosa of the small intestine undergoes dramatic changes at weaning. The long slender villi are drastically reduced in height (30 to 63% reduction) and the crypt depth is increased by 76 to 180% (Hampson, 1986; Miller et al., 1986; Cranwell and Moughan, 1989; Kelly et al., 1991a, b; van Beers-Schreurs et al., 1998; Hedemann et al., 2003) with mitotic counts showing increased proliferative activity from the third day post-weaning (Hedemann et al., 2003). Villous atrophy was not prevented by relatively high nutrient supply to the gut (Kelly et al., 1991a) although Pluske et al. (1996c) reported that feeding whole cow's milk to weaned pigs for five days maintained villous height and crypt depth.

The inclusion of SDPP in weanling pig diet has been shown to increase villous height (Spencer et al., 1997; Torrallardona et al., 2003). Jiang et al. (2000b) did not observe these effects while Van Dijk et al. (2001b, 2002c) reported no significant effect on villous length, but observed less mitotic activity in

SDPP-fed pigs on d 4 and 7 post-weaning. Spray-dried porcine plasma has also been reported to reduce small intestinal growth (Jiang et al., 2000b).

Objectives

Results from previous experiments suggest that CP source may affect ME utilization in early-weaned pigs. Therefore, as part of a larger study to examine the effects of CP source (soy protein concentrate, SPC vs spray-dried porcine plasma, SDPP) and fat level (3523 vs. 3323 kcal/kg) on organ and pancreatic enzyme development, this experiment was conducted to determine the effects of these factors on the growth of the pancreas, stomach, and small intestine, and morphology of the small intestine in weanling pigs.

Materials and Methods

The effects of CP source and fat level on the growth of the pancreas, stomach, and small intestine, and morphology of the small intestine in weanling pigs were determined using a 2 x 2 factorial in RCBD.

Animals, Treatments, and Diets

A total of 80 crossbred pigs with an average body weight of 5.2 kg were weaned at approximately 18 d and allotted to four dietary treatments in a 2 x 2 factorial with two CP sources (SPC vs SDPP) and two ME levels (3,523 vs

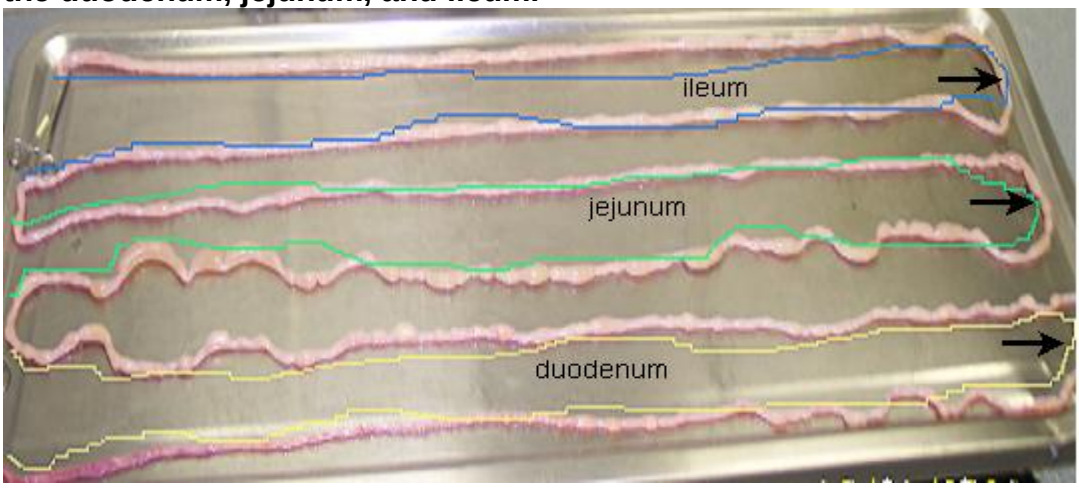
3,323). Weight, sex, and breed were equalized across treatment groups. The composition of the diets is the same as that of Exp 2 and shown in Table 4.1 which was as follows: 1) diet containing SPC with ME = 3525 kcal/kg, 2) SPC diet with ME = 3325 kcal/kg, 3) SDPP diet with ME = 3525 kcal/kg, and 4) SDPP diet with ME = 3325 kcal/kg. Substitutions were made on an equal lysine basis. To lower the ME concentration, soybean oil was replaced by cornstarch and in part by corn grain. All diets were formulated to contain 1.35% digestible lysine, 0.90 % Ca, and 0.75% P. Feed and water were provided on an ad libitum basis. The pigs were housed in metabolic chambers (5 chambers/trt) with a common feeder and nipple waterer in an environmentally regulated room. The temperature of the room was maintained at 31 to 32°C throughout the experimental period. All procedures were approved by the OSU Institutional Animal Care and Use Committee (IACUC).

Sample and data collection

Four pigs (1 pig/trt) were euthanized on d 0, 3, 7, and 14. A midline incision exposed the abdominal cavity and the pancreas was located, carefully removed, and quickly weighed. The small intestine was separated from the mesentery after clamps were secured at the junction between the stomach and duodenum and at the ileo-cecal junction. Once separated, the small intestine was washed to remove blood clots and mesenteric debris, and weighed. The whole length was returned to the table and the small intestine was divided into three sections as shown in Figure 5.1. Each section was weighed before and

after it was emptied of all contents. After emptying, it was excised and the inner surface was rinsed with physiologic saline. About 5 cm length of sample was cut off from the center portion of each section (shown by arrows in Figure 5.1), preserved in 10% formalin, then submitted to the Oklahoma Animal Disease Diagnostic Lab (OADDL). The sections were mounted and stained with hematoxylin & eosin (H&E) for morphologic measurement.

Figure 5.1. The small intestine divided into three sections to approximate the duodenum, jejunum, and ileum.



The stomach was also removed, cleaned of debris, excised and emptied, then weighed. All empty organ weights were computed in g per kg body weight (g/kg BW). Morphology of the small intestine was measured under a light microscope with an ocular micrometer. Villous height, crypt depth, and villous width were measured as shown in Figure 5.2. The villous height to crypt depth ratio was computed.

Figure 5.2. Morphologic measurement of the small intestine.

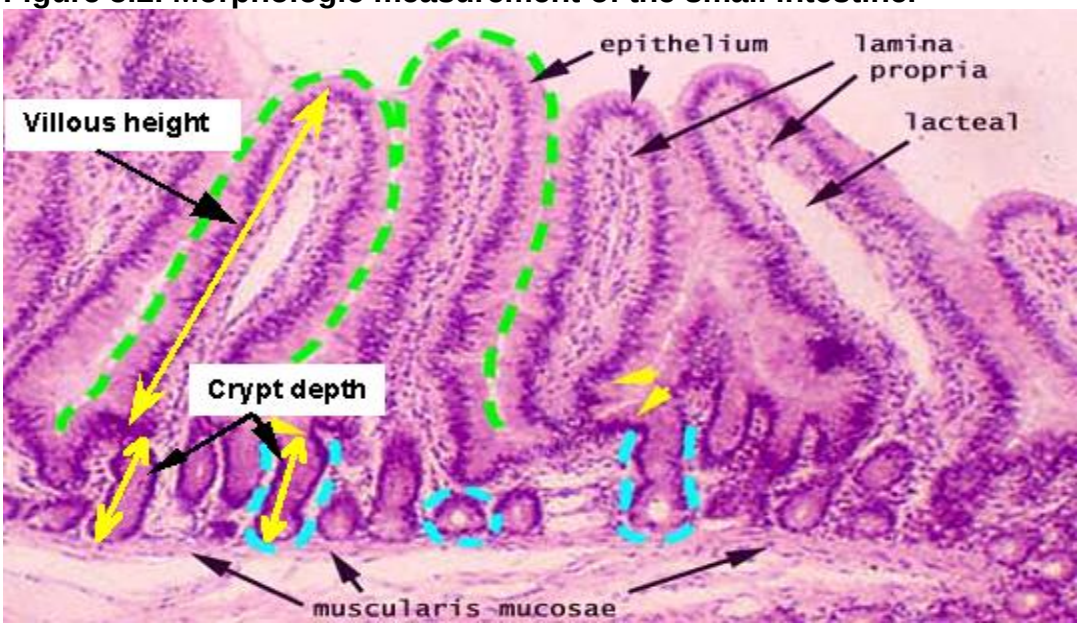


Image from Dr. David G. King, Southern Illinois University School of Medicine, used with permission (copyright 2006).

Statistical analysis

Data were analyzed as a 2 x 2 factorial over time and the main effects of CP source, fat level, and day, and their interactions were tested using PROC GLM (SAS, 2003). The pig served as experimental unit.

Results and Discussion

Growth of Pancreas, Stomach, and Small Intestine

The relative weights (g/kg BW) of the different organs are shown in Table 5.1 while the actual weights (g) are shown in Table 5.2. There was a highly significant ($P < 0.0001$) increase in the weight of the pancreas, stomach, small

intestine, and the combined weights of the stomach and small intestine from d 3 to d 14. There were no day x treatment interactions ($P > 0.10$).

Overall, the growth of the pancreas (Figure 5.3), stomach (Figure 5.4), and small intestine (Figure 5.5), were highly correlated ($r > 0.85$, $P < 0.01$) with BW. Furthermore, the weights of these organs were associated with increasing BW and age ($R^2 > 0.70$, $P < 0.01$). The weights of the pancreas, stomach, and small intestine, have been described as a function of body weight (Jensen et al., 1997) and age (Lindemann et al., 1986).

From d 3 to 7, the relative weight of the pancreas increased by 19 to 40% (average for all pigs, 27%), the stomach by 19 to 23% (average 21%), the small intestine by 15 to 35% (average 23%), and the combined stomach and small intestine relative weight by 5 to 32% (average 18%). From d 8 to 14, the relative weight of the pancreas increased by 10 to 29% (average 22%), the stomach increased by 19 to 35% (average 28%), the small intestine by 21 to 36% (average 30%), and the combined stomach and small intestine relative weight by 25 to 36% (average 30%). From d 3 to 14, the average increase in relative weight was 43% for the stomach and pancreas, and 46% for the small intestine. Combined, the relative weight of the stomach and small intestine increased by 43%. There was great variation in the relative increase in organ weights among the different treatments. Pigs fed SDPP had a much lower increase in the relative weight of the small intestine (16%) from d 3 to 7, compared to the increase in relative weight from d 8 to 14 (31%). From d 3 to 7, pigs gained an average body weight of 152.8 g while weight gain was 211.9 from d 8 to 14.

Table 5.1. Relative organ weights (g/kgBW) of weanling pigs[a], Exp 3.

Protein source	SPC	SPC	SDPP	SDPP		Probability, P <:			
ME concentration, kcal/kg	3,523	3,323	3,523	3,323	SE	SPC vs SDPP	Fat Level	CP*ME	Day
Fat inclusion, %	5.0	0.47	5.74	1.21					
Pancreas									< 0.001
d 0	- - - - - - - - - - - - - - - - - 1.08 - - - - - - - - - - - - -								
d 3	1.09	1.12	1.04	1.30	0.15	0.67	0.35	0.44	
d 7	1.81	1.48	1.36	1.61	0.09	0.12	0.66	0.01	
d 14	2.02	2.09	1.88	2.02	0.12	0.40	0.40	0.77	
Stomach									< 0.001
d 0	- - - - - - - - - - - - - - - - - 4.60 - - - - - - - - - - - - -								
d 3	4.38	4.78	4.56	4.13	0.19	0.27	0.93	0.07	
d 7	5.56	5.89	5.73	5.35	0.28	0.53	0.94	0.69	
d 14	8.57	8.59	7.06	7.17	0.59	0.04	0.91	0.95	
Small Intestine									0.013
d 0	- - - - - - - - - - - - - - - - - 32.12 - - - - - - - - - - - - -								
d 3	23.33	25.03	24.96	23.91	1.43	0.86	0.82	0.35	
d 7	35.95	31.75	29.27	28.78	2.08	0.04	0.28	0.39	
d 14	45.63	49.26	42.08	42.37	2.49	0.06	0.45	0.51	
Stomach+ Small intestine									0.001
d 0	- - - - - - - - - - - - - - - - - 36.72- - - - - - - - - - - - -								
d 3	27.81	30.82	32.24	27.77	1.47	0.65	0.64	0.04	
d 7	40.64	36.75	33.93	33.63	2.77	0.11	0.47	0.53	
d 14	54.00	57.06	46.65	48.71	3.26	0.04	0.45	0.88	

[a]Least squares means for 5 pigs per treatment per day.

Table 5.2. Organ weights (g) of weanling pigs[a], Exp 3.

Protein source	SPC	SPC	SDPP	SDPP		Probability, P <:			
ME concentration, kcal/kg	3,523	3,323	3,523	3,323	SE	SPC vs SDPP	Fat Level	CP*ME	Day
Fat inclusion, %	5.0	0.47	5.74	1.21					
Pancreas									< 0.001
d 0	- - - - - - - - - - - - - - - - 5.17 - - - - - - - - - - - - -								
d 3	5.74	5.45	5.64	6.66	0.68	0.43	0.60	0.36	
d 7	10.64	8.38	7.80	9.82	0.77	0.38	0.88	0.02	
d 14	15.70	17.18	15.20	16.24	1.16	0.55	0.30	0.85	
Stomach									< 0.001
d 0	- - - - - - - - - - - - - - - - 24.32 - - - - - - - - - - - - -								
d 3	22.6	22.7	25.3	21.8	1.51	0.56	0.27	0.28	
d 7	32.8	33.6	31.2	33.4	2.78	0.75	0.60	0.79	
d 14	66.9	71.9	58.4	59.9	4.46	0.05	0.48	0.70	
Small Intestine									0.013
d 0	- - - - - - - - - - - - - - - 155.49 - - - - - - - - - - - - -								
d 3	122.4	113.8	138.9	125.6	9.8	0.18	0.29	0.82	
d 7	211.1	180.0	166.8	176.6	16.6	0.18	0.53	0.24	
d 14	346.6	404.1	334.4	338.7	21.1	0.09	0.17	0.23	
Stomach+ Small intestine									0.001
d 0	- - - - - - - - - - - - - - - 192.62 - - - - - - - - - - - - -								
d 3	116.0	113.1	149.8	119.8	13.2	0.15	0.23	0.34	
d 7	243.0	210.0	184.7	212.5	21.3	0.22	0.91	0.19	
d 14	427.4	490.1	391.3	409.6	28.2	0.07	0.18	0.45	

[a]Least squares means for 5 pigs per treatment per day.

Figure 5.3. The weight of the of the pancreas (g) in relation to the body weight (kg) of weanling pigs, Exp 3.

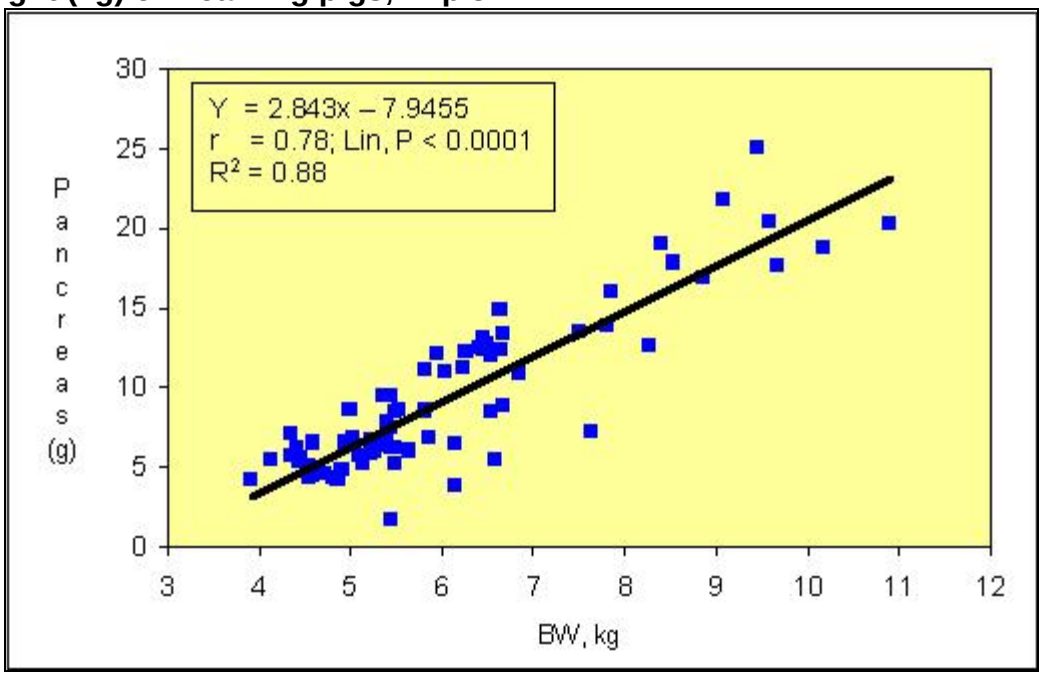


Figure 5.4. The weight of the of the stomach (g) in relation to the body weight (kg) of weanling pigs, Exp 3.

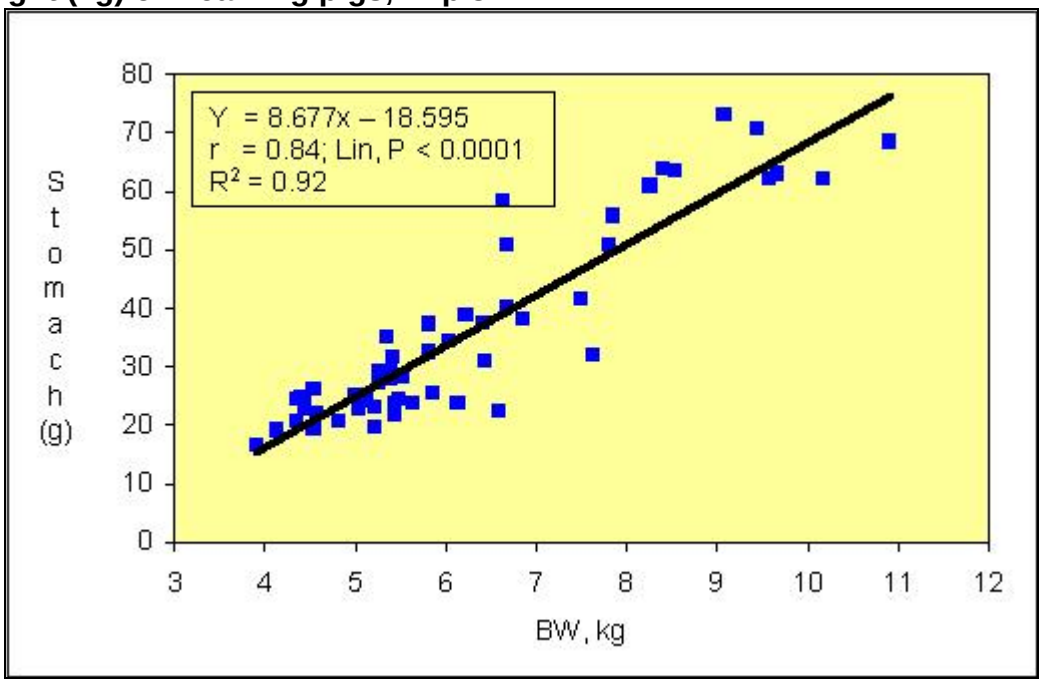
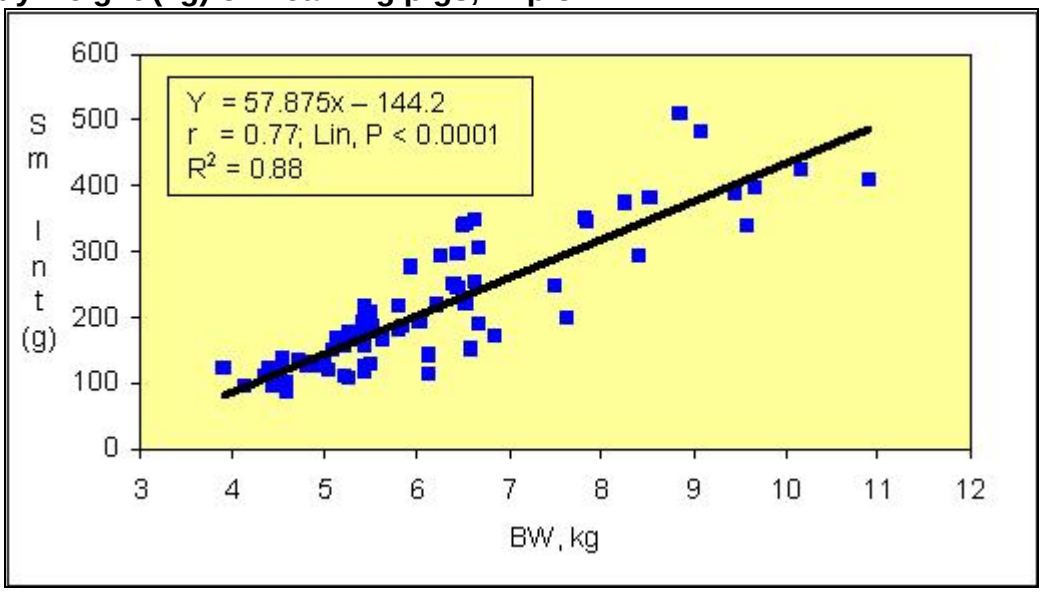


Figure 5.5. The weight of the of the small intestine (g) in relation to the body weight (kg) of weanling pigs, Exp 3.



The significant increase in organ weights post-weaning is consistent with other published data. After weaning, the growth rate and weight of the pancreas, stomach, and small intestine in relation to body weight is much greater especially the small intestine (Cranwell and Moughan, 1989). Regardless of weaning age, the relative weight of the small intestine increases by 25% from three to seven days post-weaning, and up to 52% at 10 to 14 days while the relative weight of the pancreas shows a corresponding 17% and 30% increase, respectively (Le Dividich and Seve, 2000).

The average increase in weight of the pancreas was greater from d 3 to 7 compared to the increase from d 8 to 14 (Table 5.1, 5.2). On the other hand, the stomach and small intestine had a much greater average increase from d 8 to 14 compared to the increase from d 3 to 7. There was no decrease in pancreas and

stomach weight from d 0 to 3 even though most of the pigs lost weight, with an average weight loss of –31.06 g. However, there was a 32% decrease in the relative weight of the small intestine on d 3 from the pre-weaning relative weight.

Cera et al. (1990a) reported a decline in pancreas weight at 3 d post-weaning (weaned at 21 d), but there was a consequent linear increase thereafter such that at 35 d, weaned pigs had greater relative pancreas weight compared to their suckling counterparts. In this experiment, there was no decrease observed in the weight of the pancreas during the first 3 d post-weaning, but there was a linear increase from 3 to 14 d post-weaning (Figure 5.3) which is consistent with the results of Cera et al. (1990a). Hartman et al. (1961) also reported that pancreas weight was greater from the second week onward in pigs weaned at 7 days of age compared to suckling pigs the same age.

The pancreas of piglets undergoes a very rapid growth and development during the 1st wk after birth (Corring et al., 1978) probably due to high amounts of growth factors present in sow milk and the high levels of glucocorticoids in the plasma of newborn (Mubiru and Xu, 1998). Corring et al. (1978) have reported a second phase of rapid pancreatic tissue growth between the 4th and 8th wk after birth, coinciding with the intake of creep feed by unweaned piglets. Cera et al. (1990a) reported a linear increase in pancreas weights from d 2 to 35 in suckling pigs. Lindemann et al. (1986) reported a linear increase in body weight and pancreas weight from birth to 6 wk with a markedly greater slope after weaning at 4 wk. During the first 4 wk after birth, growth of the pancreas was due to the increase in number (hyperplasia) of pancreatic cells but after the 4th wk, growth

was due to both hyperplasia and hypertrophy (increase in size) of pancreatic cells (Corring et al., 1978).

Marion et al. (2002) weaned pigs at 7 d and reported a dramatic decrease in small intestine and mucosa weights immediately post-weaning. By 14 d of age (wk post-weaning), the pigs had recovered from the growth check and had similar small intestine and mucosa weights as the 21 d old suckling pigs. They noted that although the mucosa of the intestine was greatly reduced at weaning, the consequent absolute weight of the small intestine and mucosa largely exceeded that in body weight. Sève et al. (1986) have reported that at weaning, the development of the digestive tract takes priority over the growth of other organs. This increase in gastrointestinal weight relative to body weight is attributed to the change in diet because growth is higher in pigs fed solid feed compared to those fed a liquid milk substitute (McCracken et al., 1995). The weaned pig apparently requires a larger digestive system (compared to the suckling pig counterpart) to properly digest and absorb the more complex post-weaning diet and the period needed to upgrade its digestive system is probably one of the limitations affecting post-weaning performance (Cranwell and Moughan, 1989).

Fat level did not affect ($P > 0.10$) the weights of any of the organs measured. There was a CP source x fat level interaction ($P < 0.04$) in pancreas weight on d 7 (Table 5.1, 5.2) and in the combined relative weight of stomach and small intestine on d 3 (Table 5.1) but these were transient and did not carry over to other d. Data were pooled to show the least square means for the

relative weights of the pancreas (Figure 5.6), stomach (Figure 5.7), small intestine (Figure 5.8), and combined weights of stomach and small intestine (Figure 5.9), as affected by CP source. The weights of the stomach (Figure 5.10) and small intestine (Figure 5.11) as affected by CP source were also pooled.

The results indicate that protein source had no effect on the growth of the pancreas (Figure 5.6, Table 5.2). However, other authors have reported that the growth of the pancreas is affected by the source of dietary protein in weaned pigs (Makkink et al., 1994; Peiniau et al., 1996). Pigs weaned at 28 d and fed diets containing fish meal had greater pancreas weight (g/kg live weight) at d 3 post-weaning, but it was then lower on the 6^{th} and 10^{th} d post-weaning compared to pigs fed diets containing soybean meal (Makkink et al., 1994). Peiniau et al. (1996) reported that 35 d after weaning, the fresh weight and protein content of the pancreas was significantly lower in pigs fed diets containing soluble fish protein concentrate compared to those fed diets containing casein, soybean meal, or soyabean concentrate. The presence of anti-nutritional factors in soyabean meal has beed reported to cause pancreatic hypertrophy. However, the refined soybean proteins in SPC contain much less anti-nutritional factors compared to soybean meal.

On d 7, the average relative weight of the small intestine was lower ($P < 0.04$) in pigs fed SDPP compared to pigs fed SPC, and this tended ($P < 0.06$) to remain lower on d 14 (Figure 5.8). The mean weight of the small intestine (Figure 5.11) was numerically higher ($P > 0.10$) in pigs fed SDPP while the combined weights of the stomach and small intestine (Table 5.2) tended to be

lower ($P < 0.09$) in pigs fed SDPP on d 14. The mean weight of the stomach (Figure 5.7, 5.10) and combined relative weight of the stomach and small intestine (Figure 5.9) were also lower ($P < 0.04$) in pigs fed SDPP on d 14.

The lower weight of the stomach of pigs fed SDPP is not in agreement with the results reported by Jiang et al. (2000b) where SDPP had no effect on the mass of the stomach. Torrallardona et al. (2003) reported that the weight (kg) of the small intestine of pigs fed SDPP tended to be greater compared to pigs fed a diet with no SDPP. However, Jiang et al. (2000b) reported a significantly lower relative small intestinal mass (g/kg BW) in pigs fed diets with SDPP compared to those fed diets with extruded soy protein by day 16 post-weaning. The pigs fed SDPP had lower jejunal protein masses, lower jejunal and ileal DNA masses, and lower intravillous lamina propria cell density with decreased numbers of macrophages (Jiang et al., 2000b). The pigs fed SDPP were either given feed ad libitum or fed an amount similar to the consumption of control pigs. Both groups had reduced intestinal growth so that they concluded that this response was a function of SDPP. They also noted that there were no differences in mucosal cell proliferation that can explain the reduction in intestinal protein and DNA masses they observed in SDPP pigs compared to pigs fed a control diet.

Figure 5.6. Relative weight of the pancreas (g/kg BW) in weaned pigs fed diets supplemented with SPC or SDPP, Exp 3.

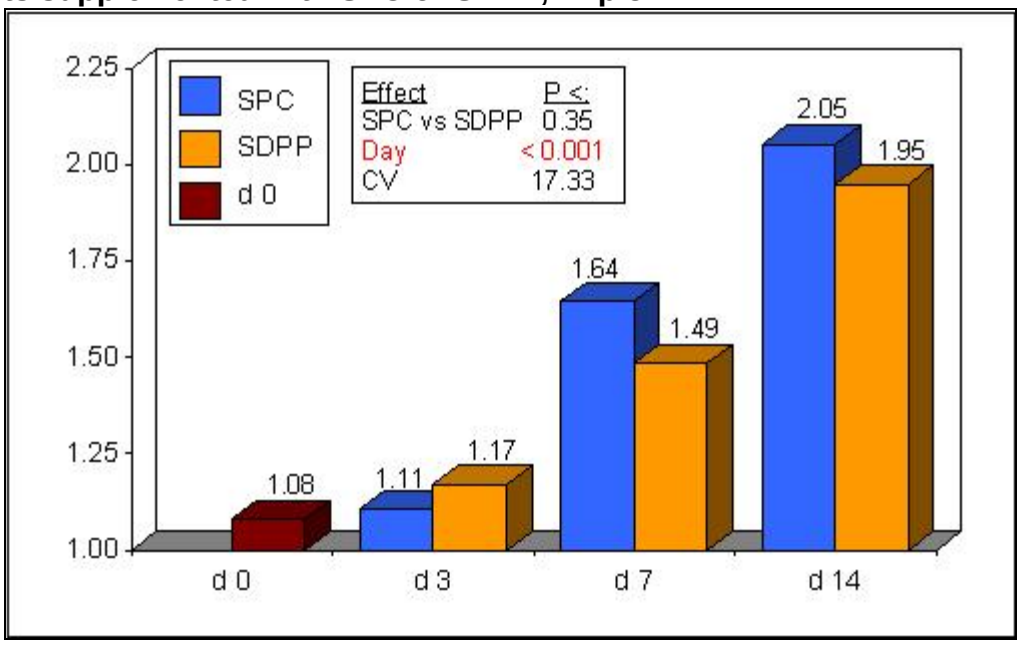


Figure 5.7. Relative weight of the stomach (g/kg BW) in weaned pigs fed diets supplemented with SPC or SDPP, Exp 3.

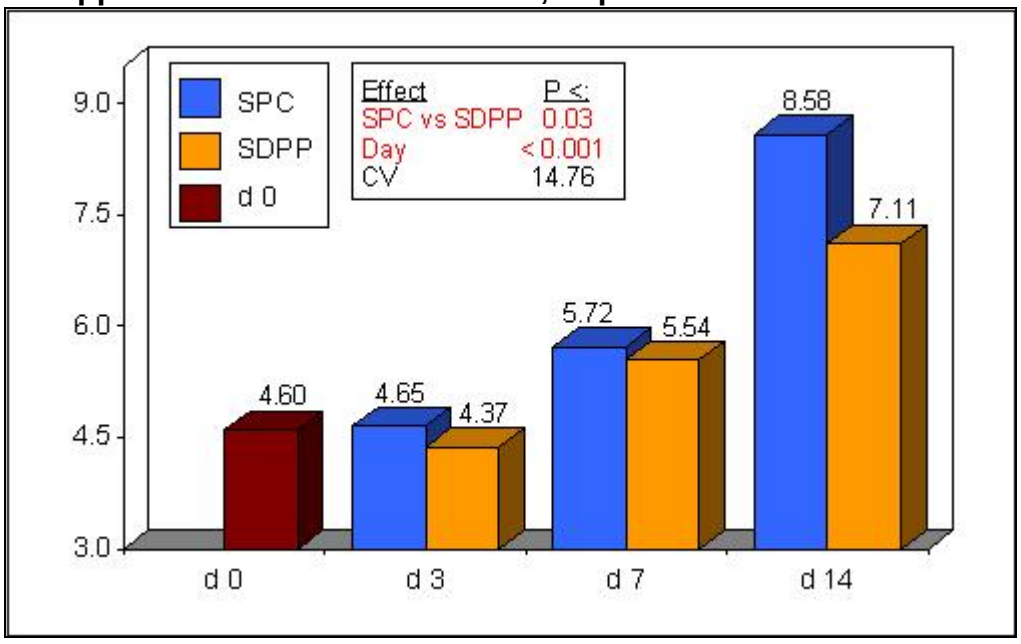


Figure 5.8. Relative weight of the small intestine (g/kg BW) in weaned pigs fed diets supplemented with SPC or SDPP, Exp 3.

