IS STARCH AN ESSENTIAL NUTRIENT FOR GROWING PIGS?

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By

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ABSTRACT

Distiller's dried grains with solubles (DDGS), a co-product of the ethanol industry, are widely used in the swine industry, partially to replace soybean meal as well as parent grain and phosphorus. The main difference between DDGS and the parent grain is the low starch content of the DDGS, which was converted to ethanol during fermentation. Diets formulated with high DDGS will therefore have reduced starch content. The overall objective of this experiment was to determine if low dietary starch impacts efficient meat production in growing pigs. Six diets, consisting of five semi-purified iso-nitrogenous and iso-caloric diets with increasing starch content (0.0, 5.5, 11.0, 16.5 and 22.0%) and one commercial reference diet were fed to growing pigs for 26 days. Diets were maintained iso-caloric with canola oil. Four blocks of 12 gilts (28±2 days, 9.8±0.2 kg) were randomly assigned within block to one of the six treatments. Two additional pigs from each block were slaughtered on d 0 for baseline carcass measurements. Pigs were fed ad libitum. Body weight and feed intake were recorded on d 0, 7, 14, 19 and 26. Fecal samples were collected on d 17 and 18. A catheter was inserted into the jugular of 4 pigs per treatment on d 20. Blood samples were taken at: -15, 0 (feeding), 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 420 and 480 min on d 24. Pigs were euthanized on d 26 and carcass composition determined. Average daily gain and feed intake improved as starch content increased ($P<0.05$) while feed efficiency was similar ($P>0.1$) among treatments. Apparent gross energy and dry matter digestibility improved linearly and apparent ether extract digestibility decreased linearly with increasing starch ($P<0.01$). Apparent crude protein digestibility was not affected as the content of dietary starch content increased ($P>0.1$). C-peptide (pro-insulin) increased ($P<0.01$) with increasing starch level. Blood glucose and blood urea nitrogen (BUN) were not affected by increasing starch concentration. Carcass crude protein, lipid, moisture, and ash accretion increased with increasing dietary starch level ($P<0.05$). The efficiency of protein

gain (crude protein accretion to energy intake ratio) tended to increase with increasing starch content ($P=0.07$). Crude protein utilization (crude protein accretion to crude protein intake ratio) increased with increasing starch level ($P<0.05$). In summary, carcass crude protein accretion improved in response to the increasing starch content from the diet. In conclusion, starch isn't an essential nutrient for growing pigs but it does require level of dietary starch for optimal protein deposition. This implies that maximizing the inclusion of DDGS or other low starch co-products in swine diets may require a consideration of the starch content of the diet to maintain optimal protein deposition.

Key words: Swine, Starch, DDGS, C-peptide, BUN, protein deposition

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DEDICATION

I would like to dedicate this thesis to my family. To my parents, Yuzhu and Zhaoan, your support is a necessity to the success of this project. To my wife Biqun and my son Qingxiao; there would be no way I could achieve my education without all of your support. I am grateful to have a wonderful family and their support behind me. Here, all I want to say is: I love you.

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LIST OF ABBREVIATIONS

AA	Amino acid
ADF	Acid detergent fiber
ADFI	Average daily feed intake
ADG	Average daily gain
ADL	Acid detergent lignin
AIA	Acid insoluble ash
AUC	Area under the curve
BUN	Blood urea nitrogen
BW	Body weight
CF	Crude fiber
CP	Crude protein
CPG	Crude protein gain
CPI	Crude protein intake
D	Day
DCP	Digestible crude protein

DDGS	Dry distiller’s grains with solubles
DE	Digestible energy
DEI	Digestible energy intake
DM	Dry matter
EBW	Empty body weight
EE	Ether extract
FC	Feed conversion
G	Gram
ISG	Initial slaughter group
Kg	Kilogram
Kcal	Kilocalorie
L	Liter
LDR	Lipid deposition rate
ME	Metabolizable energy
Mg	Milligram
Min	Minute
Mmol	Millimolar

MMT	Million metric tonne
N	Numbers
NDF	Neutral detergent fiber
NSP	Non starch polysaccharides
NE	Net energy
Nmol	Nanomolar
PDR	Protein deposition rate
WG	Water gain

1 General introduction

The demand for ethanol as an alternative energy supply has increased substantially (Valdes 2007). Cellulose, sugar (cane and beet) and grains such as wheat, corn (maize), sorghum, rye and barley are utilized for ethanol production. In competition with biofuel industries, energy (i.e., starch) from cereal grains will become an increasingly-expensive nutrient for swine (Beaulieu et al., 2009). Not only has ethanol relieved the shortage of energy supply for countries such as China and India (Yan et al., 2009), but it is also a clean and renewable energy source compared to conventional fossil derived fuels (Weisz 2004; Goldemberg 2007). Distillers dried grains with solubless (DDGS) are a co-product of the ethanol industry (Shurson and Noll, 2005). According to Wisner (2008), the production of DDGS from corn was between 38 and 40 MMT in 2011 in the USA. The estimated production of wheat DDGS from January 2009 to March 2009 in Saskatchewan was about 0.3 MMT and yearly ethanol production reached 2.1 billion liters of ethanol in gasoline (1.7 MMT of DDGS) in 2011 (McKinnon, 2008). The European Union has set up biofuel inclusion targets of 6% to 10% into gasoline within 2010 to 2020 (Turpeinen, 2007). Other nations such as China and Brazil are setting up their own standards to regulate the usage of grains for ethanol production (Elobeid and Tokgoz 2008). This will increase the use of grain for biofuel production and therefore it will contribute to an increase in DDGS production. Nutrients in grains used for biofuel production are concentrated by fermentation process with exception of starch that is converted into ethanol. Generally speaking, DDGS has higher concentrations of protein, lipids, minerals, and fiber compared with the original grain (Widyaratne and Zijlstra, 2007; Widyaratne et al., 2009). Consequently, high inclusion of DDGS means starch levels in diets can be decreased significantly, and starch

provides a smaller proportion of the total NE compared to typical diets (providing 60% of total NE) using mostly cereal grains as energy (Lammers and Honeyman, 2009).

Nutritionists must accurately provide energy and amino acids to modern genotype pigs and for lean growth. Optimal protein deposition is affected by a combination of protein quality (ie, amino acid availability, profile of amino acid, etc) and in an optimal balance with non-protein energy sources. Since protein sources and amount were kept the same in all five-semi purified diets, non-protein energy sources (starch and lipid) play crucial role in maximizing protein deposition. Incorporating DDGS into swine diets has had the attention of nutritionists for decades. However, the new focus as turned to how much DDGS can be included in the diets for different stages of growth to optimize muscle deposition. Little attention has focused on the starch concentration in diets. As mentioned previously, the starch content of DDGS is low relative to the parent grain. Starch typically contributes more than half of the energy required by swine. Furthermore, the higher level of fiber in DDGS serves to dilute the reduced starch in these diets. Protein sparing effects of carbohydrate were reported more than two decades ago (Fuller et al., 1977; McCargar et al., 1989) and has since been verified in humans (Kashyap et al., 2001) and animals (Vazquez et al., 1986; Stone et al., 2003). Since starch is important for protein accretion, our overall objective was to determine if will low starch content in high DDGS incorporation swine diets influence protein deposition (i.e., lean meat)? Or will level of starch in growing pig diet determine the level of protein accretion?

2 Literature review

2.1 Distiller's dried grains with solubles (DDGS)

Distiller's dried grains with solubles is a good source of nutrients for the animal feed industry (de Godoy et al., 2009). The CP content varies from 24.1% to 30.9% in corn DDGS (Stein, 2007) and from 33.8% to 44.5% in wheat DDGS (Losada et al., 2009). DDGS has replaced some of the soybean meal as well as parent grain and phosphorus in poultry and swine diets and reduced feed costs (Gaines et al., 2006; Spencer et al., 2007; Barbosa et al., 2008). A comparison of the fat concentration in wheat and corn and their co-products (DDGS) is shown in Table 2.1. The fat concentration in DDGS is much higher than the original grain and high fat content may contribute to the high DE (NRC 1998). However, some ethanol plants in the USA started to harvest fat after fermentation which could have negative impact on the energy level of DDGS and make it more as a CP source rather than an energy source (Saunders and Rosentrater, 2009). In addition, the high fiber content of DDGS compared with the original grain limits its inclusion in swine diets (Leterme et al., 1996; Noblet and Le Goff, 2001). Several studies have reported that the digestibility of DM, CP, and DE were negatively affected by the high fiber content of DDGS (Bindelle et al., 2008; Noblet and Le Goff, 2001; Thacker, 2006). Even though GE is similar between the original grain and DDGS, Pedersen et al. (2007) reported that DE was slightly lower in corn DDGS than in corn.

Table 2.1. Ether extract and phosphorus concentration in wheat and corn and their by products (DDGS).

Item	Concentration (%)			
	Corn	Wheat	Corn DDGS	Wheat DDGS
Ether extract (% fat)	2.8	1.9	7.0	4.6
Phosphorus	0.2	0.4	0.4	1.0

(Adapted from Pedersen et al., 2007; Widyaratne et al., 2009).

2.1.1 Use of DDGS in swine diets

Despite extensive research, the optimum DDGS inclusion levels for different stages of swine growth are still controversial. Considerable research has been conducted to examine the nutrient concentration and digestibility, feeding value, and impact of high DDGS on carcass characteristics.

2.1.2 The effect of the inclusion of DDGS in the diet on performance of pigs

Anticipated future availability of large quantities of DDGS plus increasing grain prices has encouraged researchers to determine how to overcome the negative factors in DDGS and maximize the inclusion of it into swine diets. Average daily gain, ADFI and FC were unaffected by the inclusion of 30% corn DDGS to diets for weanling pigs (Gaines et al., 2006; Spencer et al., 2007; Barbosa et al., 2008; Burkey et al., 2008; Jones et al., 2010). Similar results for ADG, ADFI and FC were seen with up to 30% sorghum DDGS inclusion compared to a control diet (no DDGS) in growing pigs (Senne et al., 1996). Similarly no differences in ADG, ADFI or FC were reported with up to 30% of wheat, corn, or sorghum DDGS in diets for growing-finishing pigs (Cook et al., 2005; DeDecker et al., 2005; Xu et al., 2010). Conversely, weanling pigs fed diets with 30% sorghum DDGS had reduced ADG relative to lower inclusion levels (Feoli et al.,

2008). Moreover, some researchers observed that ADG was reduced by 25% wheat DDGS or 30% corn DDGS incorporation in the diets (Fu et al., 2004; Thacker, 2006; Widyaratne and Zijlstra, 2007). Furthermore, high levels of corn DDGs in finisher diets result in negative meat quality characteristics (e.g. soft belly/bacon and/or high back fat (Xu et al., 2010)). Reproductive performance of sows was unaffected by the addition of 20% corn DDGS in lactation and 40% in gestation rations (Monegue and Cromwell, 1995; Wilson et al., 2003; and Hill et al., 2005). Stein and Shurson (2009) concluded from a review that up to 30% DDGS can be used in lactation diets and 50% during gestation with no effect on sows' performance.

2.2 Introduction of experiment

Factors which may influence lean tissue growth in swine include genotype (Quiniou et al., 1995), nutritional history (Bikker, 1994), and environment (Le Bellego et al., 2002). Protein deposition in pigs was determined by the protein to energy ratio in the diet (Kyriazakis and Emmans, 1992), body lipid to body protein deposition ratio (de Greef, 1992), and level of energy intake (Black et al., 1986). It has been suggested that with sufficient mineral and vitamins in the diet, the efficiency of protein utilization for protein accretion is determined by a combination of protein quality (availability, balance and profile of amino acid), protein concentration and level of dietary lipid and carbohydrate (Fuller and Crofts, 1977). The following review will emphasize the combination of protein quality and non-protein energy source influence on protein accretion in growing pigs.

Energy is produced in the body when organic molecules undergo oxidation. Dietary energy is derived from carbohydrates, lipids and proteins (NRC 1998). Unfortunately, it is difficult to distinguish the amount of energy contributed from lipid, carbohydrate or protein in a

diet (Halas and Babinszky, 2010). Starch is a major component of carbohydrate and a major contributor to dietary energy for swine (Camp et al., 2003; Vicente et al., 2009; Regmi et al., 2010). Dietary starch, not dietary fat, contributes to the change of blood glucose and insulin in monogastric animals. Because of the high starch content of grains, a deficiency of energy (i.e., glucose) from starch is unlikely in diets based on cereal grains. In other words, starch concentration in swine diets is not a typical concern and consequences of this are rarely researched.

Since most of the starch in grain is removed for ethanol production, DDGS is limited in starch content. Incorporation of high DDGS into swine diets will therefore result in reduced starch content. Protein accretion is affected by dietary carbohydrate level and available energy (Fuller et al., 1977). So, at this point, it is unclear whether reduced dietary starch content will alter optimal protein accretion in growing pigs.

2.3 Starch

2.3.1 Definition

Starch consists of glucose units joined together by glycosidic bonds and it is one of the most digestible polysaccharides existing in plants (Englyst et al., 1983). Pure starch is white, tasteless and odorless (Visakh and Thomas 2010). It does not dissolve in cold water or ethanol (Trufakina 2008).

2.3.2 Composition

Most grains contain 50 to 65% starch (Table 2.2). The starch molecule consists of linear amylose and branched amylopectin (Cummings and Englyst, 1995). Amylose is linked by alpha

(1, 4) glucosidic bonds and amylopectin contains both alpha (1, 4) and alpha (1, 6) linkages (Cummings and Englyst, 1995). In most starches, amylose comprises 20-30% of total starch; the remaining 70-80% starch is amylopectin. Amylose, due to its tighter structure is less digestible than amylopectin and this restricts enzyme access (Zhou and Kaplan, 1997). Corn and pea starch contain 25% amylose (75% amylopectin) and 35% amylose (65% amylopectin) respectively, resulting in faster starch digestibility of corn than pea starch (Chung et al., 2009). High amylose food slows the digestion and absorption of starch and was used to facilitate blood glucose regulation, especially in diabetes prevention, because of its low glycemic response after meals (Jenkins et al., 1988). Conversely, high amylopectin proportion food has a high glycemic index and stimulates insulin response (Liljeberg et al., 1992).

Table 2.2. Starch content of typical grains.

Grains	Starch (%)	Amylose : amylopectin ratio
Wheat	58.8	25:75
Corn	62.0	30:70
Barley	61.9	25:75
Sorghum	64.8	27:73
Rye	52.7	24:76

(Adapted from Mahmood, et al., 2007; Losada et al., 2009)

2.3.3 Starch content

Corn and wheat contain 59 to 62% starch (Table 2.3). During ethanol production, most of the starch in a grain is fermented into ethanol. Depending on the fermentation technique used, starch remaining in corn DDGs varies from 8.7 to 15.4% and in wheat DDGS from 5.8 to 8.7%

(Table 2.3). The remaining starch is in bran and germ (Armentano et al., 2007) and may still be available to animals.

Table 2.3. Concentration of starch in corn, wheat, corn DDGS and wheat DDGS.

	Starch, %			
Item	Average	Min. value	Max. value	SEM
Wheat	58.8	55.0	61.0	1.5
Wheat DDGS	7.4	5.8	8.7	1.4
Corn	61.9	59.6	63.9	1.2
Corn DDGS	11.9	8.7	15.4	3.1

(Adapted from Losada et al. 2009)

2.4 Glucose

Glucose is derived from the Greek word "glukus", meaning "sweet". Glucose is a monosaccharide and is the principal sugar found in blood (Marks et al., 1996). Glucose is a critical metabolite and thus animals may have evolved to conserve glucose when necessary (Lepine et al., 1989).

2.4.1 Glucose production

Exogenous and endogenous glucose contribute to maintain blood glucose within a normal range (2.6-6.5 mmol/l) in pigs (Magarian and Sterling, 2009). The normal concentration of blood glucose is affected by diet composition and stage of absorption such as fasting or feeding (Balasubramanyam et al., 1999). Whether glucose is oxidized for energy, or converted to glycogen or fat for storage is determined by glucose availability, hormone status and energy requirements (Layman et al., 2003).

2.4.2 Exogenous and endogenous glucose

Exogenous glucose is derived from the digestion of carbohydrate (mainly starch) in the digestive tract (Battilana et al., 2001). Starch digestion begins in the mouth with the secretion of salivary amylase which is deactivated in the acidic environment of the stomach. When the digesta enters the small intestine, secretin is secreted from duodenal and jejunal mucosa, leading to the release of pancreatic enzymes (Marks et al., 1996). Pancreatic amylase cleaves polysaccharides, such as starch, into mono- and di-saccharides (Newey, 1967). The monosaccharides, are absorbed through the epithelium of the small intestine by either active transport or facilitated diffusion and then transported to the hepatic portal system (Marks et al., 1996). Rising blood glucose triggers the secretion of insulin from the beta cells of the pancreas causing the conversion of excessive glucose into glycogen, which is stored in the liver and skeletal muscle (Newgard et al., 1983). Insulin also facilitates protein synthesis (Layman et al., 2003) or reduces protein degradation (Mortimore and Mondon 1970; Morgan et al., 1972). Additional glucose is converted to lipid for storage in adipose tissues once the glycogen storage capacity in liver and muscle has been met (Flatt, 1970).

Animals regulate blood glucose to maintain normal body functions (Magarian and Sterling, 2009). When blood glucose levels decline, the α-pancreatic cells secrete the hormone glucagon, which inhibits the secretion of insulin and facilitates gluconeogenesis in the liver utilizing the gluconeogenic amino acids such as leucine, isoleucine, valine and alanine (Wagenmakers, 1998) and glycerol. Gluconeogenesis occurs primarily during fasting or starvation (Salway, 2004). During the catabolism of the branched chain amino acids, the amino nitrogen is transferred to keto-glutarate for glutamate formation. Aminotranserease converts glutamate to alanine or glutamine; gluconeogenic amino acids for hepatic gluconeogenesis

(Layman et al., 2003). Glycogen will be catabolized to replenish the glucose supply through glycogenolysis (Mayes, 2000).

2.5 Functions of glucose in the body

There are two major functions associated with glucose: provision of energy and acting as a metabolic intermediate.

2.5.1. Glucose as an energy source

Glucose is the major metabolic fuel that pigs relies on to fulfill their energy requirements (Wu et al., 1991). Some tissues such as the brain, red blood cells and kidney show a preference for glucose (Young, 1991). A portion of the absorbed glucose is metabolized in the intestinal cells where it makes an important contribution to meeting the energy requirements of the intestinal epithelial cells (Britton and Krehbiel, 1993; Wu et al., 1995; Beaulieu et al., 2002). Through glycolysis, a metabolic breakdown of glucose, ATP is mainly produced under aerobic conditions (Figure 2.1).

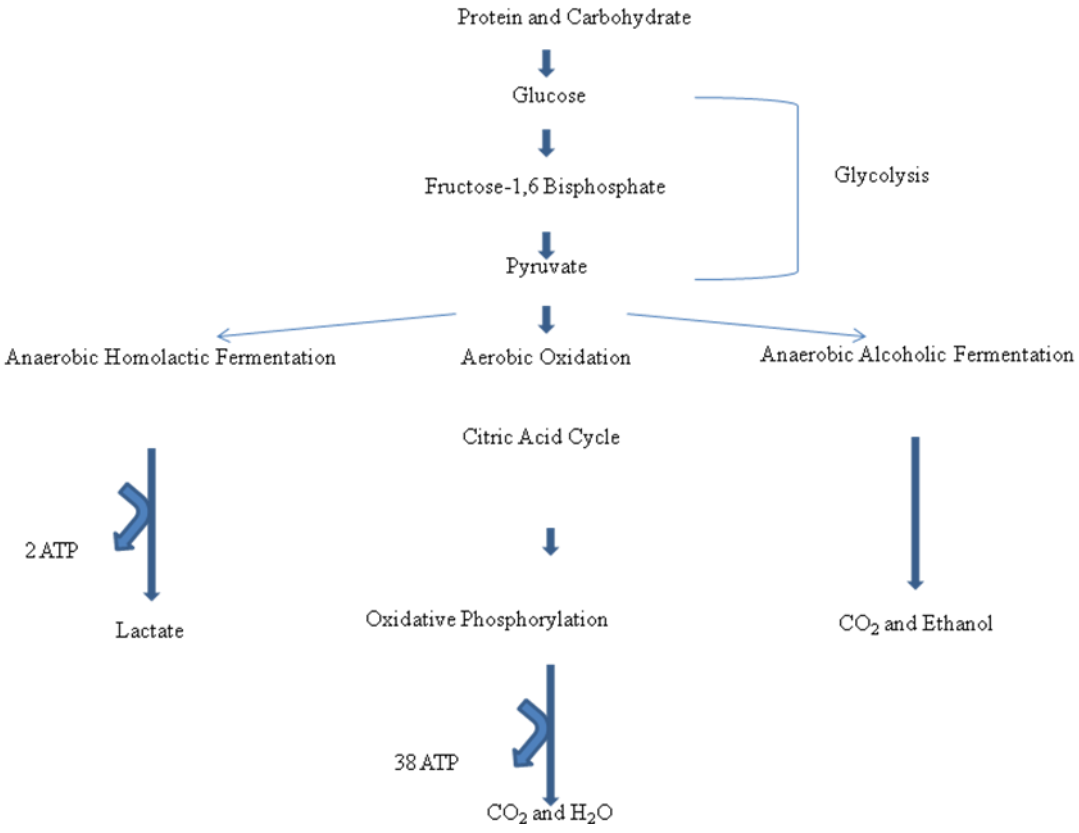


Figure 2.1. ATP production from protein and carbohydrate (redrawn by Xianjian Zeng based on Marks et al., 1996).

The end product of glycolysis is pyruvate, which yields acetyl CoA, an energy rich compound. When oxygen is not present, pyruvate is converted to lactate and produces two ATP. In aerobic situations, pyruvate is metabolized to acetyl CoA, which enters the citric acid cycle to generate 38 ATP (Marks et al., 1996). ATP generated by glucose is used as an energy substrate and brain cells, erythrocytes, and renal medulla cells show a preference for glucose (Cerasi, 1975; Jacquez, 1984; Magnani et al., 1983).

2.5.2 Glucose as an metabolic intermediate

Glucose also serves as a metabolic intermediate. It is the precursor for the synthesis of other carbohydrates in the body, including lactose for milk synthesis and glycogen for energy storage in skeletal muscles and liver. It is also the precursor for the synthesis of ribose and deoxyribose; components of DNA and RNA (Lee et al., 1998). Furthermore, glucose is a substrate for glycolysis and a precursor for vitamin C (Roberts et al., 1995). In monogastric animals, glucose is a precursor for lipid (Winegrad, 1965).

2.6 Glucose, insulin and protein synthesis

Glucose homeostasis in the blood is controlled by insulin and glucagon (Koo et al., 2005). A minor change in blood glucose concentration will be detected by the pancreatic cells which release insulin or glucagon into the circulation in response to an increasing or decreasing blood glucose concentration. When glucose is infused into the blood, the insulin response takes a few seconds to a couple of minutes (Blackard and Nelson, 1970). Grodsky et al. (1968) discovered that the insulin response in vivo is proportional over a wide range of blood glucose concentrations. In general, insulin is released to facilitate the up-take of glucose by skeletal muscle and liver cells to store as glycogen after a meal. Excessive glucose is converted to lipid for storage (Flatt, 1995). Once the plasma glucose concentration decreases, insulin secretion decreases to reduce glucose uptake to maintain blood glucose concentration (Figure 2.2).