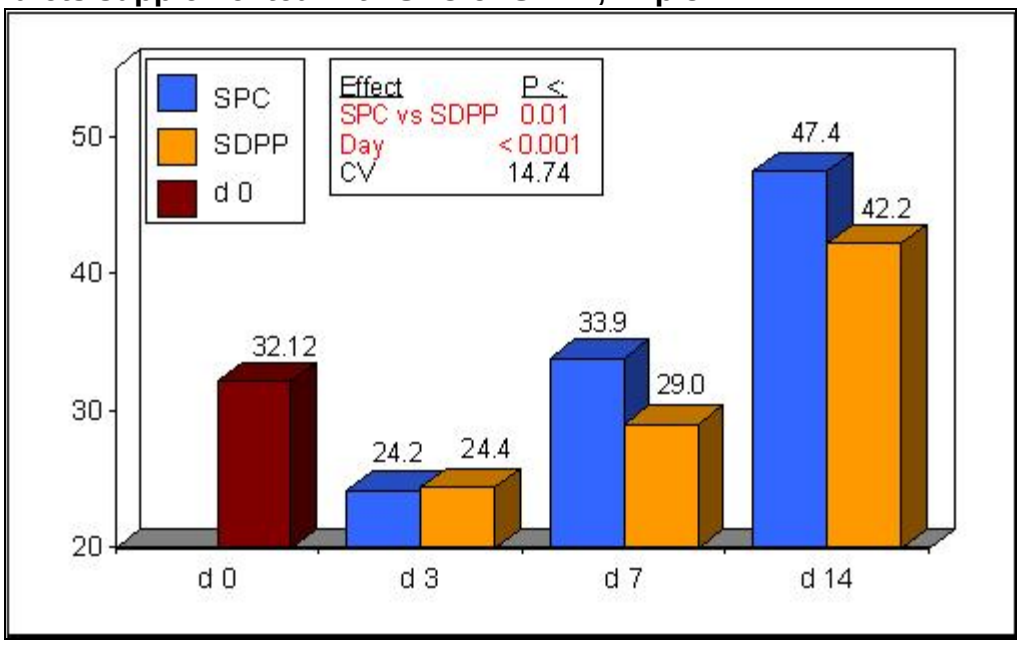


Figure 5.9. Relative combined weight of the stomach and small intestine (g/kg BW) in weaned pigs fed diets supplemented with SPC or SDPP, Exp 3.

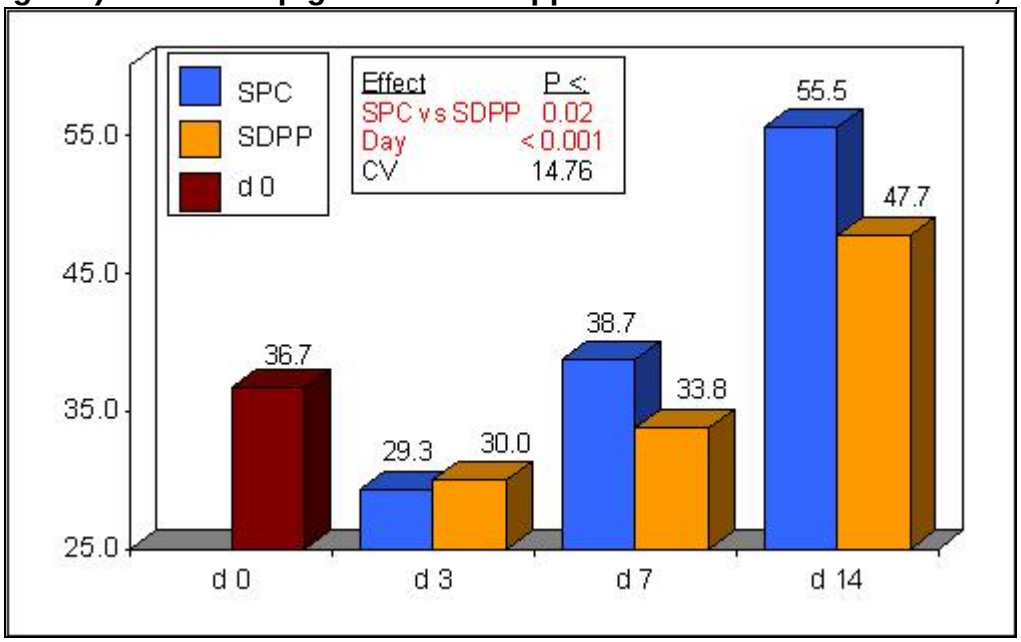


Figure 5.10. Weight of the stomach (g) in weaned pigs fed diets supplemented with SPC or SDPP, Exp 3.

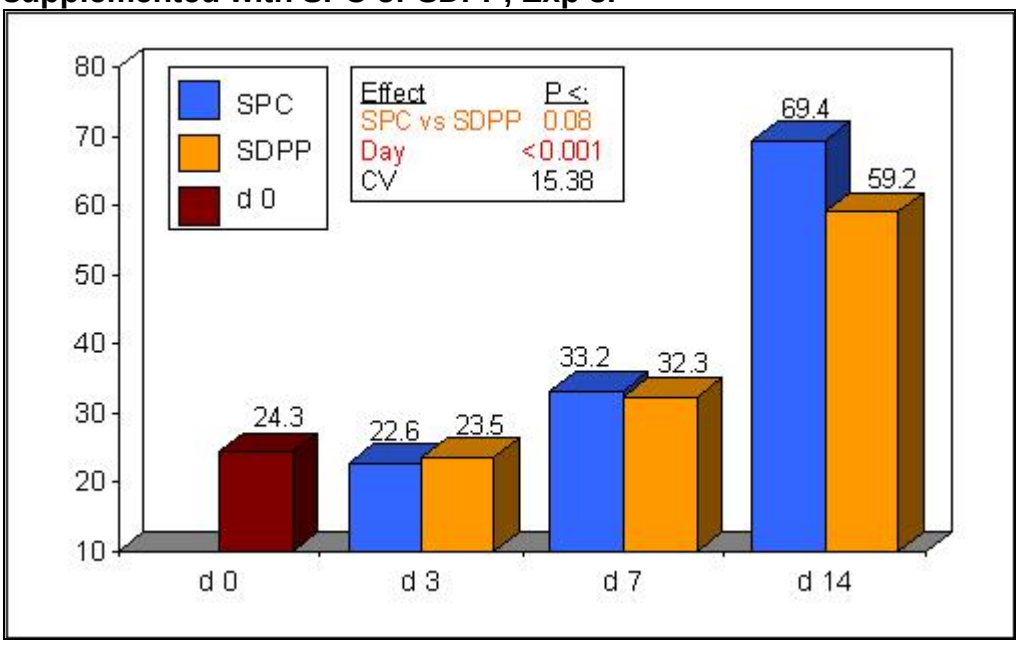
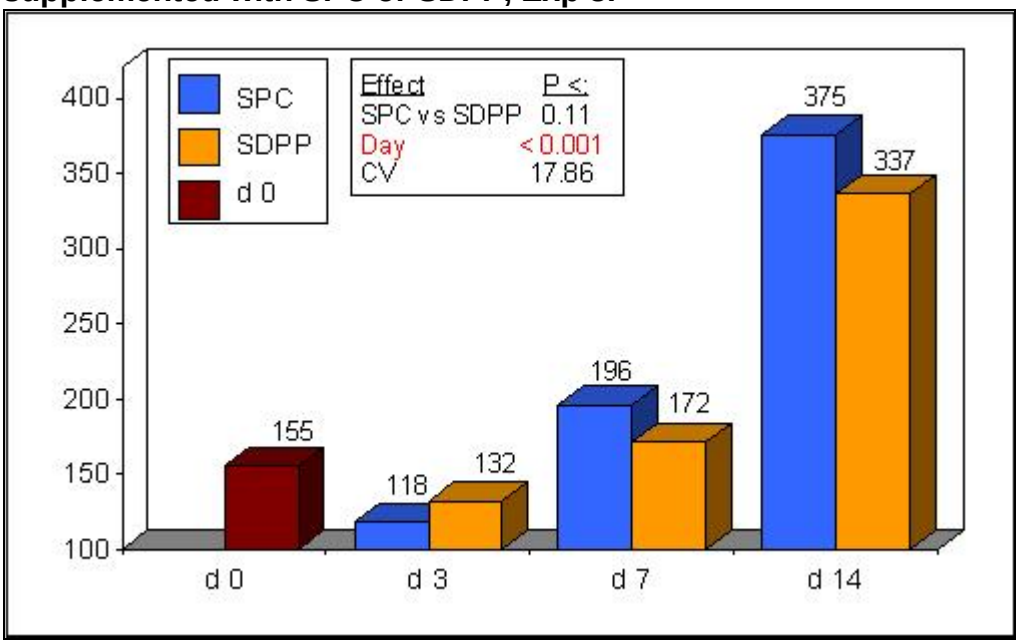


Figure 5.11. Weight of the small intestine (g) in weaned pigs fed diets supplemented with SPC or SDPP, Exp 3.



Effects on Morphology of the Small Intestine

Effect of weaning on morphology. The measurements of small intestinal morphology are presented in Table 5.3. There is a highly significant ($P < 0.001$) day effect such that villous height (V), crypt depth (C), and villous width are greater on d 14 compared to d 3. There was no day by treatment interactions ($P > 0.10$). Villous height and villous height:crypt depth (V:C) ratio decreased dramatically after weaning (Figure 5.12, Figure 5.14) while crypt depth and villous width increased (Figure 5.13, Figure 5.15) after weaning.

From d 0 to 3, villous height decreased by 16 to 50% (average for all pigs, 36%) and V:C ratio decreased by 45 to 80% (average 63%). After d 3, villous height started to increase and by d 14, villous height was similar to that observed on d 0. Crypt depth increased by 7 to 16% (average 13%) from d 0 to 3, increasing to 30% (average for all pigs) from d 0 to 7, and 36% from d 0 to 14. Because of the drastic decrease of villous height and accompanying increase in crypt depth, the V:C ratio decreased by up to 140% from d 0 to 7.

Previous studies have described dramatic changes in the small intestinal mucosa taking place at weaning. These include drastic reductions of the villous height (30 to 63% reduction) and increase in crypt depth by 76 to 180% (Hampson, 1986; Miller et al., 1986; Cranwell and Moughan, 1989; Kelly et al., 1991a; van Beers-Schreurs et al., 1998; Hedemann et al., 2003). These changes are also observed in this experiment except the increase in crypt depth is lower than previously reported. Marion et al. (2002) reported marginal

weaning effect on crypt depth. They cited that the reduced energy intake restricted crypt cell proliferation. Hedemann et al. (2003) have reported increased mitotic counts in the crypts with increased proliferative activity from the 3rd d post-weaning.

Van Beers-Schreurs et al. (1998) attempted to determine whether villous atrophy was caused by separation of the pigs from the sow or by changes in the quantity and composition of the diet. They reported that the degree of villous atrophy was more closely associated with the level of feed intake rather than the composition of the diet (sow milk vs commercial weanling diet) and that villous atrophy was partly caused by separation from the sow. Earlier, Kelly et al. (1991a) reported that villous atrophy was not prevented by relatively high nutrient supply to the gut.

Dietary composition affects small intestinal morphology. Although digestible carbohydrate composition (lactose vs. glucose vs. starch) did not prevent villous atrophy and an increase in crypt depth at weaning (Vente-Spreeuwenberg et al., 2003), the increase in villous height was greater in pigs fed diets with lactose between d 3 to 10, (Vente-Spreeuwenberg et al., 2003). Pluske et al. (1996c) reported that feeding whole cow's milk to weaned pigs for 5 d maintained villous height and crypt depth.

Pluske et al. (1997) performed an extensive review of factors that influence the intestinal structure and function in the newly-weaned pig and listed five major factors that contribute to the changes in gut structure and function at

weaning including: 1) the presence of pathogenic bacteria, 2) maladaptation to weaning stressors, 3) withdrawal of sow's milk that contains high levels of epidermal growth factor, polyamines, insulin, insulin-like growth factors, and glutamine, 4) dietary change (causing decreased feed intake and possible exposure to anti-nutritional factors), and 5) cytokines and the role they play in epithelial cell growth. There is a great interplay of these factors and aside from level of feed intake, the health status of the gut also plays a major role in maintenance of mucosal structure.

Recently, Mei and Xu (2005) reported a significant decrease in transforming growth factor-β1 (TGF-β1) at the apical membrane of the intestinal villi epithelium on d 4 post-weaning; this was transient, returning to the pre-weaning level by the 8^{th} d. This decrease in TGF-β1 was associated with atrophy of the villi and a marked reduction in digestive enzyme activities (Mei and Xu, 2005). Thus, there are many varied influences on the small intestinal epithelium that can have an effect on its morphology. Many of these influences are altered at weaning, thus, the drastic changes in small intestinal morphology.

Table 5.3. Morphology of the small intestine of weanling pigs[a], Exp 3.

Protein source	SPC	SPC	SDPP	SDPP		Probability, P <:			
ME concentration, kcal/kg	3,523	3,323	3,523	3,323	SE	SPC vs SDPP	Fat Level	CP*ME	Day
Fat inclusion, %	5.0	0.47	5.74	1.21					
Villous height (V), mm									< 0.001
d 0	- - - - - - - - - - - - - - - - 0.450 - - - - - - - - - - - - -								
d 3	0.312	0.302	0.388	0.330	0.03	0.09	0.25	0.41	
d 7	0.314	0.281	0.357	0.299	0.03	0.33	0.15	0.68	
d 14	0.375	0.404	0.464	0.645	0.03	0.02	0.61	0.63	
Crypt depth, (C) mm									< 0.001
d 0	- - - - - - - - - - - - - - - - 0.142 - - - - - - - - - - - - -								
d 3	0.170	0.162	0.167	0.153	0.01	0.28	0.06	0.001	
d 7	0.227	0.210	0.198	0.181	0.01	0.04	0.20	0.99	
d 14	0.252	0.283	0.226	0.256	0.02	0.13	0.09	0.98	
V:C ratio									0.013
d 0	- - - - - - - - - - - - - - - - 3.425 - - - - - - - - - - - - -								
d 3	1.957	1.910	2.371	2.231	0.21	0.11	0.67	0.83	
d 7	1.408	1.398	1.922	1.724	0.13	0.01	0.44	0.49	
d 14	1.530	1.485	2.300	1.948	0.21	0.01	0.36	0.48	
Villous width, mm									0.001
d 0	- - - - - - - - - - - - - - - -- 0.098 - - - - - - - - - - - - -								
d 3	0.107	0.102	0.098	0.097	0.004	0.16	0.53	0.68	
d 7	0.116	0.105	0.102	0.098	0.002	0.003	0.01	0.23	
d 14	0.119	0.110	0.110	0.114	0.005	0.61	0.61	0.27	

[a]Least squares means for 5 pigs per treatment per day.

Effect of CP source and fat level on morphology. There was a significant CP x fat level interaction ($P < 0.001$) for d 3 crypt depth, but this was not carried over to d 7 or d 14. There were no other CP x fat level interactions ($P > 0.10$). In general, fat level did not affect small intestinal morphology ($P > 0.10$). However, when data were analyzed by day, villous width was reduced ($P < 0.01$) when fat was removed from the diet (d 7). Crypt depth tended to decrease ($P < 0.06$) in pigs fed diets with lower fat, then tending to increase ($P < 0.09$) on d 14.

Fat supplementation has been demonstrated to decrease jejunal villi and reduce absorptive area (Cera et al., 1988b). Similarly, villous height was numerically lower ($P > 0.10$) in pigs fed diets with lower fat inclusion in this experiment. Li et al. (1990) also demonstrated shorter and more slender villi in pigs fed diets supplemented with coconut or soybean oil alone. Li et al. (1990) supplemented weanling pig diets with soybean oil, coconut oil, or a combination (50:50) of the two and reported that pigs fed the 50% soybean oil:50% coconut oil combination had longer villi that coincided with greater net ileal disappearance of unsaturated fatty acids compared to those supplemented with soybean oil or coconut oil alone. Pigs fed this combination also had better growth performance compared to pigs fed diets with coconut oil alone (Li et al., 1990).

While fat level had inconsistent effects on intestinal morphology, the source of protein had a marked effect. Pigs fed diets containing SDPP had greater ($P < 0.01$) villous height, lower ($P < 0.005$) crypt depth, increased V:C ratio ($P < 0.001$), and lower ($P < 0.01$) villous width. Data were pooled to show the least squares means for villous height (Figure 5.12), crypt depth (Figure

5.13), V:C ratio (Figure 5.14), and villous width (Figure 5.15) in weanling pigs fed diets supplemented with either SDPP or SPC. Villous height and V:C were higher ($P < 0.02$) in pigs fed SDPP and on d 14, villous height was the same as pre-weaning values in pigs fed SDPP (Figure 5.12, Figure 5.14). On d 7, crypt depth was lower ($P < 0.04$) (Figure 5.13) and villous width was lower ($P < 0.003$) (Figure 5.15) in pigs fed SDPP. However, pigs fed SDPP had a consistent numerically higher villous height and V:C, and numerically lower crypt depth and villous width on all d compared to pigs fed SPC.

Jiang et al. (2000b) and Torrallardona et al. (2003) reported that inclusion of SDPP in weanling pigs diet tended to increase villous height. Jiang et al. (2000b) did not observe effects on crypt depth or cell proliferation index in pigs fed diets with SDPP compared to those fed diets with extruded soy protein by d 16 post-weaning. Van Dijk et al. (2001b, 2002c) used SDPP vs. casein and reported no significant effect on villous length, but observed less mitotic activity in pigs fed SDPP on d 4 and 7 post-weaning. Compared to casein, SDPP had no effect on crypt depth, enterocyte mitotic activity, and intestinal brushborder disaccharidases in piglets kept under low infection pressure (Van Dijk et al., 2002c). However, they noted that the pigs in their experiment were raised in cleaner environments with low infection pressure. The effects of SDPP are minimal on pigs raised under cleaner environments. In addition, Marion et al. (2002) have cited that pigs weaned to a cleaner environment had shorter crypts compared to pigs weaned to a conventional environment suggesting that pathogen exposure may play a role on epithelial cell renewal.

Figure 5.12. Small intestinal villous height (mm) in weaned pigs fed diets supplemented with SPC or SDPP, Exp 3.

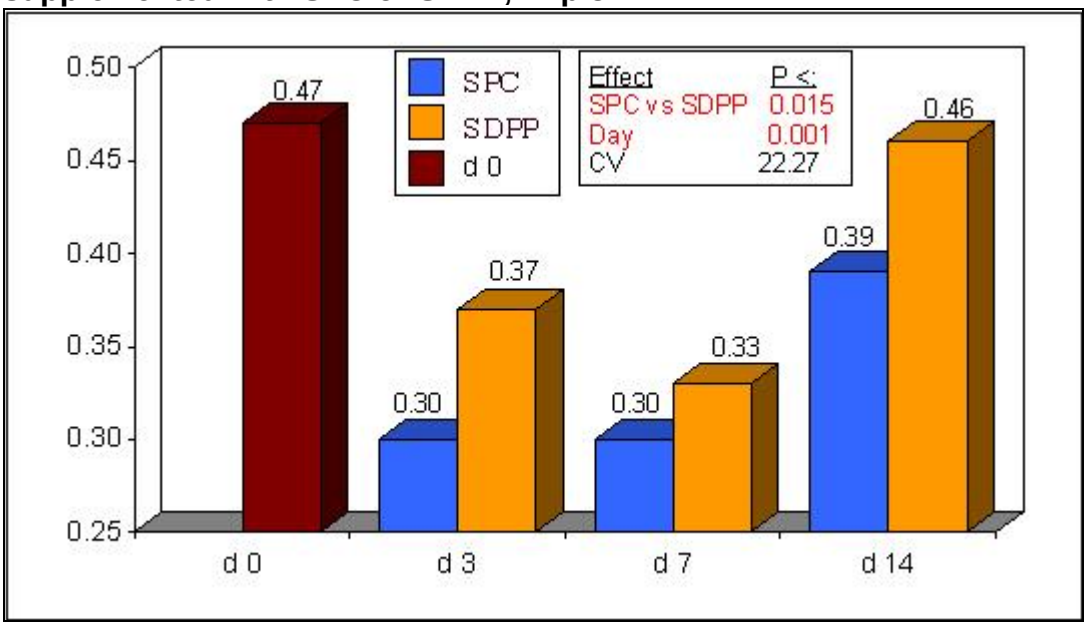


Figure 5.13. Crypt depth (mm) of the small intestine in weaned pigs fed diets supplemented with SPC or SDPP, Exp 3.

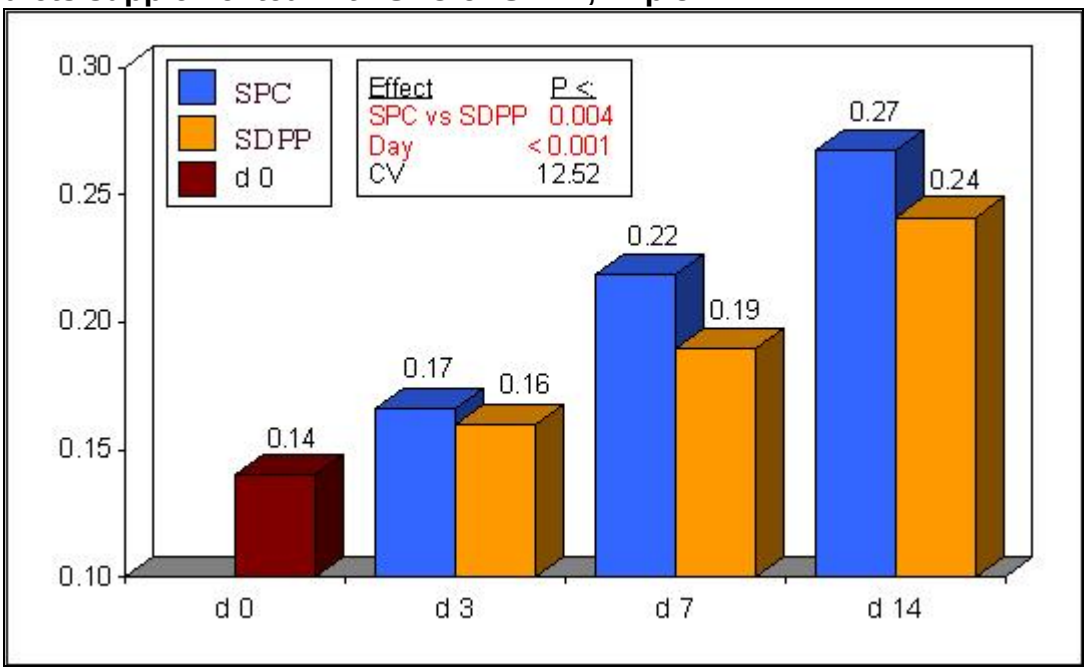


Figure 5.14. The small intestinal villous length:crypt depth ratio in weaned pigs fed diets supplemented with SPC or SDPP, Exp 3.

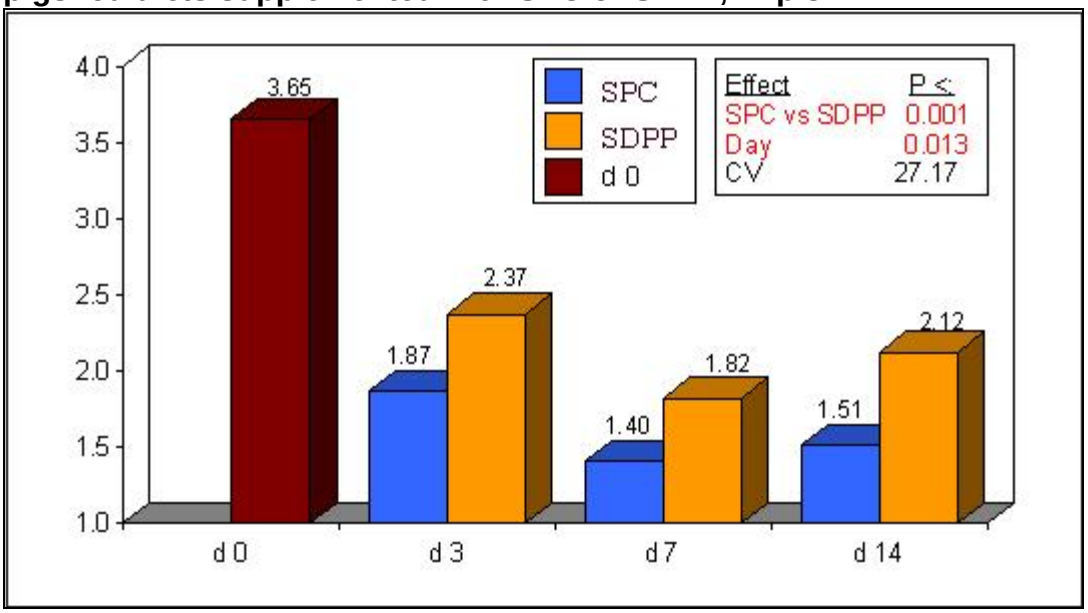
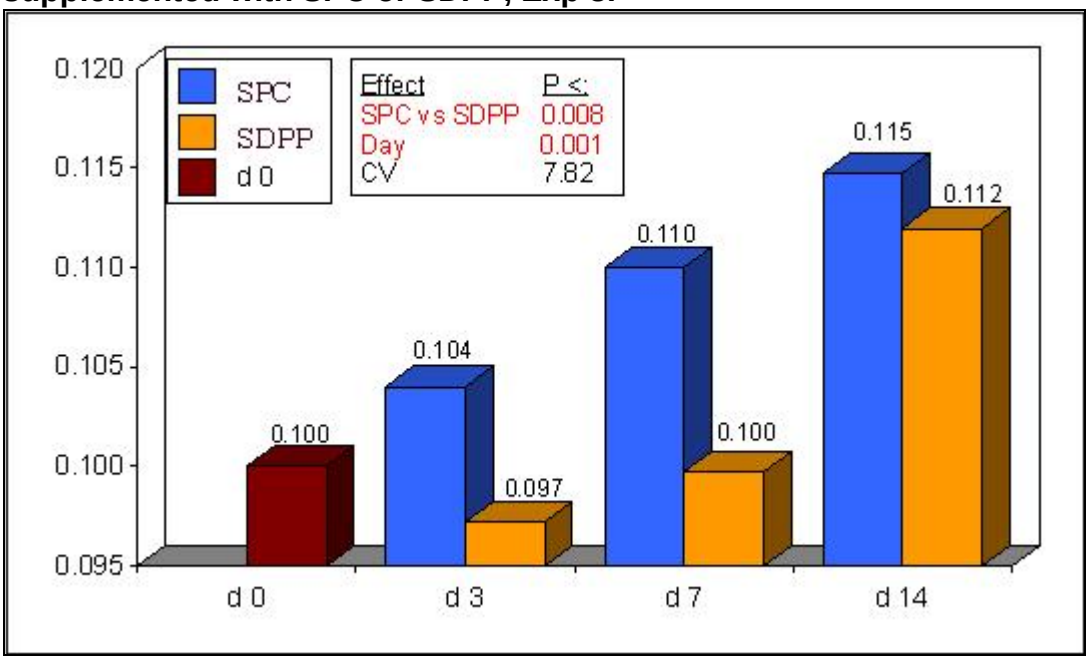


Figure 5.15. Small intestinal villous width (mm) in weaned pigs fed diets supplemented with SPC or SDPP, Exp 3.



Implications

The growth of the pancreas, stomach, and SI were highly correlated with BW and the weights of these organs were associated with increasing BW and age. However, CP source affected organ growth such that the relative weight of the stomach and small intestine was lower in pigs fed SDPP compared to pigs fed SPC. In addition, villi were longer, villous height:crypt depth ratio was greater, and crypt depth and villous width were lower in pigs fed SDPP. These results suggest that CP source (SPC vs SDPP), but not fat level, can dramatically affect the growth of the stomach and small intestine, as well as the morphology of the small intestine. Fat inclusion had no significant effect on small intestinal morphology although fat supplementation has been demonstrated to decrease jejunal villi and reduce absorptive area. Further investigation is needed to elucidate the mechanism behind greater organ growth in pigs fed SPC and improved small intestinal morphology in pigs fed SDPP.

CHAPTER VI

EXPERIMENT 3

EFFECTS OF PROTEIN SOURCE AND FAT LEVEL ON IMMUNOGLOBULINS IN SERUM AND CHYME, AND PANCREATIC ENZYME ACTIVITY AND GENE EXPRESSION IN EARLY-WEANED PIGS

ABSTRACT: As part of a continuing study to examine the effects of CP source on ME (fat) utilization in early-weaned pigs, the effects on pancreatic enzyme activity and gene expression were determined. A total of 80 pigs (avg 5.2 kg BW, 18 d) were allotted to four dietary treatments (4 pigs/trt) in a 2 x 2 factorial, with two CP sources (soy protein concentrate, SPC vs. spray-dried porcine plasma, SDPP) and two ME levels (3,323 vs. 3,523 kcal/kg). Spray-dried pocine plasma replaced SPC on a digestible Lys basis and ME level was adjusted using soybean oil. All diets had equal amounts of corn, soybean meal, dried whey, lactose, and fishmeal. One pig/trt was euthanized on d 0, 3, 7, and 14. The pancreas and small intestine were excised and intestinal contents (chyme) collected. RNA was extracted from the pancreas and enzyme gene expression was quantified with real-time PCR. Enzymatic activity of the pancreas and chyme, and immunoglobulin G (IgG) in chyme were quantified. Chyme IgG was higher ($P < 0.03$) in pigs fed SDPP vs. those fed SPC on d 3, 7, and 14

Amylase activity in pancreas and chyme and gene expression of amylase increased ($P < 0.001$) from d 3 to 14, and gene expression was higher ($P < 0.005$) in pigs fed SDPP. Source of protein had no effect ($P > 0.01$) on lipase and trypsinogen gene expression, but these were numerically higher in pigs fed SDPP. Lipase activity in pancreas and chyme decreased numerically from d 0 to 14, and activity in chyme was higher ($P < 0.02$) in pigs fed SDPP on d 7 and tended to be greater ($P < 0.06$) on d 14. Increasing the fat level in the diet did not affect pancreatic enzyme activity and gene expression. The increase in pancreatic enzyme activity and gene expression may be another mechanism by which SDPP improves growth performance of early-weaned pigs.

Key words: pigs, protein, pancreatic enzymes

Introduction

The secretion of enzymes and hormones by the pancreas is affected by changes in the diet. Weaning is characterized by a drastic change in diet; therefore, we expect major changes in pancreatic function in the immediate post-weaning period. Indeed, several authors have reported that weaning results in a general decrease in the activity of pancreatic enzymes (Hartman et al., 1961; Lindemann et al., 1986; Cera et al., 1990a; Marion, 2003), regardless of weaning age. Marion et al. (2003) reported that trypsin and lipase mRNA levels decreased 3 d after weaning and this decrease in gene expression was enhanced with low feed intake.

It has been reported that the IgG-rich fraction of SDPP is responsible for the improvements in the early-weaned pig growth performance (Owen et al., 1995; Pierce et al., 1995; Weaver et al., 1995; Pierce et al., 2005) regardless of origin (Pierce et al., 2005). Secreted immunoglobulins in the gut lumen serve as a first line of defense against antigens and pathogens (Fagarasan and Honjo, 2003). McClelland at al. (1972) demonstrated that secreted IgA agglutinated a wide range of organisms in the intestinal tract. Secretory IgA is a more efficient immunologic agent compared to serum IgG, but this might be due to the abundance of IgA, being the major secretory immunoglobulin in mucosal surfaces. Immunoglobulin G may be as effective if available in sufficient amounts (Blum et al., 1981).

Hypothesis and Objectives

Our previous results suggested that the source of dietary protein may affect energy (fat) utilization in the weanling pig. Dietary protein source and feed intake have been reported to affect pancreatic enzyme activity (Makkink et al., 1994) and gene expression (Marion et al., 2003). Pigs fed diets supplemented with SDPP have higher feed intake and both SDPP as a protein source and the associated increase in feed intake may play a role in pancreatic exocrine function. Thus, this experiment was performed to determine the effects of protein source (SPC vs. SDPP) and fat level (3,523 vs. 3,323 kcal/kg) of diets on pancreatic enzyme activity and gene expression of weanling pigs. Also, IgG is a protein and can be digested by proteases in the intestinal lumen. But because

the IgG-rich fraction of SDPP is responsible for the improvements in the growth performance of early-weaned pigs (Owen et al., 1995; Pierce et al., 1995; Weaver et al., 1995; Pierce et al., 2005), the levels of IgA and IgG in serum and small intestinal chyme were quantified to determine the immunoglobulin profile especially along the intestinal sections.