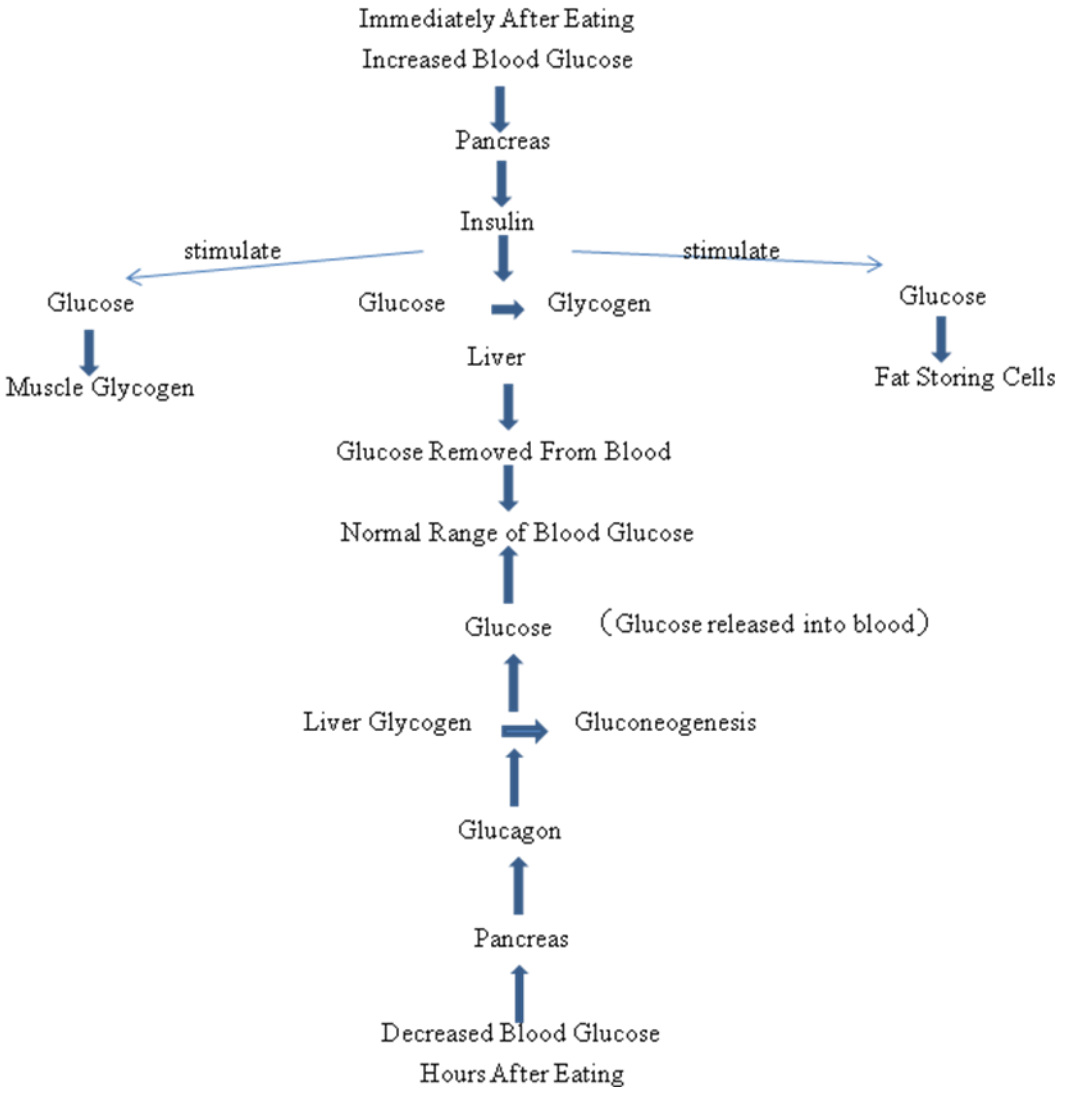


Figure 2.2. Blood glucose is regulated by insulin and glucagon (redrawn by Xianjian Zeng based on Marks et al., 1996)

Although glucose is the primary physiological stimulator for insulin release, certain essential amino acids such as leucine, also trigger insulin secretion from beta pancreatic cells, however, the mechanism is unclear (Zhou et al., 1997).

Protein synthesis requires three steps; initiation, elongation and termination. Insulin regulates initiation and elongation (Proud, 2006). Whole body protein synthesis is the net result of the oxidation of amino acids from the diet and muscle protein catabolism and protein

anabolism (Tesseraud et al., 2007). Protein accretion and proteolysis are regulated by physiological, environmental and genetic factors (Tesseraud et al., 2007). Among those factors, insulin is considered the major regulator (Tesseraud et al., 2007). Insulin stimulates cellular responses and activates the protein kinases, which triggers glucose transport and induces repression of specific genes, phosphorylation of proteins, reversal of glucagon-stimulated phosphorylation and stimulation of protein synthesis (Figure 2.3). High blood glucose stimulates insulin secretion. Both glucose and insulin stimulate protein synthesis, but via different mechanisms. Although glucose can stimulate protein accretion directly, the amount produced is insignificant compared with insulin in terms of total protein gain (Tokudome et al., 2004). When leucine was infused into young adults the addition of dietary glucose (food) resulted in a greater passage of leucine to peripheral tissues for protein synthesis than when it was infused alone because of higher insulin with the additional glucose (Young, 1991).

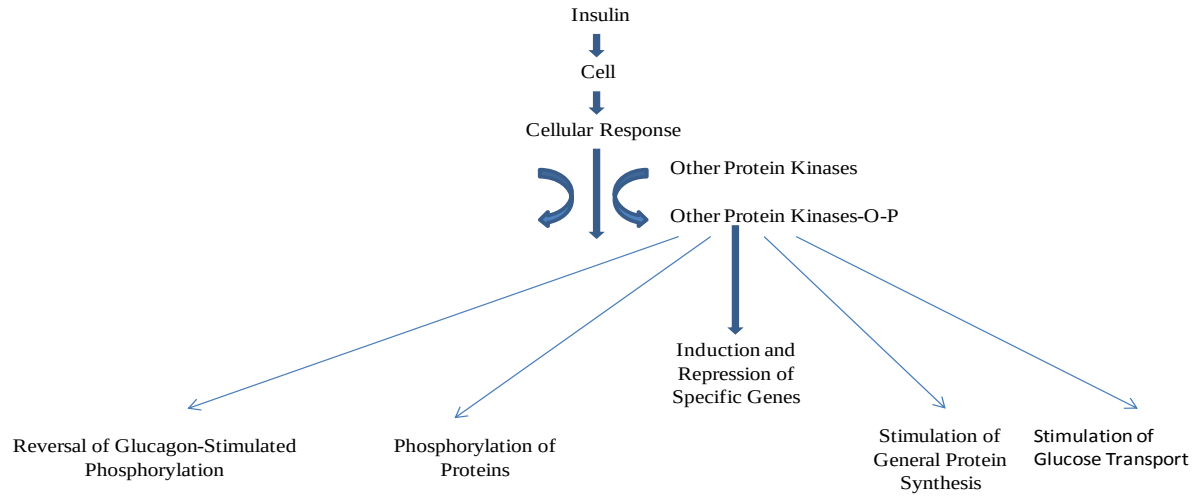


Figure 2.3. Insulin initiates phosphorylation and cellular responses involved in protein synthesis. (redrawn by Xianjian Zeng based on Marks et al., 1996)

2.7 Factors influencing protein deposition

2.7.1 The quality and the amount of protein consumed

The essential amino acid composition, the availability of amino acids, the anti-nutritional factors and fiber content determine protein quality (Gatel, 1994). A balanced amino acid profile results in ideal protein utilization and fewer amino acids will be used for oxidization. Some anti-nutritional factors damage proteolytic enzymes leading to reduced enzyme availability in the digestive tract which cause depressed protein digestibility and more endogenous nitrogen loss (Grala et al., 1998). Animal protein is superior to plant protein in terms of amino acids profile and digestibility (Aumaitre and Seve, 1978). However, plant protein is a major protein source in swine diets because of its lower cost. DDGS has been utilized in swine diets as a protein source to replace some of the soybean meal. Although DDGS has a high protein concentration, its amino acid profile and amino acids digestibilities are lower than the original grain (Stein 2007).

As shown in Table 2.3, the CP content of DDGS is approximately 27% in corn and 44% in wheat. It is suggested that the ratio of lysine to CP be 2.8% or greater when formulating swine diets using DDGS (Stein 2007). The digestibility of amino acids in DDGS is variable (Urriola et al., 2007; Lan et al., 2008). Amino acid digestibility of DDGS, either apparent or ileal, was reduced by 10% compared with the original grain (Stein and Shurson 2009). Reduced digestibility of lysine may be due to heat damage during the drying of the DDGS (Cromwell et al., 1993; Stein et al., 2006). The color of DDGS has been used to predict the degree of damage and near infrared spectroscopy can be used to measure color in DDGS (Cromwell et al., 1993; Fastinger and Mahan, 2006). Light brown and yellow DDGS are considered good quality; while darker colors indicate heat damage (Cromwell et al., 1993). Another reason for reduced amino acid digestibility was the high fiber content of DDGS (Stein, 2008). To overcome the shortage of

lysine in DDGS, synthetic lysine may be required to maintain the growth rate in swine (Stein, 2007).

Table 2.4. Concentration and standardized ileal digestibility (SID) of CP and amino acids (AA) in DDGS fed to growing pigs[1]

	Concentration of CP and AA. % (DM)				SID of CP and AA			
	Corn		Wheat		Corn		Wheat	
Item	DDGS	Corn	DDGS	Wheat	DDGS	Corn	DDGS	Wheat
CP	27.38	10.5	40.67	13.8	72.8	79.6	72.2	84.8
Essential AA								
Arg	1.14	0.59	1.53	0.68	81.1	88.6	83.4	90.4
His	0.71	0.32	0.92	0.32	77.1	85.1	74.2	88.0
Ile	1.00	0.38	1.35	0.45	75.3	84.3	77.2	88.0
Leu	3.11	1.25	2.66	0.90	83.5	89.7	81.4	85.5
Lys	0.76	0.35	0.65	0.39	60.6	77.5	47.7	84.2
Met	0.54	0.23	0.53	0.22	81.8	87.7	79.5	89.9
Phe	1.32	0.52	1.92	0.64	80.8	90.6	85.1	92.4
Thr	1.04	0.40	1.21	0.39	70.4	72.4	72.5	77.5
Trp	0.21	0.06	0.40	0.12	69.6	69.6	80.4	81.4
Val	1.34	0.53	1.70	0.60	74.4	83.7	80.1	86.0

[1]Adapted from Lin et al., 1987; Stein et al., 2006; Widyaratne and Zijlstra, 2007; Widyaratne et al., 2009

Fiber content of DDGS can also influence the protein digestibility. As Table 2.4 shows, the concentration of fiber whether characterized as CF, NDF, or ADF increased almost three times in DDGS compared with the original grain (Losada et al., 2009). The higher fiber concentration of DDGS has a negative impact on nutrient digestibility (Wenk, 2001). Higher fiber inclusion in the diet increases endogenous nitrogen loss (Leterme, et al., 1996; Souffrant, 2001) and damages the gastrointestinal tract (Varel and Yen, 1997). As a consequence, intestinal damage or reduced passage rate associated with high fiber in the diet caused reduced digestibility of energy and protein (Noblet and Le Goff, 2001). However, some researchers (Montagene et al.,

2003; Aarnink and Vertegen, 2007) have reported positive results on reduction of ammonia emission by converting some of ammonia to urea and improvement of gut health because of fiber. Although there are negative effects of phytate and fiber, the phytase and xylanase addition to the wheat based feed are beneficial to animals (Partridge et al. 2009).

Table 2.5. Fiber content of feed ingredients as fed basis (%).

Ingredient	N	Moisture	Crude fiber	NDF[1]	ADF[2]	ADL[3]
Wheat grain	13					
Mean		13.0	2.5	13.1	3.3	1.0
SD		0.9	0.4	1.1	0.4	0.1
Min		10.9	1.9	10.6	2.8	0.8
Max		14.4	3.2	14.7	4.0	1.1
Corn grain	12					
Mean		13.7	2.1	11.3	2.6	0.4
SD		1.2	0.2	0.8	0.3	0.1
Min		12.9	1.8	10.4	2.1	0.2
Max		16.7	2.5	12.9	3.1	0.6
Wheat DDGS	4					
Mean		7.9	6.6	27.6	8.8	4.4
SD		0.3	0.2	1.0	0.8	0.6
Min		7.5	6.4	26.2	7.9	3.4
Max		8.2	6.8	28.8	9.8	4.8
Corn DDGS	6					
Mean		9.7	6.9	33.6	10.3	2.9
SD		1.8	0.4	2.6	1.3	1.0
Min		7.2	6.3	29.9	8.5	1.4
Max		11.4	7.2	36.1	11.8	4.2

[1]NDF: neutral detergent fiber
[2]ADF: acid detergent fiber
[3]ADL: acid detergent ligin
(Adapted from Losada et al., 2009)

Many researchers reported that swine fed a high crude protein diet gained more lean muscle than those fed a low crude protein diet (Kerr and Easter, 1995). More protein deposition was associated with higher availability of amino acids. However, when dietary protein concentration is above requirement, increased daily gain or protein deposition stops (Rosebrough

and Steele, 1987; Campbell and Taverner., 1988). Higher dietary protein level might not stimulate more protein deposition due to the energetic cost of disposing excess amino acids through urea synthesis (ARC, 1981).

2.7.2 The amount of non-protein energy (carbohydrate and lipid)

Energy is the most expensive nutrient to provide in swine diets (King and Taverner, 1975; Noblet and Henry, 1993; Holden et al., 1996). The carbon backbone of protein can be degraded to amino acids for oxidation or converted to glycogen or fat for energy storage (Patel and Woodgett, 2008). Oxidation of carbohydrate, protein and lipids contribute to the energy pool. Formulating diets to meet energy requirement for swine is very challenging, not only from a cost perspective, but also to reduce the risk of utilizing excess proteins for energy which is not efficient (van Milgen et al., 2001). Additionally, utilization of protein as an energy source results in excess nitrogen excreted into the environment (Dourmad and Henry, 1994).

2.7.3 Protein sparing effects

The concept of protein sparing suggests that protein will not be oxidized if sufficient non-protein energy is available (Munro, 1951; Fuller et al., 1977).

2.7.3.1 Protein sparing effect of lipid

High fat diets exert their mechanism for sparing protein, or preventing protein catabolism (Blackburn et al., 1973; Cho and Kaushik, 1985; Helland and Grisdale-Helland, 1998). Newsholme and Leech (1986) explained that high fat diets result in increased fatty acids oxidation and decreased glucose utilization for energy production, which slowed down the

process of amino acids being used for energy production since the energy supply was met by oxidation of fatty acids. Li and Sauer (1994) concluded that additional fat promoted protein utilization which implied more dietary protein used for protein synthesis.