Materials and Methods

The effects of CP source and ME level on the levels of immunoglobulins A and G in serum and chyme and on pancreatic enzyme gene expression and activity were investigated using a 2 x 2 factorial in a randomized complete block design.

Animals, Treatments, and Diets

A total of 80 crossbred pigs with an average body weight of 5.2 kg were weaned at approximately 18 d and allotted to four dietary treatments in a 2 x 2 factorial with two CP sources (SPC vs SDPP) and two ME levels (3,523 vs 3,323). Weight, sex, and breed were equalized across treatment groups. The composition of the diets is the same as that of Exp. 2 and shown in Table 4.1 which was as follows: 1) diet containing SPC with ME = 3,525 kcal/kg, 2) SPC diet with ME = 3,325 kcal/kg, 3) SDPP diet with ME = 3,525 kcal/kg, and 4) SDPP diet with ME = 3,325 kcal/kg. Substitutions were made on an equal lysine

basis. To lower the ME concentration, soybean oil was replaced by cornstarch and in part by corn grain. All diets were formulated to contain 1.35% digestible lysine, 0.90 % Ca, and 0.75% P. Feed and water were provided on an ad libitum basis. The pigs were housed in metabolic chambers (5 chambers/trt) with a common feeder and nipple waterer in an environmentally regulated room. The temperature of the room was maintained at 31 to 32°C throughout the experimental period. All procedures were approved by the OSU Institutional Animal Care and Use Committee (IACUC).

Sample Collection

Four pigs (1 pig/trt) were euthanized on d 0, 3, 7, and 14. Blood was collected from each pig via the vena cava using vacutainer tubes without anticoagulant. Collected blood was centrifuged and serum was frozen at –20^{o}C for IgG and IgA determination. After the abdominal cavity was exposed, the pancreas was located and carefully removed. After weighing, it was quickly sliced into pieces and approximately 200 mg of pancreas tissue were mixed with 2 mL of RNAwiz®, an RNA isolation reagent. This was thoroughly homogenized using a Tekmar® Tissumizer (Tekmar Co., USA), then the sample was transferred to microcentrifuge tubes, the lid secured, and frozen in liquid nitrogen. The remaining pieces of pancreas tissue were quickly wrapped separately in foil and frozen in liquid nitrogen. Samples in liquid nitrogen were then transferred to a –80^{o}C freezer and stored until assays were performed.

Next, the small intestine was separated from the mesentery after clamps were secured at the junction between the stomach and duodenum and at the ileo-cecal junction. Most of the intestinal content was liquid (fluid) so care was taken to keep the intestine level, because raising any section could cause backflow and mixing of contents. Once removed, the small intestine was washed to remove blood clots and mesenteric debris and then weighed. Then, the whole length was returned to the table and the small intestine was divided into three sections as shown in Figure 5.1. Two clamps were placed at the end of the first third and two more at the end of the second third and the three sections were separated by cutting between each pair of clamps. The sections were weighed before and after they were emptied. Each section was emptied by carefully squeezing out the contents into vials or cups (depending on the amount) and the weight and volume of chyme was recorded. The samples were centrifuged, supernatant transferred to microcentrifuge tubes, and frozen at -20^{o}C until pancreatic α-amylase and triglyceride lipase activity, and IgG and IgA content were determined.

Data Collection

Immunoglobulin assay. The levels of immunoglobulins (IgG and IgA) in serum and small intestinal chyme were quantified by sandwich Enzyme Linked ImmunoSorbent Assay (ELISA) method. This type of assay is called “sandwich’ because the sample antigen to be measured is bound between two antibodies, the capture antibody and the detection antibody. Pig IgG and Pig IgA ELISA

Quantitation kits (Bethyl Laboratories, Inc., USA) were used. The kits contained the specific coating antibody (goat anti-Pig IgG-affinity purified for IgG assay, goat anti-Pig IgA-affinity purified for IgA assay), a calibrator (pig reference serum), and the horseradish peroxidase (HRP) detection antibody (goat anti-Pig IgG-HRP conjugate for IgG assay, goat anti-Pig IgA- HRP conjugate for IgA assay). All steps were performed at room temperature; the reagents, buffers, and solutions were common for both IgG and IgA assays and were purchased from Bethyl Laboratories, Inc (USA).

An ELISA plate (96-well) was first coated with the capture antibody then the coated plate was incubated for 1 hr. The coating antibody was then aspirated and the plate washed three times using a wash solution (50mM Tris, 0.14 M NaCl, 0.05% Tween 20, pH 8.0). A blocking (postcoat) solution (50mM Tris, 0.14 M NaCl, 1% BSA, pH 8.0) was then added and the plate incubated for 30 min. After washing the wells three times, the standards or properly diluted samples were added to designated wells in duplicates and incubated for 1 hr. The standards and samples were then aspirated, the wells washed five times, then the HRP detection antibody was added to the wells and incubated for 1 hr. After five more washes, the TMB enzyme substrate (3,3',5,5'-tetramethyl-benzidine) was added to each well. After 10 min, the reaction was stopped by adding 2M H_2SO_4 to the wells in the same order as the enzyme substrate and the absorbencies in each well were determined at 450 nm. The standards were used to graph a standard curve from which, and along with the blanks, the concentrations of IgG or IgA were determined using the AD LD Analysis Software

1.6 (Beckman Coulter, Inc., USA). The results were expressed as mg/mL for serum IgG or μg/mL for serum IgA and intestinal chyme IgG and IgA.

Pancreatic lipase and α-amylase activity. Pancreatic amylase and pancreatic lipase activity in both pancreatic tissue and intestinal chyme were determined using the ACE® Chemistry Analyzer (Alfa Wassermann, Inc., USA).

CelLytic™ MT (Sigma-Aldrich, Inc., USA), a mammalian tissue lysis/extraction reagent, was used to extract cell proteins in pancreatic tissue samples A protease inhibitor cocktail (Sigma-Aldrich, Inc., USA) for mammalian cell and tissue extracts was added to the CelLytic™ MT reagent to inhibit the activity of endogenous enzymes (such as proteases and phosphatases) which are capable of degrading proteins in the extracts. Approximately 50 mg frozen pancreatic tissue was used per 1 mL CelLytic™ MT reagent and homogenized inside a cold room (4°C) using a tissue homogenizer. The homogenate was then centrifuged for 10 min at 14,000 x g to pellet the debris and supernatant was collected into microcentrifuge tubes and maintained in ice. Tissue samples were weighed and homogenized on the day they were analyzed for enzyme activity. Initial tests were performed on the lysates to determine the optimum dilutions using phosphate buffered saline. Intestinal chyme samples were thawed in ice on the day of assay and dilutions were also performed as needed.

ACE® Amylase reagent (Alfa Wassermann, Inc., USA) was used to determine α-amylase levels; α-amylase in the sample hydrolyzes the 2-chloro-p-

nitrophenyl-α-D-maltotrioside (CNPG3) in the reagent to release 2-chloro-p-nitrophenol (CNP) and form 2-chloro-p-nitrophenyl-α-D-maltoside (CNPG2), maltotriose (G3) and glucose. The rate of increase in absorbance is directly proportional to the α-amylase activity in the sample. Amylase activity in the sample is expressed in International Units per liter (U/L).

DCL Lipase (Diagnostic Chemicals Limited, USA) was used to assay lipase activity. Lipase acts on the substrate 1,2-diglyceride to free 2-monoglyceride which is hydrolyzed by monoglyceride lipase into glycerol and fatty acid. Glycerol is acted upon by glycerol kinase to form glycerol-3-phosphate which is oxidized to generate hydrogen peroxide. A quinone dye is produced by the reaction of hydrogen peroxide with 4-aminoantipyretine and sodium N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine and the rate of increase in absorbance at 550 n, is proportional to the lipase activity in the sample. Lipase activity in the sample is expressed in International Units per liter (U/L).

Pancreatic enzyme gene expression. The mRNA expression levels for the pancreatic enzymes lipase, amylase, and trypsinogen, were quantified with real-time polymerase chain reaction (real-time PCR) using the two-step method.

RNA isolation. Frozen homogenates of pancreas tissue in RNAwiz® (Ambion, Inc., USA) were thawed in ice just before RNA was extracted. RNA extraction was carried out according to the RNAwiz® manufacturer's protocol. Approximately 1mL homogenate in a 2 mL microcentrifuge tube was incubated

for 5 min at room temperature then 200 μL chloroform was added, and after vigorous shaking, incubated again for 10 min at room temperature. The mixture was then centrifuged at 14,000 x g for 15 min at 4^{o}C, separating the mixture into three phases: the colorless upper aqueous phase containing the RNA, the semi-solid interphase containing most of the DNA, and the lower organic phase. The tubes were then placed on ice and the clear aqeous phase was extracted very carefully (without disturbing the interphase or the organic phase) and transferred into clean RNase-free tubes. RNase-free water (500 μL) was added to each tube and mixed well. One mL of isopropanol was also added, mixed well, and incubated at room temperature for 10 min, then centrifuged at 14,000 x g for 15 min at 4^{o}C to pellet the RNA. The supernatant was decanted and the pellet washed with 1 mL cold 75% ethanol by vortexing, then centrifuged again at 14,000 x g for 15 mins at 4^{o}C. Finally, the supernatant was discarded and the pellet air-dried for approximately 10 min. Each RNA pellet was then resuspended in 150 μL RNase-free water and the total RNA concentration in each sample was determined using a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Inc., USA). Aliquots were collected and total RNA was diluted to 1 μg/μL. All samples was stored at –80^{o}C until RNA samples were run on a 1.25 % denaturation gel or used for cDNA synthesis.

Primer design and optimization. Forward and reverse primers for pancreatic triglyceride lipase (E.C. 3.1.1.3), amylase (E.C. 3.2.1.1), and trypsinogen (E.C. 3.4.21.4) were designed using ABI Primer Express®. Table 6.1 lists the primers and their sequences. Primers were purchased from Integrated

DNA Technologies (IDT), Inc. The primers were optimized with PCR reactions performed in a MJ Dyad® thermal cycler (MJ Research Inc., USA). Each 20 uL reaction mix contained 1 uL DNA, 1X PCR buffer (Applied Biosystems, Foster City, CA), 1.5 mM $MgCl_2$, 500 nM of each primer, 200 uM dNTP's, and 3 U Taq DNA Polymerase (Pomega, Madison, WI). Amplification conditions for trypsinogen were: one cycle for 1 min at 95°C, 20 sec at 94°C, 30 sec at 55°C, 30 sec at 72°C followed by 34 additional cycles of 20 sec at 94°C, 30 sec at 55°C, 30 sec at 72°C, after which the temperature was made constant at 4°C. Amplification conditions for lipase and amylase were the same except that annealing temperature was set to 60°C.

Table 6.1. Primers used for PCR amplification of pancreatic triglyceride lipase, α-amylase, and trypsinogen.

Primer	Accession No. (Source)	Sequence 5'-3'	Product length
α-Amylase	AF064742 (NCBI)		79
Forward		CGTTGGGCAAGAAATTTTGTG	
Reverse		CCTTAATGACCCCGTTATTATTTGG	
Triglyceride Lipase	BI345373 (NCBI)		113
Forward		GACTCTTACAATGTTTTCACTGCAAAT	
Reverse		TGGCTCACTCCGTTTGTCTTC	
Trypsinogen	TC101491 (TIGR)		100
Forward		GCGGGTCCCTCATCAGTGA	
Reverse		TCCAGGACGTCAATGTTGTTCTC	

cDNA synthesis. All cDNA were synthesized using the QuantiTect® Reverse Transcription Kit (Qiagen, Inc., USA). Template RNA was thawed in ice as well as the necessary kit components. The procedure involved two reaction components, the genomic DNA elimination, and the reverse-transcription reaction. To remove genomic DNA, 2 μL gDNA wipeout buffer (7x) was added to 1 μg template RNA, and RNase-free water was added for a total volume of 14 μL. The tubes were incubated for 2 min at 42°C, then placed immediately on ice, and 6 μL of the reverse-transcription master mix was added making a total volume of 20 μL. The reverse-transcription master mix contained 1 μL Quantiscript reverse transcriptase (which contained an RNase inhibitor), 4 μL Quantiscript RT buffer (5x), and 1 μL RT primer mix (containing Mg^{2+} and dNTPs). The tubes were incubated for 30 min at 42 °C, then finally for 3 min at 95 °C to inactivate the reverse transcriptase. All synthesized cDNAs were frozen at –20°C until real-time PCR was performed.

Gene quantitation using Real-time PCR. Real-time PCR was performed using QuantiTect® SYBR Green RT-PCR kit (Qiagen, Inc., USA), which included 2x QuantiTect® SYBR Green PCR master mix (the master mix contained HotStarTaq® DNA polymerase, QuantiTect® SYBR Green PCR buffer, dNTP mix including dUTP, SYBR Green I, ROX passive reference dye, and 5 mM $MgCl_2$) and RNase-free water.

For each gene, a 96-well PCR plate was used and duplicates were analyzed using a second plate. Each well contained 7.5 μL QuantiTect SYBR

Green PCR master mix, 500 nM primer for each target gene (i.e. triglyceride lipase, α-amylase, or trypsinogen), the appropriate amount of cDNA (for α-amylase and trypsinogen, 100 ng cDNA was used; 400 ng was used for lipase), and RNase-free water for a 15 μL total volume.

Real-time PCR was performed using a MyiQ® RealTime PCR Detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Figure 6.1 shows a representative PCR Amp/Cycle graph for a particular gene (e.g. pancreatic α–amylase) while Figure 6.2 shows the corresponsing melt curve. Figure 6.3 shows the gel electrophoresis picture of the real-time PCR products for the pancreatic enzymes triglyceride lipase, α–amylase, and trypsinogen.

Ribosomal 18S RNA was used as a housekeeping gene to normalize samples for any variation in cDNA loading. Quantification of pancreatic triglyceride lipase, α-amylase, and trypsinogen mRNA expression was performed using the comparative $\Delta\Delta C_T$ method. The ΔC_T was computed by subtracting the 18S C_T from the C_T value of the corresponding gene (lipase, amylase, or trypsinogen). Lipase, amylase, and trypsinogen $\Delta\Delta C_T$ was determined by subtracting the highest ΔC_T from all other ΔC_T values. Fold changes in mRNA expression of the target genes were calculated as $2^{-\Delta\Delta Ct}$.

Statistical Analysis

Data were analyzed as a 2 x 2 factorial over time and the main effects of CP source, fat level, and day, and their interactions were tested using PROC GLM (SAS, 2003). The pig served as experimental unit. Data showing heterogeneous variances (intestinal IgG and IgA levels, and enzyme activity) were analyzed after transformation to log (x+1) and mean squares and P values were used while least squares means from untransformed data were reported.

Figure 6.1. A representative PCR Amp/Cycle graph for a gene.

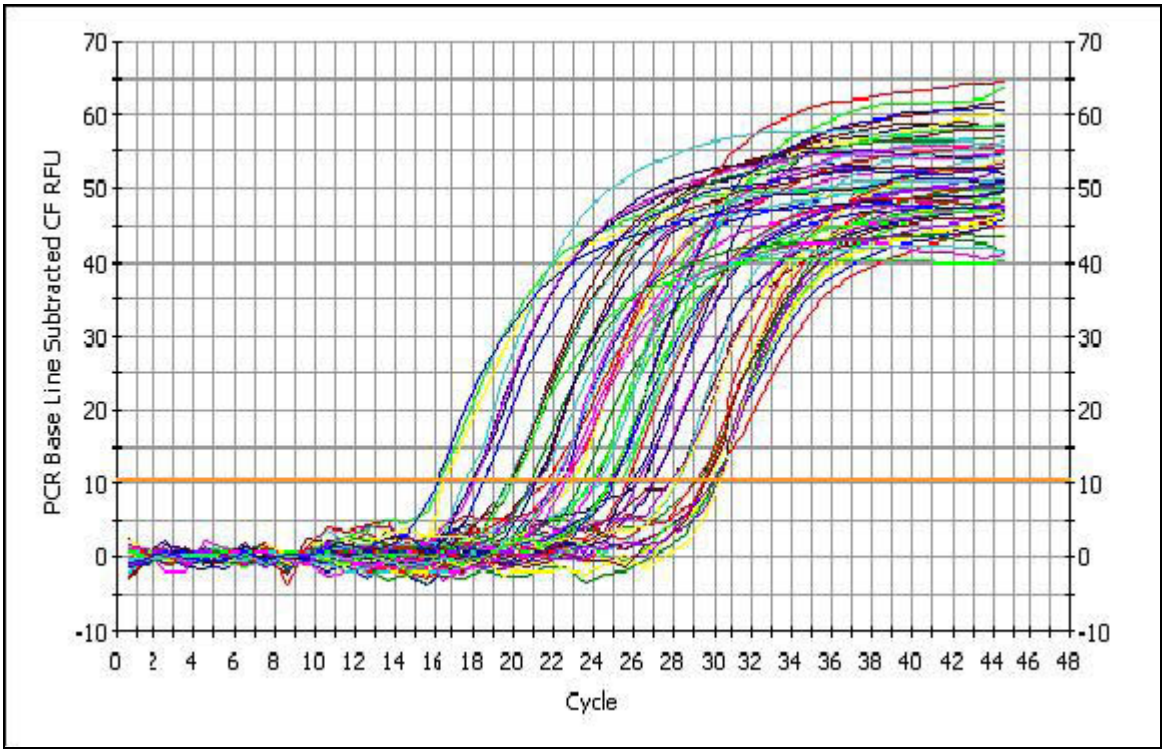


Figure 6.2. Melt curve graph for pancreatic α-amylase gene.

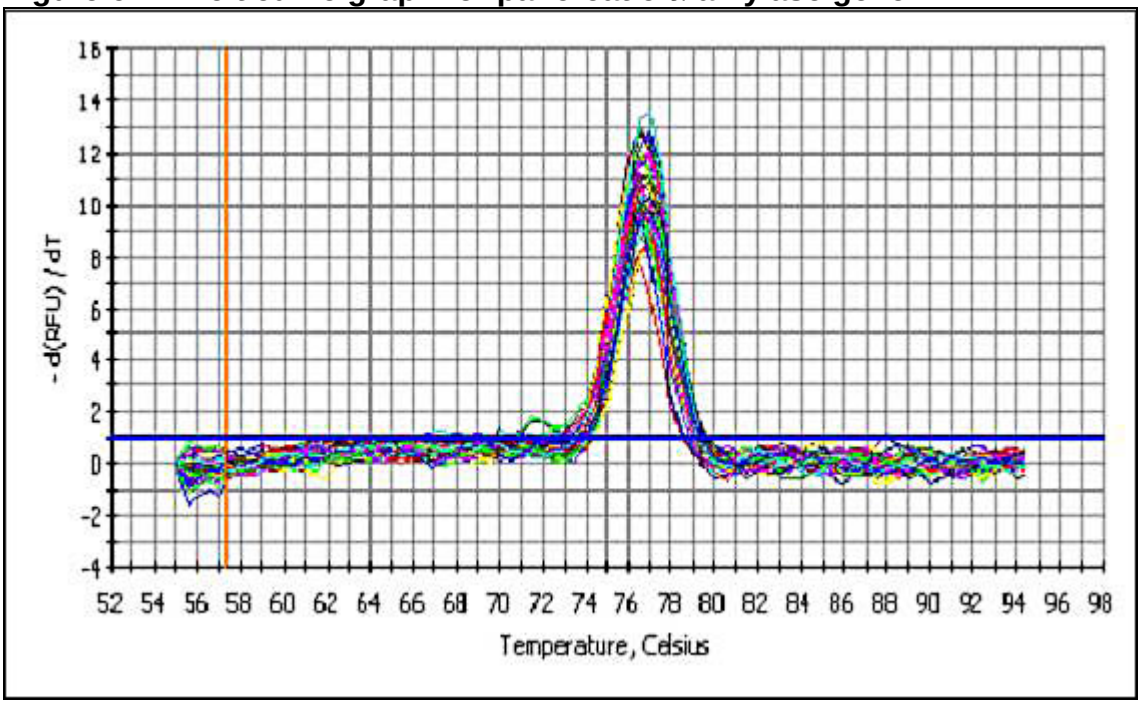
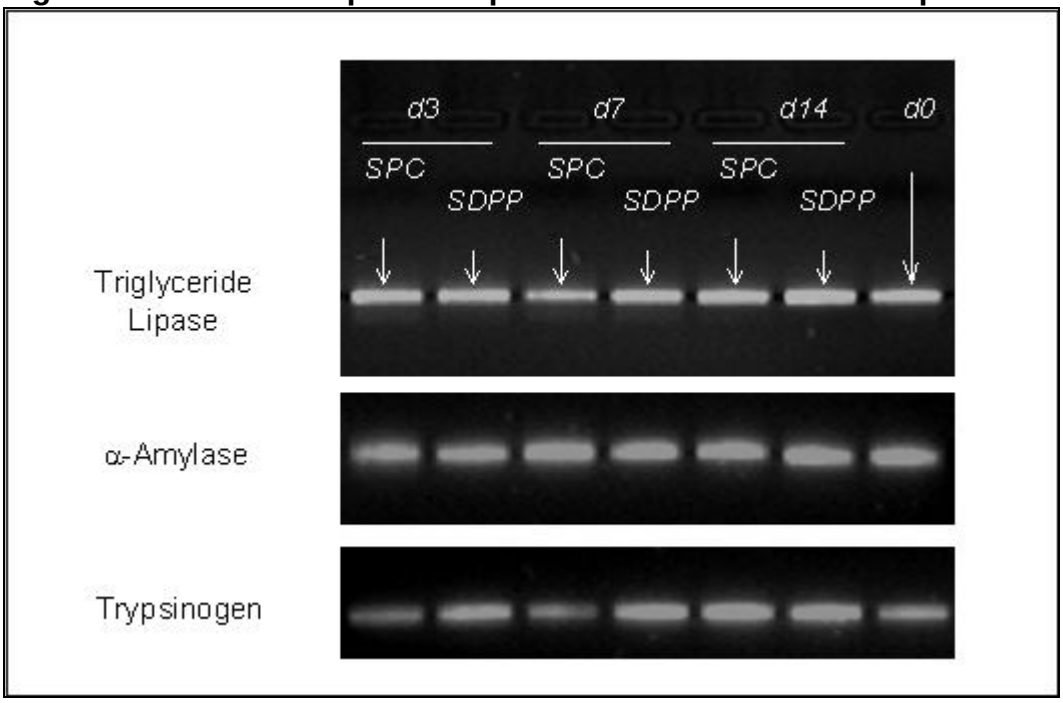


Figure 6.3. Gel electrophoresis picture of the real-time PCR products.



Results and Discussion

Immunoglobulins in Serum and Chyme

The levels of immunoglobulins in serum and intestinal chyme are summarized in Table 6.2 (IgG) and Table 6.3 (IgA). There was a decrease ($P < 0.01$) in serum IgG and an increase in serum IgA ($P < 0.0001$) from d 0 to 14.

Immunoglobulin G is the predominant immunoglobulin in the serum and in colostrum of sows (Bourne and Curtis, 1973) so that newborn pigs have high serum IgG. Curtis and Bourne (1971) quantified immunoglobulins in serum of young pigs from birth until 16 wk of age as well as in sow serum, colostrum, and milk. They reported that at 16 wk of age, the pigs had not attained the mature pig's serum immunoglobulin profile. They noted that IgG fell slowly after birth reaching a minimum of 6.23 mg/mL at 36 to 40 d of age, and which, when plotted on a logarithmic scale, is a straight line decrease in concentration between 2 and 28 d of age (Curtis and Bourne, 1971). Figure 6.4 shows the numerically decreasing IgG levels from d 0 to 14 when pigs were, on average, 18 to 32 days of age. Curtis and Bourne (1971) also noted that 24 hr after birth, there was a rapid fall in IgA concentration reaching 0.13 mg/mL between 17 and 22 d, then slowly rose to levels three-fifths of the adult serum by 16 wk (Curtis and Bourne, 1971). Figure 6.6 illustrates the same numerically decreasing trend for d 0 to 7 when pigs were approximately 18 to 25 d of age.

Table 6.2. IgG in serum (mg/mL) and chyme (μg/mL) of weanling pigs[a], Exp 3.

Protein source	SPC	SPC	SDPP	SDPP		Probability, P <:			
ME concentration, kcal/kg	3,523	3,323	3,523	3,323	SE	SPC vs SDPP	Fat Level	CP*ME	Day
Fat inclusion, %	5.0	0.47	5.74	1.21					
Serum IgG, mg/mL									0.006
d 0	- - - - - - - - - - - - - 11.1 - - - - - - - - - - - - -								
d 3	9.07	9.36	10.87	11.14	1.33	0.20	0.84	0.64	
d 7	8.54	8.17	7.72	9.86	2.10	0.84	0.68	0.56	
d 14	6.42	6.41	6.63	6.66	0.85	0.79	0.99	0.98	
Duodenum, μg/mL									0.139
d 0	- - - - - - - - - - - - - 309.9 - - - - - - - - - - - - -								
d 3	179.5	171.8	843.4	784.2	245.3	0.03	0.89	0.92	
d 7	120.6	77.9	647.8	580.8	269.9	0.08	0.84	0.97	
d 14	112.5	138.1	279.4	414.4	111.5	0.08	0.49	0.64	
Jejunum, μg/mL									0.088
d 0	- - - - - - - - - - - - - 258.6 - - - - - - - - - - - - -								
d 3	138.5	184.2	1,987.7	1,521.5	699.1	0.04	0.77	0.72	
d 7	100.8	20.6	716.1	1,394.5	416.5	0.03	0.52	0.17	
d 14	263.6	40.0	3,90.5	1,231.3	355.7	0.10	0.41	0.17	
Ileum, μg/mL									0.001
d 0	- - - - - - - - - - - - - 472.2 - - - - - - - - - - - - -								
d 3	160.9	103.9	4,832.4	3,867.1	1,158.8	0.01	0.67	0.70	
d 7	34.3	88.6	1,435.6	1,170.6	491.5	0.02	0.70	0.89	
d 14	355.5	29.5	284.6	982.1	466.7	0.37	0.70	0.30	
Intestinal average, μg/mL									0.033
d 0	- - - - - - - - - - - - - 358.0 - - - - - - - - - - -								
d 3	159.6	170.9	2,175.5	2,057.6	579.9	0.01	0.93	0.91	
d 7	84.8	99.3	933.1	1048.6	323.1	0.02	0.84	0.88	
d 14	50.0	70.7	346.4	1003.2	254.7	0.03	0.21	0.24	

[a]Least squares means for 5 pigs per treatment per day.

Table 6.3. IgA (μg/mL) in serum and chyme of weanling pigs[a], Exp 3.

Protein source	SPC	SPC	SDPP	SDPP		Probability, P <:			
ME concentration, kcal/kg	3,523	3,323	3,523	3,323	SE	SPC vs SDPP	Fat Level	CP*ME	Day
Fat inclusion, %	5.0	0.47	5.74	1.21					
Serum									< 0.001
d 0	- - - - - - - - - - - - 118.6 - - - - - - - - - -								
d 3	89.3	90.9	96.9	110.3	15.8	0.41	0.64	0.72	
d 7	86.3	95.3	90.1	85.5	12.8	0.82	0.86	0.61	
d 14	161.3	195.2	195.4	123.9	34.1	0.60	0.59	0.15	
Duodenum									0.056
d 0	- - - - - - - - - - - - 269.5 - - - - - - - - - -								
d 3	262.2	113.6	235.8	148.0	57.4	0.95	0.06	0.61	
d 7	101.0	177.2	193.0	193.5	49.3	0.30	0.45	0.46	
d 14	348.4	169.9	309.1	247.2	65.9	0.78	0.09	0.39	
Jejunum									0.331
d 0	- - - - - - - - - - - - 494.3 - - - - - - - - - -								
d 3	469.4	207.3	317.4	157.7	78.8	0.23	0.02	0.53	
d 7	169.4	327.3	237.6	404.5	90.9	0.44	0.10	0.96	
d 14	251.8	224.1	279.4	429.6	56.5	0.06	0.30	0.14	
Ileum									0.001
d 0	- - - - - - - - - - - - 1,013.2 - - - - - - - - - - -								
d 3	591.6	354.5	575.7	288.2	104.4	0.70	0.03	0.81	
d 7	143.3	502.9	289.5	224.3	145.0	0.66	0.33	0.17	
d 14	371.5	174.1	199.6	387.9	61.2	0.74	0.94	0.01	
Intestinal chyme average									0.041
d 0	- - - - - - - - - - - - 621.5 - - - - - - - - - -								
d 3	441.0	236.9	376.3	197.9	69.4	0.47	0.02	0.86	
d 7	143.9	335.8	240.0	274.1	83.5	0.84	0.20	0.36	
d 14	323.9	189.3	262.7	350.4	46.9	0.31	0.63	0.04	

[a]Least squares means for 5 pigs per treatment per day.

In general, for the levels of IgG (Table 6.2) and IgA (Table 6.3), in serum and intestinal chyme, there were no day x treatment interactions ($P > 0.10$), no CP source x fat level interactions ($P > 0.10$), and serum immunoglobulin levels were not affected ($P > 0.10$) by CP source or fat level. However, IgA in jejunum and ileum was lower ($P < 0.03$) and tended to be lower ($P < 0.06$) in the duodenum on d 3. On d 14, IgA ileal levels were greater in pigs fed SDPP with lower fat inclusion while those in pigs fed SPC with low fat inclusion were lower on the same d (CP x fat level, $P < 0.01$); the same interaction (CP x fat level, $P < 0.04$) was also observed for the average IgA levels in the intestinal chyme on d 14. While fat level seemed to affect IgA in intestinal chyme, CP source had a consistent impact on the intestinal chyme IgG levels.

Because the effects of fat inclusion on immunoglobulin levels in serum and intestinal chyme were inconsistent, data were pooled to show the least square means of immunoglobulin levels as affected by SDPP or SPC and to relate these with the impact of immunoglobulins in the diet on the growth of weanling pigs. Thus, the main effect of protein source is shown for IgG in serum (Figure 6.4), intestinal chyme (Figure 6.5), serum IgA (Figure 6.6), and intestinal chyme (Figure 6.7).

Figure 6.4. Serum IgG (mg/mL) in weaned pigs fed diets containing SPC or SDPP, Exp. 3.

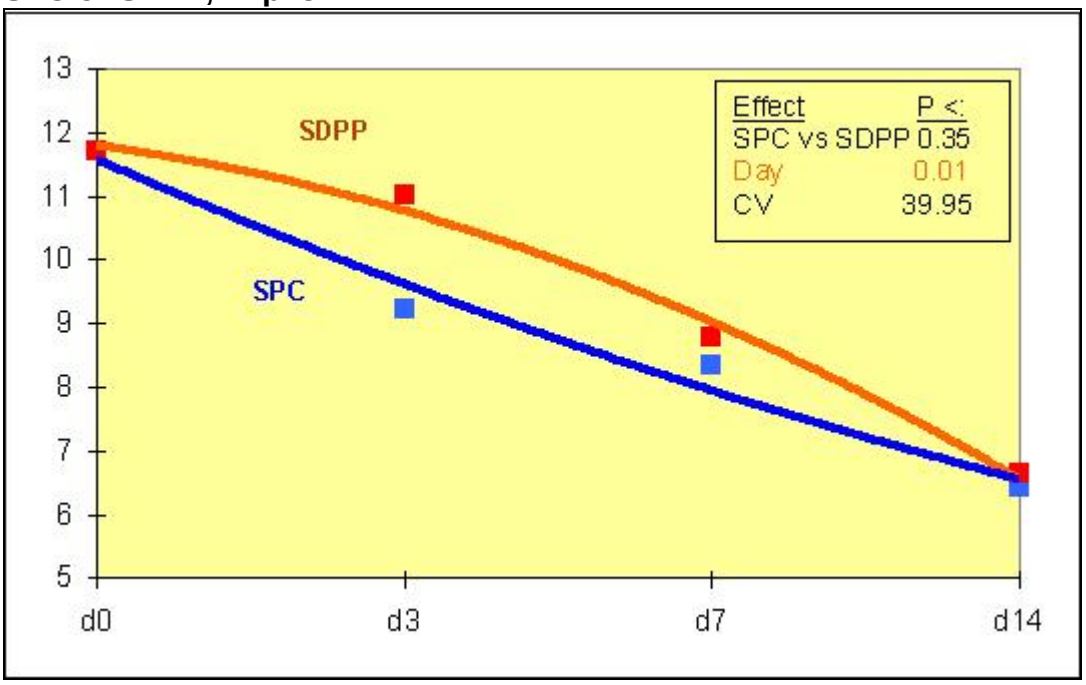


Figure 6.5. Intestinal chyme IgG (μg/mL) in weaned pigs fed diets containing SPC or SDPP, Exp. 3.