2.7.3.2 Protein sparing effect of carbohydrate

Numerous authors have determined that carbohydrates spare dietary protein for protein synthesis (Fuller et al., 1977; McCargar et al., 1989; Stone et al., 2003; Satpathy and Ray, 2009). A protein sparing effect of carbohydrate was hypothesized at the beginning of the 20th century by Cathcart (1909), who found that 400 kcal/day of carbohydrate, but not fat, reduced N loss in fasted humans. Similar protein sparing effects of carbohydrate results were obtained by Fuller et al. (1977), who conducted experiments on growing pigs and found that protein was spared with increasing dietary starch. Further, the leucine oxidation rate was reduced with the addition of carbohydrate to the diet while fat addition increased leucine catabolism under marginal caloric intake (Vazquez et al., 1986). When low-birth infants were fed iso-nitrogenous and iso-caloric diets, higher protein deposition with carbohydrate inclusion was observed (Kashyap et al., 2001).

Carbohydrate was more effective than fat in sparing protein (Richardson et al., 1979; Schneeman, 1994; Mariotti et al., 2000). Richardson et al. (1979) found a diet containing two times the non-protein energy from carbohydrate as from fat had a significantly improved overall N balance than a diet supplying equal non-protein energy from carbohydrate and fat in young healthy men. Munro (1964) proposed that the protein sparing effect is tightly associated with the insulin level, which means that glucose stimulates insulin secretion and insulin stimulates protein synthesis. Fuller et al. (1977) simultaneously infused physiological amounts of exogenous insulin and glucose into healthy pigs. They administrated glucose with insulin at a rate to

maintain the plasma glucose level within the normal physiological range and tried to prevent the hypoglycemic and counter-regulatory response to insulin. Over 3-7 days, plasma glucose declined 50%, plasma urea N decreased 30%, urinary urea excretion declined 30% while there was a 2 to 7 times increase in plasma insulin concentration. Fuller et al. (1977) concluded that the protein-sparing effect obtained by administration of carbohydrate, was regulated by insulin. Other researchers also reported that insulin secretion is dependent on the glucose concentration in the blood (Byrnes et al., 1975), but does not occur in response to fat (Richardson et al., 1979; Storlien et al., 1986). Insulin is important for skeletal muscle growth through transporting amino acids for protein synthesis (Kipnis and Noall, 1958), the initiation of peptide chain formation (Jefferson et al., 1974), and reducing muscle protein catabolism (Pozefsky et al., 1969). Decreased BUN indicated more protein synthesis (deposition) with increasing starch inclusion (Zhang et al., 2010).

Some others found that carbohydrate and lipid were considered equal in promoting nitrogen retention at a fixed protein intake (Nakano and Ashida, 1975). They observed the phenomenon that similar protein retention from carbohydrate and lipid.

2.8 The effect of starch on performance

Starch effects on animal growth have been intensively investigated in different species (McCargar et al., 1989; Camp et al., 2003; Zhang et al., 2009; Pirgozliev et al., 2010; Zhang et al., 2010). In all studies, increasing dietary starch increased weight gain. In most of the trials, increases in average daily gain were associated with increases in average daily feed intake (Mateos et al., 2007; Vicente et al., 2008). Increasing dietary starch from 30.3%, 37.3%, and 42.9% resulted in significantly increased feed intake from 2.36 kg, 2.44 kg, and 2.48 kg per day

respectively in grower-finisher pigs although diets were not isocaloric (O'Doherty et al., 2002). Feed intake did not differ when isonitrogenous and isocaloric diets containing 44.1% or 53.4% starch were fed to weanling pigs (Bengala-Freire et al., 1988). Average daily gain and feed intake were not affected with increasing dietary carbohydrate in pigs (Beech et al., 1991 ab; Knowles et al., 1998) but feed conversion improved in a different study (Gutierrez et al., 2002). Beech et al., (1991 ab) used lactose in the experimental diets which may explain why average daily gain and average feed intake were not affected. A portion of lactose escapes from the small intestine to large intestine and can not be utilized for energy and does not act as a stimulate for insulin secretion.

2.9 The effect of starch on nutrient digestibility

The digestibility of DM, DE and CP improved in pigs fed diets with increased starch content (Noblet and Shi, 1992; Mateos et al., 2007; Vicente et al., 2008). The improved DM and DE digestibility with increased dietary starch may be due to the highly digestible starch and its high digestible energy value (Dreher et al., 1984). O'Doherty et al. (2002) stated increased starch content was associated with lower fiber inclusion in iso-caloric formulation. Reduced fiber and high glucose availability in the gut led to a longer retention time in the digestive tract allowing more time for the action of digestive enzymes (Degen et al., 2009). Improved CP digestibility with increased starch inclusion was explained by the availability of glucose, as an energy supply, which aided the absorption of amino acids by transporters in intestinal walls (Young 1991). Van der Meulen et al. (1997) and Li et al. (2008) observed that the digestibility of starch and increased availability of glucose is closely related to the absorption of amino acids. Higher starch digestibility resulted in increased glucose released to the small intestine and higher amino acid

absorption (Li et al., 2008). Beside higher glucose and amino acid absorption, improved nutrient utilization associated with starch was reported in pigs (Van den Borne et al., 2007). Total tract apparent digestibility of lipid declined linearly with increased dietary starch at a constant lipid content in the diets (Tao and Li, 2006; Morsy et al., 2009). Typically a diet low in lipid has a high starch content to maintain energy balance. Endogenous fat loss may contribute to a low fat digestibility. Changes in fiber content and type may affect endogenous fat loss (Bach Knudsen and Hansen, 1991). In iso-caloric diets, increasing dietary carbohydrate content was associated with reduced fiber content, which may lower endogenous fat loss due to low dietary fiber inclusion (Degen et al., 2007).

2.10 The effect of starch on blood metabolites

Blood metabolites such as glucose, insulin, C-peptide, BUN, and free fatty acids provide an indication of an animal's metabolic status. C-peptide and insulin are cleaved from pro-insulin. However, hepatic disappearance of C-peptide is negligible (Guan et al., 2000; Cabioglu and Ergene, 2006). Higher availability of glucose led to higher insulin production and higher insulin facilitated blood urea absorption for protein synthesis and leads to decreased BUN in humans (Deutz et al., 1995). Glucagon secretion will contribute to increased glucose release from glycogen or through gluconeogenesis if the diet is low in starch. Measuring insulin concentration in blood is not accurate, however, because the half-life of insulin is only 3-5 minutes as it is rapidly hydrolyzed. C-peptide provides an estimate of insulin secretion and its half-life of 30 minutes allows for easier measurement (Ruberstein et al., 1969; Horwitz et al., 1975; Polonsky et al., 1983; Cauter et al., 1991).

2.11 The effect of starch on nitrogen retention and body composition

Many trials have indicated that a high carbohydrate diet results in increased nitrogen retention compared with a high fat diet (Thomson and Munro, 1955; Munro, 1964; Richardson et al., 1979; McCargar et al., 1989). There are a series of mechanisms involved in nitrogen retention. Glucose induces insulin secretion (Leahy et al., 1992) which facilitates increased nitrogen uptake from the blood into muscle tissue and reduces nitrogen output through feces or urine (Munro, 1964). Higher insulin led to increased protein deposition in the muscle and more fat accretion in adipose tissues and increased protein retention was higher in pigs fed high carbohydrate diets (Munro, 1964). Fuller et al. (1977) hypothesized that this could be explained by the protein sparing effect of carbohydrate and less dietary protein catabolized for energy. Increased lipid deposition in the carcass may be the result of the excess carbohydrate converted to lipid for storage (Satpathy and Ray, 2009). In preterm infants, nitrogen retention did not change with increased carbohydrate inclusion in an iso- nitrogenous and iso-caloric diet, but fat deposition increased with increased fat inclusion (Romera et al., 2004). Others observed decreased nitrogen retention with increased carbohydrate intake in the newborn infant (Salassalvado et al., 1993), while others reported that carbohydrate and fat have the same effect on nitrogen sparing in newborn infants given an iso-nitrogenous and iso-caloric intravenous infusion (Pineault et al., 1988).

Further research may need to be done to clarify those above ambiguities particularly in light of future diets containing higher protein, lower carbohydrate and variable levels of fat due to variations in sources of available co-products for feeding.

2.12 Summary

Distiller's dried grain with solubless (DDGS), a co-product of the ethanol industry, has a high nutritional value, especially with respect to protein and phosphorus. The amino acid profile is excellent but not the availability of lysine. It has been widely used in the swine industry, partially to replace soybean meal as well as added oil and phosphorus. One of the main differences between DDGS and the parent grain is the low starch content of the DDGS, which was converted to ethanol during fermentation. Diets formulated with high DDGS will have reduced starch content. Starch is one of two non-protein energy sources and is an essential precursor for glucose for tissues such as brain cells and erythrocytes. In addition, a protein sparing effect of carbohydrate has been shown in human, swine and fish. Starch concentration in swine diets may contribute to different aspects in terms of performance, digestibility, blood metabolites, carcass protein deposition and nitrogen retention despite different results mentioned above. In order to incorporate maximum DDGS into swine rations, the starch "requirement" needs to be addressed to find out if low starch diets have an impact on protein deposition in pigs although there are other health and environment sustainability concerns such as higher nitrogen and phosphorus excretion that is associated with improved nutrient retention (Jenkin et al., 2007).

3. Nutritional value of starch for swine

3.1 Introduction

In farm animals, dietary starch provides more than 50 % of the energy needs for maintenance, growth and reproduction (Nafikov and Beitz, 2007). Although glucose is the most important carbohydrate and is irreplaceable for many functions (Marks et al., 1996), deficiency symptoms are not expected if swine consume diets devoid of glucose or glucose precursors such as starch. If dietary glucose is insufficient, the body will synthesize glucose through gluconeogenesis (Exton and Park, 1966). However, if the diet lacks sufficient carbohydrate, then, dietary and muscle amino acids along with dietary and tissue lipids will be used directly for energy. Oxidation of dietary and muscle amino acids for energy are an inefficient source of energy for animals (van Milgen and Noblet, 2003).

Starch is not listed as a required nutrient for pigs in commonly used requirement tables (NRC, 1998). Typical swine diets are based on grains, which are high in starch and thus the starch content has not been an issue for nutritionists when formulating swine diets. The co-product of ethanol production, DDGS are very low in starch (Stein and Shurson, 2008). Diets formulated with high DDGS content will therefore have reduced starch concentration. For example, a normal commercial swine diet based on corn and soybean meal will typically have 40% starch, but a swine diet with 30% DDGS inclusion will have about 26% starch (Whitney et al., 2006). There are a few published papers that have measured the importance of starch on carcass protein deposition in pigs (Fuller et al., 1977; Kyriazakis and Emmans, 1992); and certainly since this time protein deposition potential of pigs has increased significantly.

Our hypothesis is that maximizing protein deposition in growing pigs requires a minimum level of dietary starch. The overall objective of this experiment was to determine if dietary starch

concentration impacts level of carcass protein deposition in growing pigs. Growth rate, nutrient digestibility, blood metabolites and carcass nutrient accretion were looked at in this experiment to determine if differences were associated with level of dietary starch.

3.2 Materials and Methods

3.2.1 Animal and housing

All animals utilized for these experiments were PIC Camborough Plus genetics (Camborough Plus females x C 337 sires, PIC Canada Ltd. Winnipeg, MB) and were housed within their thermo-neutral zone on a 12-hour light (0700 h to 1900 h) and 12-hour dark cycle (1900 h to 0700 h). The trial was conducted in accordance with the recommendations of the Canadian Council on Animal Care (CCAC, 1993) and followed Animal Care Protocol (No, 20080069) approved by the University of Saskatchewan Committee on Animal Care and Supply.

The gilts were housed in individual pens measuring (1.3 m x 1.55 m) in a nursery room at the Prairie Swine Center. Room temperature was maintained at 23^0C and integrated controllers (model PEC; Phason, Winnipeg, MB) regulated heating and ventilation systems. Each pen had a fully slatted floor, multi-space dry feeder located at the front of pen and a nipple drinker providing with ad libitum water access located on the rear wall. Doors were stainless steel with slats allowing the pigs to see each other.

Every two weeks for eight weeks, 20 gilts of similar body weight were selected at weaning (28±2 days of age) from the farrowing group (about 160 pigs). They were housed in a group pen (1.3m x 3.1m) and fed a pelleted commercial phase 1 starter diet (Ultra-wean, Co-op Feeds, Saskatoon, SK) for 7 days (medicated). The 20 gilts were then reweighed and the 14 closest in body weight and rate of gain were selected. The 14 gilts were randomized to seven groups, six

treatment diets and one initial slaughter group (ISG). They were housed 2 pigs per pen (starting at 19.6±0.4 kg / pen). All pigs were fed twice a day at 0800 h and 1600 h.