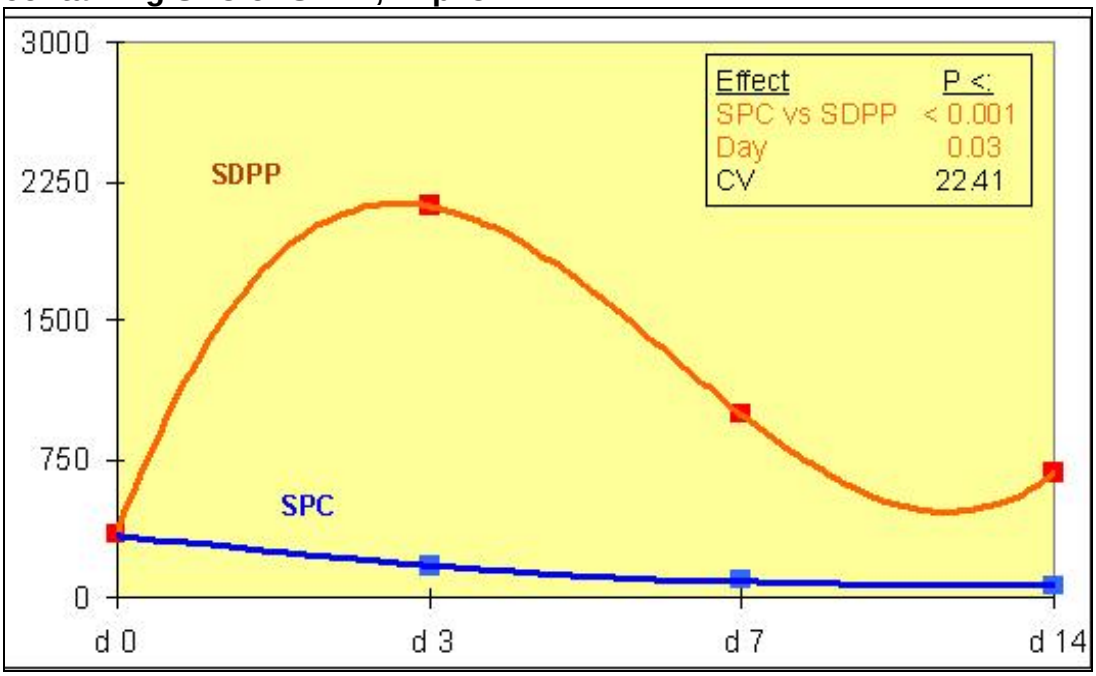


Figure 6.6. Serum IgA (μg/mL) in weaned pigs fed diets containing SPC or SDPP, Exp. 3.

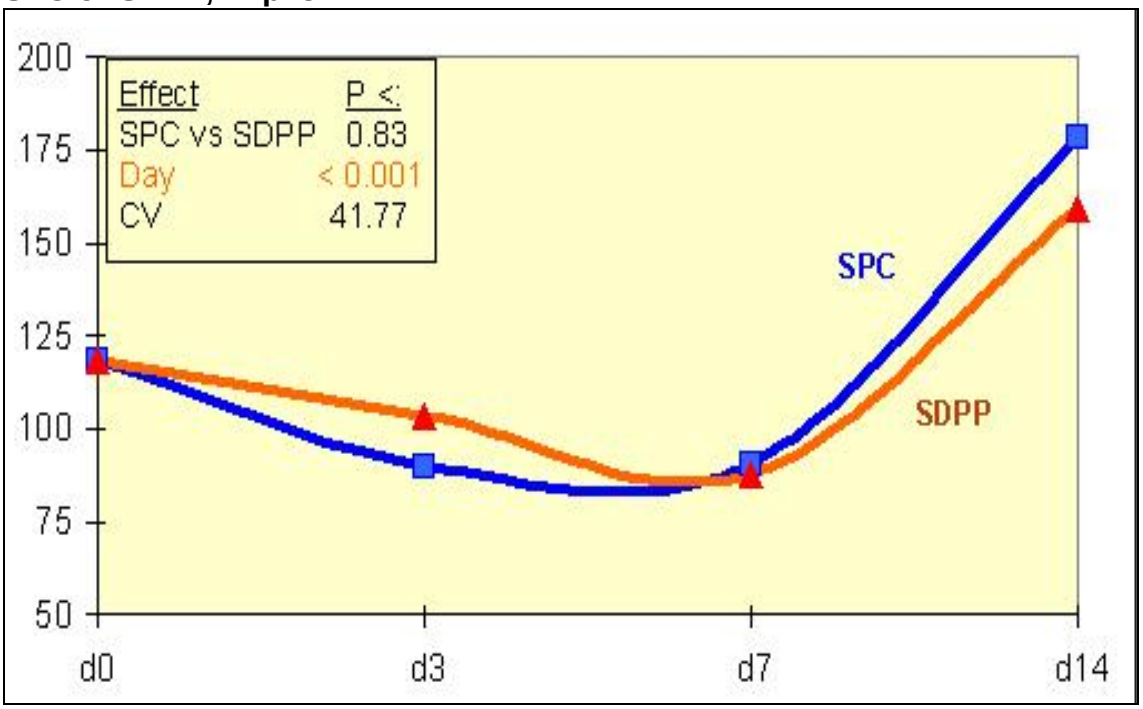
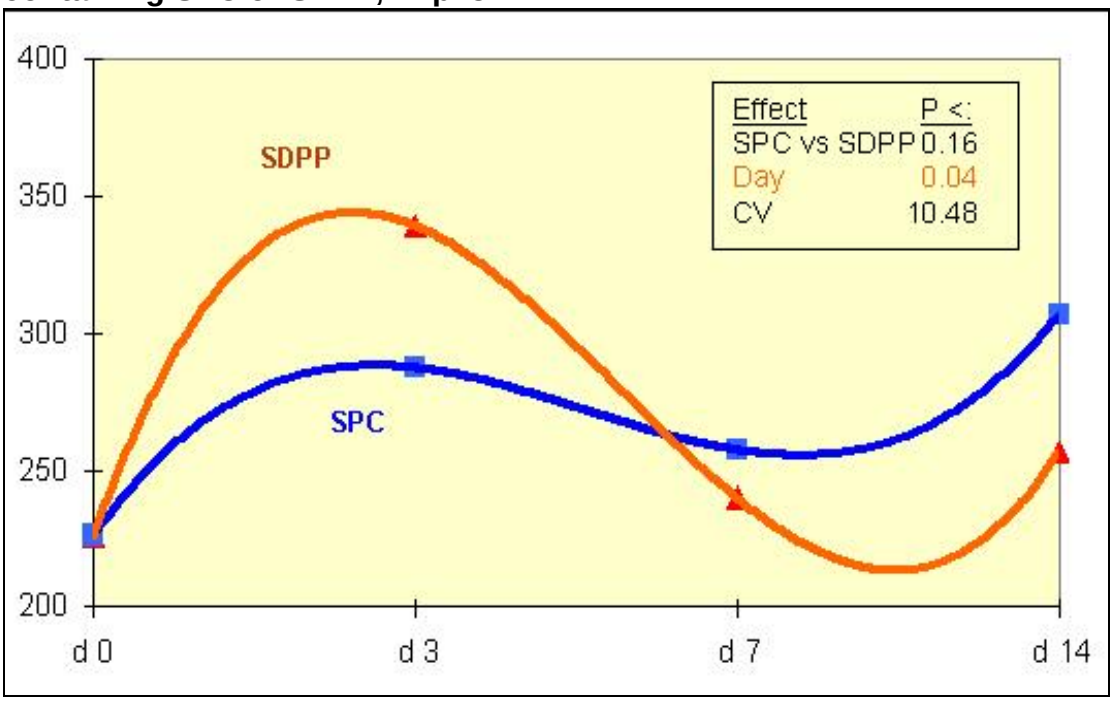


Figure 6.7. Intestinal chyme IgA (μg/mL) in weaned pigs fed diets containing SPC or SDPP, Exp. 3.



The levels of IgG in intestinal chyme were higher ($P < 0.03$) in pigs fed SDPP on d 3, 7, and 14 (Table 6.3). This was expected because of the IgG content in SDPP. The IgG levels in chyme are much higher on d 3 compared to d 7 and d 14 (Figure 6.5). Likewise, there is a numerically higher IgG level in serum on d 3 (Figure 6.4). This may imply absorption of IgG. The follicle-associated epithelium of the Peyer's patches contains M cells ('microfold' or 'membranous') (Brandtzaeg and Pabst, 2004). Microfold or M cells are epithelial cells that possess the capability of transcytotic transport of antigens (Gebert et al., 1996). If IgG is absorbed, it would be through the M cells.

The higher IgG levels in chyme on d 3, compared to d 7 and 14, may be due to the lower activity of proteases such as trypsin and chymotrypsin, at this time. As the activities of proteases increase, there may be a consequent increase in breakdown of IgG, thus decreasing its level in chyme and also decreasing its impact to gut health. This may be why the effects of SDPP on growth is observed only during the first 10 to 14 d post-weaning.

Most of the IgA in the body is found in epithelial secretions such as in the lumen of the gut where it forms the first line of defense against food-borne antigens and pathogens (Hanson & Brandtzaeg, 1993; Fagarasan and Honjo, 2003). McClelland et al. (1972) demonstrated that secreted IgA agglutinated a wide range of organisms in the intestinal tract. Walker (1976) considered secretory IgA to be a more efficient immunologic agent compared to serum IgG. However, this might be due to the abundance of IgA, being the major secretory

immunoglobulin in mucosal surfaces, and IgG may be as effective if available in sufficient amounts (Blum et al., 1981). Svanborg-Eden et al. (1978) have shown that both IgG and secretory IgA antibodies were effective in blocking adhesion of *E. coli* to human urinary tract epithelial cells in vitro. Thus, it is possible that the abundance of IgG in chyme creates the first line of defense in the intestinal lumen, blocking adhesion of *E. coli*, or other antigens, to the intestinal epithelial cells.

Torrallardona et al. (2003) and Bosi et al. (2004) have reported positive effects of SDPP on growth and immune status of pigs weaned at 21 to 24 d and challenged with *E. coli*. Torrallardona et al. (2003) also reported some interactions between colistin sulfate and SDAP in contrast to earlier reports that had suggested that the effects of SDPP and antibiotics are independent and non-additive (Coffey and Cromwell, 1995; Torrallardona et al., 2002). However, Coffey and Cromwell (1995) and Torrallardona et al. (2002) did not challenge the newly-weaned pigs with *E. coli*. Torrallardona et al. (2003) concluded that SDAP may be a good alternative to antibiotics because the magnitude of the response to SDAP was similar to that observed with colistin. Colistin is a polymyxin antibiotic with bactericidal effect on Gram-negative bacilli especially on *Pseudomonas* and coliform organisms.

The health status and degree of pathogen exposure of weanling pig is probably the major determinant of the level of response to SDAP. Health status is also a factor that influences the response to antibiotics. Coffey and Cromwell (1995) and Bergstrom et al. (1997) reported that the response to SDPP was

greater in pigs reared in a conventional, on-farm nursery setting compared to those in a cleaner, off-site nursery. Van Dijk et al. (2002b) reported that the magnitude of improvement in growth performance due to SDPP was not as high under typical Northern European research station conditions with high hygiene status. The impact of IgG antibodies in the intestinal lumen may not be significant if these were not utilized for binding antigens and combatting disease. Thus, for healthier pigs in a cleaner environment, IgG in the lumen may not have a significant impact.

Jiang et al. (2000b) earlier reported that pigs fed diets with SDPP had lower intravillous lamina propria cell density with decreased numbers of macrophages indicative of reduced inflammation. Results from Touchette et al. (2002) and Carroll et al. (2002) indicated that early-weaned pigs fed diets supplemented with SDAP are 'naive' pigs with lower immune system activation. Immunoglobulin A-containing cells in the lamina propria of weaned pigs increase in proportion, reaching 90% by 12 wk of age; thus, weaning appears to influence the relative population of immunoglobulin-containing cells (Allen and Porter, 1977). Antigenic challenge is also a factor that may influence the extent of plasma cell (immunoglobulin-bearing cell) proliferation because very few of these cells occurred in gnotobiotic animals compared to those given oral *E. coli* antigen (Porter et al., 1974). It is possible that the increased IgG levels in the intestinal lumen of pigs fed SDPP may decrease the proliferation of immunoglobulin-bearing cells in the lamina propria thus contributing to the lower immune system activation.

Pancreatic Enzyme Activity and Gene Expression

The least squares means for pancreatic triglyceride lipase in pancreas tissue and intestinal chyme are summarized in Table 6.4, while those for pancreatic α-amylase are summarized in Table 6.5. There were no day effects ($P > 0.10$) on triglyceride lipase activity in pancreas tissue, but there was a significant decrease (Day, $P < 0.0001$) in the total activity of triglyceride lipase in intestinal chyme from d 3 to 14. There was a marked increase ($P < 0.0001$) in α-amylase activity in pancreas tissue and in the intestinal chyme from d 3 to 14. There were no day x treatment interactions ($P > 0.10$).

Several authors have reported that weaning results in a general decrease in the activity of pancreatic enzymes (Hartman et al., 1961; Lindemann et al., 1986; Cera et al., 1990a; Jensen et al., 1997; Marion, 2003) regardless of weaning age. In this experiment, there was a decrease in lipase and amylase activities from d 0 to 3. Lindemann et al. (1986) reported that the total activity of lipase in pancreas tissue continued to decrease 2 wk after weaning, while the total activities of amylase increased to near pre-weaning levels 2 wk after weaning. Similarly, Marion et al. (2003) weaned pigs at 7 d and reported that relative lipase activity decreased linearly during the 2-wk period post-weaning. There is a similar decrease in lipase activity and an increase in amylase activity from d 3 to 14 in pancreas and chyme of weaned pigs in this experiment.

For the activities of pancreatic triglyceride lipase and α-amylase in pancreas tissue and intestinal chyme, there were no CP source x fat level

interactions (P > 0.10). Furthermore, fat level had no effect (P > 0.10) on lipase and amylase activities. Thus, data were pooled and the least square means for the main effect of protein source on the activity of lipase and α-amylase activity in pancreas and intestinal chyme was tested. The trendlines for pancreatic lipase activity as affected by the protein source in weanling pigs are shown in Figure 6.8 (pancreas tissue) and Figure 6.9 (intestinal chyme), while that of pancreatic α-amylase are shown in Figure 6.10 (pancreas tissue) and Figure 6.11 (intestinal chyme). The source of protein had no effect on lipase and amylase activity in pancreatic tissue (P > 0.10). However, activity of lipase (d 7) and amylase (d 14) in intestinal chyme were greater (P < 0.02) for pigs fed SDPP compared to pigs fed SPC (Figure 6.9 and Figure 6.11).

Dietary protein source and feed intake affect pancreatic enzyme activity (Efird et al., 1982; Makkink et al., 1994; Peiniau et al., 1996). Peiniau et al. (1996) reported that the pancreas of weanling pigs fed diets containing soluble fish protein concentrate had a lower weight and had decreased total enzyme activity compared to pigs fed diets containing casein, soybean meal, or soyabean concentrate. Makkink et al. (1994) reported a similar lower pancreas tissue weight and lowest chymotrypsin activities in chyme of pigs fed diets containing fishmeal compared with pigs fed skimmed milk powder or soya-bean-protein concentrate. Makkink et al. (1994) explained that pigs fed fishmeal had the highest feed intakes and their low digesta weights implied a higher rate of gastric emptying. Thus, there is a continuous supply of a more alkaline chyme to the duodenum, causing a decrease in the pancreatic secretion of bicarbonate. They

also noted that when gastric emptying is gradual, there may be a lower need for pancreatic enzymes. This may explain the low enzyme activities in chyme of pigs fed fishmeal (Makkink et al., 1994). Peiniau et al. (1996) noted that soluble fish protein concentrate contained more soluble proteins that resulted in a weak stimulation of the exocrine pancreas, thus, limiting the digestive adaptation of the piglet to the weaning diet.

Trypsin inhibitors in soybean meal cause pancreatic hypertrophy and hypersecretion of trypsin and chymotrypsin (Liener, 1976). Soy protein concentrate, the soy form used in this experiment, contains refined soybean proteins containing much less anti-nutritional factors compared to soybean meal. Peiniau et al. (1996) reported that proteins of soyabean concentrate, but not soyabean meal, are utilized almost as well as casein by newly weaned piglets. Van Dijk et al. (2001a) compared the amino acid composition and apparent ileal digestibility of SDAP preparations with casein and soybean meal. These authors noted that the protein content and apparent ileal digestibility of amino acids were lower in SDAP compared to casein but that the essential amino acid content of SDAP is superior to soybean protein. Direct comparison between SDPP and SPC digestibility or solubility has not been performed. Thus, the differences in pancreatic enzyme activity observed in chyme of weanling pigs fed diets containing SPC or SDPP cannot be explained based on digestibility or solubility. In addition, the decreased relative weight of the pancreas generally resulted in lower exocrine output (Makkink et al., 1994; Peiniau et al., 1996). Pigs fed SDPP

had numerically lower pancreas relative weight, yet, there is a generally higher pancreatic enzyme activity in pancreas and chyme of these pigs.

The level of feed intake has been identified as a factor influencing pancreatic enzyme secretion. Makkink et al., (1994) reported that feed intake may affect trypsin synthesis because it was positively related to trypsin and chymotrypsin activity. Braude et al. (1970) noted increased proteolytic enzyme activity in intestinal chyme with increased feed intake. However, Efird et al. (1982) reported differences in trypsin and chymotrypsin activity in the intestine of pigs fed soy flour and pigs fed nonfat dry milk even though feed intakes were similar. The pigs used in this experiment were group-fed so that individual feed intake data are not available and as such, cannot be related to enzyme activity. However, as a group, pigs fed SDPP have higher feed intake and may explain the generally higher enzyme activity in chyme.

Table 6.4. Triglyceride lipase activity (1000 IU/L) in pancreas tissue and chyme of weanling pigs[a], Exp 3.

Protein source	SPC	SPC	SDPP	SDPP		Probability, P <:			
ME concentration, kcal/kg	3,523	3,323	3,523	3,323	SE	SPC vs SDPP	Fat Level	CP*ME	Day
Fat inclusion, %	5.0	0.47	5.74	1.21					
Pancreas									0.197
d 0	- - - - - - - - - - - -		158.4	- - - - - - - - - -					
d 3	87.42	69.29	54.82	57.04	19.05	0.26	0.68	0.60	
d 7	28.99	45.80	48.82	35.70	11.57	0.68	0.88	0.22	
d 14	28.08	33.32	28.52	33.01	8.81	0.99	0.59	0.97	
Duodenum									0.099
d 0	- - - - - - - - - - - - -		47.9	- - - - - - - - - -					
d 3	25.29	40.33	28.13	20.04	7.98	0.30	0.67	0.17	
d 7	13.01	23.21	30.30	16.90	6.55	0.42	0.81	0.10	
d 14	13.80	8.15	21.42	22.28	7.79	0.19	0.76	0.68	
Jejunum									0.243
d 0	- - - - - - - - - - - - -		70.1	- - - - - - - - - -					
d 3	27.84	34.52	36.34	24.28	8.47	0.92	0.76	0.29	
d 7	15.25	21.46	61.62	44.39	13.61	0.03	0.69	0.41	
d 14	12.17	9.04	24.21	26.71	5.55	0.02	0.96	0.62	
Ileum									< 0.0001
d 0	- - - - - - - - - - - - -		48.8	- - - - - - - - - -					
d 3	39.18	41.63	35.63	41.29	7.69	0.80	0.61	0.84	
d 7	13.70	20.35	46.47	29.47	8.83	0.04	0.57	0.21	
d 14	10.69	7.18	14.67	11.75	3.42	0.24	0.37	0.93	
Total Intestinal activity									0.0001
d 0	- - - - - - - - - - - -		142.3	- - - - - - - - - -					
d 3	92.30	116.48	100.10	85.59	16.10	0.49	0.77	0.25	
d 7	37.56	63.54	138.38	90.76	24.01	0.02	0.66	0.15	
d 14	36.66	24.37	60.28	60.73	14.86	0.07	0.70	0.68	

[a]Least squares means for 5 pigs per treatment.

Table 6.5. Pancreatic α-amylase activity (1000 IU/L) in pancreas tissue and chyme of weanling pigs[a], Exp 3.

Protein source	SPC	SPC	SDPP	SDPP		Probability, P <:			
ME concentration, kcal/kg	3,523	3,323	3,523	3,323	SE	SPC vs SDPP	Fat Level	CP*ME	Day
Fat inclusion, %	5.0	0.47	5.74	1.21					
Pancreas									< 0.0001
d 0	- - - - - - - - - - - - - 385.9 - - - - - - - - - - -								
d 3	149.65	169.47	105.76	152.96	55.07	0.60	0.56	0.81	
d 7	189.20	120.91	324.78	119.50	61.82	0.30	0.05	0.29	
d 14	426.00	582.40	416.84	455.20	134.51	0.62	0.48	0.67	
Duodenum									< 0.0001
d 0	- - - - - - - - - - - - - 47.3 - - - - - - - - - -								
d 3	14.96	12.20	15.05	10.53	3.92	0.84	0.37	0.83	
d 7	28.18	56.12	65.54	59.52	24.93	0.43	0.67	0.51	
d 14	44.98	59.71	90.42	151.92	45.66	0.16	0.42	0.62	
Jejunum									< 0.0001
d 0	- - - - - - - - - - - - - 71.7 - - - - - - - - - -								
d 3	42.13	24.91	19.06	19.46	8.05	0.10	0.32	0.30	
d 7	62.84	31.27	119.24	84.20	25.21	0.05	0.21	0.95	
d 14	72.89	127.23	213.79	223.85	38.00	0.01	0.41	0.57	
Ileum									0.0001
d 0	- - - - - - - - - - - - - 48.2 - - - - - - - - - -								
d 3	68.84	41.99	29.66	25.01	13.01	0.19	0.67	0.13	
d 7	98.08	51.61	247.07	101.16	59.05	0.12	0.13	0.42	
d 14	87.31	159.28	211.64	249.30	49.86	0.05	0.30	0.74	
Intestinal total									< 0.0001
d 0	- - - - - - - - - - - - - 175.7 - - - - - - - - - -								
d 3	104.01	49.94	63.77	277.62	123.57	0.46	0.54	0.31	
d 7	149.44	171.84	431.84	244.87	111.27	0.14	0.48	0.37	
d 14	205.18	346.22	515.85	631.73	115.36	0.02	0.29	0.91	

[a]Least squares means for 5 pigs per treatment per day

Figure 6.8. Triglyceride lipase activity (1000 IU/L) in pancreas tissue of weaned pigs fed diets containing SPC or SDPP, Exp. 3.

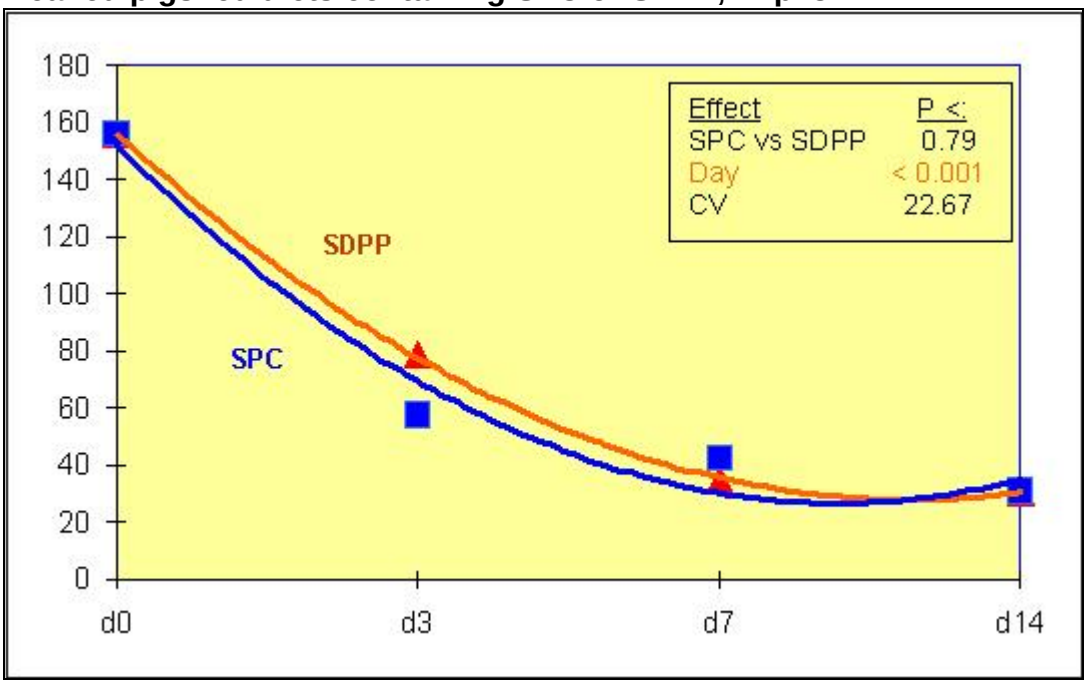


Figure 6.9. Triglyceride lipase activity (1000 IU/L) in intestinal chyme of weaned pigs fed diets containing SPC or SDPP, Exp. 3.

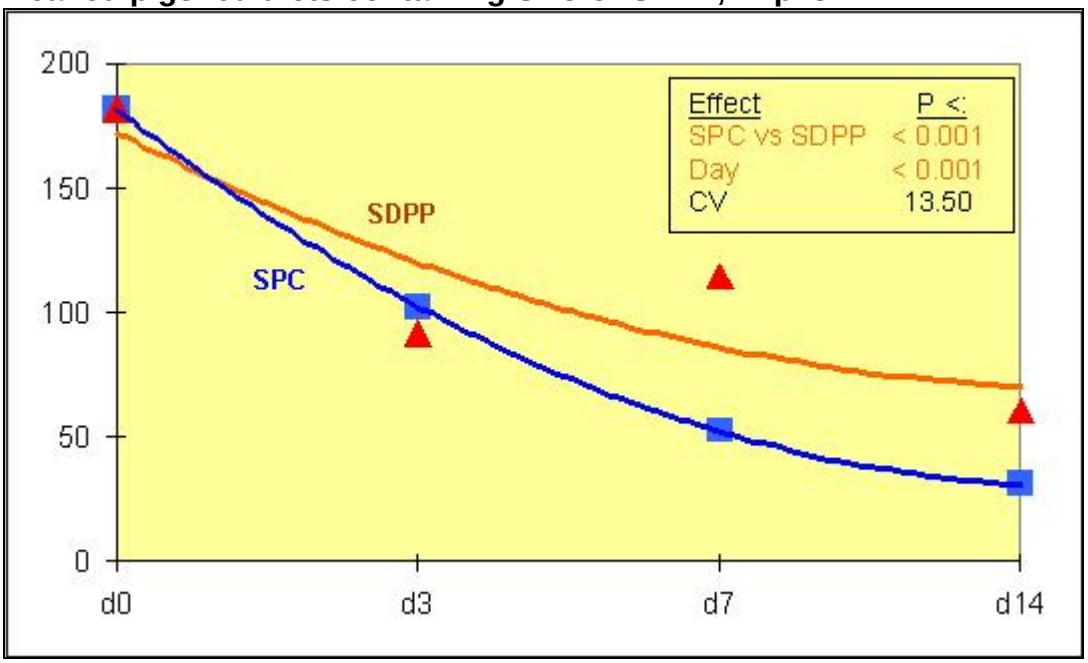


Figure 6.10. Amylase activity (1000 IU/L) in pancreas tissue of weaned pigs fed diets containing SPC or SDPP, Exp. 3.

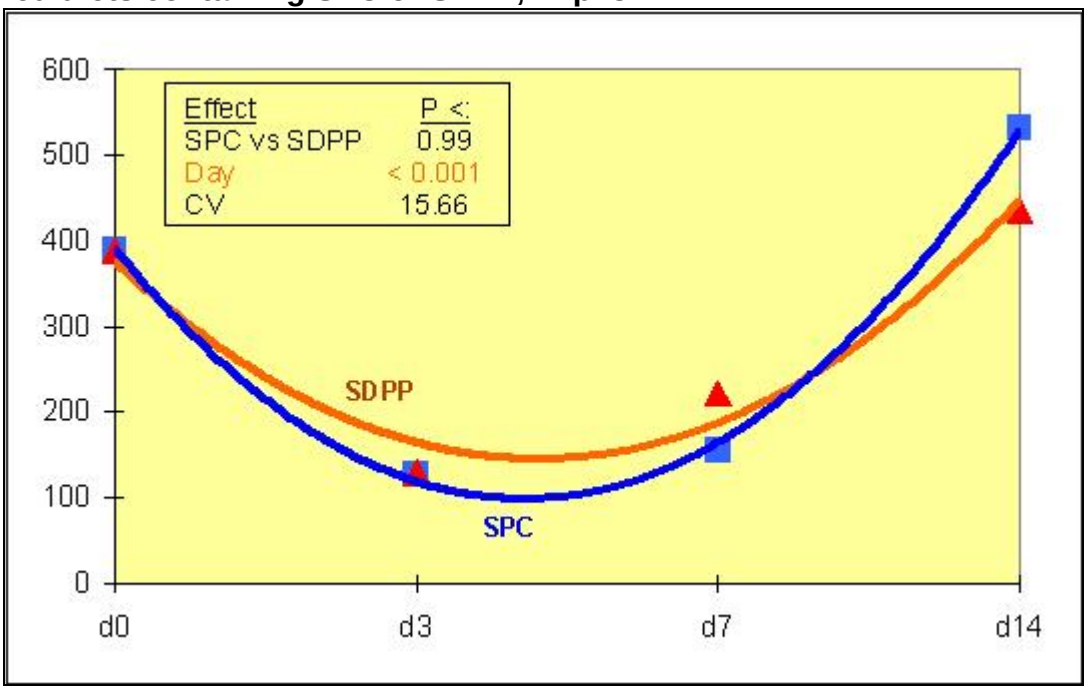
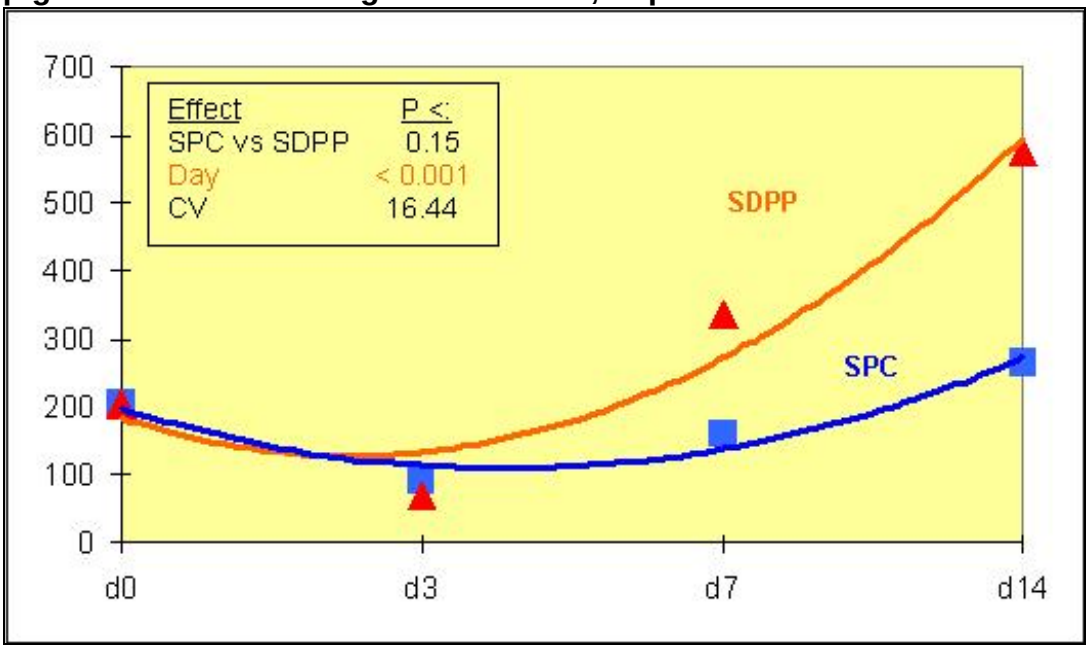


Figure 6.11. Amylase activity (1000 IU/L) in intestinal chyme of weaned pigs fed diets containing SPC or SDPP, Exp. 3.



The fold change in gene expression of the pancreatic enzymes are shown in Figure 6.12 (triglyceride lipase), Figure 6.13 (α-amylase), and Figure 6.14 (trypsinogen). There was an increase (Day, $P < 0.005$) in amylase gene expression from d 3 to 14. The numerical decrease in lipase and increase in trypsinogen gene expression were not significant (Day, $P > 0.10$). There were no day x treatment interactions ($P > 0.10$). Also, there were no CP source x fat level interactions ($P > 0.10$) and fat level did not affect ($P > 0.10$) gene expression. The source of protein did not affect ($P > 0.10$) lipase and trypsinogen gene expression, but increased ($P < 0.005$) amylase gene expression.

Gene expression of both pancreatic α-amylase and trypsinogen were low at weaning and numerically increased from d 3 to 14 post-weaning. The decrease in mRNA levels on d 3 parallels the reported general decrease in the activity of pancreatic enzymes at weaning (Hartman et al., 1961; Lindemann et al., 1986; Cera et al., 1990a; Jensen et al., 1997; Marion, 2003). Likewise, the amylase gene expression profile corresponds to the amylase activity in chyme (Figure 6.11).

Marion et al. (2003) reported decreased trypsin mRNA levels 3 d after weaning that increased linearly in the two-wk period post-weaning; this is similar to the trend observed here (Figure 6.14). Trypsin activity was not measured in this study but the increase in mRNA from d 3 to 14 corresponds to a similar increase in trypsin activity in pancreas and chyme reported by Makkink et al. (1994).

Peirzynowski et al. (1993) reported that the maturation of the exocrine pancreas function in terms of volume, protein content, and trypsin activity, was induced by weaning, and by the shift to solid feed at weaning either at 4 or 6 wk of age. A major event at weaning is the drastic reduction in feed intake during the first 24 to 48 hr. Marion et al. (2003) reported that low feed intake enhanced the decrease in pancreatic enzyme gene expression, just as low feed intake enhanced the decrease in pancreatic enzyme activity. Pigs fed diets containing SDPP have higher feed intake and generally greater pancreatic gene expression compared to pigs fed SPC.

Figure 6.12. Fold change in pancreatic triglyceride lipase gene expression in pancreas of weaned pigs fed diets containing SPC or SDPP, Exp. 3.

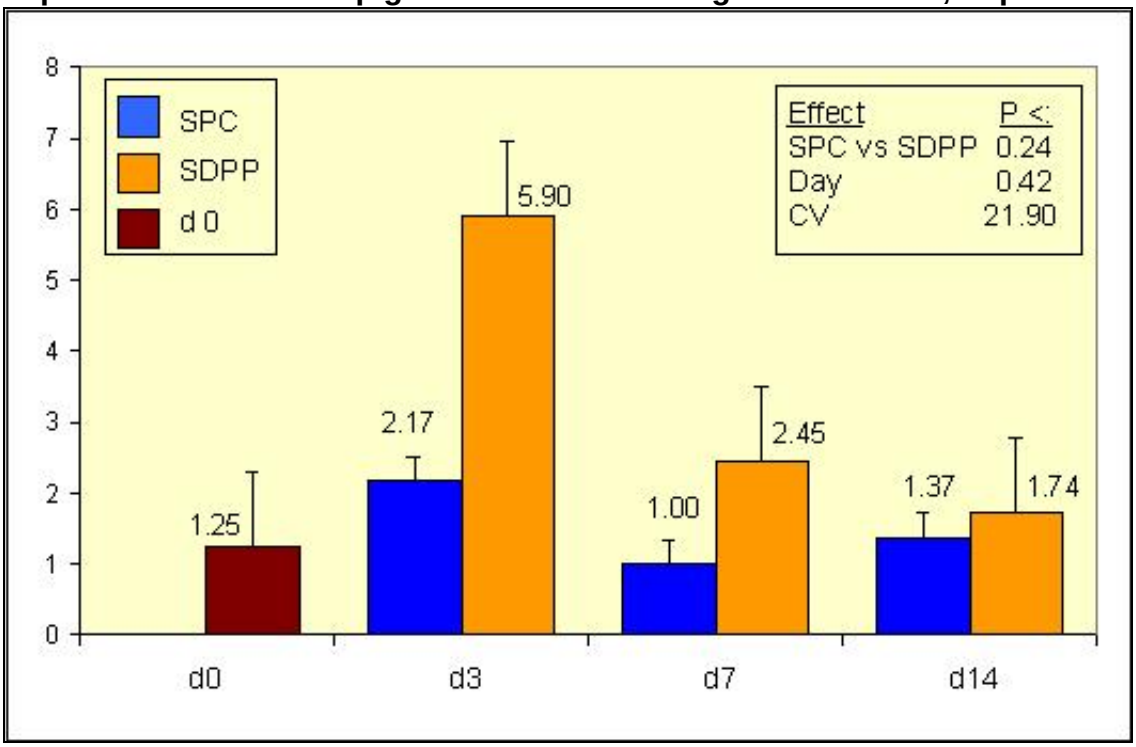


Figure 6.13. Fold change in pancreatic α-amylase gene expression in pancreas of weaned pigs fed diets containing SPC or SDPP, Exp. 3.

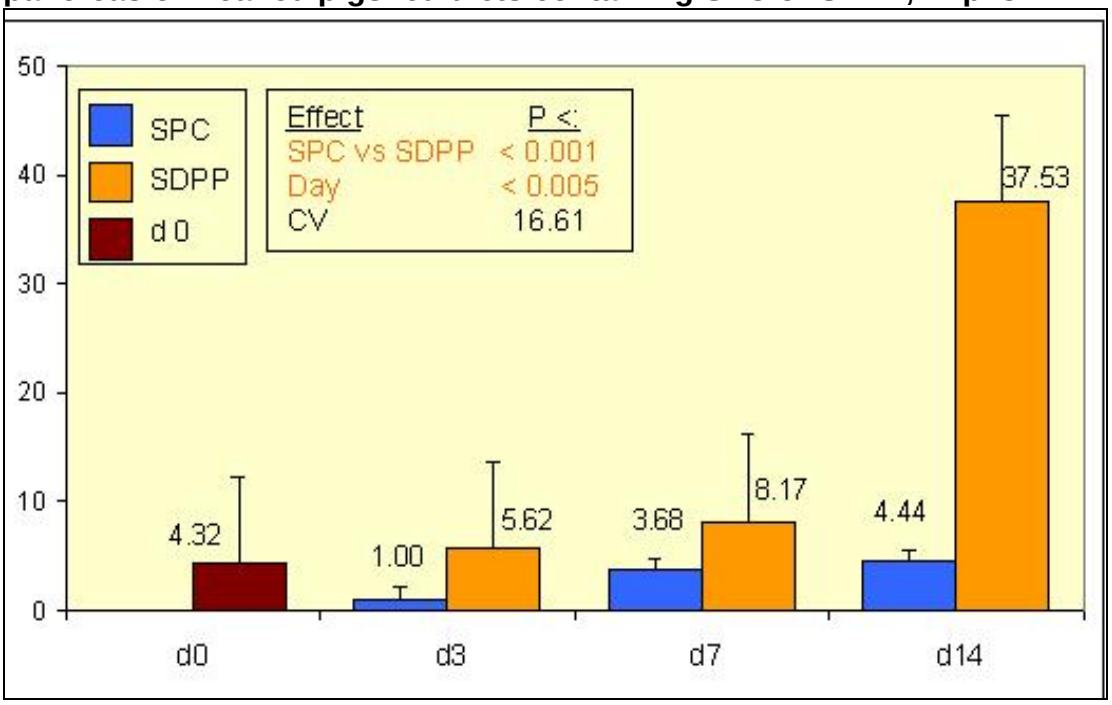
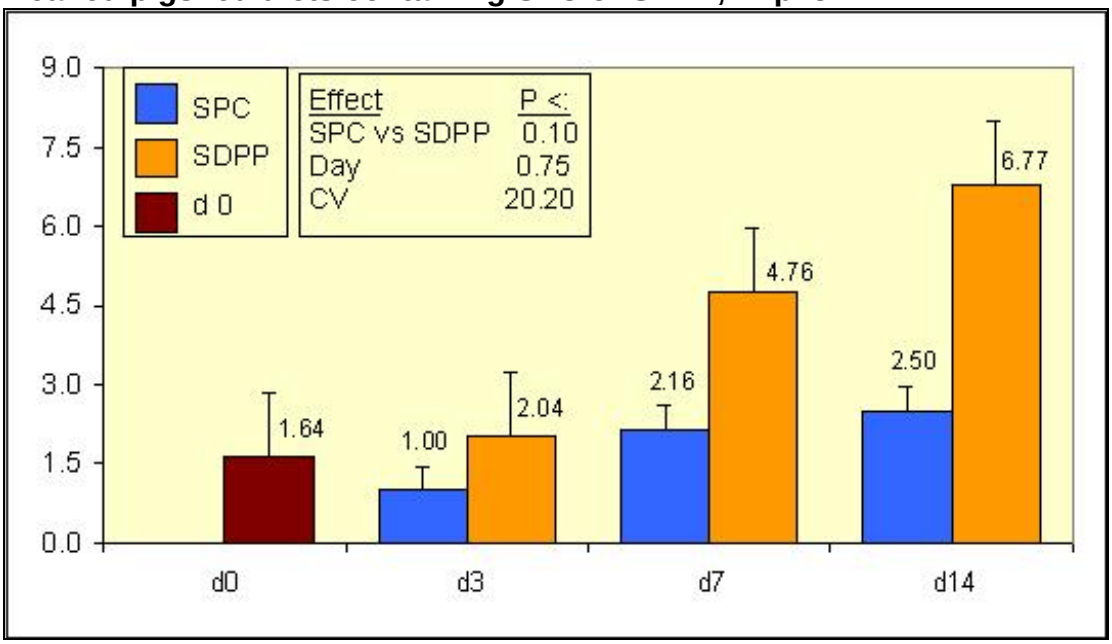


Figure 6.14. Fold change in trypsinogen gene expression in pancreas of weaned pigs fed diets containing SPC or SDPP, Exp. 3.



There was a numerically higher ($P > 0.10$) lipase mRNA level on d 3 post-weaning compared to d 14. This corresponds to a greater lipase enzyme activity in pancreas and chyme on d 3 compared to d 14 (Figure 6.8, Figure 6.9, Figure 6.12). However, on d 0 (pre-weaning), lipase mRNA is very low while activity in pancreas and chyme is very high. The amount of lipase protein in pancreas and chyme was not measured. However, the low lipase mRNA on d 0 may signify that lipase mRNA had been translated to lipase enzyme protein resulting in the high level of activity. This may have depleted lipase mRNA in pancreas and therefore the low gene expression. Increased synthesis of lipase may be due to the high fat level in milk. The higher mRNA levels post-weaning (compared to d 0 level) may imply less translation into lipase enzyme. The higher lipase mRNA levels on d 3 compared to d 0 implies that gene expression may not the factor limiting lipase synthesis in the immediate post-weaning period.

Changes in synthesis of proteases, amylase, and lipase parallel the changes in their mRNA levels in response to their respective substrates (Brannon, 1990). While sow milk has very high fat content (around 40% on dry matter basis), the weanling pig diet contains more complex carbohydrate and protein. The drastic decrease in percentage of fat in the diet may be the reason for the decreased lipase activity in the immediate post-weaning period. On the other hand, the presence of more complex carbohydrate and proteins may be the reason for the increase in amylase activity and gene expression, and increase in trypsinogen gene expression from d 3 to 14.

Implications

Immunoglobulins in SDPP significantly increased IgG levels in chyme from the three sections of the small intestine while fat level had inconsistent effects on IgA levels in intestinal chyme of weanling pigs. The significantly high levels of IgG in intestinal chyme may also form a strong line of defense against antigens from pathogenic bacteria in the intestinal lumen of weanling pigs fed diets containing SDPP. In addition, increased IgG levels in the intestinal lumen of pigs fed SDPP may decrease the proliferation of immunoglobulin-bearing cells in the lamina propria thus contributing to the lower immune system activation previously reported in pigs fed SDPP. Protein source or fat level did not affect pancreatic lipase and amylase activity in pancreas tissue, but activities in intestinal chyme were increased in pigs fed SDPP. In addition, fat level had no effect on pancreatic gene expression, but CP source increased amylase gene expression and numerically increased lipase and trypsinogen gene expression. Low feed intake at weaning enhances the decrease in pancreatic enzyme gene expression, and because SDPP increased feed intake, this may be one mechanism that may explain the apparently higher pancreatic enzyme gene expression in pigs fed SDPP.

CHAPTER VII

SUMMARY AND CONCLUSIONS

Early weaning technology, weaning as early as 10 to 21 d, has been implemented in the swine industry since the 1980's. Weaning is a very stressful period that alters piglet behavior as well as neuroendocrine and metabolic responses. However, most of the adaptive changes taking place in the immediate post-weaning period are, for the most part, dictated by the level of nutrient intake. Many of the problems observed post-weaning are exacerbated by the drastic reduction in feed intake at weaning. Spray-dried porcine plasma (SDPP) has become a popular protein source in post-weaning pig diets because it has consistently been reported to improve average daily gain (ADG) and average daily feed intake (ADFI) during the first 2 wk post-weaning. The effect of SDPP on growth and performance is well documented, but the mechanism behind it is only just evolving. Spray-dried plasma protein increases feed intake and, thus, probably decreases the problems associated with reduced feed intake at weaning. It also contains immunoglobulins that have been identified as a component behind the improvement in performance. Spray-dried porcine plasma has also been shown to reduce intestinal growth and it was this report that prompted this investigation.

The gastrointestinal system develops functionally and grows rapidly soon after enteral feeding starts at birth. Weaning to a more complex diet causes an even greater growth rate and increase in weight of the gastrointestinal and associated digestive organs in relation to body weight and this has been reported to be more pronounced in the small intestine. Regardless of weaning age, the relative weight of the small intestine increases by 25% from d 3 to 7 post-weaning, and up to 52% at 10 to 14 d while the relative weight of the pancreas has a corresponding 17% and 30% increase, respectively. This growth takes place even though metabolizable energy (ME) intake at the end of the first wk post weaning is only 60 to 70% of the pre-weaning milk ME intake.

Thus, it was hypothesized that reduced intestinal growth in pigs fed spray dried porcine plasma may lower ME requirement for maintenance in the gut therefore increasing ME available for lean tissue deposition. Exp. 1 was performed to determine the effect of reducing the ME (fat) concentration in weanling pig diets supplemented with SDPP. A total of 232 crossbred pigs (avg BW = 5.8 kg; avg 21 d were randomly allotted to four dietary treatments (9 pens/trt): 1) Control diet containing soy protein concentrate (SPC) with ME = 3,471 kcal/kg, 2) SDPP replaced SPC, ME = 3,471 kcal/kg, 3) SDPP diet with ME level reduced by 100 kcal/kg, and 4) SDPP diet with ME level reduced by 200 kcal/kg.

The inclusion of SDPP in weanling pig diets improved ADG, ADFI, and G:F. However, reducing ME concentration in SDPP diets did not affect growth

performance of weanling pigs, but linearly increased weight gain/ME intake. In the course of reducing the ME level of the diet, the added fat in the form of soybean oil was reduced. The apparent digestibility of fat is generally low at weaning and the use of fat in weanling pig diets is controversial. The low activity of lipase at weaning has been identified as the probable cause for this low digestibility. However, the digestibility coefficients for fat were reportedly higher for diets containing casein compared to soybean protein diets, implying that protein source may influence fat utilization.

To determine whether the linear increase in body weight gain/100 kcal ME intake in Exp. 1 was due to the decrease in fat content or due to the protein source (SDPP), Exp. 2 was performed to compare the effects of reduced ME concentration of diets containing either soy protein concentrate (SPC) or SDPP on weanling pig performance. A total of 168 crossbred pigs (avg BW = 5.8 kg; avg 21 d) were randomly allotted four dietary treatments: 1) diet containing SPC with ME = 3,525 kcal/kg, 2) SPC diet with ME = 3,325 kcal/kg, 3) SDPP diet with ME = 3,525 kcal/kg, and 4) SDPP diet with ME = 3,325 kcal/kg.

As in Exp. 1, SDPP improved ADG and ADFI and tended to improve gain:feed ratio. Similarly, reducing ME had no effect on growth performance of weanling pigs, but it increased weight gain/ME intake. The improvement in weight gain/ME intake associated with reducing the ME of the diet tended to be greater for pigs fed SPC than for pigs fed SDPP. Also, pigs fed SPC with reduced ME tended to have a better G:F ratio (d 0 to 18) and higher weight

gain/Lys intake, but there was no effect for pigs fed SDPP. These results suggest that the source of dietary protein may affect energy (fat) utilization.

Thus, Exp. 3 was performed to determine the effects of SPC and SDPP with two ME levels, 3,523 vs 3,323 kcal/kg (the same dietary treatments described in Exp. 2), on the growth of the pancreas, stomach, and small intestine, morphology of the small intestine, levels of immunoglobulins (IgG and IgA) in serum and small intestinal contents (chyme), pancreatic α-amylase and triglyceride lipase activities in pancreatic tissue and intestinal chyme, and gene expression of pancreatic triglyceride lipase, α-amylase and trypsinogen. To accomplish these objectives, a total of 80 crossbred pigs (avg BW= 5.2 kg, avg 18 d) were euthanized on d 0, 3, 7, and 14 (4 pigs/day, 5 pigs/trt).

The growth of the pancreas, stomach, and small intestine were highly correlated with BW and the weights of these organs were associated with increasing BW and age. However, CP source affected organ growth such that the relative weight of the stomach and small intestine was lower in pigs fed SDPP compared to pigs fed SPC. In addition, villi were longer, villous height:crypt depth ratio was greater, and crypt depth and villous width were lower in pigs fed SDPP. These results suggest that CP source, but not ME (fat) level, can dramatically affect the growth of the stomach and small intestine, as well as the morphology of the small intestine. Longer villi with lower crypt depths imply a healthier intestinal epithelium with slow turnover of cells. This is advantageous

because the enterocyte needs time to mature into a fully functional cell that is equipped with brushborder digestive enzymes with greater absorptive capacity.

Immunoglobulins in SDPP significantly increased IgG levels in chyme from the three sections of the small intestine, while fat level had inconsistent effects on IgA levels in intestinal chyme of weanling pigs. Immunoglobulin A secreted by the epithelial cells forms the first line of defense along the intestinal mucosa. However, the significantly high levels of IgG in intestinal chyme may form an even stronger line of defense against antigens from pathogenic bacteria in the intestinal lumen of weanling pigs fed diets containing SDPP. In addition, increased IgG levels in the intestinal lumen of pigs fed SDPP may decrease the proliferation of immunoglobulin-bearing cells in the lamina propria. This may contribute to the lower immune system activation previously reported in pigs fed SDPP.

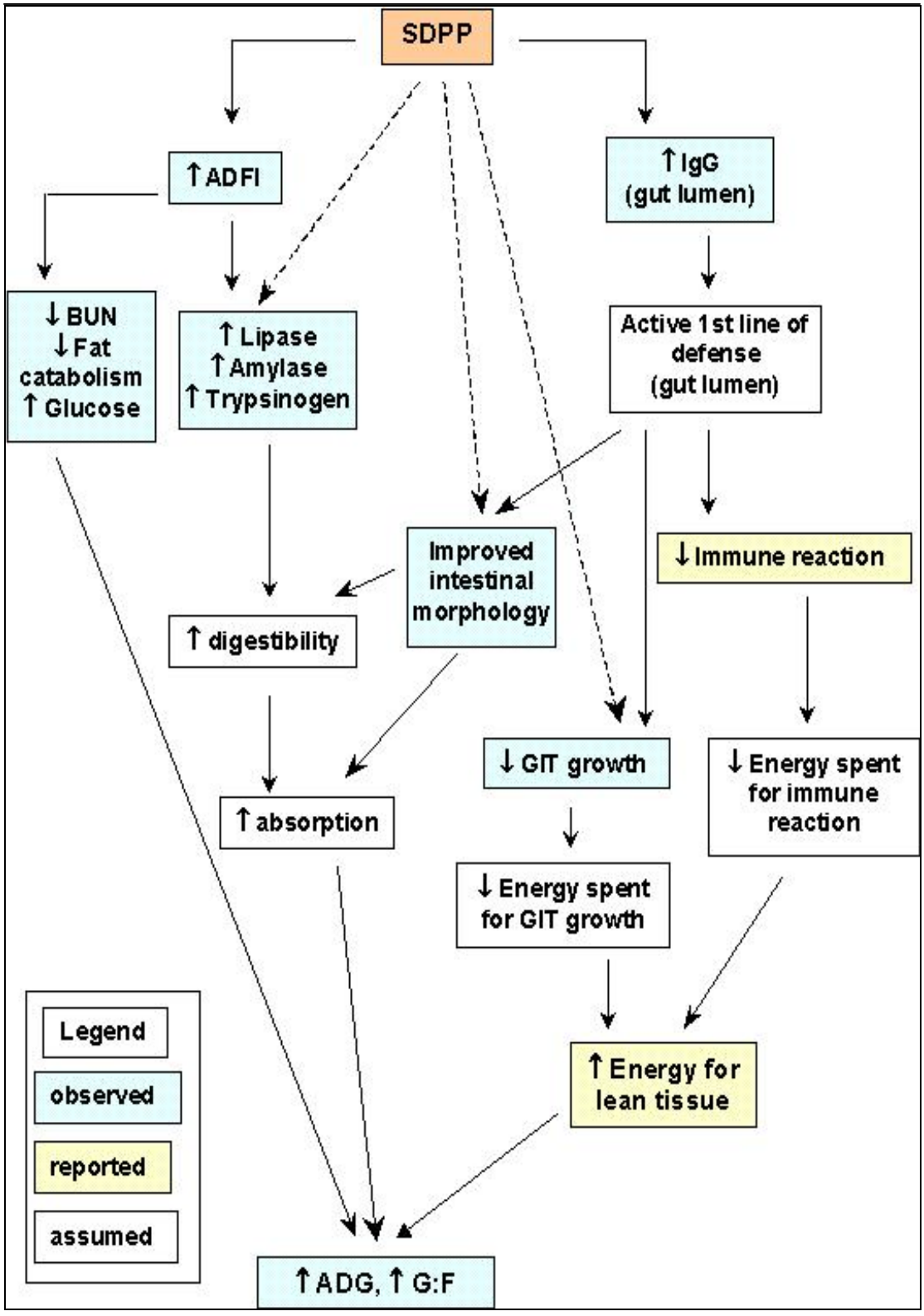
Protein source or fat level did not affect pancreatic lipase and amylase activity in pancreas tissue, but SDPP increased these activities in intestinal chyme. In addition, fat level had no effect on pancreatic gene expression, but CP source increased amylase gene expression and numerically increased lipase and trypsinogen gene expression. Low feed intake at weaning enhances the decrease in pancreatic enzyme gene expression and since SDPP increased feed intake, this may be one mechanism that explains the apparently higher pancreatic enzyme gene expression in pigs fed SDPP. Consequently, the increased pancreatic enzyme activity and gene expression in pigs fed SDPP may

be another mechanism by which SDPP improves growth performance in weanling pigs.

There is a drastic decrease in pancreatic enzyme activity and gene expression at weaning accompanied by drastic changes in intestinal morphology that may decrease nutrient digestion and absorption. Although not observed in this experiment, fat supplementation to weanling pig diets has been demonstrated to decrease jejunal villi and reduce absorptive area. Therefore, the supplementation of oil during this phase may not be necessary because it does not improve weanling pig performance. A positive response to fat supplementation has been reported to occur only after 2 to 3 wk post-weaning. This improvement in growth was observed even though fat was added only on the 3rd wk post-weaning (no adaptation). In addition, fat supplementation in the starter stage had no effect on the subsequent grow-finish stages. However, fat is importat in the pelleting process especially for diets containing high levels of milk products to reduce dustiness and improve pellet quality.

Figure 7.1 illustrates the proposed mode of action of SDPP in early-weaned pigs based on observations from the previous three experiments (blue boxes) and from other reported data (yellow boxes). There may also be direct effects exerted by SDPP (broken arrows) on gastrointestinal growth, small intestinal morphology, and pancreatic enzyme activity and gene expression.

Figure 7.1. Flowchart showing the proposed mode of action of SDPP.



In conclusion, the inclusion of SDPP to weanling pig diets improves the growth performance of the newly weaned pig by varied mechanisms. It provides abundant IgG that may serve as a first line of defense for the intestinal mucosa (improving morphology and decreasing energy spent to mount an immune response); decreases stomach and small intestine growth (that may provide more energy towards lean tissue deposition); improves small intestinal morphology (that may increase digestive and absorptive capacity); and increases feed intake that increases pancreatic enzyme activity and gene expression (that may enhance digestion and absorption of nutrients). All these lead to an increase in ADG and G:F. On the other hand, the use of fat in weanling pig diets during the first 2 wk post-weaning may have no benefit to pig growth.

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APPENDIX

Pen and Pig Means and Analysis of Variance Tables

Appendix Table 1. Average initial and final weights of pigs in experimental pens, Exp. 1.

Pen	Trt	Rep	Initial Wt, kg	Final Wt, kg
1	1	1	6.85	18.29
2	1	2	5.66	15.34
3	1	3	4.39	12.69
4	1	4	6.94	17.89
5	1	5	5.97	16.09
6	1	6	4.71	15.17
7	1	7	7.11	17.95
8	1	8	5.93	15.76
9	1	9	4.77	13.88
10	2	1	6.81	18.84
11	2	2	5.60	15.64
12	2	3	4.36	12.65
13	2	4	7.04	17.43
14	2	5	6.00	17.41
15	2	6	4.62	15.53
16	2	7	7.16	19.04
17	2	8	5.91	15.32
18	2	9	4.88	15.50
19	3	1	6.85	17.76
20	3	2	5.58	15.32
21	3	3	4.44	14.08
22	3	4	6.87	18.75
23	3	5	5.93	17.35
24	3	6	4.81	15.71
25	3	7	7.05	17.71
26	3	8	5.86	16.47
27	3	9	4.84	15.84
28	4	1	6.87	17.19
29	4	2	5.75	16.22
30	4	3	4.41	13.94
31	4	4	6.84	18.90
32	4	5	5.92	17.49
33	4	6	4.79	15.76
34	4	7	7.17	18.96
35	4	8	5.91	17.36
36	4	9	4.72	15.67

Appendix Table 2. Analysis of variance for initial and final weights of pigs in experimental pens, Exp. 1.

Source	df	Mean Squares	
		Initial Wt, kg	Final Wt, kg
Total	35		
Error	24	0.00410	0.37144
Replication	8	4.12575	11.32749
C1: 1 vs 234	1	0.00000	3.22403
C2: Linear ME	1	0.00000	0.94761
C3: Quadratic ME	1	0.00167	0.01402
C.V., %		1.10	3.71

Appendix Table 3. Average daily gain, average daily feed intake, and gain:feed of pigs in experimental pens (d 0-7), Exp. 1.

Pen	Trt	Rep	ADG, g	ADFI, g	G:F
1	1	1	59.55	161.04	0.37
2	1	2	94.09	112.55	0.84
3	1	3	53.18	97.40	0.55
4	1	4	46.36	88.13	0.53
5	1	5	14.09	66.79	0.21
6	1	6	50.91	95.24	0.53
7	1	7	57.36	126.62	0.45
8	1	8	51.02	103.90	0.49
9	1	9	64.01	107.61	0.59
10	2	1	112.73	172.62	0.65
11	2	2	115.91	154.76	0.75
12	2	3	68.18	110.39	0.62
13	2	4	8.64	77.92	0.11
14	2	5	86.36	102.97	0.84
15	2	6	80.00	94.16	0.85
16	2	7	160.17	196.97	0.81
17	2	8	145.64	160.48	0.91
18	2	9	166.05	182.75	0.91
19	3	1	107.27	156.93	0.68
20	3	2	101.82	135.82	0.75
21	3	3	111.36	146.65	0.76
22	3	4	71.36	86.27	0.83
23	3	5	83.64	100.19	0.83
24	3	6	89.55	97.40	0.92
25	3	7	96.32	135.28	0.71
26	3	8	71.43	114.10	0.63
27	3	9	168.83	179.96	0.94
28	4	1	54.09	107.14	0.50
29	4	2	119.09	142.32	0.84
30	4	3	94.09	127.71	0.74
31	4	4	42.73	82.56	0.52
32	4	5	84.55	109.46	0.77
33	4	6	100.45	117.97	0.85
34	4	7	91.99	142.86	0.64
35	4	8	153.06	179.04	0.85
36	4	9	104.82	146.57	0.72

Appendix Table 4. Analysis of variance for average daily gain, average daily feed intake, and gain:feed of pigs in experimental pens (d 0-7), Exp. 1.

Source	df	Mean Squares		
		ADG, g	ADFI, g	G:F
Total	35			
Error	24	806.6701	498.3649	0.02523
Replication	8	2,476.5135	2,815.1493	0.04496
C1: 1 vs 234	1	13,745.8083	4,324.5299	0.36169
C2: Linear ME	1	542.4120	526.9340	0.00002
C3: Quadratic ME	1	3.9528	198.1834	0.02756
C.V., %		32.15	17.78	23.35

Appendix Table 5. Average daily gain, average daily feed intake, and gain:feed of pigs in experimental pens (d 7-14), Exp. 1.

Pen	Trt	Rep	ADG, g	ADFI, g	G:F
1	1	1	237.66	225.97	1.05
2	1	2	202.38	234.85	0.86
3	1	3	95.24	175.32	0.54
4	1	4	269.02	298.70	0.90
5	1	5	230.98	259.74	0.89
6	1	6	232.47	277.92	0.84
7	1	7	244.59	306.28	0.80
8	1	8	192.95	221.71	0.87
9	1	9	206.86	199.44	1.04
10	2	1	287.88	326.84	0.88
11	2	2	208.87	262.99	0.79
12	2	3	151.52	190.48	0.80
13	2	4	198.05	240.26	0.82
14	2	5	298.70	337.66	0.88
15	2	6	306.28	325.76	0.94
16	2	7	254.33	278.14	0.91
17	2	8	153.99	195.73	0.79
18	2	9	212.43	218.00	0.97
19	3	1	212.12	266.23	0.80
20	3	2	175.32	228.35	0.77
21	3	3	192.64	226.19	0.85
22	3	4	274.58	328.39	0.84
23	3	5	294.99	339.52	0.87
24	3	6	268.83	306.49	0.88
25	3	7	218.61	247.84	0.88
26	3	8	209.65	260.67	0.80
27	3	9	209.65	242.12	0.87
28	4	1	164.50	220.78	0.75
29	4	2	209.96	251.62	0.83
30	4	3	203.46	251.08	0.81
31	4	4	317.25	348.79	0.91
32	4	5	294.37	334.42	0.88
33	4	6	250.00	299.78	0.83
34	4	7	307.36	348.48	0.88
35	4	8	219.85	244.90	0.90
36	4	9	197.59	188.31	1.05

Appendix Table 6. Analysis of variance for average daily gain, average daily feed intake, and gain:feed of pigs in experimental pens (d 7-14), Exp. 1.

Source	df	Mean Squares		
		ADG, g	ADFI, g	G:F
Total	35			
Error	24	1,359.8737	1,168.0229	0.00672
Replication	8	6,560.3104	6,999.7569	0.01527
C1: 1 vs 234	1	2,865.7692	4,667.9870	0.00033
C2: Linear ME	1	473.1913	700.6272	0.00020
C3: Quadratic ME	1	282.9524	14.0862	0.00463
C.V., %		16.18	12.94	9.53

Appendix Table 7. Average daily gain, average daily feed intake, and gain:feed of pigs in experimental pens (d 0-18), Exp. 1.

Pen	Trt	Rep	ADG, g	ADFI, g	G:F
1	1	1	201.01	251.01	0.80
2	1	2	191.50	234.43	0.82
3	1	3	127.10	193.60	0.66
4	1	4	203.10	270.92	0.75
5	1	5	185.79	232.32	0.80
6	1	6	186.04	238.99	0.78
7	1	7	186.20	269.94	0.69
8	1	8	149.01	212.58	0.70
9	1	9	142.17	187.63	0.76
10	2	1	260.52	332.28	0.78
11	2	2	204.55	271.89	0.75
12	2	3	148.57	210.23	0.71
13	2	4	185.19	244.53	0.76
14	2	5	234.85	287.16	0.82
15	2	6	232.74	259.68	0.90
16	2	7	210.13	286.68	0.73
17	2	8	159.60	214.97	0.74
18	2	9	214.29	249.15	0.86
19	3	1	221.38	289.98	0.76
20	3	2	181.82	236.74	0.77
21	3	3	202.44	249.79	0.81
22	3	4	247.84	288.60	0.86
23	3	5	256.49	301.95	0.85
24	3	6	229.29	267.68	0.86
25	3	7	180.22	244.42	0.74
26	3	8	210.87	242.99	0.87
27	3	9	211.89	279.56	0.76
28	4	1	167.93	223.91	0.75
29	4	2	207.91	255.89	0.81
30	4	3	207.07	251.68	0.82
31	4	4	260.82	305.56	0.85
32	4	5	234.78	282.61	0.83
33	4	6	230.22	266.84	0.86
34	4	7	233.25	308.21	0.76
35	4	8	219.75	267.60	0.82
36	4	9	186.43	225.85	0.83

Appendix Table 8. Analysis of variance for average daily gain, average daily feed intake, and gain:feed of pigs in experimental pens (d 0-18), Exp. 1.