All the animals from the trial were subjected to a 3-day period of training to ensure a consistent DE intake and meal pattern of feeding. The same DE intake is important in terms of interpretation of blood metabolites responses of pigs fed different diets. In order to get the same DE intake, the combination of pig body weight and gross energy digestibility of each treatment were involved in DE intake determination. To ensure meals could be consumed within one hour, pigs were fed at 0800 h, 1500 h and 2200 h (lights on for allowing pigs to see and eat feed) which was different from regular feeding (two meals daily). The feeders were removed after one hour feeding. All the animals could finish the meal within an hour period after three days training.

3.2.2 Experimental design

The experiment was conducted in four blocks of 14 pigs each (including 12 pigs assigned to five semi-purified diets and a reference diet, the other 2 pigs assigned to the initial slaughter group (ISG)). Treatments were randomly assigned to pens within the room. The whole experiment was designed to measure if low dietary starch has an impact on protein accretion on growing pigs.

3.2.3 Experimental diets

Five semi-purified experimental diets with increasing levels of added starch (0.0, 5.5, 11.0, 16.5 and 22.0%) and challenging amount inclusion of wheat DDGS and corn DDGS were formulated. The GE and CP content of wheat DDGS and corn DDGS were 4524 vs 4750

kcal/kg, 40.3 vs 28.3 % respectively. Canola oil and pure cellulose (Sokafloc) were added to balance calories and energy density and the respective iso-caloric and iso-nitrogenous semi-purified diets. Purified lysine was added to diets to provide the same levels of digestible lysine, other amino acids were added according to the ideal protein ratio (NRC, 1998). The reference diet was a commercial phase II diet (Co-op Feeds, Saskatoon, SK) and used for comparison with five-semi purified diets. All diets contained 0.5% Celite^{TM} (as an acid insolubles ash digestibility marker, McCarthy et al., 1974). Five semi-purified diets were on the form of mash and reference diet was on the form of pellet. Diets composition of the experimental and commercial reference diets are shown in Table 3.1 and Table 3.2, respectively.

Table 3.1. Diet composition and chemical analysis of the semi-purified treatment diets[1]

Item	Starch Level (%)				
	2.0	6.3	10.2	14.2	18.6
Ingredients (g/kg)					
Corn DDGS	240	240	240	240	240
Solka-floc[2]	210	175	140	105	70
Wheat DDGS	150	150	150	150	150
Soybean meal, 46% CP	150	150	150	150	150
Canola oil	90	70	50	30	10
Menhaden fish meal	70	70	70	70	70
Spray dried plasma	50	50	50	50	50
Corn starch[3]	0	55	110	165	220
Dicalcium phosphate	7	7	7	7	7
Limestone	10	10	10	10	10
Minerals[4]	6	6	6	6	6
Vitamins[5]	6	6	6	6	6
DL-methionine	1	1	1	1	1
Salt	3	3	3	3	3
Endox[6]	0.13	0.13	0.13	0.13	0.13
Celite[7]	5	5	5	5	5
LS20[8]	1	1	1	1	1
Copper Sulfate	0.4	0.4	0.4	0.4	0.4
Choline Chloride	0.8	0.8	0.8	0.8	0.8
Formulated nutrients as fed					
DE (kcal/kg)	3376	3393	3409	3426	3442
ADF (%)	26.3	23.3	20.3	17.3	14.2
NDF (%)	33.9	30.6	27.3	23.9	20.6
Analyzed values (%)					
DM	94.3	93.3	93.3	93.1	92.6
CP	29.3	29.4	29.1	29.0	29.1
Ether extract	14.1	12.3	10.4	8.4	6.6
Starch	2.0	6.3	10.2	14.2	18.6

[1]Diets (mash) mixed at the University of Saskatchewan.
[2]Solka-floc, wood cellulose (Canada Colors and Chemical Ltd, Ontario, Canada).
[3]Corn starch, (National Starch, Brampton, Ontario, Canada).
[4]Provided per kg of diet : zinc, 100 mg as zinc sulphate; iron, 80 mg as ferrous sulphate; copper, 50 mg as copper sulphate; manganese, 25 mg as manganous sulphate; iodine, 0.50 mg as calcium iodate; selenium, 0.10 mg as sodium selenite.
[5]Provided per kg of diet : vitamin A, 8250 IU; vitamin D, 825 IU; vitamin E, 40 IU; vitamin B12, 25 ug; niacin, 35 mg; D-pantothenic acid, 15 mg; riboflavin, 5 mg; menadione, 4 mg; folacin, 2 mg; thiamine, 1 mg; D-biotin, 0.2 mg.
[6]Endox, antioxidant (Kemin Industries, Des Moines, IA).
[7]Celite 545, Celite Corporation, Lompoc, CA, USA.
[8]LS20 contains lincomycin at 22g/kg and spectinomycin at 22g/kg (Pfizer Animal Health, Canada).

Table 3.2. Composition and chemical analysis of the reference diet[1] (g/kg).

Ingredients (g/kg)	
Wheat	458
Soybean meal	180
Spray Dried Whey	143
Oatgroats	100
Fishmeal	50
Spray Dried Plasma	16
Canola oil	18
Vitamins	6
Minerals	6
Limestone	3
Celite[2]	5
Dicalcium Phosphate	10
Salt	3
Lysine	3
Methionine	1
LS20	1
Choline chloride	0.8
Copper Sulfate	0.4
Formulated nutrients	
DE (kcal/kg)	3378
ADF (%)	3.7
NDF (%)	8.9
Analyzed values (%)	
DM	91.2
CP	22.4
Ether extract	6.8
Starch	28.5

[1]The reference diet (pelleted): Co-op Feed Plant, Saskatoon.
[2]Celite 545, Celite Corporation, Lompoc, CA, USA (was added as a marker separately).

3.2.4 Growth performance

Pigs were gradually introduced to experimental diets at the rate of 25, 50 and 75% over the first 3 days, then allowed ad libitum intake with 5% extra for the remainder of the 26 day trial. Body weight of pigs was measured at 2 hours post feeding and feed consumption was determined on day 0 (35±2 d of age, mean±SD), 7, 14, and 19. These data were used to calculate ADG, ADFI, and G: F (FC). Feed samples were taken daily at the time of feeding and pooled by diet.

3.2.4.1 Calculations

Average daily gain (ADG) = BW1- BW0 / days

Where BW1 and BW0 are final and initial body weight respectively within the weight period, the days are the number of days between initial and final body weight.

Average daily feed intake (ADFI) = (FI1- FI0) / days

Where FI1 and FI0 are total feed addition for period days, feed leftover on weighing day (d 0, 7, 14 and 19), days are the days between two weighing period.

Feed Conversion (FC) or feed efficiency (Gain : Feed) = ADG/ADFI, g/g.

3.2.5 Digestibility

Faecal samples were collected on d 17 and 18 directly from each pig to ensure that fresh, uncontaminated samples were obtained. Feces were pooled by pen and day and mixed. Three samples of about 200g each were weighed and kept frozen at -20^0C until analysis. Faecal samples were freeze dried at Prairie Swine Centre (VirTis Model # 25L Genesis SQ Super ES-55). It took 3 to 4 days for fecal samples and 5 days for carcass samples to dry. Shelf temperature was maintained at 25^0C while the condenser ranged from -56.1^0C to -63.9^0C. Freeze-dried

samples were then ground through a 1 mm screen in a Retsch mill (Retsch Model ZM1; Brinkman Instrument of Canada Ltd, Rexdale, ON) prior to the lab analysis.

3.2.5.1 Calculations

The apparent total tract digestibility (ATTD) of energy, CP, crude fat and DM was calculated for each treatment based on the ratio of the indigestible marker (Celite) in feed and faecal samples using the equation:

$$ATTD = [1-(I_D \times A_F)/(A_D \times I_F)] \times 100$$

Where I_D, I_F, and A_D, A_F stand for acid insolubles ash in diet, acid insolubles ash in feces, nutrient in diet and nutrient in feces respectively.

Digestible energy (DE) content of the diet was calculated by using the equation:

$$DE = ATTDGE \times GE$$

Where ATTDGE is the apparent total tract digestibility of energy and GE is the GE content of the diet.

3.2.6 Blood metabolites

3.2.6.1 Surgery and animal housing

Jugular catheters were inserted into half of the pigs from each block (one pig per pen) on day 19 (n=24). After an overnight fast, pigs were pre-medicated with ketamine (11mg/kg BW), stresnil (2.2 mg/kg BW) and atropine sulfate (0.04 mg/kg BW) to anaesthetize. The pig was masked and received 4-5% halothane for about 1 min. The halothane was then reduced to approximately 3.0% at 1.5 L/min O_2 flow. Heart rate was monitored throughout the surgery. The ventral left side of the neck was shaved and disinfected for surgery. After tubing (1.78mm OD,

1.02mm ID) was inserted into the jugular vein (10-12cm) and prior to closing the wound, blood was withdrawn and flushed with heparinized sterile saline to ensure tubing was in the right place. Pigs were housed individually after the surgery and returned to their room. All the pigs had free access to water but on a 3 meal per day schedule. All the surgery pigs were maintained on an anaelgesic (Cronyxin) for 3 days and were monitored closely and body temperature was measured daily. Tubing was flushed twice per day using EDTA saline (2.5mg/ml) to prevent clotting. The detailed information associating with jugular vein catheterization surgery can be read in Appendix I.

3.2.6.2 Blood samples collection

On d 3, following recovery and resumption of normal feed intake, blood samples of 10 ml were taken from each pig at -15, 0 (feeding), 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 420, 480 min and transferred to tubes (10 ml heparinized and disinfected). Those tubes were placed immediately on ice and centrifuged for 10 min at 1000 g within 30 min after blood was collected. Plasma was transferred to a separate tube and immediately frozen at -20^{0}C.

3.2.6.3 Calculations

Cumulative glucose, C-peptide and BUN concentration or area under curve (AUC) was calculated using the trapezoid method (Wolever, et al., 2008).

3.2.7 Carcass data

3.2.7.1 Animals and slaughter procedure

The 8 ISG pigs (total 4 blocks and 2 pigs per block) were slaughtered prior to the experiment on day 0 after an overnight fast using captive bolt stunning following by ex-sanguination to determine the initial body composition of experimental pigs. The remaining 48 pigs were fasted overnight on day 26 after the experiment, then were weighed and humanely slaughtered by captive bolt stunning following by ex-sanguination. Blood was drained into poly bags (6 ml Poly Bags, Uline, Waukegan). The gastrointestinal tract was cleaned of contents and patted dry then returned to the carcass. The urinary bladder and bile ducts were drained of their contents and returned to the carcass. The empty carcass was then weighed. Carcasses were frozen at -20^0C for later analysis.

3.2.7.2 Carcass samples procedure

The carcass includes the entire body including the hair, head, organs, hooves and blood. The frozen carcass was sawed into small pieces and passed through a commercial grinder (Autio GHP grinder (Model 801 GHP-25; Autio Company, Astoria, OR). Before sub-samples were taken, the whole carcass was ground five times through a 6mm die and mixed thoroughly

between each grinding. Sub-samples were composited again before final samples were taken. Three samples were taken from each pig, one was used for freeze-drying, one was frozen and archived and the third was analyzed fresh to determine DM content. The lyophilized carcass samples were pre-ground (coffee grinder) then ground through a 1 mm mesh screen (Christy Norris) in a Retsch mill. Ground freeze-dried carcass samples were analyzed for dry matter, crude protein, ether extract, and ash.

3.2.7.3 Calculations

TND, TNC, TNS, ADND and TPN were measured to determine body nutrient deposition.

TND (kg) = TNC (kg) – TNS (kg)

ADND (g/d) = TND/26

Where TND, TNC, TNS, ADND and 26 stands for total carcass nutrient deposition, total nutrient in carcass, total nutrient in initial slaughter pig, average daily nutrient deposition and total 26 days on trial. Nutrient includes protein, ether extract, ash and moisture content.

3.3 Chemical analysis

All laboratory analyses were conducted in the Department of Animal and Poultry Science, University of Saskatchewan. Analysis was repeated if percentage difference between duplicates was above 3%.