Source	df	Mean Squares		
		ADG, g	ADFI, g	G:F
Total	35			
Error	24	677.7483	709.5217	0.00197
Replication	8	1,513.8795	1,724.1177	0.00539
C1: 1 vs 234	1	9,729.5278	7,043.3380	0.01763
C2: Linear ME	1	530.5110	55.4053	0.00436
C3: Quadratic ME	1	136.5810	63.8091	0.00060
C.V., %		12.82	10.38	5.62

Appendix Table 9. Average daily ME intake (kcal) and weight gain (g)/100 kcal ME intake of pigs in experimental pens, Exp. 1.

Pen	Trt	Rep	Avg daily ME intake, kcal	Wt gain (g)/100 kcal ME intake
1	1	1	871.08	23.05
2	1	2	813.53	23.51
3	1	3	671.86	18.90
4	1	4	940.19	21.58
5	1	5	806.23	23.02
6	1	6	829.36	22.40
7	1	7	936.76	19.85
8	1	8	737.71	20.17
9	1	9	651.13	21.81
10	2	1	1,153.12	22.57
11	2	2	943.52	21.65
12	2	3	729.55	20.34
13	2	4	848.59	21.80
14	2	5	996.52	23.54
15	2	6	901.17	25.80
16	2	7	994.88	21.10
17	2	8	746.01	21.37
18	2	9	864.61	24.75
19	3	1	977.34	22.62
20	3	2	797.90	22.76
21	3	3	841.87	24.02
22	3	4	972.68	25.45
23	3	5	1,017.66	25.17
24	3	6	902.16	25.39
25	3	7	823.77	21.85
26	3	8	818.97	25.72
27	3	9	942.22	22.46
28	4	1	732.25	22.91
29	4	2	836.86	24.81
30	4	3	823.09	25.13
31	4	4	999.27	26.07
32	4	5	924.23	25.37
33	4	6	872.64	26.35
34	4	7	1,007.96	23.11
35	4	8	875.15	25.08
36	4	9	738.61	25.21

Appendix Table 10. Analysis of variance for average daily ME intake (kcal) and weight gain (g)/100 kcal ME intake of pigs in experimental pens, Exp. 1.

Source	df	Mean Squares	
		Avg daily ME intake, kcal	Wt gain (g)/100 kcal ME intake
Total	35		
Error	24	6,616.5246	1.68362
Replication	8	14,264.9957	4.54803
C1: 1 vs 234	1	31,779.9219	32.81316
C2: Linear ME	1	1,764.8704	24.78080
C3: Quadratic ME	1	2,914.6867	0.28456
C.V., %		9.53	5.58

Appendix Table 11. Average daily lysine intake (g) and weight gain (g)/lysine intake of pigs in experimental pens, Exp. 1.

Pen	Trt	Rep	Avg daily lysine intake, g	Wt gain (g)/lysine intake (g)
1	1	1	3.76	53.39
2	1	2	3.52	54.46
3	1	3	2.90	43.77
4	1	4	4.06	49.98
5	1	5	3.48	53.31
6	1	6	3.58	51.90
7	1	7	4.05	45.99
8	1	8	3.19	46.73
9	1	9	2.81	50.52
10	2	1	4.98	52.27
11	2	2	4.08	50.15
12	2	3	3.15	47.11
13	2	4	3.67	50.49
14	2	5	4.31	54.52
15	2	6	3.89	59.75
16	2	7	4.30	48.86
17	2	8	3.22	49.50
18	2	9	3.74	57.34
19	3	1	4.35	50.90
20	3	2	3.55	51.20
21	3	3	3.75	54.03
22	3	4	4.33	57.25
23	3	5	4.53	56.63
24	3	6	4.01	57.11
25	3	7	3.67	49.16
26	3	8	3.64	57.85
27	3	9	4.19	50.53
28	4	1	3.36	50.00
29	4	2	3.84	54.17
30	4	3	3.77	54.85
31	4	4	4.58	56.91
32	4	5	4.24	55.38
33	4	6	4.00	57.52
34	4	7	4.62	50.45
35	4	8	4.01	54.75
36	4	9	3.39	55.03

Appendix Table 12. Analysis of variance for average daily lysine intake (g) and weight gain (g)/lysine intake of pigs in experimental pens, Exp. 1.

Source	df	Mean Squares	
		Avg daily lysine intake, g	Wt gain (g)/lysine intake (g)
Total	35		
Error	24	0.15907	8.80162
Replication	8	0.38920	23.53058
C1: 1 vs 234	1	1.59384	81.05068
C2: Linear ME	1	0.01227	20.20361
C3: Quadratic ME	1	0.01467	1.95320
C.V., %		10.37	5.64

Appendix Table 13. Average plasma urea nitrogen (mg/dL) of pigs in experimental pens on d 0, 7, and 18 (Exp. 1).

Pen	Trt	Rep	d 0	d 7	d 18
1	1	1	12.35	8.60	9.49
2	1	2	8.43	9.64	11.33
3	1	3	7.15	9.60	8.83
4	1	4	7.04	10.68	9.08
5	1	5	9.08	10.58	9.27
6	1	6	7.25	9.74	7.66
7	1	7	5.27	12.46	11.21
8	1	8	5.31	15.31	15.13
9	1	9	7.64	11.61	11.85
10	2	1	8.93	8.75	8.24
11	2	2	9.18	14.22	7.25
12	2	3	9.12	9.66	8.18
13	2	4	7.17	8.98	6.99
14	2	5	7.56	9.21	8.19
15	2	6	5.44	5.59	5.15
16	2	7	4.30	10.62	11.35
17	2	8	4.77	8.72	8.53
18	2	9	5.89	8.23	8.54
19	3	1	9.44	11.69	7.49
20	3	2	6.41	8.87	7.71
21	3	3	9.74	10.67	9.08
22	3	4	7.04	7.24	6.57
23	3	5	5.45	7.11	6.21
24	3	6	10.86	3.42	10.00
25	3	7	6.26	11.37	10.67
26	3	8	9.51	11.59	10.14
27	3	9	8.31	10.17	9.27
28	4	1	8.40	9.41	8.56
29	4	2	8.74	5.80	6.37
30	4	3	7.08	13.84	9.02
31	4	4	5.51	6.17	7.68
32	4	5	7.63	5.27	6.60
33	4	6	5.63	8.15	6.61
34	4	7	7.43	9.77	8.54
35	4	8	5.87	8.26	8.39
36	4	9	8.18	7.31	7.45

Appendix Table 14. Analysis of variance for average plasma urea nitrogen (mg/dL) of pigs in experimental pens on d 0, 7, and 18 (Exp. 1).

Source	df	Mean Squares		
		d 0	d 7	d 18
Total	35			
Error	24	2.5850	4.9459	1.8549
Replication	8	5.5495	8.9841	5.7420
C1: 1 vs 234	1	0.7024	27.5730	36.4822
C2: Linear ME	1	0.2473	5.5556	0.5689
C3: Quadratic ME	1	6.8338	0.7350	2.9587
C.V., %		21.49	23.66	15.68

Appendix Table 15. Average blood glucose (mg/dL) of pigs in experimental pens on d 0, 7, and 18 (Exp. 1).

Pen	Trt	Rep	d 0	d 7	d 18
1	1	1	157.00	119.00	133.75
2	1	2	173.00	115.50	126.25
3	1	3	171.00	110.25	118.00
4	1	4	168.50	109.75	142.25
5	1	5	181.25	95.50	116.75
6	1	6	153.00	114.25	111.50
7	1	7	151.50	116.50	101.50
8	1	8	140.00	84.00	80.25
9	1	9	179.50	94.50	91.00
10	2	1	196.00	116.50	127.50
11	2	2	154.25	132.50	152.50
12	2	3	138.50	114.50	127.50
13	2	4	131.00	108.25	110.25
14	2	5	164.00	101.75	102.75
15	2	6	121.50	127.00	116.75
16	2	7	136.25	124.50	129.00
17	2	8	167.00	123.00	119.75
18	2	9	139.00	118.50	119.75
19	3	1	165.75	143.00	132.50
20	3	2	171.25	118.00	127.75
21	3	3	129.25	119.50	138.50
22	3	4	150.75	108.25	118.25
23	3	5	154.00	109.50	127.50
24	3	6	108.50	88.00	148.50
25	3	7	141.50	105.25	96.25
26	3	8	139.50	119.25	104.00
27	3	9	115.75	110.75	100.25
28	4	1	164.75	136.75	118.25
29	4	2	142.25	138.00	133.25
30	4	3	180.75	123.25	146.75
31	4	4	147.25	114.25	130.25
32	4	5	176.00	101.50	144.00
33	4	6	114.50	106.75	111.25
34	4	7	142.25	130.50	106.75
35	4	8	120.25	103.25	101.00
36	4	9	119.25	117.25	109.75

Appendix Table 16. Analysis of variance for average blood glucose (mg/dL) of pigs in experimental pens on d 0, 7, and 18 (Exp. 1).

Source	df	Mean Squares		
		d 0	d 7	d 18
Total	35			
Error	24	290.9495	110.7669	202.4660
Replication	8	914.5161	318.7739	596.6593
C1: 1 vs 234	1	2,252.7367	735.0284	518.9867
C2: Linear ME	1	90.0035	1.3889	1.1250
C3: Quadratic ME	1	193.6123	167.1296	7.4074
C.V., %		11.36	9.20	11.85

Appendix Table 17. Average triglycerides (mg/dL) in plasma of pigs in experimental pens on d 0, 7, and 18 (Exp. 1).

Pen	Trt	Rep	d 0	d 7	d 18
1	1	1	39.25	32.75	35.75
2	1	2	49.00	18.50	38.75
3	1	3	83.00	31.50	39.00
4	1	4	53.25	68.75	44.25
5	1	5	69.00	63.25	39.50
6	1	6	76.25	64.00	32.25
7	1	7	30.75	39.75	31.75
8	1	8	55.50	69.25	57.00
9	1	9	53.00	63.50	52.75
10	2	1	75.00	40.00	46.00
11	2	2	59.25	29.00	77.50
12	2	3	59.75	27.25	47.50
13	2	4	52.75	53.25	27.25
14	2	5	72.75	48.00	26.75
15	2	6	73.25	89.50	33.75
16	2	7	40.75	41.00	34.75
17	2	8	56.50	34.75	31.00
18	2	9	53.25	33.00	29.75
19	3	1	61.50	29.75	38.75
20	3	2	42.00	21.50	48.75
21	3	3	52.75	27.00	43.50
22	3	4	53.25	51.00	34.00
23	3	5	53.00	53.75	33.75
24	3	6	64.50	43.50	45.50
25	3	7	85.50	48.00	40.00
26	3	8	43.50	42.50	31.00
27	3	9	31.00	34.50	26.50
28	4	1	45.00	34.25	40.50
29	4	2	53.25	25.25	38.50
30	4	3	63.50	36.50	57.50
31	4	4	58.75	47.25	28.50
32	4	5	69.75	43.25	35.50
33	4	6	58.00	42.75	22.75
34	4	7	47.25	35.75	27.00
35	4	8	45.00	48.50	41.75
36	4	9	56.00	26.50	21.50

Appendix Table 18. Analysis of variance for average triglycerides (mg/dL) in plasma of pigs in experimental pens on d 0, 7, and 18 (Exp. 1).

Source	df	Mean Squares		
		d 0	d 7	d 18
Total	35			
Error	24	166.0146	117.4796	112.6457
Replication	8	233.7687	586.8859	180.2661
C1: 1 vs 234	1	0.0006	657.6134	99.1875
C2: Linear ME	1	121.4201	172.6701	92.2535
C3: Quadratic ME	1	80.0567	19.8623	4.5938
C.V., %		22.78	25.36	27.68

Appendix Table 19. Average initial and final weights of pigs in experimental pens, Exp. 2.

Pen	CP Source	ME Level	Rep	Initial Wt, kg	Final Wt, kg
1	SPC	3523	1	7.32	19.60
2	SPC	3523	2	5.95	15.25
3	SPC	3523	3	4.82	15.60
4	SPC	3523	4	7.02	17.58
5	SPC	3523	5	5.91	16.14
6	SPC	3523	6	5.21	14.38
7	SPC	3523	7	4.45	14.03
8	SPC	3323	1	7.37	18.98
9	SPC	3323	2	6.03	17.17
10	SPC	3323	3	4.76	13.54
11	SPC	3323	4	6.95	17.56
12	SPC	3323	5	5.95	16.60
13	SPC	3323	6	5.20	14.74
14	SPC	3323	7	4.45	11.95
15	SDPP	3523	1	7.33	19.39
16	SDPP	3523	2	5.95	17.14
17	SDPP	3523	3	4.83	14.85
18	SDPP	3523	4	7.03	18.73
19	SDPP	3523	5	5.93	17.67
20	SDPP	3523	6	5.15	14.46
21	SDPP	3523	7	4.45	13.48
22	SDPP	3323	1	7.34	20.00
23	SDPP	3323	2	5.99	15.63
24	SDPP	3323	3	4.76	15.51
25	SDPP	3323	4	7.05	18.18
26	SDPP	3323	5	5.93	16.47
27	SDPP	3323	6	5.14	14.06
28	SDPP	3323	7	4.48	13.41

Appendix Table 20. Analysis of variance for initial and final weights of pigs in experimental pens, Exp. 2.

Source	df	Mean Squares	
		Initial Wt, kg	Final Wt, kg
Total	27		
Error	18	0.00116	0.50944
Replication	6	4.73197	18.82704
CP source	1	0.00003	1.22641
ME level	1	0.00009	0.72321
CP x ME Interaction	1	0.00000	0.00630
C.V., %		0.58	4.42

Appendix Table 21. Average daily gain, average daily feed intake, and gain:feed of pigs in experimental pens (d 0-7), Exp. 2.

Pen	CP Source	ME Level	Rep	ADG, g	ADFI, g	G:F
1	SPC	3523	1	115.80	149.35	0.78
2	SPC	3523	2	74.03	115.58	0.64
3	SPC	3523	3	127.71	192.64	0.66
4	SPC	3523	4	41.13	101.73	0.40
5	SPC	3523	5	57.36	111.47	0.51
6	SPC	3523	6	81.17	134.20	0.60
7	SPC	3523	7	91.99	132.03	0.70
8	SPC	3323	1	114.72	127.71	0.90
9	SPC	3323	2	114.72	166.67	0.69
10	SPC	3323	3	88.74	147.19	0.60
11	SPC	3323	4	36.80	98.48	0.37
12	SPC	3323	5	91.99	122.29	0.75
13	SPC	3323	6	70.35	115.80	0.61
14	SPC	3323	7	48.70	90.91	0.54
15	SDPP	3523	1	154.76	222.94	0.69
16	SDPP	3523	2	167.75	201.30	0.83
17	SDPP	3523	3	124.46	190.48	0.65
18	SDPP	3523	4	59.52	123.38	0.48
19	SDPP	3523	5	142.86	172.08	0.83
20	SDPP	3523	6	91.99	136.36	0.67
21	SDPP	3523	7	79.00	116.88	0.68
22	SDPP	3323	1	208.87	215.37	0.97
23	SDPP	3323	2	102.81	160.17	0.64
24	SDPP	3323	3	168.83	200.22	0.84
25	SDPP	3323	4	95.24	158.01	0.60
26	SDPP	3323	5	97.40	137.45	0.71
27	SDPP	3323	6	82.25	122.29	0.67
28	SDPP	3323	7	116.88	138.53	0.84

Appendix Table 22. Analysis of variance for average daily gain, average daily feed intake, and gain:feed of pigs in experimental pens (d 0-7), Exp. 2.

Source	df	Mean Squares		
		ADG, g	ADFI, g	G:F
Total	27			
Error	18	647.2751	512.8892	0.00886
Replication	6	3,773.4784	2,968.8388	0.04918
CP source	1	10,314.6253	8,554.3624	0.06509
ME level	1	29.5612	352.3732	0.01329
CP x ME Interaction	1	201.4826	47.7630	0.00260
C.V., %		25.01	15.46	13.98

Appendix Table 23. Average daily gain, average daily feed intake, and gain:feed of pigs in experimental pens (d 7-14), Exp. 2.

Pen	CP Source	ME Level	Rep	ADG, g	ADFI, g	G:F
1	SPC	3523	1	306.28	359.31	0.85
2	SPC	3523	2	223.38	281.82	0.79
3	SPC	3523	3	251.08	270.56	0.93
4	SPC	3523	4	269.48	341.99	0.79
5	SPC	3523	5	275.97	316.02	0.87
6	SPC	3523	6	273.81	292.21	0.94
7	SPC	3523	7	272.73	282.47	0.97
8	SPC	3323	1	304.11	351.73	0.86
9	SPC	3323	2	332.25	327.92	1.01
10	SPC	3323	3	240.26	225.11	1.07
11	SPC	3323	4	294.37	308.44	0.95
12	SPC	3323	5	312.77	335.50	0.93
13	SPC	3323	6	282.47	296.54	0.95
14	SPC	3323	7	219.70	230.52	0.95
15	SDPP	3523	1	306.28	336.58	0.91
16	SDPP	3523	2	281.39	344.16	0.82
17	SDPP	3523	3	249.35	246.75	1.01
18	SDPP	3523	4	378.79	365.80	1.04
19	SDPP	3523	5	330.09	363.64	0.91
20	SDPP	3523	6	298.70	301.95	0.99
21	SDPP	3523	7	231.60	224.03	1.03
22	SDPP	3323	1	360.39	409.09	0.88
23	SDPP	3323	2	260.82	277.06	0.94
24	SDPP	3323	3	301.95	293.29	1.03
25	SDPP	3323	4	390.69	376.62	1.04
26	SDPP	3323	5	321.43	349.57	0.92
27	SDPP	3323	6	295.45	337.66	0.88
28	SDPP	3323	7	261.90	258.66	1.01

Appendix Table 24. Analysis of variance for average daily gain, average daily feed intake, and gain:feed of pigs in experimental pens (d 7-14), Exp. 2.

Source	df	Mean Squares		
		ADG, g	ADFI, g	G:F
Total	27			
Error	18	989.3242	695.0064	0.00352
Replication	6	4,092.0488	7,809.9380	0.01026
CP source	1	6,008.5510	2,502.7385	0.01080
ME level	1	1,883.2120	90.7920	0.01160
CP x ME Interaction	1	0.3726	1,257.7241	0.01243
C.V., %		10.84	8.48	6.33

Appendix Table 25. Average daily gain, average daily feed intake, and gain:feed of pigs in experimental pens (d 0-18), Exp. 2.

Pen	CP Source	ME Level	Rep	ADG, g	ADFI, g	G:F
1	SPC	3523	1	278.20	318.60	0.87
2	SPC	3523	2	182.32	238.38	0.76
3	SPC	3523	3	221.80	279.88	0.79
4	SPC	3523	4	218.43	280.72	0.78
5	SPC	3523	5	217.59	294.19	0.74
6	SPC	3523	6	211.70	273.99	0.77
7	SPC	3523	7	208.33	261.78	0.80
8	SPC	3323	1	268.52	305.13	0.88
9	SPC	3323	2	252.95	312.71	0.81
10	SPC	3323	3	197.81	234.01	0.85
11	SPC	3323	4	237.37	292.51	0.81
12	SPC	3323	5	275.67	322.81	0.85
13	SPC	3323	6	234.85	281.14	0.84
14	SPC	3323	7	168.35	217.17	0.78
15	SDPP	3523	1	278.20	335.86	0.83
16	SDPP	3523	2	262.63	321.55	0.82
17	SDPP	3523	3	197.28	241.33	0.82
18	SDPP	3523	4	303.45	346.80	0.88
19	SDPP	3523	5	302.19	351.01	0.86
20	SDPP	3523	6	248.74	292.09	0.85
21	SDPP	3523	7	196.13	246.21	0.80
22	SDPP	3323	1	322.39	362.79	0.89
23	SDPP	3323	2	227.71	270.54	0.84
24	SDPP	3323	3	249.16	284.51	0.88
25	SDPP	3323	4	307.66	364.06	0.85
26	SDPP	3323	5	273.15	326.18	0.84
27	SDPP	3323	6	237.37	312.71	0.76
28	SDPP	3323	7	218.01	286.62	0.76

Appendix Table 26. Analysis of variance for average daily gain, average daily feed intake, and gain:feed of pigs in experimental pens (d 0-18), Exp. 2.

Source	df	Mean Squares		
		ADG, g	ADFI, g	G:F
Total	27			
Error	18	637.7778	630.5938	0.00119
Replication	6	4,034.1298	3,911.4635	0.00278
CP source	1	7,237.9297	6,580.2492	0.00438
ME level	1	740.3657	292.5089	0.00260
CP x ME Interaction	1	90.4322	106.5480	0.00438
C.V., %		10.40	8.52	4.19

Appendix Table 27. Average daily ME intake (kcal) and weight gain (g)/100 kcal ME intake of pigs in experimental pens, Exp. 2.

Pen	CP Source	ME Level	Rep	Avg daily ME intake, kcal	Wt gain (g)/100 kcal ME intake
1	SPC	3523	1	1122.21	24.76
2	SPC	3523	2	839.66	21.69
3	SPC	3523	3	985.83	22.47
4	SPC	3523	4	988.79	22.06
5	SPC	3523	5	1036.23	20.97
6	SPC	3523	6	965.07	21.91
7	SPC	3523	7	922.08	22.57
8	SPC	3323	1	1013.76	26.46
9	SPC	3323	2	1038.93	24.32
10	SPC	3323	3	777.45	25.41
11	SPC	3323	4	971.81	24.40
12	SPC	3323	5	1072.49	25.67
13	SPC	3323	6	934.06	25.11
14	SPC	3323	7	721.52	23.30
15	SDPP	3523	1	1182.99	23.49
16	SDPP	3523	2	1132.59	23.16
17	SDPP	3523	3	850.04	23.18
18	SDPP	3523	4	1221.54	24.81
19	SDPP	3523	5	1236.36	24.41
20	SDPP	3523	6	1028.82	24.15
21	SDPP	3523	7	867.23	22.59
22	SDPP	3323	1	1205.32	26.72
23	SDPP	3323	2	898.83	25.30
24	SDPP	3323	3	945.24	26.33
25	SDPP	3323	4	1209.52	25.41
26	SDPP	3323	5	1083.67	25.18
27	SDPP	3323	6	1038.93	22.82
28	SDPP	3323	7	952.23	22.87

Appendix Table 28. Analysis of variance for average daily ME intake (kcal) and weight gain (g)/100 kcal ME intake of pigs in experimental pens, Exp. 2.

Source	df	Mean Squares	
		Avg daily ME intake, kcal	Wt gain (g)/100 kcal ME intake
Total	27		
Error	18	7,386.8115	0.99925
Replication	6	45,667.2144	2.50372
CP source	1	76,485.6463	3.10223
ME level	1	9,497.3522	26.19023
CP x ME Interaction	1	740.7772	3.15571
C.V., %		8.52	4.17

Appendix Table 29. Average daily lysine intake (g) and weight gain (g)/lysine intake of pigs in experimental pens, Exp. 2.

Pen	CP Source	ME Level	Rep	Avg daily lysine intake, g	Wt gain (g)/lysine intake (g)
1	SPC	3523	1	4.78	58.21
2	SPC	3523	2	3.58	50.99
3	SPC	3523	3	4.20	52.83
4	SPC	3523	4	4.21	51.87
5	SPC	3523	5	4.41	49.31
6	SPC	3523	6	4.11	51.51
7	SPC	3523	7	3.93	53.05
8	SPC	3323	1	4.58	58.67
9	SPC	3323	2	4.69	53.93
10	SPC	3323	3	3.51	56.35
11	SPC	3323	4	4.39	54.10
12	SPC	3323	5	4.84	56.93
13	SPC	3323	6	4.22	55.69
14	SPC	3323	7	3.26	51.68
15	SDPP	3523	1	5.04	55.22
16	SDPP	3523	2	4.82	54.45
17	SDPP	3523	3	3.62	54.50
18	SDPP	3523	4	5.20	58.33
19	SDPP	3523	5	5.26	57.39
20	SDPP	3523	6	4.38	56.77
21	SDPP	3523	7	3.69	53.11
22	SDPP	3323	1	5.44	59.24
23	SDPP	3323	2	4.06	56.11
24	SDPP	3323	3	4.27	58.38
25	SDPP	3323	4	5.46	56.34
26	SDPP	3323	5	4.89	55.83
27	SDPP	3323	6	4.69	50.61
28	SDPP	3323	7	4.30	50.71

Appendix Table 30. Analysis of variance for average daily lysine intake (g) and weight gain (g)/lysine intake of pigs in experimental pens, Exp. 2.

Source	df	Mean Squares	
		Avg daily lysine intake, g	Wt gain (g)/lysine intake (g)
Total	27		
Error	18	0.14142	5.17095
Replication	6	0.87730	12.73152
CP source	1	1.46743	17.08203
ME level	1	0.06703	10.35789
CP x ME Interaction	1	0.02460	17.49060
C.V., %		8.50	4.16

Appendix Table 31. Average daily gain of weaned pigs, Exp. 3.

Pig	CP Source	ME Level	Rep	Day	ADG1[a], g	ADG2[b], g
1	SPC	3523	1	3	75.76	75.76
2	SPC	3323	1	3	-30.30	-30.30
3	SDPP	3523	1	3	106.06	106.06
4	SDPP	3323	1	3	-136.36	-136.36
5	SPC	3523	2	3	-136.36	-136.36
6	SPC	3323	2	3	60.61	60.61
7	SDPP	3523	2	3	-151.52	-151.52
8	SDPP	3323	2	3	-60.61	-60.61
9	SPC	3523	3	3	-15.15	-15.15
10	SPC	3323	3	3	45.45	45.45
11	SDPP	3523	3	3	45.45	45.45
12	SDPP	3323	3	3	90.91	90.91
13	SPC	3523	4	3	-106.06	-106.06
14	SPC	3323	4	3	-121.21	-121.21
15	SDPP	3523	4	3	-151.52	-151.52
16	SDPP	3323	4	3	-121.21	-121.21
17	SPC	3523	5	3	-75.76	-75.76
18	SPC	3323	5	3	-257.58	-257.58
19	SDPP	3523	5	3	212.12	212.12
20	SDPP	3323	5	3	106.06	106.06
21	SPC	3523	1	7	97.40	136.36
22	SPC	3323	1	7	58.44	34.09
23	SDPP	3523	1	7	123.38	147.73
24	SDPP	3323	1	7	-32.47	90.91
25	SPC	3523	2	7	155.84	329.55
26	SPC	3323	2	7	149.35	318.18
27	SDPP	3523	2	7	136.36	318.18
28	SDPP	3323	2	7	246.75	511.36
29	SPC	3523	3	7	58.44	170.45
30	SPC	3323	3	7	103.90	193.18
31	SDPP	3523	3	7	-77.92	-79.55
32	SDPP	3323	3	7	136.36	329.55
33	SPC	3523	4	7	19.48	125.00
34	SPC	3323	4	7	-97.40	-102.27
35	SDPP	3523	4	7	-51.95	-11.36

[a]ADG computed from d 0 to d pig was killed.
[b]ADG from d 0-d 3 for pigs killed on d 3, d 4-d 7 for pigs killed on d 7, and d 8-14 for pigs killed on d 14.

Appendix Table 31. Continued.

Pig	CP Source	ME level	Rep	Day	ADG1[a], g	ADG2[b], g
36	SDPP	3323	4	7	-116.88	-113.64
37	SPC	3523	5	7	77.92	193.18
38	SPC	3323	5	7	32.47	136.36
39	SDPP	3523	5	7	116.88	136.36
40	SDPP	3323	5	7	142.86	193.18
41	SPC	3523	1	14	100.65	181.82
42	SPC	3323	1	14	181.82	246.75
43	SDPP	3523	1	14	178.57	220.78
44	SDPP	3323	1	14	126.62	233.77
45	SPC	3523	2	14	246.75	357.14
46	SPC	3323	2	14	305.19	448.05
47	SDPP	3523	2	14	250.00	357.14
48	SDPP	3323	2	14	155.84	246.75
49	SPC	3523	3	14	207.79	324.68
50	SPC	3323	3	14	279.22	409.09
51	SDPP	3523	3	14	214.29	266.23
52	SDPP	3323	3	14	253.25	383.12
53	SPC	3523	4	14	162.34	285.71
54	SPC	3323	4	14	103.90	253.25
55	SDPP	3523	4	14	155.84	240.26
56	SDPP	3323	4	14	142.86	279.22
57	SPC	3523	5	14	220.78	383.12
58	SPC	3323	5	14	262.99	428.57
59	SDPP	3523	5	14	331.17	428.57
60	SDPP	3323	5	14	357.14	428.57

[a]ADG computed from d 0 to d pig was killed.
[b]ADG from d 0-d 3 for pigs killed on d 3, d 4-d 7 for pigs killed on d 7, and d 8-14 for pigs killed on d 14.

Appendix Table 32. Analysis of variance for average daily gain of weaned pigs, Exp. 3.

Source	df	Mean Squares	
		ADG1, kg	ADG2, kg
Total	59		
Error	44	8,310.708	12,984.660
Replication	4	40,419.691	64,080.403
CP source	1	5,257.699	1,082.475
ME level	1	421.668	731.015
Day	2	299,688.448	617,133.740
CP*ME	1	295.349	4,929.910
CP*Day	2	3,868.614	7,077.699
ME*Day	2	1,359.929	3,745.061
CP*ME*Day	2	4,539.007	18,088.964
C.V., %		111.74	77.36

[a]ADG computed from d 0 to d pig was killed.
[b]ADG from d 0-d 3 for pigs killed on d 3, d 4-d 7 for pigs killed on d 7, and d 8-14 for pigs killed on d 14.

Appendix Table 33. Relative weights (g/kg BW) of pancreas and small intestine in weaned pigs, Exp. 3.

Pig	CP Source	ME level	Rep	Day	Pancreas	Small intestine
1			1	0	0.94	26.05
2			2	0	1.10	31.62
3			3	0	1.12	31.32
4			4	0	1.16	33.42
5			1	0	0.94	26.05
6			2	0	1.10	31.62
7			3	0	1.12	31.32
8			4	0	1.16	33.42
9			1	0	0.94	26.05
10			2	0	1.10	31.62
11			3	0	1.12	31.32
12			4	0	1.16	33.42
13			1	0	0.94	26.05
14			2	0	1.10	31.62
15			3	0	1.12	31.32
16			4	0	1.16	33.42
17	SPC	3523	1	3	1.13	22.95
18	SPC	3523	2	3	1.14	28.24
19	SPC	3523	3	3	0.89	25.70
20	SPC	3523	4	3	1.26	21.46
21	SPC	3523	5	3	1.04	18.32
22	SPC	3323	1	3	0.31	20.99
23	SPC	3323	2	3	1.06	29.35
24	SPC	3323	3	3	1.31	22.58
25	SPC	3323	4	3	1.19	25.73
26	SPC	3323	5	3	1.72	26.50
27	SDPP	3523	1	3	0.62	23.05
28	SDPP	3523	2	3	1.28	21.10
29	SDPP	3523	3	3	0.95	27.59
30	SDPP	3523	4	3	1.41	27.29
31	SDPP	3523	5	3	0.94	25.79
32	SDPP	3323	1	3	1.31	25.01
33	SDPP	3323	2	3	1.38	22.63
34	SDPP	3323	3	3	1.35	23.77
35	SDPP	3323	4	3	1.63	25.33
36	SDPP	3323	5	3	0.82	22.82
37	SPC	3523	1	7	1.74	39.37
38	SPC	3523	2	7	1.95	38.84
39	SPC	3523	3	7	2.03	37.65
40	SPC	3523	4	7	1.77	30.37

Appendix Table 33. Continued.