Moisture content of feed, freeze-dried feces and carcass samples was determined following drying at 135^0C in an airflow oven for 2 hours (Method 930.15; AOAC, 1990). Ash content of

carcass samples was determined following incineration in a muffle furnace at 600^0C for 12 hours (Method 942.05; AOAC, 1990). Nitrogen content of freeze-dried feces, feed, and freeze-dried carcass samples were measured by combustion (Method 968.06; AOAC, 1990) using a Leco protein/nitrogen analyzer (Model FP-528, Leco Co.; St. Joseph, MI, USA) and crude protein was calculated as N x 6.25. Ethylenediaminetetraacetic acid (EDTA) was used to calibrate the standard. Gross energy of freeze-dried feces and feed were determined using an adiabatic bomb calorimeter with benzoic acid as the standard (Model C5003, IKA Calorimeter, Wilmington, NC., USA). Ether extract of feed and carcass samples were determined by ether extract method (Method 920.39; AOAC, 1990) and ether extract in faecal samples were determined by using 9 N HCL to acidify followed by extraction with ethyl ether and petroleum ether. Acid insolubles ash (AIA) was measured in feed and faecal samples by gravimetry after digestion with 4 N HCL (modified method; McCarthy et al., 1974). Starch content in feed samples was determined using enzymatic and colourimetric methods (Megazyme International Kit, Wicklow, Ireland; Method 996.11; AOAC, 2002). Plasma glucose was determined spectrophotometrically at 520 nm using hexokinase (Autokit Glucose Wako USA). Plasma C-peptide was determined by radioimmunoassay (RIA) using a commercial kit (Porcine C-peptide RIA, Millipore Corporation, USA). Blood urea nitrogen (BUN) concentration was determined by an enzymatic method and measured spectrophotometrically at 520 nm (QuantiChromTM Urea Assay Kit, Hayward, USA).

3.4 Statistical analysis

Growth performance, digestibility, total area under the curve of blood metabolites, and carcass nutrient accretion data were analyzed using the Proc. Regression procedure and concentrations of blood metabolites over times were analyzed using Mixed procedure (Repeated

measures) of SAS (SAS/STAT Version 9.2, SAS Institute, 2004) with pen as the experimental unit. Total area under the curve of blood metabolites from 0-8 hours was estimated from the sum of successive trapezoids between each analyzed data point (Morishita et al., 2007).

With respect to significance, P values less than 0.05 were considered significant, P values $0.05 < P < 0.1$ were discussed as a trend and P values larger than 0.1 were considered non-significant. Values are reported as mean ± SEM.

3.5 Results

All the pigs remained healthy during the 26 day experimental period and no pigs were removed from the trial. The final starch concentrations in the experimental diet were 2.0, 6.3, 10.2, 14.2 and 18.6% as fed, which is similar to intended when the moisture content of the starch ingredient is considered.

3.5.1 Performance of pigs fed diets containing increasing starch levels and a reference Diet

The effects of different dietary starch levels (five semi-purified diets) and one reference diet on pig performance from day 0 to day 19 (prior to the surgery) are shown in Table 3.3., Figure 3.1.

Initial body weights (19.6±0.2 kg) were similar among treatments. Final body weight, average daily gain and average daily feed intake increased with increasing dietary starch level in five semi-purified diets ($P < 0.05$). Treatment had no effect on gain to feed ratio ($P > 0.10$).

Table 3.3. The effect of the dietary starch level on the linear and quadratic effect of growth performance of growing pigs[1]. Growth parameters are least square means ± SEM

	Dietary starch level (%)					P-value[2]		
Growth parameter	2.0	6.3	10.2	14.2	18.6	Linear	Quadratic	Reference diet
Initial BW, kg	19.51±0.143	19.41±0.173	19.49±0.163	19.53±0.170	20.00±0.143	0.872	0.914	19.33±0.073
Final BW, kg	36.90±0.398	36.49±0.095	37.80±0.570	38.55±0.538	39.95±0.828	0.021	0.438	40.91±0.583
ADG, kg	0.92±0.023	0.90±0.013	0.97±0.026	1.00±0.025	1.05±0.039	0.034	0.561	1.14±0.027
ADFI, kg	1.27±0.037	1.31±0.027	1.34±0.038	1.48±0.057	1.48±0.061	0.048	0.959	1.55±0.037
G:F, g/g	0.73±0.008	0.69±0.007	0.73±0.020	0.68±0.012	0.72±0.010	0.795	0.457	0.74±0.012

[1]Diets were formulated to be iso-caloric and the starch was added at the expense of calories from canola oil; and also solka-floc was added. Where pen (two piglets per pen) is the experimental unit. N = 4 pens/treatment. Pen average data from day 0 to day 19. Data points represent means of four observations.

[2]P-values from the five semi-purified diets

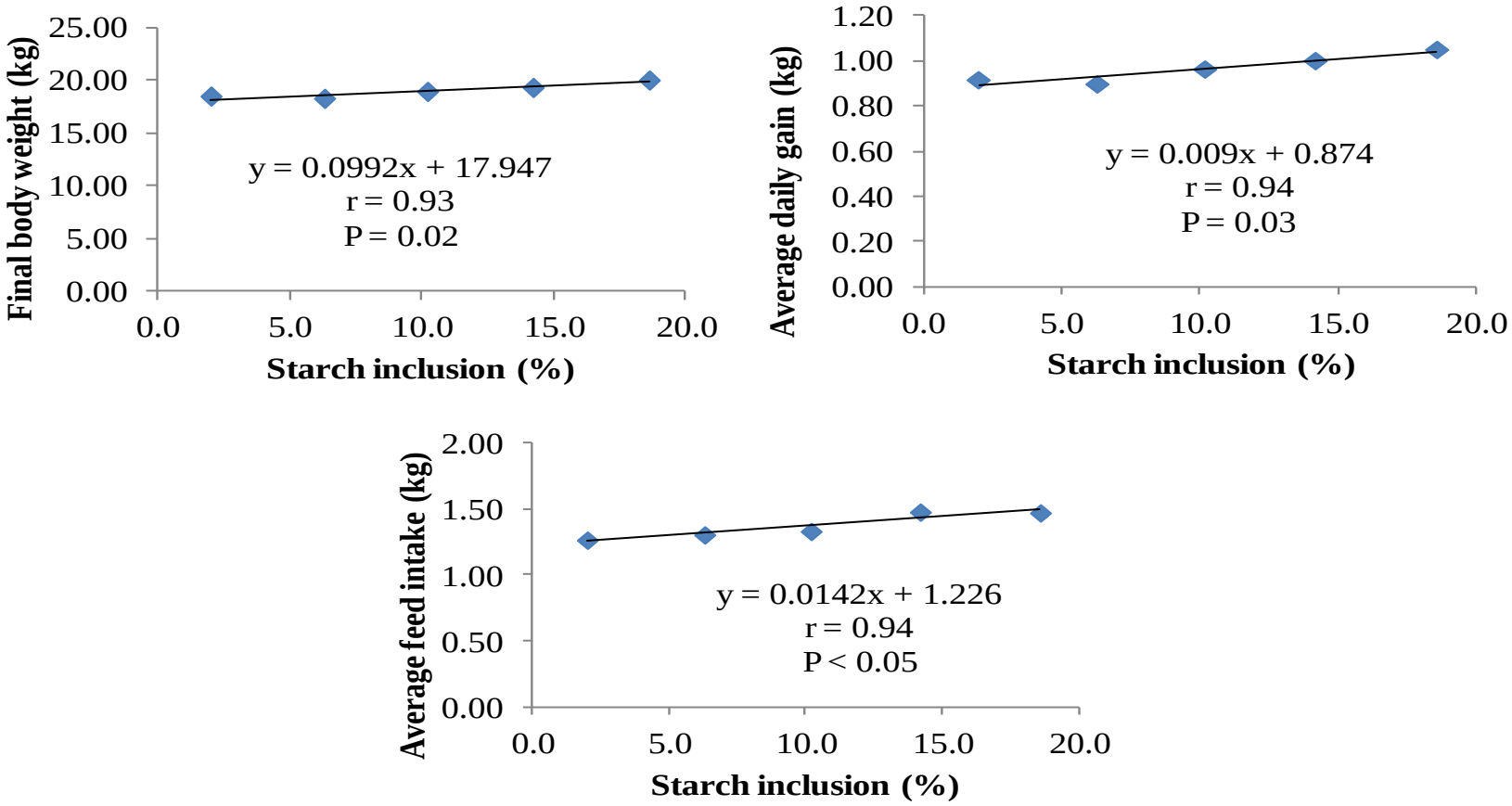


Figure 3.1. Final body weight, Average daily gain and average daily feed intake increased with increasing dietary starch level in growing pigs with regression analysis. Diets were formulated to be iso-caloric and the starch was added at the expense of calories from canola oil. Where pen (two piglets per pen) is the experimental unit. N=4 pens/treatment. Pen average data from day 0 to day 19. Data points represent means of four observations. P values from the five semi-purified diets.

3.5.2 Apparent total tract digestibility of pigs fed diets containing increasing starch levels and a reference diet

The apparent total tract digestibility of DM, CP, EE and GE for diets containing 2.0, 6.3, 10.2, 14.2 and 18.6% starch are shown in Table 3.4 and Figure 3.2.

Among the five semi-purified diets, DM digestibility increased from 62.4% to 74.2% with increasing starch concentration ($P < 0.01$). Apparent DE digestibility increased from 64.5% to 72.4% ($P < 0.01$), while apparent EE digestibility decreased from 70.6 to 44.0% with increasing starch concentration ($P < 0.01$). Apparent CP digestibility did not differ among diets ($P > 0.10$). Apparent DM, CP and GE digestibility was greater in the reference diet (85.3, 83.2, and 84.9% respectively), compared to the five semi-purified diets which averaged 68.1, 75.5, and 68.1% for DM, CP and GE digestibility respectively.

Table 3.4. The effect of the dietary starch level on the linear and quadratic effect of apparent nutrient digestibility of growing Pigs[1]. Nutrient digestibility parameters are least square means ± SEM

Digestibility parameter	Dietary starch level (%)					P-value[2]		Reference diet
	2.0	6.3	10.2	14.2	18.6	Linear	Quadratic	
DE digestibility, %	64.5±0.71	65.8±1.41	68.2 ±0.71	69.4±0.59	72.4±0.63	0.001	0.729	84.9±0.39
DM digestibility, %	62.4±0.69	65.2±1.47	68.9±0.69	70.1±0.70	74.2±0.67	<0.001	0.982	85.3±0.03
CP digestibility, %	74.3±0.77	75.2±1.30	76.7±0.54	74.0±0.42	77.4±0.67	0.316	0.871	83.2±0.52
EE digestibility, %	70.6±1.31	68.2±1.76	65.0±0.94	56.5±1.78	44.0±1.96	<0.001	0.040	63.9±1.52

[1]Diets were formulated to be iso-caloric and the starch was added at the expense of calories from canola oil.
Where pen (two piglets per pen) is the experimental unit. N = 4 pens/treatment. Fecal samples were collected on day 17 and 18. Data points represent means of four observations.
[2]P-values from the five semi-purified diets.