Pig	CP Source	ME level	Rep	Day	Pancreas	Small intestine
41	SPC	3523	5	7	1.55	33.51
42	SPC	3323	1	7	1.53	35.33
43	SPC	3323	2	7	1.80	34.96
44	SPC	3323	3	7	1.32	28.09
45	SPC	3323	4	7	1.31	25.14
46	SPC	3323	5	7	1.44	35.24
47	SDPP	3523	1	7	1.28	33.57
48	SDPP	3523	2	7	1.82	31.65
49	SDPP	3523	3	7	1.14	20.31
50	SDPP	3523	4	7	1.12	29.79
51	SDPP	3523	5	7	1.46	31.03
52	SDPP	3323	1	7	1.31	30.80
53	SDPP	3323	2	7	1.59	24.73
54	SDPP	3323	3	7	1.80	32.70
55	SDPP	3323	4	7	1.42	18.47
56	SDPP	3323	5	7	1.91	37.21
57	SPC	3523	1	14	1.95	46.38
58	SPC	3523	2	14	2.64	41.04
59	SPC	3523	3	14	2.03	43.79
60	SPC	3523	4	14	1.95	51.93
61	SPC	3523	5	14	1.52	45.00
62	SPC	3323	1	14	2.23	52.44
63	SPC	3323	2	14	1.86	37.50
64	SPC	3323	3	14	1.91	57.44
65	SPC	3323	4	14	2.03	46.19
66	SPC	3323	5	14	2.40	52.75
67	SDPP	3523	1	14	1.82	52.04
68	SDPP	3523	2	14	2.13	35.14
69	SDPP	3523	3	14	1.77	44.59
70	SDPP	3523	4	14	1.85	37.96
71	SDPP	3523	5	14	1.82	40.68
72	SDPP	3323	1	14	1.91	45.69
73	SDPP	3323	2	14	2.08	44.57
74	SDPP	3323	3	14	2.26	34.68
75	SDPP	3323	4	14	1.99	45.31
76	SDPP	3323	5	14	1.85	41.61

Appendix Table 34. Analysis of variance for relative weights (g/kg BW) of pancreas and small intestine in weaned pigs, Exp. 3.

Source	df	Mean Squares	
		Pancreas	Small intestine
Total	59		
Error	44	0.07379	24.40783
Replication	4	0.13766	24.52376
CP source	1	0.06600	159.64228
ME level	1	0.06868	0.00542
Day	2	3.72395	2,172.21796
CP*ME	1	0.31828	2.37208
CP*Day	2	0.06748	46.61504
ME*Day	2	0.04773	23.60234
CP*ME*Day	2	0.08080	19.12426
C.V., %		17.33	14.74

Appendix Table 35. Relative weights (g/kg BW) of stomach and the combined weights of stomach and small intestine in weaned pigs, Exp. 3.

Pig	CP Source	ME level	Rep	Day	Stomach	Stomach + Small intestine
1			1	0	4.83	36.45
2			2	0	4.27	35.59
3			3	0	4.70	38.11
4			4	0	4.83	36.45
5			1	0	4.27	35.59
6			2	0	4.70	38.11
7			3	0	4.83	36.45
8			4	0	4.27	35.59
9			1	0	4.70	38.11
10			2	0	4.83	36.45
11			3	0	4.27	35.59
12			4	0	4.70	38.11
13	SPC	3523	1	3	4.33	32.56
14	SPC	3523	2	3	4.26	29.95
15	SPC	3523	3	3	5.05	26.51
16	SPC	3523	4	3	3.88	22.20
17	SPC	3323	1	3	4.19	33.54
18	SPC	3323	2	3	4.59	27.18
19	SPC	3323	3	3	5.30	31.03
20	SPC	3323	4	3	5.02	31.52
21	SDPP	3523	1	3	.	.
22	SDPP	3523	2	3	4.22	31.82
23	SDPP	3523	3	3	5.56	32.84
24	SDPP	3523	4	3	4.18	29.96
25	SDPP	3323	1	3	3.92	26.55
26	SDPP	3323	2	3	4.50	28.27
27	SDPP	3323	3	3	4.72	30.05
28	SDPP	3323	4	3	3.38	26.21
29	SPC	3523	1	7	5.80	44.64
30	SPC	3523	2	7	4.79	42.44
31	SPC	3523	3	7	6.53	36.90
32	SPC	3523	4	7	5.09	38.59
33	SPC	3323	1	7	6.22	41.18
34	SPC	3323	2	7	5.97	34.07
35	SPC	3323	3	7	5.55	30.69
36	SPC	3323	4	7	5.82	41.06
37	SDPP	3523	1	7	5.69	37.34
38	SDPP	3523	2	7	5.14	25.45
39	SDPP	3523	3	7	5.72	35.51
40	SDPP	3523	4	7	6.38	37.40

Appendix Table 35. Continued.

Pig	CP Source	ME level	Rep	Day	Stomach	Stomach + Small intestine
41	SDPP	3323	1	7	5.52	30.25
42	SDPP	3323	2	7	5.52	38.22
43	SDPP	3323	3	7	4.79	23.27
44	SDPP	3323	4	7	5.57	42.78
45	SPC	3523	1	14	7.47	48.51
46	SPC	3523	2	14	7.07	50.86
47	SPC	3523	3	14	12.39	64.31
48	SPC	3523	4	14	7.34	52.33
49	SPC	3323	1	14	6.26	43.76
50	SPC	3323	2	14	9.23	66.67
51	SPC	3323	3	14	10.87	57.05
52	SPC	3323	4	14	8.01	60.76
53	SDPP	3523	1	14	6.47	41.61
54	SDPP	3523	2	14	6.49	51.08
55	SDPP	3523	3	14	8.77	46.73
56	SDPP	3523	4	14	6.49	47.17
57	SDPP	3323	1	14	7.41	51.97
58	SDPP	3323	2	14	7.59	42.27
59	SDPP	3323	3	14	7.57	52.88
60	SDPP	3323	4	14	6.09	47.70

Appendix Table 36. Analysis of variance for relative weights (g/kg BW) of stomach and the combined weights of stomach and small intestine in weaned pigs, Exp. 3.

Source	df	Mean Squares	
		Stomach	Stomach + Small intestine
Total	46		
Error	32	0.92741	33.58682
Replication	3	4.57901	3.11025
CP source	1	4.64758	202.80682
ME level	1	0.00208	0.03898
Day	2	45.81586	1,999.11974
CP*ME	1	0.66608	5.19688
CP*Day	2	2.08689	64.08296
ME*Day	2	0.00904	21.97676
CP*ME*Day	2	0.23513	24.91933
C.V., %		16.01	14.76

Appendix Table 37. Weights (g) of stomach and small intestine in weaned pigs, Exp. 3.

Pig	CP Source	ME level	Rep	Day	Stomach	Small intestine
1			1	0	.	151.2
2			2	0	24.7	.
3			3	0	16.5	99.0
4			4	0	27.6	131.9
5			1	0	.	132.1
6			2	0	.	135.1
7			3	0	23.1	168.2
8			4	0	19.4	149.6
9			1	0	.	156.7
10			2	0	29.1	185.9
11			3	0	25.3	121.7
12			4	0	24.4	184.7
13			1	0	.	173.5
14			2	0	.	159.5
15			3	0	23.9	206.3
16			4	0	29.2	176.9
17	SPC	3523	1	3	.	126.2
18	SPC	3523	2	3	23.6	154
19	SPC	3523	3	3	20.5	123.8
20	SPC	3523	4	3	22.5	95.6
21	SPC	3523	5	3	23.8	112.4
22	SPC	3323	1	3	.	114.5
23	SPC	3323	2	3	23.6	165.4
24	SPC	3323	3	3	19.0	93.4
25	SPC	3323	4	3	23.6	114.6
26	SPC	3323	5	3	25.1	132.5
27	SDPP	3523	1	3	.	141.4
28	SDPP	3523	2	3	.	110.3
29	SDPP	3523	3	3	19.2	125.4
30	SDPP	3523	4	3	24.5	120.3
31	SDPP	3523	5	3	31.9	196.9
32	SDPP	3323	1	3	.	123.9
33	SDPP	3323	2	3	21.4	123.4
34	SDPP	3323	3	3	22.7	119.9
35	SDPP	3323	4	3	20.6	110.5
36	SDPP	3323	5	3	22.3	150.4
37	SPC	3523	1	7	.	214.7
38	SPC	3523	2	7	37.2	248.9
39	SPC	3523	3	7	30.9	243.0
40	SPC	3523	4	7	35.0	162.9

Appendix Table 37. Continued.

Pig	CP Source	ME level	Rep	Day	Stomach	Small intestine
41	SPC	3523	5	7	28.2	185.8
42	SPC	3323	1	7	.	194.3
43	SPC	3323	2	7	38.7	217.7
44	SPC	3323	3	7	39.9	187.7
45	SPC	3323	4	7	24.2	109.7
46	SPC	3323	5	7	31.5	190.6
47	SDPP	3523	1	7	.	219.7
48	SDPP	3523	2	7	34.4	191.3
49	SDPP	3523	3	7	27.1	107.1
50	SDPP	3523	4	7	26.0	135.4
51	SDPP	3523	5	7	37.1	180.5
52	SDPP	3323	1	7	.	166.6
53	SDPP	3323	2	7	37.9	169.7
54	SDPP	3323	3	7	41.4	245.2
55	SDPP	3323	4	7	22.0	84.8
56	SDPP	3323	5	7	32.4	216.5
57	SPC	3523	1	14	.	290.9
58	SPC	3523	2	14	70.6	388.0
59	SPC	3523	3	14	55.6	344.3
60	SPC	3523	4	14	80.5	337.5
61	SPC	3523	5	14	60.7	372.2
62	SPC	3323	1	14	.	348.0
63	SPC	3323	2	14	68.3	409.0
64	SPC	3323	3	14	81.8	509.1
65	SPC	3323	4	14	64.7	275.0
66	SPC	3323	5	14	72.8	479.5
67	SDPP	3523	1	14	.	340.6
68	SDPP	3523	2	14	62.0	337.0
69	SDPP	3523	3	14	50.7	348.6
70	SDPP	3523	4	14	58.2	251.9
71	SDPP	3523	5	14	62.8	393.8
72	SDPP	3323	1	14	.	294.9
73	SDPP	3323	2	14	63.3	380.8
74	SDPP	3323	3	14	63.8	291.6
75	SDPP	3323	4	14	50.6	302.7
76	SDPP	3323	5	14	62.0	423.6

Appendix Table 38. Analysis of variance for weights (g) of stomach and small intestine in weaned pigs, Exp. 3.

Source	Stomach		Small intestine	
	df	Mean squares	df	Mean squares
Total	45		58	
Error	31	39.2354	43	1,592.3448
Replication	3	48.0119	4	10,500.8253
CP source	1	131.9563	1	4,170.3398
ME level	1	11.7181	1	213.5468
Day	2	6,933.1237	2	279,952.9544
CP*ME	1	15.7827	1	182.6657
CP*Day	2	136.1484	2	3,280.4569
ME*Day	2	23.6284	2	2,736.9241
CP*ME*Day	2	11.3089	2	2,765.1288
C.V., %		15.38		17.86

Appendix Table 39. Villous height (mm), villous width (mm), crypt depth (mm), and villous height:crypt depth (V:C) in weaned pigs, Exp. 3.

Pig	CP Source	ME level	Rep	Day	Villous height, mm	Crypt depth, mm	V:C	Villous width, mm
1			1	0	0.333	0.183	1.861	.
2			1	0	0.370	0.170	2.250	.
3			1	0	0.257	0.157	1.711	.
4			1	0	0.178	0.213	0.904	.
5			2	0	0.387	0.132	2.949	0.098
6			2	0	0.448	0.152	2.959	0.112
7			2	0	0.417	0.108	3.832	0.088
8			2	0	0.466	0.144	3.275	0.099
9			3	0	0.537	0.130	4.194	0.090
10			3	0	0.502	0.114	4.407	0.094
11			3	0	0.534	0.146	4.033	0.098
12			3	0	0.576	0.144	4.083	0.092
13			4	0	0.730	0.108	6.700	0.096
14			4	0	0.560	0.126	4.550	0.091
15			5	0	0.445	0.141	3.161	0.100
16			5	0	0.543	0.151	3.605	0.099
17			5	0	0.375	0.100	3.750	0.110
18	SPC	3523	1	3	0.267	0.222	1.236	.
19	SPC	3323	1	3	0.221	0.195	1.170	.
20	SDPP	3523	1	3	0.270	0.189	1.444	.
21	SDPP	3323	1	3	0.282	0.179	1.627	.
22	SPC	3523	2	3	0.482	0.142	3.450	.
23	SPC	3323	2	3	0.489	0.162	3.027	0.113
24	SDPP	3523	2	3	0.583	0.160	3.648	0.097
25	SDPP	3323	2	3	0.369	0.159	2.365	0.089
26	SPC	3523	3	3	0.281	0.178	1.592	0.107
27	SPC	3323	3	3	0.223	0.158	1.415	0.110
28	SDPP	3523	3	3	0.352	0.165	2.118	0.108
29	SDPP	3323	3	3	0.356	0.144	2.481	0.102
30	SPC	3523	4	3	0.236	0.147	1.609	0.101
31	SPC	3323	4	3	0.325	0.146	2.235	0.091
32	SDPP	3523	4	3	0.310	0.167	1.884	0.093
33	SDPP	3323	4	3	0.222	0.144	1.576	0.085
34	SPC	3523	5	3	0.296	0.159	1.900	0.111
35	SPC	3323	5	3	0.254	0.150	1.702	0.093
36	SDPP	3523	5	3	0.425	0.155	2.759	0.093
37	SDPP	3323	5	3	0.424	0.138	3.105	0.111
38	SPC	3523	1	7	0.312	0.284	1.104	.
39	SPC	3323	1	7	0.313	0.287	1.120	.
40	SDPP	3523	1	7	0.340	0.255	1.351	.

Appendix Table 39. Continued.

Pig	CP Source	ME level	Rep	Day	Villous height, mm	Crypt depth, mm	V:C	Villous width, mm
41	SDPP	3323	1	7	0.205	0.226	0.912	.
42	SPC	3523	2	7	0.468	0.207	2.238	0.120
43	SPC	3323	2	7	0.344	0.198	1.805	0.105
44	SDPP	3523	2	7	0.535	0.179	3.068	0.110
45	SDPP	3323	2	7	0.385	0.162	2.438	0.106
46	SPC	3523	3	7	0.312	0.229	1.380	0.123
47	SPC	3323	3	7	0.295	0.203	1.460	0.107
48	SDPP	3523	3	7	0.233	0.137	1.697	0.095
49	SDPP	3323	3	7	0.358	0.211	1.761	0.095
50	SPC	3523	4	7	0.262	0.209	1.268	0.103
51	SPC	3323	4	7	0.209	0.139	1.513	0.098
52	SDPP	3523	4	7	0.305	0.206	1.722	0.100
53	SDPP	3323	4	7	0.181	0.135	1.364	0.092
54	SPC	3523	5	7	0.218	0.208	1.051	0.116
55	SPC	3323	5	7	0.242	0.224	1.086	0.108
56	SDPP	3523	5	7	0.374	0.212	1.772	0.103
57	SDPP	3323	5	7	0.364	0.171	2.147	0.097
58	SPC	3523	1	14	0.300	0.264	1.173	.
59	SPC	3323	1	14	0.321	0.359	0.902	.
60	SDPP	3523	1	14	0.333	0.319	1.052	.
61	SDPP	3323	1	14	0.334	0.324	1.058	.
62	SPC	3523	2	14	0.405	0.235	1.734	0.100
63	SPC	3323	2	14	0.415	0.239	1.755	0.097
64	SDPP	3523	2	14	0.440	0.244	1.804	0.113
65	SDPP	3323	2	14	0.400	0.300	1.318	0.118
66	SPC	3523	3	14	0.321	0.262	1.241	0.143
67	SPC	3323	3	14	0.464	0.282	1.654	0.117
68	SDPP	3323	3	14	0.422	0.209	2.044	0.118
69	SPC	3523	4	14	0.467	0.246	1.949	0.116
70	SPC	3323	4	14	0.405	0.243	1.683	0.113
71	SDPP	3523	4	14	0.533	0.159	3.420	0.100
72	SDPP	3323	4	14	0.525	0.201	2.612	0.103
73	SPC	3523	5	14	0.381	0.254	1.555	0.118
74	SPC	3323	5	14	0.413	0.290	1.431	0.114
75	SDPP	3523	5	14	0.563	0.196	2.928	0.106
76	SDPP	3323	5	14	0.645	0.245	2.706	0.116

Appendix Table 40. Analysis of variance for measurements of small intestine morphology in weaned pigs, Exp. 3.

Source	df	Mean Squares			
		Villous height	Crypt depth	Villous width[a]	V:C
Total	58				
Error	43	0.00629	0.00067	0.00007	0.26866
Replication	4	0.03993	0.01089	0.00028	2.32984
CP source	1	0.04018	0.00606	0.00055	3.16031
ME level	1	0.00672	0.00001	0.00019	0.24253
Day	2	0.07038	0.04065	0.00056	1.29082
CP*ME	1	0.00395	0.00003	0.00019	0.12903
CP*Day	2	0.00235	0.00079	0.00004	0.07449
ME*Day	2	0.00516	0.00302	0.00004	0.01263
CP*ME*Day	2	0.00020	0.00002	0.00003	0.01116
C.V., %		22.27	12.51	7.82	28.15

[a]Villous width Total df = 45, Error df = 31, Replication df = 3.

Appendix Table 41. Serum immunoglobulin levels in weaned pigs, Exp. 3.

Pig	CP Source	ME level	Rep	Day	IgG, mg/mL	IgA, µg/mL
1			1	0	13.66	.
2			1	0	13.09	57.12
3			1	0	12.78	57.87
4			1	0	0.00	.
5			2	0	17.20	144.30
6			2	0	12.60	109.60
7			2	0	8.57	60.84
8			2	0	19.58	74.12
9			3	0	9.32	70.15
10			3	0	13.86	100.70
11			3	0	10.46	88.96
12			3	0	12.42	376.20
13			4	0	17.67	305.70
14			4	0	8.25	92.97
15			5	0	10.14	95.26
16			5	0	5.71	75.31
17			5	0	9.32	49.86
18			5	0	4.39	139.00
19	SPC	3523	1	3	8.66	115.45
20	SPC	3323	1	3	8.37	112.83
21	SDPP	3523	1	3	12.94	102.27
22	SDPP	3323	1	3	8.95	43.84
23	SPC	3523	2	3	9.01	48.08
24	SPC	3323	2	3	2.80	91.19
25	SDPP	3523	2	3	13.43	89.09
26	SDPP	3323	2	3	12.94	182.00
27	SPC	3523	3	3	7.46	75.62
28	SPC	3323	3	3	10.56	56.13
29	SDPP	3523	3	3	9.55	116.80
30	SDPP	3323	3	3	11.77	111.80
31	SPC	3523	4	3	10.92	100.90
32	SPC	3323	4	3	14.38	107.70
33	SDPP	3523	4	3	12.64	81.26
34	SDPP	3323	4	3	10.90	102.50
35	SPC	3523	5	3	9.29	106.40
36	SPC	3323	5	3	10.68	86.59
37	SDPP	3523	5	3	5.78	95.34
38	SDPP	3323	5	3	11.14	111.60
39	SPC	3523	1	7	7.41	102.81
40	SPC	3323	1	7	8.48	52.24

Appendix Table 41. Continued.

Pig	CP Source	ME level	Rep	Day	IgG, mg/mL	IgA, μg/mL
41	SDPP	3523	1	7	7.93	63.29
42	SDPP	3323	1	7	8.27	37.14
43	SPC	3523	2	7	5.06	107.30
44	SPC	3323	2	7	8.03	66.99
45	SDPP	3523	2	7	8.68	65.39
46	SDPP	3323	2	7	23.89	98.82
47	SPC	3523	3	7	13.34	67.37
48	SPC	3323	3	7	5.99	126.10
49	SDPP	3523	3	7	7.29	103.50
50	SDPP	3323	3	7	7.35	112.90
51	SPC	3523	4	7	10.71	88.90
52	SPC	3323	4	7	9.57	85.11
53	SDPP	3523	4	7	8.79	86.34
54	SDPP	3323	4	7	4.68	95.34
55	SPC	3523	5	7	6.17	65.15
56	SPC	3323	5	7	8.77	146.30
57	SDPP	3523	5	7	5.91	131.80
58	SDPP	3323	5	7	5.11	83.33
59	SPC	3523	1	14	8.11	89.45
60	SPC	3323	1	14	3.68	195.05
61	SDPP	3523	1	14	10.71	199.97
62	SDPP	3323	1	14	8.28	124.23
63	SPC	3523	2	14	3.62	91.95
64	SPC	3323	2	14	3.63	131.70
65	SDPP	3523	2	14	4.15	141.80
66	SDPP	3323	2	14	4.73	146.00
67	SPC	3523	3	14	8.35	97.14
68	SPC	3323	3	14	7.51	281.80
69	SDPP	3523	3	14	5.35	345.60
70	SDPP	3323	3	14	7.38	125.00
71	SPC	3523	4	14	7.52	286.80
72	SPC	3323	4	14	9.39	239.40
73	SDPP	3523	4	14	8.97	119.00
74	SDPP	3323	4	14	8.65	111.90
75	SPC	3523	5	14	4.52	241.10
76	SPC	3323	5	14	7.84	128.10
77	SDPP	3523	5	14	3.96	170.70
78	SDPP	3323	5	14	4.28	112.40

Appendix Table 42. Analysis of variance for serum immunoglobulin levels in weaned pigs, Exp. 3.

Source	df	Mean Squares	
		IgG, mg/mL	IgA, μg/mL
Total	59		
Error	44	11.27023	2,445.0986
Replication	4	11.87986	2,271.4497
CP source	1	10.06141	108.5415
ME level	1	2.30888	136.6249
Day	2	64.39633	38,657.7910
CP*ME	1	2.69240	4,791.1258
CP*Day	2	3.59702	1,292.4928
ME*Day	2	1.00132	967.7086
CP*ME*Day	2	2.59313	4,754.2107
C.V., %		39.95	41.77

Appendix Table 43. Average IgG and IgA (μg/mL) levels in intestinal chyme of weaned pigs, Exp. 3.

Pig	CP Source	ME level	Rep	Day	Ave IgG	Ave IgA
1			1	0	539.02	.
2			1	0	426.96	2,420.85
3			1	0	702.03	4,564.35
4			1	0	625.65	.
5			2	0	187.95	196.17
6			2	0	314.89	64.98
7			2	0	190.00	363.23
8			2	0	267.38	55.43
9			3	0	469.76	224.50
10			3	0	512.55	166.87
11			3	0	170.42	548.70
12			3	0	165.38	213.88
13			4	0	171.85	93.10
14			4	0	296.57	138.51
15			5	0	261.82	278.52
16			5	0	722.89	139.43
17			5	0	382.62	257.47
18			5	0	37.03	218.60
19	SPC	3523	1	3	50.48	221.17
20	SPC	3323	1	3	51.99	167.17
21	SDPP	3523	1	3	828.40	229.88
22	SDPP	3323	1	3	57.27	134.38
23	SPC	3523	2	3	465.08	297.17
24	SPC	3323	2	3	124.81	119.88
25	SDPP	3523	2	3	3,518.68	521.30
26	SDPP	3323	2	3	5,976.67	115.86
27	SPC	3523	3	3	58.11	643.40
28	SPC	3323	3	3	177.86	184.17
29	SDPP	3523	3	3	4,242.50	682.50
30	SDPP	3323	3	3	2,937.00	174.58
31	SPC	3523	4	3	122.07	639.33
32	SPC	3323	4	3	278.70	324.17
33	SDPP	3523	4	3	41.85	169.46
34	SDPP	3323	4	3	204.85	311.45
35	SPC	3523	5	3	102.34	404.17
36	SPC	3323	5	3	220.99	388.87
37	SDPP	3523	5	3	2,246.00	278.37
38	SDPP	3323	5	3	1,112.17	253.60
39	SPC	3523	1	7	15.59	77.98
40	SPC	3323	1	7	52.56	23.61

Appendix Table 43. Continued.

Pig	CP Source	ME level	Rep	Day	Ave IgG	Ave IgA
41	SDPP	3523	1	7	351.79	85.88
42	SDPP	3323	1	7	240.06	75.19
43	SPC	3523	2	7	100.57	214.53
44	SPC	3323	2	7	155.17	85.54
45	SDPP	3523	2	7	2,598.90	161.18
46	SDPP	3323	2	7	2,227.17	376.60
47	SPC	3523	3	7	24.17	185.40
48	SPC	3323	3	7	33.99	496.30
49	SDPP	3523	3	7	38.48	481.47
50	SDPP	3323	3	7	442.65	209.83
51	SPC	3523	4	7	240.78	208.93
52	SPC	3323	4	7	144.12	978.80
53	SDPP	3523	4	7	1,570.05	332.48
54	SDPP	3323	4	7	128.68	425.07
55	SPC	3523	5	7	42.97	32.73
56	SPC	3323	5	7	110.70	94.79
57	SDPP	3523	5	7	106.42	139.17
58	SDPP	3323	5	7	2,204.50	283.80
59	SPC	3523	1	14	23.71	58.39
60	SPC	3323	1	14	65.84	102.14
61	SDPP	3523	1	14	116.77	171.74
62	SDPP	3323	1	14	79.99	125.53
63	SPC	3523	2	14	50.95	611.44
64	SPC	3323	2	14	81.89	243.50
65	SDPP	3523	2	14	193.83	211.93
66	SDPP	3323	2	14	187.33	333.93
67	SPC	3523	3	14	94.97	309.90
68	SPC	3323	3	14	39.85	217.87
69	SDPP	3523	3	14	67.62	252.97
70	SDPP	3323	3	14	172.78	466.50
71	SPC	3523	4	14	66.27	395.40
72	SPC	3323	4	14	147.30	329.50
73	SDPP	3523	4	14	499.88	347.67
74	SDPP	3323	4	14	1,966.00	477.60
75	SPC	3523	5	14	14.27	244.33
76	SPC	3323	5	14	18.73	53.73
77	SDPP	3523	5	14	853.67	329.07
78	SDPP	3323	5	14	2,609.83	348.20

Appendix Table 44. Analysis of variance for log of average IgG and IgA (μg/mL) levels in intestinal chyme of weaned pigs, Exp. 3.

Source	df	Mean Squares	
		Ave IgG	Ave IgA
Total	59		
Error	44	1.39609	0.32136
Replication	4	4.08050	2.91145
CP source	1	53.69166	0.66636
ME level	1	1.41407	0.41151
Day	2	5.14525	1.10251
CP*ME	1	0.04428	0.16480
CP*Day	2	0.00631	0.59193
ME*Day	2	0.40476	0.87672
CP*ME*Day	2	0.25438	0.24037
C.V., %		22.41	10.48

Appendix Table 45. IgG levels (ug/mL) in chyme from duodenum, jejunum, and ileum of weaned pigs, Exp. 3.

Pig	CP Source	ME level	Rep	Day	Duodenum	Jejunum	Ileum
1			1	0	476.36	516.49	624.22
2			1	0	376.78	207.16	696.94
3			1	0	533.01	611.02	962.05
4			1	0	.	357.58	893.73
5			2	0	74.50	263.60	225.75
6			2	0	608.50	209.88	126.29
7			2	0	242.75	171.40	155.85
8			2	0	.	347.70	187.05
9			3	0	.	175.12	764.40
10			3	0	615.60	.	409.50
11			3	0	153.60	135.50	222.15
12			3	0	194.70	160.65	140.80
13			4	0	172.30	.	171.40
14			4	0	391.10	258.60	240.00
15			5	0	285.20	216.90	283.35
16			5	0	130.92	190.35	1,847.40
17			5	0	330.20	295.80	521.85
18			5	0	63.52	20.05	27.53
19	SPC	3523	1	3	51.23	55.12	45.08
20	SPC	3323	1	3	76.93	32.70	46.33
21	SDPP	3523	1	3	259.54	731.08	1,494.57
22	SDPP	3323	1	3	59.08	39.08	73.64
23	SPC	3523	2	3	476.40	497.55	421.30
24	SPC	3323	2	3	61.84	194.00	118.60
25	SDPP	3523	2	3	369.35	284.70	9,902.00
26	SDPP	3323	2	3	1,683.00	4,539.00	11,708.00
27	SPC	3523	3	3	92.66	44.53	37.15
28	SPC	3323	3	3	210.70	224.10	98.77
29	SDPP	3523	3	3	2,239.50	5,787.00	4,701.00
30	SDPP	3323	3	3	1,102.00	2,415.00	5,294.00
31	SPC	3523	4	3	119.46	20.74	226.00
32	SPC	3323	4	3	204.40	.	353.00
33	SDPP	3523	4	3	17.02	17.14	91.38
34	SDPP	3323	4	3	100.88	90.68	423.00
35	SPC	3523	5	3	157.58	74.46	74.98
36	SPC	3323	5	3	305.00	116.98	241.00
37	SDPP	3523	5	3	742.00	1,608.00	4,388.00
38	SDPP	3323	5	3	976.00	523.50	1,837.00
39	SPC	3523	1	7	.	14.77	16.40
40	SPC	3323	1	7	27.47	125.61	4.60

Appendix Table 45. Continued.

Pig	CP Source	ME level	Rep	Day	Duodenum	Jejunum	Ileum
41	SDPP	3523	1	7	173.47	303.84	578.07
42	SDPP	3323	1	7	695.26	22.77	2.14
43	SPC	3523	2	7	186.60	93.26	21.85
44	SPC	3323	2	7	217.00	216.90	31.62
45	SDPP	3523	2	7	2,251.00	2,581.70	2,964.00
46	SDPP	3323	2	7	446.50	3,219.00	3,016.00
47	SPC	3523	3	7	47.57	15.14	9.81
48	SPC	3323	3	7	63.59	17.99	20.39
49	SDPP	3523	3	7	71.52	17.39	26.52
50	SDPP	3323	3	7	227.25	431.20	669.50
51	SPC	3523	4	7	283.95	336.50	101.90
52	SPC	3323	4	7	151.80	153.15	127.41
53	SDPP	3523	4	7	670.10	546.04	3,494.00
54	SDPP	3323	4	7	55.38	196.46	134.20
55	SPC	3523	5	7	70.32	42.65	15.93
56	SPC	3323	5	7	34.39	152.20	145.50
57	SDPP	3523	5	7	72.77	131.30	115.20
58	SDPP	3323	5	7	1,479.50	3,103.00	2,031.00
59	SPC	3523	1	14	52.79	10.44	7.92
60	SPC	3323	1	14	123.18	.	8.50
61	SDPP	3523	1	14	175.46	101.14	73.715
62	SDPP	3323	1	14	136.33	94.22	9.41
63	SPC	3523	2	14	110.51	20.71	21.624
64	SPC	3323	2	14	162.40	61.76	21.52
65	SDPP	3523	2	14	261.10	203.20	117.2
66	SDPP	3323	2	14	216.20	164.70	181.1
67	SPC	3523	3	14	52.70	108.10	124.1
68	SPC	3323	3	14	74.81	33.76	10.97
69	SDPP	3523	3	14	98.78	78.96	25.11
70	SDPP	3323	3	14	63.74	196.10	258.5
71	SPC	3523	4	14	101.80	78.16	18.86
72	SPC	3323	4	14	302.60	79.69	59.61
73	SDPP	3523	4	14	256.80	439.35	803.5
74	SDPP	3323	4	14	1,072.50	.	2,859.5
75	SPC	3523	5	14	14.92	21.59	6.305
76	SPC	3323	5	14	27.43	16.31	12.46
77	SDPP	3523	5	14	618.50	1,314.50	628
78	SDPP	3323	5	14	583.00	2,842.00	4,404.5

Appendix Table 46. Analysis of variance for log of IgG levels (ug/mL) in chyme from duodenum, jejunum, and ileum of weaned pigs, Exp. 3.

Source	df	Mean Squares		
		Duodenum	Jejunum	Ileum
Total	55			
Error	40	1.00165	1.67329	2.16449
Replication	4	2.31178	6.32924	6.87912
CP source	1	20.36528	54.07941	83.90708
ME level	1	0.04884	0.36718	0.98114
Day	2	2.08004	4.32664	18.71248
CP*ME	1	0.06327	0.05587	0.92227
CP*Day	2	0.23220	0.27731	2.91945
ME*Day	2	0.27035	0.49097	0.99266
CP*ME*Day	2	0.21574	2.35434	0.66238
C.V., %		19.12	25.21	29.30

Appendix Table 47. IgA levels (ug/mL) in chyme from duodenum, jejunum, and ileum of weaned pigs, Exp. 3.