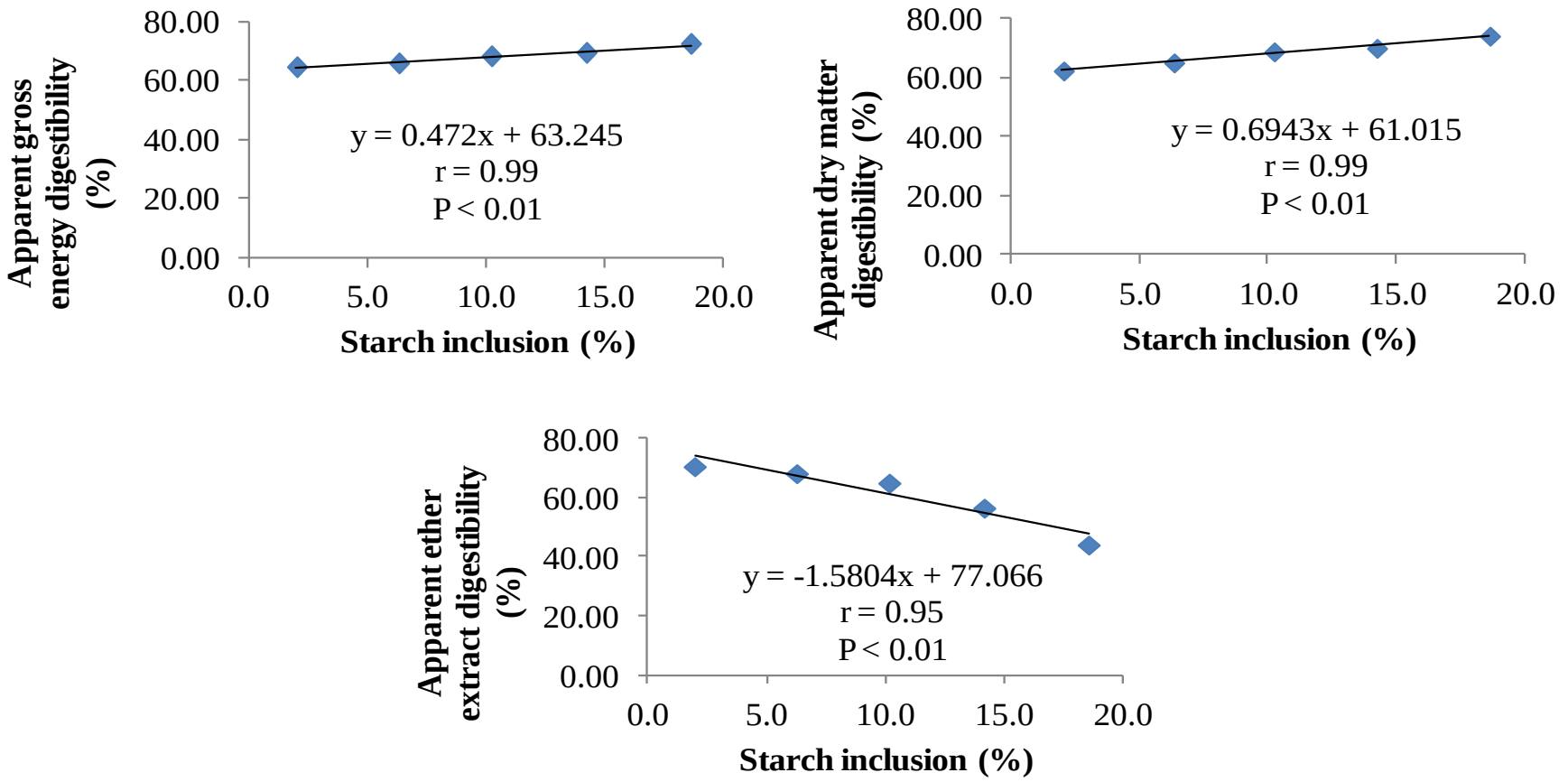
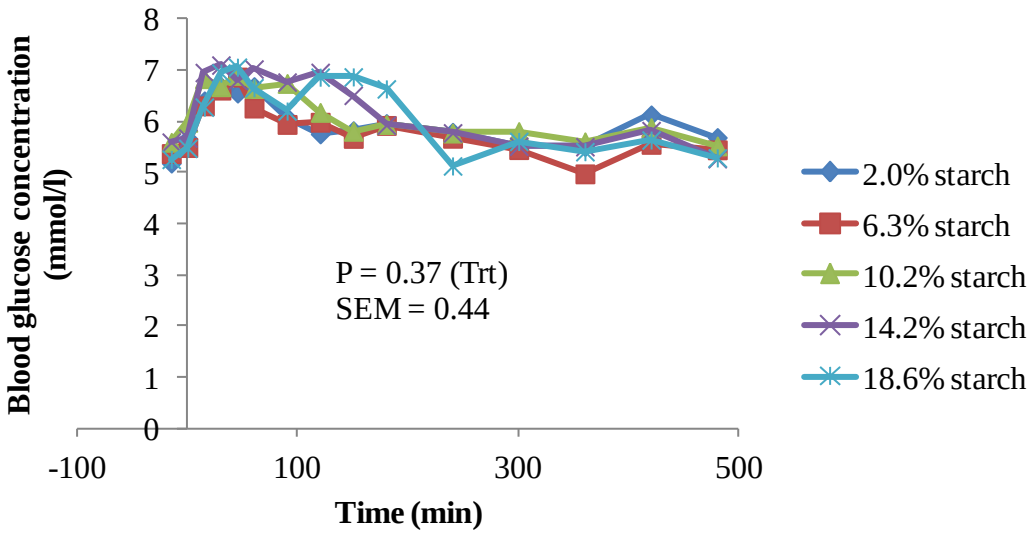
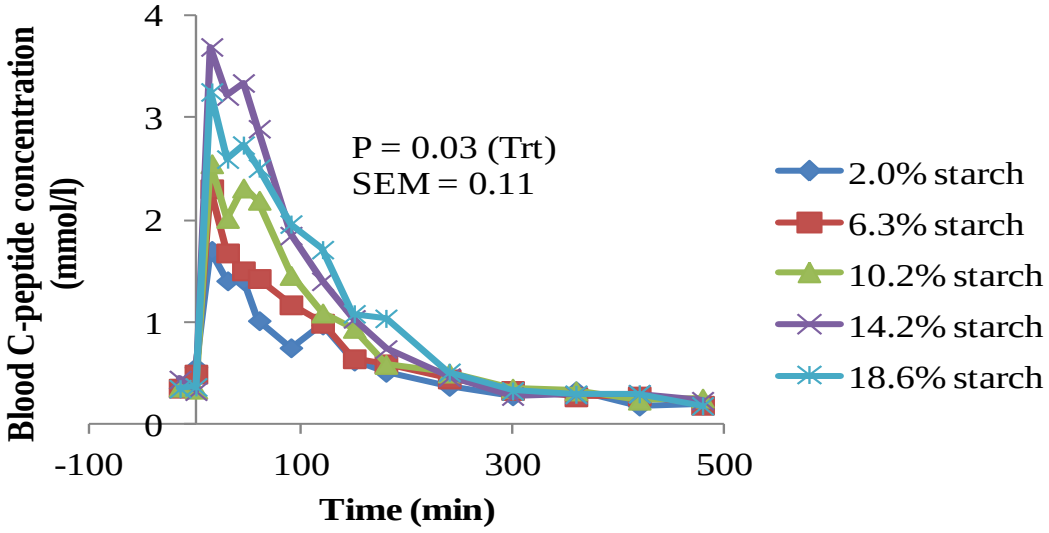


Figure 3.2. Apparent gross energy and dry matter increased, apparent ether extract decreased with increasing dietary starch level in growing pigs with regression analysis. Diets were formulated to be iso-caloric and the starch was added at the expense of calories from canola oil. Where pen (two piglets per pen) is the experimental unit. N=4 pens/treatment. Faecal samples were collected on day 17 and 18. Data points represent means of four observations. P values from five semi-purified diets.

3.5.3 Blood metabolites of pigs fed diets containing increasing starch levels and a reference diet

The response of blood glucose, C-peptide, and BUN concentration measured from 15 min before feeding to 8 hours after feeding to increasing dietary starch is shown in Figures 3.3., 3.4., and 3.5. The total area under the curve for blood glucose, C-peptide, and BUN is described in Table 3.5. Blood glucose concentration was similar among the five semi-purified diets with averaging 5.99 mmol/l (Figure 3.3.). C-peptide concentration increased linearly with increasing starch concentration from a low of 0.71 mmol/l to a high of 1.37 mmol/l (Figure 3.4., $P < 0.01$). BUN concentration wasn't affected by increasing starch inclusion in the semi-purified diets with average 16.22 nmol/l (Figure 3.5.). Cumulative glucose and BUN concentrations measured as total area under the curve was similar among semi-purified diets (Table 3.5.). Cumulative output of C-peptide increased linearly with increasing starch concentration (Figure 3.6., $P < 0.01$).


Blood glucose concentration (mmol/l)
8
7
6
5
4
3
2
1
0
-100
100
300
500
Time (min)
P = 0.37 (Trt)
SEM = 0.44
2.0% starch
6.3% starch
10.2% starch
14.2% starch
18.6% starch


Blood C-peptide concentration (mmol/l)
4
3
2
1
0
-100
100
300
500
Time (min)
P = 0.03 (Trt)
SEM = 0.11
2.0% starch
6.3% starch
10.2% starch
14.2% starch
18.6% starch

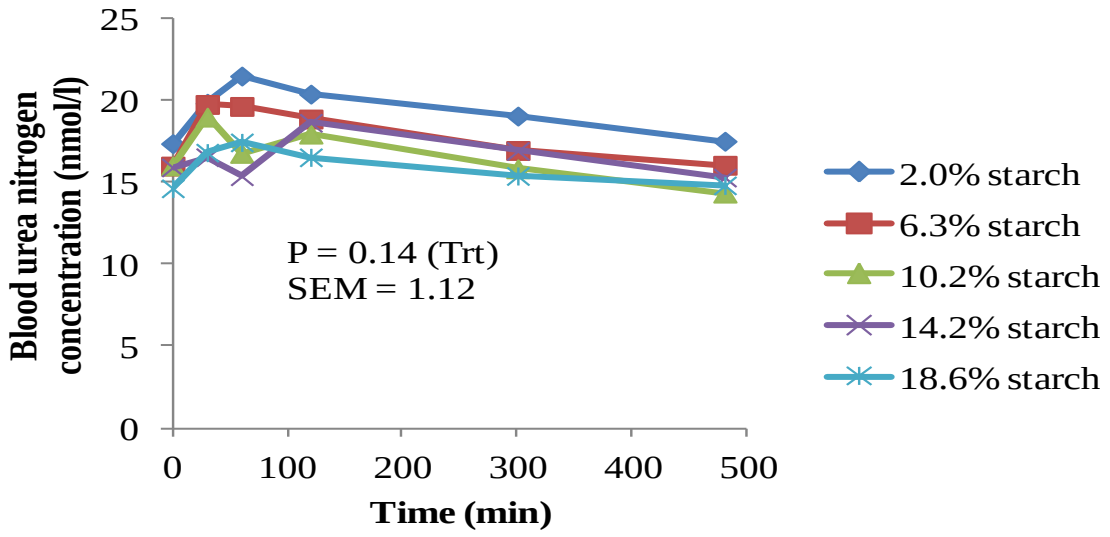


Figure 3.3. Blood glucose, C-peptide and blood urea nitrogen concentration with increasing dietary starch level in growing pigs. Diets were formulated to be iso-caloric and the starch was added at the expense of calories from canola oil. Blood samples were taken at: -15, 0 (feeding), 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 420 and 480 min on d 24. Blood glucose and C-peptide samples were analyzed at every specific time and blood urea nitrogen samples were analyzed at time 0, 30, 60, 120, 300, 480 min. Data points represent means of four observations.

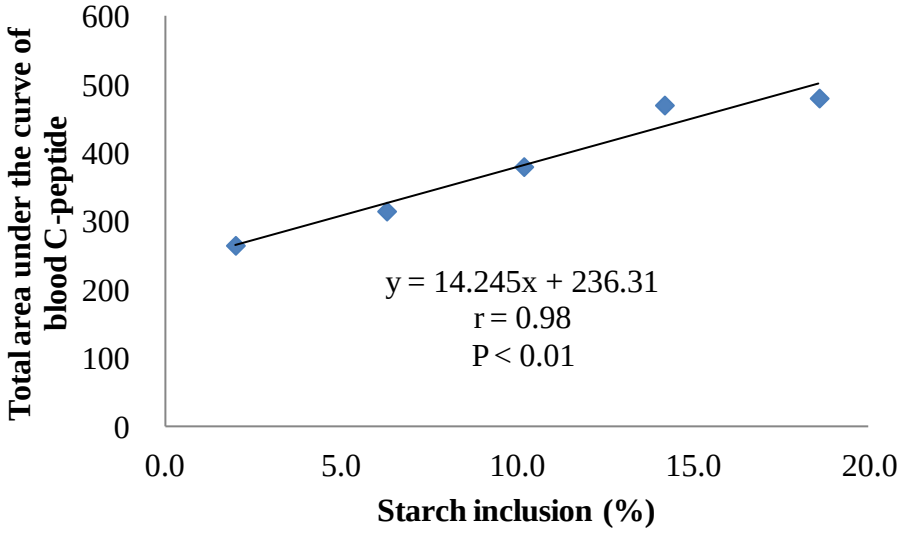


Figure 3.4. Total area under the curve of blood C-peptide increased with increasing dietary starch level in growing pigs with regression analysis. Blood samples were taken at: -15, 0 (feeding), 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 420 and 480 min on d 24. Data points represent means of four observations. Diets were formulated to be iso-caloric and the starch was added at the expense of calories from canola oil.

Table 3.5. The effect of the dietary starch level on the linear and quadratic effect of total area under the curve of blood metabolites of growing pigs[1]. Total area under the curve of blood metabolite parameters are least square means ± SEM

Blood metabolites parameter	Dietary starch level (%)					P-value[2]		Reference diet
	2.0	6.3	10.2	14.2	18.6	Linear	Quadratic	
Glucose	2741±22.6	2659±51.7	2786±8.8	2826±41.1	2766±31.4	0.335	0.864	2879±52.7
C-peptide	249±22.1	309±9.7	375±24.0	465±81.9	475±20.5	<0.001	0.561	371±18.1
BUN	1446±86.4	1322±86.8	1225±68.4	1261±89.4	1185±96.6	0.258	0.713	1039±66.5

[1]Diets were formulated to be iso-caloric and the starch was added at the expense of calories from canola oil. Where pen (two piglets per pen) is the experimental unit. N = 4 pens/treatment. Blood samples were taken at: -15, 0 (feeding), 15, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 420 and 480 min on d 24. Data points represent means of four observations.

[2]P-values from the five semi-purified diets.