Pig	CP Source	ME level	Rep	Day	Duodenum	Jejunum	Ileum
1			1	0	159.55	587.92	6,515.08
2			1	0	2,338.72	3,893.17	7,461.16
3			1	0	70.95	260.90	256.65
4			1	0	66.35	91.28	37.32
5			2	0	223.90	366.00	499.80
6			2	0	.	42.62	68.25
7			2	0	.	233.00	216.00
8			2	0	120.88	.	212.85
9			3	0	293.70	643.50	708.90
10			3	0	200.70	267.30	173.64
11			3	0	81.18	.	105.02
12			3	0	78.35	188.68	148.50
13			4	0	150.36	368.55	316.65
14			4	0	44.18	142.52	231.60
15			5	0	88.40	335.55	348.45
16			5	0	40.90	136.90	478.00
17			5	0	232.18	218.59	212.75
18			5	0	121.24	132.60	247.68
19	SPC	3523	1	3	111.50	237.29	340.86
20	SPC	3323	1	3	67.49	218.88	116.77
21	SDPP	3523	1	3	167.20	303.10	421.20
22	SDPP	3323	1	3	94.55	122.30	142.80
23	SPC	3523	2	3	324.20	568.50	671.20
24	SPC	3323	2	3	74.64	76.94	196.00
25	SDPP	3523	2	3	313.50	744.50	872.20
26	SDPP	3323	2	3	87.31	165.40	299.80
27	SPC	3523	3	3	449.85	402.30	1,195.35
28	SPC	3323	3	3	66.23	170.70	286.80
29	SDPP	3523	3	3	447.40	588.60	882.00
30	SDPP	3323	3	3	56.14	.	592.20
31	SPC	3523	4	3	51.99	116.20	340.20
32	SPC	3323	4	3	287.40	63.96	583.00
33	SDPP	3523	4	3	150.70	492.20	569.60
34	SDPP	3323	4	3	209.00	467.40	490.20
35	SPC	3523	5	3	241.50	262.80	330.80
36	SPC	3323	5	3	244.20	258.10	258.50
37	SDPP	3523	5	3	.	37.95	118.01
38	SDPP	3323	5	3	29.42	23.15	18.26
39	SPC	3523	1	7	60.22	77.96	119.45
40	SPC	3323	1	7	135.40	67.82	22.35

Appendix Table 47. Continued.

Pig	CP Source	ME level	Rep	Day	Duodenum	Jejunum	Ileum
41	SDPP	3523	1	7	160.90	234.20	248.50
42	SDPP	3323	1	7	107.30	57.39	91.92
43	SPC	3523	2	7	181.30	167.75	134.50
44	SPC	3323	2	7	238.40	549.10	342.30
45	SDPP	3523	2	7	175.30	165.40	215.50
46	SDPP	3323	2	7	264.20	500.60	724.10
47	SPC	3523	3	7	438.70	509.10	496.60
48	SPC	3323	3	7	218.10	170.10	241.30
49	SDPP	3523	3	7	161.10	370.10	95.59
50	SDPP	3323	3	7	427.80	917.40	1,591.20
51	SPC	3523	4	7	165.78	265.76	565.90
52	SPC	3323	4	7	118.30	917.20	239.70
53	SDPP	3523	4	7	19.73	39.43	39.03
54	SDPP	3323	4	7	57.28	138.20	88.90
55	SPC	3523	5	7	118.90	167.50	131.10
56	SPC	3323	5	7	257.20	318.50	275.70
57	SDPP	3523	5	7	49.12	42.80	83.24
58	SDPP	3323	5	7	79.10	82.82	144.49
59	SPC	3523	1	14	231.24	150.58	133.41
60	SPC	3323	1	14	146.62	99.66	130.31
61	SDPP	3523	1	14	719.04	464.64	650.64
62	SDPP	3323	1	14	230.80	335.40	164.30
63	SPC	3523	2	14	197.50	277.50	160.80
64	SPC	3323	2	14	301.70	331.40	368.70
65	SDPP	3523	2	14	446.60	279.80	203.30
66	SDPP	3323	2	14	299.60	182.40	171.60
67	SPC	3523	3	14	304.60	233.90	220.40
68	SPC	3323	3	14	403.30	521.40	474.80
69	SDPP	3523	3	14	318.70	234.90	632.60
70	SDPP	3323	3	14	198.50	465.00	325.00
71	SPC	3523	4	14	289.40	491.10	262.50
72	SPC	3323	4	14	137.10	818.10	.
73	SDPP	3523	4	14	208.40	237.10	287.50
74	SDPP	3323	4	14	41.55	54.74	64.89
75	SPC	3523	5	14	522.60	243.70	220.90
76	SPC	3323	5	14	247.50	377.50	419.60
77	SDPP	3523	5	14	719.04	464.64	650.64
78	SDPP	3323	5	14	230.80	335.40	164.30

Appendix Table 48. Analysis of variance for log of IgA levels (ug/mL) in chyme from duodenum, jejunum, and ileum of weaned pigs, Exp. 3.

Source	df	Mean Squares		
		Duodenum	Jejunum	Ileum
Total	58			
Error	43	0.44353	0.49340	0.43401
Replication	4	1.61613	3.17744	3.57745
CP source	1	1.02686	0.63768	0.23141
ME level	1	1.14208	0.15893	0.49199
Day	2	1.36989	0.55912	3.30378
CP*ME	1	0.16741	0.12244	0.05465
CP*Day	2	0.46062	1.50929	0.26111
ME*Day	2	1.00346	1.34076	0.63785
CP*ME*Day	2	0.11858	0.08339	1.24122
C.V., %		13.00	13.07	11.98

Appendix Table 49. Pancreatic α-amylase activity (1000 IU/L) in pancreas tissue and small intestinal chyme of weaned pigs, Exp. 3.

Pig	CP Source	ME level	Rep	Day	Pancreas tissue	Intestinal chyme
1			1	0	253.9	62.3
2			1	0	113.7	.
3			1	0	28.7	32.8
4			1	0	.	.
5			2	0	264.0	23.4
6			2	0	155.0	90.2
7			2	0	468.0	.
8			2	0	257.3	.
9			3	0	371.0	.
10			3	0	490.0	.
11			3	0	401.0	63.2
12			3	0	766.0	763.7
13			4	0	302.0	0.0
14			4	0	392.0	467.0
15			5	0	708.0	217.1
16			5	0	692.0	37.7
17			5	0	581.0	.
18			5	0	702.0	.
19	SPC	3523	1	3	27.7	56.1
20	SPC	3323	1	3	32.3	41.1
21	SDPP	3523	1	3	28.8	29.8
22	SDPP	3323	1	3	172.8	53.1
23	SPC	3523	2	3	317.0	150.2
24	SPC	3323	2	3	39.0	43.0
25	SDPP	3523	2	3	174.0	145.6
26	SDPP	3323	2	3	146.0	72.6
27	SPC	3523	3	3	175.0	184.5
28	SPC	3323	3	3	287.0	.
29	SDPP	3523	3	3	100.0	22.3
30	SDPP	3323	3	3	73.0	128.5
31	SPC	3523	4	3	81.0	99.1
32	SPC	3323	4	3	166.0	78.5
33	SDPP	3523	4	3	167.0	55.1
34	SDPP	3323	4	3	333.0	.
35	SPC	3523	5	3	394.0	139.8
36	SPC	3323	5	3	323.0	73.9
37	SDPP	3523	5	3	59.0	66.2
38	SDPP	3323	5	3	40.0	29.9
39	SPC	3523	1	7	117.0	.
40	SPC	3323	1	7	24.5	60.2

Appendix Table 49. Continued.

Pig	CP Source	ME level	Rep	Day	Pancreas tissue	Intestinal chyme
41	SDPP	3523	1	7	343.9	197.8
42	SDPP	3323	1	7	43.5	24.4
43	SPC	3523	2	7	381.0	369.9
44	SPC	3323	2	7	277.0	289.3
45	SDPP	3523	2	7	433.0	1,149.3
46	SDPP	3323	2	7	157.0	236.8
47	SPC	3523	3	7	163.0	235.7
48	SPC	3323	3	7	111.0	151.0
49	SDPP	3523	3	7	58.0	230.1
50	SDPP	3323	3	7	129.0	302.3
51	SPC	3523	4	7	99.0	96.7
52	SPC	3323	4	7	33.0	39.9
53	SDPP	3523	4	7	22.0	31.8
54	SDPP	3323	4	7	35.0	28.2
55	SPC	3523	5	7	186.0	84.2
56	SPC	3323	5	7	159.0	.
57	SDPP	3523	5	7	767.0	550.2
58	SDPP	3323	5	7	233.0	632.7
59	SPC	3523	1	14	47.0	54.5
60	SPC	3323	1	14	466.0	422.5
61	SDPP	3523	1	14	461.2	279.5
62	SDPP	3323	1	14	246.0	327.5
63	SPC	3523	2	14	1,152.0	230.8
64	SPC	3323	2	14	1,014.0	454.2
65	SDPP	3523	2	14	417.0	564.7
66	SDPP	3323	2	14	272.0	369.1
67	SPC	3523	3	14	419.0	265.7
68	SPC	3323	3	14	302.0	200.5
69	SDPP	3523	3	14	228.0	346.9
70	SDPP	3323	3	14	983.0	1,269.9
71	SPC	3523	4	14	123.0	112.0
72	SPC	3323	4	14	319.0	212.9
73	SDPP	3523	4	14	262.0	691.5
74	SDPP	3323	4	14	282.0	.
75	SPC	3523	5	14	389.0	362.9
76	SPC	3323	5	14	811.0	441.1
77	SDPP	3523	5	14	716.0	696.7
78	SDPP	3323	5	14	493.0	577.5

Appendix Table 50. Analysis of variance for log of pancreatic α-amylase activity (1000 IU/L) in pancreas tissue and small intestinal chyme of weaned pigs, Exp. 3.

Source	Pancreas tissue		Intestinal chyme	
	df	Mean squares	df	Mean squares
Total	58		54	
Error	43	0.65052	39	0.70052
Replication	4	2.72838	4	1.94777
CP source	1	0.00005	1	1.50660
ME level	1	0.03156	1	0.00816
Day	2	9.08232	2	9.54790
CP*ME	1	0.02518	1	0.07313
CP*Day	2	0.05841	2	0.83331
ME*Day	2	1.40495	2	0.76065
CP*ME*Day	2	0.34554	2	1.41686
C.V., %		15.66		16.44

Appendix Table 51. Pancreatic α-amylase activity (1000 IU/L) in chyme from duodenum, jejunum, and ileum of weaned pigs, Exp. 3.

Pig	CP Source	ME level	Rep	Day	Duodenum	Jejunum	Ileum
1			1	0	17.31	20.93	24.03
2			1	0	.	11.45	27.32
3			1	0	4.18	11.77	16.80
4			1	0	.	6.15	12.28
5			2	0	6.45	10.90	6.00
6			2	0	14.75	60.88	14.55
7			2	0	.	47.05	44.33
8			2	0	.	29.33	27.23
9			3	0	.	.	80.40
10			3	0	.	.	83.85
11			3	0	6.38	29.55	27.23
12			3	0	63.98	408.30	291.40
13			4	0	31.30	0.00	22.60
14			4	0	214.05	173.70	79.20
15			5	0	20.40	102.60	94.13
16			5	0	9.30	12.60	15.83
17			5	0	153.40	194.55	0.00
18			5	0	25.70	27.45	0.00
19	SPC	3523	1	3	11.78	12.85	31.43
20	SPC	3323	1	3	7.79	3.66	29.68
21	SDPP	3523	1	3	6.51	8.68	14.57
22	SDPP	3323	1	3	21.40	21.35	10.32
23	SPC	3523	2	3	12.20	52.90	85.05
24	SPC	3323	2	3	4.80	9.75	28.45
25	SDPP	3523	2	3	34.65	37.85	73.05
26	SDPP	3323	2	3	7.70	21.45	43.40
27	SPC	3523	3	3	13.20	50.20	121.10
28	SPC	3323	3	3	15.29	76.50	.
29	SDPP	3523	3	3	4.83	16.70	0.75
30	SDPP	3323	3	3	3.17	27.94	97.40
31	SPC	3523	4	3	17.70	37.40	44.00
32	SPC	3323	4	3	10.30	13.45	54.70
33	SDPP	3523	4	3	9.30	19.15	26.60
34	SDPP	3323	4	3	13.70	.	63.20
35	SPC	3523	5	3	19.90	57.30	62.60
36	SPC	3323	5	3	22.80	21.20	29.90
37	SDPP	3523	5	3	19.95	12.90	33.35
38	SDPP	3323	5	3	6.70	12.45	10.75
39	SPC	3523	1	7	.	35.93	137.20
40	SPC	3323	1	7	13.96	17.90	28.33

Appendix Table 51. Continued.

Pig	CP Source	ME level	Rep	Day	Duodenum	Jejunum	Ileum
41	SDPP	3523	1	7	26.73	43.52	127.55
42	SDPP	3323	1	7	13.64	3.78	6.99
43	SPC	3523	2	7	32.65	164.15	173.10
44	SPC	3323	2	7	64.85	72.75	151.70
45	SDPP	3523	2	7	120.45	298.60	730.30
46	SDPP	3323	2	7	6.20	89.05	141.55
47	SPC	3523	3	7	61.72	55.08	118.85
48	SPC	3323	3	7	63.20	39.15	48.60
49	SDPP	3523	3	7	26.05	110.90	93.10
50	SDPP	3323	3	7	66.41	121.35	114.50
51	SPC	3523	4	7	48.68	20.45	27.60
52	SPC	3323	4	7	10.80	16.28	12.84
53	SDPP	3523	4	7	6.70	9.52	15.60
54	SDPP	3323	4	7	2.75	16.90	8.55
55	SPC	3523	5	7	11.95	38.60	33.65
56	SPC	3323	5	7	.	10.25	16.60
57	SDPP	3523	5	7	147.75	133.65	268.80
58	SDPP	3323	5	7	208.60	189.90	234.20
59	SPC	3523	1	14	23.37	9.69	21.48
60	SPC	3323	1	14	40.32	175.10	207.10
61	SDPP	3523	1	14	25.50	161.85	92.10
62	SDPP	3323	1	14	39.37	49.71	238.40
63	SPC	3523	2	14	23.16	112.98	94.68
64	SPC	3323	2	14	75.65	109.95	268.60
65	SDPP	3523	2	14	236.55	146.55	181.60
66	SDPP	3323	2	14	81.10	174.50	113.45
67	SPC	3523	3	14	87.25	59.50	118.95
68	SPC	3323	3	14	56.55	60.50	83.45
69	SDPP	3523	3	14	36.25	193.40	117.25
70	SDPP	3323	3	14	452.30	393.50	424.10
71	SPC	3523	4	14	16.80	38.30	56.90
72	SPC	3323	4	14	79.40	83.95	49.50
73	SDPP	3523	4	14	132.05	269.25	290.20
74	SDPP	3323	4	14	40.10	312.65	.
75	SPC	3523	5	14	74.30	144.00	144.55
76	SPC	3323	5	14	46.65	206.65	187.75
77	SDPP	3523	5	14	21.75	297.90	377.05
78	SDPP	3323	5	14	146.75	188.90	241.80

Appendix Table 52. Analysis of variance for log of pancreatic α-amylase activity (1000 IU/L) in chyme from duodenum, jejunum, and ileum of weaned pigs, Exp. 3.

Source	df	Mean Squares		
		Duodenum	Jejunum[a]	Ileum
Total	57			
Error	42	0.75884	0.53469	0.84027
Replication	4	1.29540	3.20146	2.28937
CP source	1	0.09682	1.90135	0.29307
ME level	1	0.00629	0.64742	0.42088
Day	2	12.51327	14.85217	2.28937
CP*ME	1	0.11744	0.01273	9.33545
CP*Day	2	0.63999	1.83114	2.42277
ME*Day	2	0.94260	1.14467	2.00525
CP*ME*Day	2	0.16529	1.01239	0.90697
C.V., %		26.10	18.64	21.77

[a]Jejunum Total df = 58, Error df = 43.

Appendix Table 53. Pancreatic triglyceride lipase activity (1000 IU/L) in pancreas tissue and intestinal chyme of weaned pigs, Exp. 3.

Pig	CP Source	ME level	Rep	Day	Pancreas tissue	Intestinal chyme
1			1	0	94.80	78.20
2			1	0	98.80	137.93
3			1	0	160.20	86.05
4			1	0	66.90	.
5			2	0	116.90	.
6			2	0	212.80	.
7			2	0	150.50	152.33
8			2	0	185.30	281.56
9			3	0	121.10	0.00
10			3	0	157.00	287.77
11			4	0	248.60	133.24
12			4	0	189.50	123.81
13			4	0	279.00	.
14			4	0	135.50	.
15	SPC	3523	1	3	13.71	8.94
16	SPC	3323	1	3	24.63	7.14
17	SDPP	3523	1	3	30.32	30.99
18	SDPP	3323	1	3	.	24.63
19	SPC	3523	2	3	129.70	154.63
20	SPC	3323	2	3	9.80	103.88
21	SDPP	3523	2	3	87.00	171.67
22	SDPP	3323	2	3	90.10	114.11
23	SPC	3523	3	3	74.80	121.40
24	SPC	3323	3	3	68.90	227.95
25	SDPP	3523	3	3	36.40	138.62
26	SDPP	3323	3	3	17.20	106.12
27	SPC	3523	4	3	78.90	89.35
28	SPC	3323	4	3	128.20	141.72
29	SDPP	3523	4	3	98.80	41.84
30	SDPP	3323	4	3	155.50	.
31	SPC	3523	5	3	140.00	87.17
32	SPC	3323	5	3	114.90	101.72
33	SDPP	3523	5	3	21.60	117.39
34	SDPP	3323	5	3	22.40	109.93
35	SPC	3523	1	7	13.34	.
36	SPC	3323	1	7	5.92	6.91
37	SDPP	3523	1	7	52.08	21.70
38	SDPP	3323	1	7	16.88	6.87
39	SPC	3523	2	7	42.20	39.47
40	SPC	3323	2	7	33.60	33.90

Appendix Table 53. Continued.

Pig	CP Source	ME level	Rep	Day	Pancreas tissue	Intestinal chyme
41	SDPP	3523	2	7	46.20	137.15
42	SDPP	3323	2	7	19.50	82.43
43	SPC	3523	3	7	12.50	33.28
44	SPC	3323	3	7	11.30	40.23
45	SDPP	3523	3	7	30.90	232.79
46	SDPP	3323	3	7	18.00	44.19
47	SPC	3523	4	7	37.60	87.26
48	SPC	3323	4	7	105.70	239.04
49	SDPP	3523	4	7	33.70	229.69
50	SDPP	3323	4	7	101.30	200.22
51	SPC	3523	5	7	39.30	21.27
52	SPC	3323	5	7	43.00	18.19
53	SDPP	3523	5	7	81.20	70.56
54	SDPP	3323	5	7	22.80	120.08
55	SPC	3523	1	14	7.38	5.43
56	SPC	3323	1	14	24.48	6.33
57	SDPP	3523	1	14	38.49	13.34
58	SDPP	3323	1	14	25.96	7.44
59	SPC	3523	2	14	61.50	54.44
60	SPC	3323	2	14	45.70	31.82
61	SDPP	3523	2	14	20.30	53.23
62	SDPP	3323	2	14	24.60	31.89
63	SPC	3523	3	14	27.60	34.21
64	SPC	3323	3	14	16.80	16.49
65	SDPP	3523	3	14	15.60	32.01
66	SDPP	3323	3	14	72.80	147.27
67	SPC	3523	4	14	13.60	48.36
68	SPC	3323	4	14	18.00	43.06
69	SDPP	3523	4	14	27.40	130.08
70	SDPP	3323	4	14	20.40	.
71	SPC	3523	5	14	30.30	40.86
72	SPC	3323	5	14	61.60	24.16
73	SDPP	3523	5	14	40.80	72.75
74	SDPP	3323	5	14	21.30	66.04

Appendix Table 54. Analysis of variance for log of pancreatic triglyceride lipase activity in pancreas tissue and intestinal chyme (1000 IU/L) of weaned pigs, Exp. 3.

Source	df	Mean Squares	
		Pancreas	Intestinal chyme
Total	58		
Error	43	0.64361	0.28515
Replication	4	2.27849	8.13971
CP source	1	0.12144	3.94954
ME level	1	0.47619	0.25254
Day	2	1.08647	3.25814
CP*ME	1	0.65014	0.21245
CP*Day	2	0.91212	0.81976
ME*Day	2	0.62433	0.09060
CP*ME*Day	2	0.08058	0.29025
C.V., %		22.67	13.50

Appendix Table 55. Pancreatic triglyceride lipase activity (1000 IU/L) in chyme from duodenum, jejunum, and ileum of weaned pigs, Exp. 3.

Pig	CP Source	ME level	Rep	Day	Duodenum	Jejunum	Ileum
1			1	0	14.69	28.04	35.48
2			1	0	45.38	79.31	13.25
3			1	0	25.26	29.64	31.16
4			1	0	.	31.36	48.27
5			2	0	.	.	73.82
6			2	0	.	.	81.59
7			2	0	52.28	55.13	44.92
8			2	0	29.87	141.14	110.56
9			3	0	51.64	.	27.24
10			3	0	104.99	137.60	45.18
11			4	0	15.80	76.23	41.21
12			4	0	44.92	46.09	32.80
13			4	0	106.76	122.96	.
14			4	0	35.69	23.24	.
15	SPC	3523	1	3	0.68	3.54	4.72
16	SPC	3323	1	3	2.67	1.78	2.69
17	SDPP	3523	1	3	2.40	2.59	26.01
18	SDPP	3323	1	3	8.03	12.30	4.31
19	SPC	3523	2	3	28.07	66.28	60.28
20	SPC	3323	2	3	48.81	22.49	32.59
21	SDPP	3523	2	3	57.52	55.50	58.65
22	SDPP	3323	2	3	22.30	39.30	52.52
23	SPC	3523	3	3	12.81	29.14	79.46
24	SPC	3323	3	3	65.93	86.25	75.78
25	SDPP	3523	3	3	26.10	62.09	50.44
26	SDPP	3323	3	3	5.48	22.72	77.93
27	SPC	3523	4	3	57.73	1.13	30.49
28	SPC	3323	4	3	31.19	33.12	77.41
29	SDPP	3523	4	3	4.29	23.03	14.53
30	SDPP	3323	4	3	23.34	.	49.84
31	SPC	3523	5	3	27.14	39.09	20.94
32	SPC	3323	5	3	53.07	28.95	19.70
33	SDPP	3523	5	3	50.36	38.53	28.50
34	SDPP	3323	5	3	41.04	47.06	21.84
35	SPC	3523	1	7	.	2.06	4.48
36	SPC	3323	1	7	1.49	3.18	2.24
37	SDPP	3523	1	7	9.51	4.96	7.23
38	SDPP	3323	1	7	1.58	4.51	0.78
39	SPC	3523	2	7	13.97	14.97	10.54
40	SPC	3323	2	7	9.57	9.49	14.84

Appendix Table 55. Continued.

Pig	CP Source	ME level	Rep	Day	Duodenum	Jejunum	Ileum
41	SDPP	3523	2	7	25.02	53.06	59.07
42	SDPP	3323	2	7	6.98	38.15	37.30
43	SPC	3523	3	7	9.35	8.69	15.24
44	SPC	3323	3	7	16.12	12.72	11.39
45	SDPP	3523	3	7	30.44	130.20	72.16
46	SDPP	3323	3	7	6.45	17.25	20.49
47	SPC	3523	4	7	36.62	36.94	13.71
48	SPC	3323	4	7	82.17	90.73	66.14
49	SDPP	3523	4	7	59.23	99.06	71.40
50	SDPP	3323	4	7	33.47	118.58	48.17
51	SPC	3523	5	7	3.07	11.18	7.03
52	SPC	3323	5	7	0.65	8.65	8.89
53	SDPP	3523	5	7	27.28	20.81	22.48
54	SDPP	3323	5	7	36.04	43.46	40.59
55	SPC	3523	1	14	4.86	0.19	0.39
56	SPC	3323	1	14	1.21	2.21	2.91
57	SDPP	3523	1	14	1.27	5.92	6.16
58	SDPP	3323	1	14	0.45	3.53	3.46
59	SPC	3523	2	14	20.46	15.07	18.91
60	SPC	3323	2	14	16.52	7.99	7.31
61	SDPP	3523	2	14	35.62	10.76	6.86
62	SDPP	3323	2	14	6.62	14.65	10.62
63	SPC	3523	3	14	15.36	9.69	9.17
64	SPC	3323	3	14	6.26	7.29	2.95
65	SDPP	3523	3	14	8.94	11.71	11.37
66	SDPP	3323	3	14	73.89	47.07	26.32
67	SPC	3523	4	14	18.41	14.72	15.24
68	SPC	3323	4	14	13.58	19.75	9.74
69	SDPP	3523	4	14	39.87	65.89	24.33
70	SDPP	3323	4	14	9.55	41.48	.
71	SPC	3523	5	14	9.93	21.20	9.74
72	SPC	3323	5	14	3.20	7.95	13.01
73	SDPP	3523	5	14	21.39	26.75	24.62
74	SDPP	3323	5	14	20.90	26.80	18.34

Appendix Table 56. Analysis of variance for log of pancreatic triglyceride lipase activity in chyme from duodenum, jejunum, and ileum of weaned pigs, Exp. 3.

Source	df	Mean Squares		
		Duodenum	Jejunum	Ileum
Total	58			
Error	43	0.64971	0.82087	0.61057
Replication	4	7.82315	6.66165	4.67765
CP source	1	0.83867	5.07446	1.68555
ME level	1	0.66566	0.07385	0.84775
Day	2	1.58523	1.20185	7.35815
CP*ME	1	0.42149	0.46442	0.43908
CP*Day	2	1.02213	1.10351	0.35129
ME*Day	2	0.93877	0.03713	0.12992
CP*ME*Day	2	0.47640	0.48324	0.11218
C.V., %		30.18	31.41	27.19

Appendix Table 57. Gene expression (Δ^{Ct}) of pancreatic triglyceride lipase, α-amylase, and trypsinogen in weaned pigs, Exp. 3.

Pig	CP Source	ME level	Rep	Day	Lipase	Amylase	Trypsinogen
1			1	0	12.99	9.04	21.18
2			2	0	14.03	8.32	18.81
3			3	0	12.07	9.64	17.79
4			4	0	15.70	10.55	21.4
5			1	0	13.33	11.89	24.52
6			2	0	13.43	.	20.31
7			3	0	17.39	9.87	17.42
8			4	0	19.75	10.03	25.21
9	1	1	1	3	13.19	.	.
10	1	1	2	3	11.68	12.00	17.59
11	1	1	3	3	13.69	12.52	20.66
12	1	1	4	3	14.38	13.48	23.56
13	1	2	1	3	17.1	.	24.71
14	1	2	2	3	10.91	12.77	16.28
15	1	2	3	3	9.75	9.36	21.31
16	1	2	4	3	16.16	11.93	19.45
17	2	1	1	3	14.56	7.61	.
18	2	1	2	3	9.86	8.92	16.32
19	2	1	3	3	15.26	.	20.58
20	2	1	4	3	8.58	6.73	13.4
21	2	2	1	3	17.42	12.75	26.23
22	2	2	2	3	3.14	5.62	6.86
23	2	2	3	3	17.03	12.22	24.86
24	2	2	4	3	9.47	8.88	14.67
25	1	1	1	7	16.58	10.78	23.12
26	1	1	2	7	16.63	11.07	20.18
27	1	1	3	7	15.46	.	22.33
28	1	1	4	7	12.56	11.91	17.16
29	1	2	1	7	15.09	8.57	16.82
30	1	2	2	7	14.01	.	17.63
31	1	2	3	7	13.58	9.14	24.85
32	1	2	4	7	11.84	9.35	15.84
33	2	1	1	7	15.61	7.08	28.23
34	2	1	2	7	8.07	8.48	14.52
35	2	1	3	7	10.27	.	19.22
36	2	1	4	7	14.65	9.62	18.15
37	2	2	1	7	11.06	9.88	15.76
38	2	2	2	7	14.75	9.79	19.06
39	2	2	3	7	15.66	9.06	19.77
40	2	2	4	7	15.38	.	17.98

Appendix Table 57. Continued.

Pig	CP Source	ME level	Rep	Day	Lipase	Amylase	Trypsinogen
41	1	1	1	14	10.47	9.29	.
42	1	1	2	14	15.56	9.43	21.17
43	1	1	3	14	14.88	.	17.17
44	1	1	4	14	14.45	9.96	20.84
45	1	2	1	14	15.86	9.78	20.29
46	1	2	2	14	12.69	9.39	12.49
47	1	2	3	14	13.32	11.30	19.73
48	1	2	4	14	14.94	.	21.66
49	2	1	1	14	13.12	.	16.05
50	2	1	2	14	12.68	7.35	15.75
51	2	1	3	14	11.67	6.96	20.44
52	2	1	4	14	13.68	6.38	16.75
53	2	2	1	14	14.4	6.47	16.96
54	2	2	2	14	16.02	7.56	19.04
55	2	2	3	14	16.11	5.96	21.11
56	2	2	4	14	11.70	.	13.32

Appendix Table 58. Analysis of variance for computed Δ^{Ct} of pancreatic triglyceride lipase, α-amylase, and trypsinogen of weaned pigs, Exp. 3.

Source	df	Mean Squares		
		Lipase	Amylase	Trypsinogen
Total	47			
Error	33	8.65779	2.53588	14.56070
Replication	3	12.45894	1.14131	61.70134
CP source	1	12.63827	40.61787	40.78475
ME level	1	2.02130	0.75570	8.94348
Day	2	7.67791	18.39037	4.23623
CP*ME	1	7.06100	5.28346	4.45250
CP*Day	2	1.40104	3.36768	5.43708
ME*Day	2	1.33875	0.73639	3.13517
CP*ME*Day	2	4.60234	3.37331	1.22891
C.V., %		21.90	16.62	20.20

VITA

TERESA ASUNCION BUHAY

Candidate for the Degree of

Doctor of Philosophy

Thesis: EFFECTS OF PROTEIN SOURCE AND FAT LEVEL ON PERFORMANCE, GROWTH OF THE PANCREAS, STOMACH, AND SMALL INTESTINE, AND ON PANCREATIC ENZYME ACTIVITY AND GENE EXPRESSION IN EARLY-WEANED PIGS

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Title of Study: EFFECTS OF PROTEIN SOURCE AND FAT LEVEL ON PERFORMANCE, GROWTH OF THE PANCREAS, STOMACH, AND SMALL INTESTINE, AND ON PANCREATIC ENZYME ACTIVITY AND GENE EXPRESSION IN EARLY-WEANED PIGS

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Major Field: Animal Nutrition

Scope and Method of Study: Two experiments were conducted to determine the effects of reducing ME (fat) level in diets containing soy protein concentrate (SPC) or spray-dried porcine plasma (SDPP) on performance of 400 crossbred pigs (avg BW = 5.8 kg; avg 21 d). In Exp.1, pigs were allotted to four dietary treatments (6-7 pigs/pen, 9 replications) in RCBD: 1) SPC, ME = 3,471 kcal/kg, 2) SDPP, ME = 3,471, 3) SDPP, ME = 3,371, and 4) SDPP, ME = 3,271. In Exp. 2, a 2 x 2 factorial in RCBD (7 pigs/pen, 6 replications) was used: 1) SPC with ME = 3,523, 2) SPC, ME = 3,323, 3) SDPP, ME = 3,523, and 4) SDPP, ME = 3,323. Pigs and feeders were weighed on d 0, 7, 14, and 18, to determine ADG, ADFI, and G:F. Exp. 3 was performed using Exp. 2 treatment design (4 pigs/ trt, 5 reps, avg BW = 6.1 kg; avg 18 d) to determine the effect on growth of the pancreas, stomach, and small intestine, morphology of the small intestine, levels of IgG and IgA in serum and intestinal chyme, pancreatic α-amylase and triglyceride lipase activities in pancreas and chyme, and gene expression of triglyceride lipase, α-amylase and trypsinogen.

Findings and Conclusions: Inclusion of SDPP in weanling pig diets improved ADG, ADFI, and G:F. Reducing ME level in SDPP diets did not affect growth performance, but linearly increased weight gain/ME intake. The improvement in weight gain/ME intake associated with reduced ME tended to be greater for pigs fed SPC than for pigs fed SDPP. Protein source or fat level did not affect lipase and amylase activity in pancreas, but SDPP increased these in intestinal chyme. CP source increased gene expression of amylase and numerically increased lipase and trypsinogen expression. Intestinal chyme IgG was significantly increased in pigs fed SDPP. Results suggest that inclusion of SDPP to weanling pig diets improves growth performance by varied mechanisms. In addition, the use of fat in weanling pig diets during the first 2 wk post-weaning may have no benefit to pig growth.

ADVISER'S APPROVAL: Dr. Scott D. Carter