3.5.4 Body composition and nutrient deposition of pigs fed diets containing increasing starch levels and a reference diet

Table 3.6. describes empty body composition, the nutrient intake and carcass nutrients deposition. Carcass CP, lipid, ash and moisture deposition increased with increasing dietary starch level. Digestible CP and DE intake increased linearly with increasing starch level in the five semi-purified diets. Accretion of CP, EE, water and ash increased linearly ($P < 0.05$) with increasing starch concentration among the five semi-purified diets (Figure 3.7 and 3.8). The efficiency of utilization of CP (crude protein accretion to digestible energy intake) tended to improve with increasing starch concentration in the diets from 34.1 to 38.0% ($P = 0.07$). Crude protein accretion to crude protein intake ratio improved with increasing starch level (Figure 3.9., $P < 0.05$).

Table 3.6. The effect of the inclusion level of starch on the linear and quadratic effect of body composition and nutrient accretion in the carcass of growing pigs[1]. Nutrient composition and accretion parameters are least square means + SEM

	Dietary starch level (%)					P-value[3]		
Body composition parameters[2]	2.0	6.3	10.2	14.2	18.6	Linear	Quadratic	Reference diet
Protein, kg	6.24±0.141	6.31±0.064	6.68±0.116	6.88±0.090	7.13±0.148	0.021	0.659	7.48±0.132
Lipid, kg	2.36±0.082	2.24±0.028	2.30±0.029	2.60±0.072	2.68±0.066	0.020	0.170	3.90±0.087
Ash, kg	1.18±0.022	1.19±0.015	1.28±0.029	1.28±0.020	1.33±0.031	0.011	0.999	1.33±0.042
Moisture, kg	26.07±0.289	26.02±0.145	27.44±0.443	28.57±0.473	28.85±0.456	0.002	0.920	29.91±0.489
Nutrient accretion parameters[4]								
Protein, g/d	123.0±5.05	127.8±2.70	140.3±4.42	148.0±3.34	154.5±5.45	0.002	0.667	171.8± 3.66
Lipid, g/d	20.0±3.69	20.7±1.84	17.0±2.30	29.3±2.30	31.0±2.98	0.021	0.169	80.3± 2.22
Moisture, g/d	508.8±9.68	508.5±3.70	562.5±15.1	605.3±14.78	603.0±16.39	0.002	0.922	662.5±11.20
Ash, g/d	24.0±0.84	24.5±0.78	28.3±0.90	28.0±0.91	29.5±0.97	0.012	0.992	30.3± 0.66
CP intake, g/d	284.1±7.56	290.4±8.03	311.2±7.46	316.4±10.49	328.2±11.91	0.041	0.674	292.3± 5.91
DE intake, Mcal/d	3.68±0.095	3.62±0.071	3.78±0.095	4.14±0.139	4.21±0.147	0.031	0.586	4.98± 0.103
CP accretion to CP intake ratio	0.43±0.012	0.44±0.015	0.45±0.016	0.47±0.013	0.47±0.018	0.042	0.673	0.59± 0.035
CP accretion to DE intake, g/Mcal	34.1±1.03	33.6±1.17	37.9±1.07	36.5±0.64	38.0±0.14	0.066	0.975	34.8± 0.61

[1]Diets were formulated to be iso-caloric and the starch was added at the expense of calories from canola oil. Where pen (two piglets per pen) is the experimental unit. N = 4 pens/treatment. Pen average data from day 0 to day 26. Data points represent means of four observations.

[2]Based on the whole empty carcass excluding bile, gallbladder, feces, urine.

[3]P-values from the five semi-purified diets

[4]Nutrient accretion is the nutrient deposition over 26 days period.

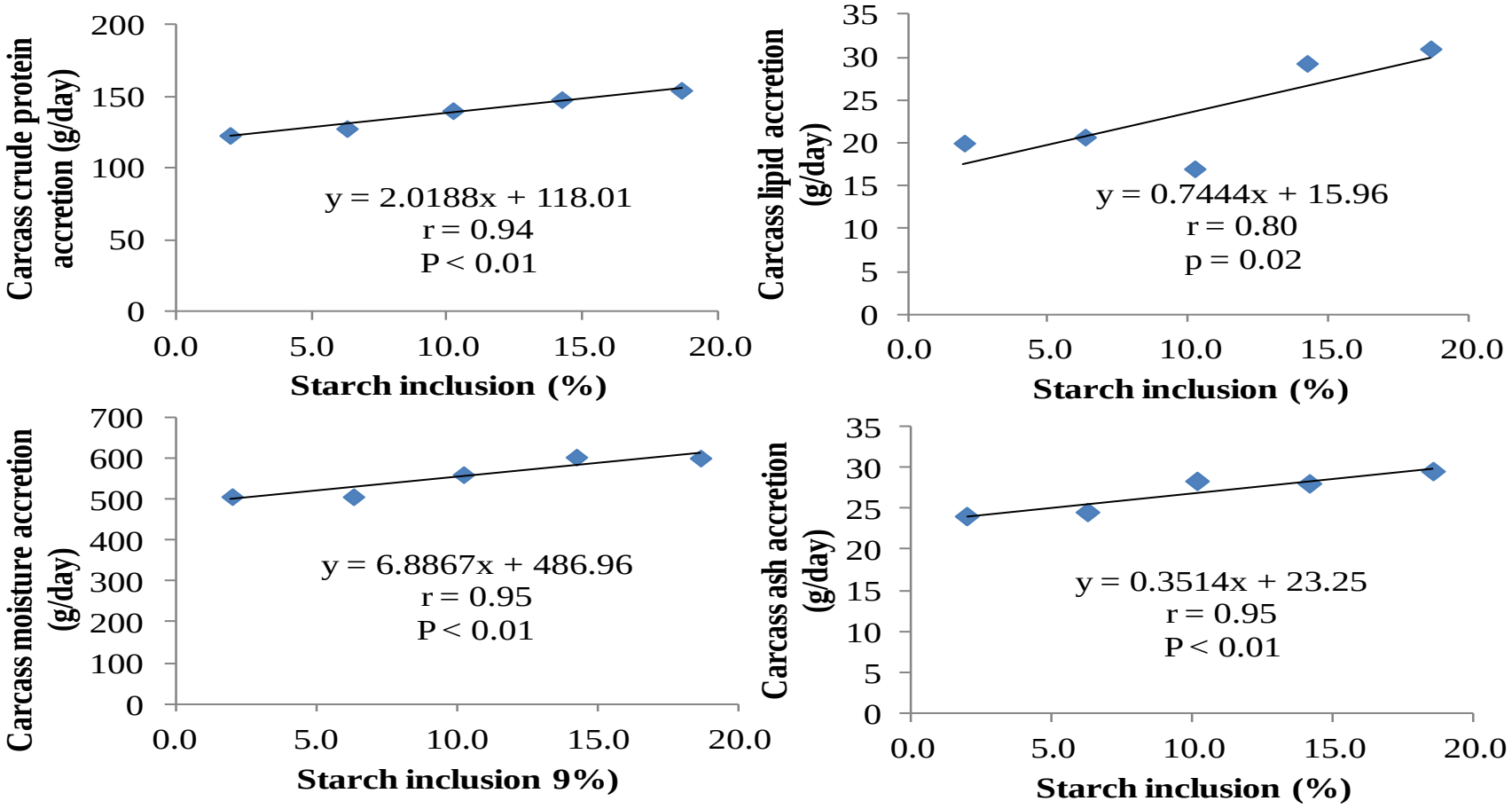


Figure 3.5. Carcass crude protein, lipid, ash and moisture increased with increasing dietary starch level in growing pigs with regression analysis. Diets were formulated to be iso-caloric and the starch was added at the expense of calories from canola oil. Where pen (two piglets per pen) is the experimental unit. N=4 pens/treatment. Pen average data from day 0 to day 26. Data points represent means of four observations. P values from the five semi-purified diets.

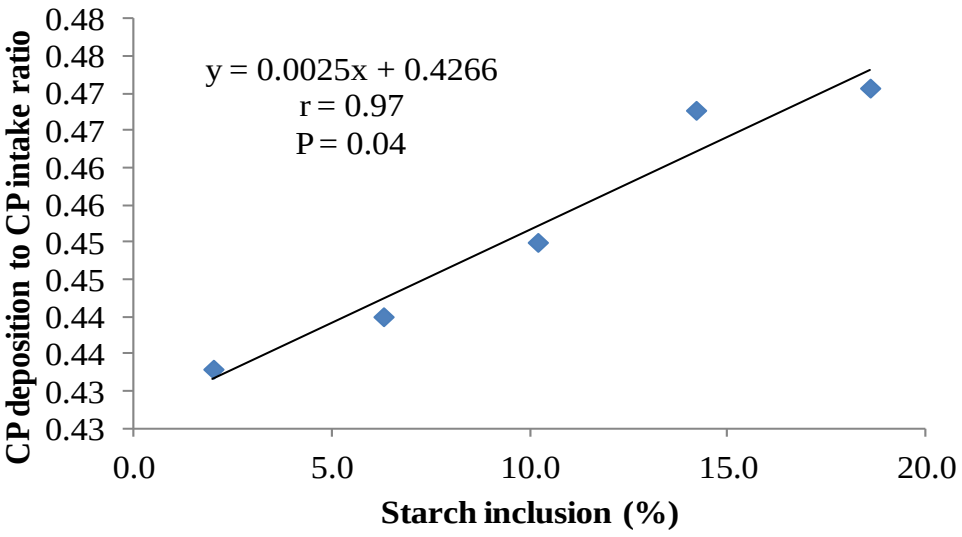


Figure 3.6. Crude protein accretion to crude protein intake ratio increased with increasing dietary starch level in growing pigs with regression analysis. Diets were formulated to be iso-caloric and the starch was added at the expense of calories from canola oil. Where pen (two piglets per pen) is the experimental unit. N=4 pens/treatment. Pen average data from day 0 to day 26. Data points represent means of four observations. P values from the five semi-purified diets.

3.6 Discussion

The overall objective of this experiment was to determine the effect of level of dietary starch on protein deposition in growing pigs. In this regard, the design of the experiment and experimental treatments are five semi-purified diets with 2.0, 6.3, 10.2, 14.2, 18.6% dietary starch and a reference diet.

With the focus on incorporation of DDGS into swine diets, whether dietary starch has an effect on protein accretion needs to be addressed due to the lower starch content with high inclusion of DDGS in those diets.

3.6.1 Performance

In the present study, the inclusion of increasing levels of starch in semi-purified diets fed to growing pigs improved ADG. The improved ADG could be due to the differences in fat, solka-floc intake and starch concentrations in diets. In our experiment, diets were formulated to be isocaloric, as starch inclusion decrease, the concentration of fat in the diet was increased to compensate. This was done to minimize the confounding due to differences in energy intake. However, it makes it difficult to separate if effects are due to changes in the fat or starch content of the diets. Adding dietary fat is a common practice to improve average daily gain and feed conversion in pigs (Stahly et al., 1981; Campbell and Taverner 1988; De la Llata et al., 2001). However, ADG decreased with increasing fat in the current experiment ($P < 0.05$). The negative effect of adding fat in our trial may be due to the piglets used in our experiment was too young to utilize fat efficiently as older pigs do. Baidoo et al. (1996) estimated that once pigs reach 40kg they have full capacity to digest fat. If this is the case, the increased ADG in current experiment may not be the effect coming from the canola oil inclusion because decreased ADG was observed with increasing fat addition in the five semi-purified diets instead of improving ADG.

Average daily gain was not affected by pure cellulose inclusion up to 15% fed to growing pigs (Partridge et al., 1982) which could imply pure cellulose does not have an impact on growth of growing pigs.

Improved ADG with increasing starch has also been shown previously in pigs (Owusu-Asiedu et al., 2006; Mateos et al., 2007; Vicente et al., 2008). Average daily gain increased with waxy corn (100% amylopection) vs. nonwaxy corn (30% amylose and 70% amylopection) fed to nursery (Perez and Aumaitre, 1979) and growing-finishing pigs (Camp et al., 2003). Although these trials were not designed to compare the amount of starch, but rather the rate of starch degradation, we can assume that more glucose would be released from waxy vs nonwaxy corn (Weurding et al., 2001). Owusu-Asiedu et al. (2006) replaced 7% cornstarch with either guar gum or cellulose or a combination of guar gum and cellulose in iso-nitrogenous and iso-caloric diets fed to growing pigs and observed that ADG decreased significantly with decreased cornstarch content. This experiment was designed to examine differences due to changes in non-starch polysaccharides (NSP) in the diet. But part of difference in decreased ADG could be due to the decreased starch in guar gum or cellulose or a combination of guar gum and cellulose diets compared with starch diet as well. Mahan et al. (2004) and Cromwell et al. (2008) observed increased growth rate with increasing lactose in diets (iso-nitrogenous and iso-caloric) for nursery pigs. Increasing dietary lactose provides more availability of lactose which can be utilized for energy production that would contribute to the more nutrients absorption and resulted in improved ADG. Kashyap et al. (2001) observed improved weight gain when high carbohydrate diet were fed to low birth body weight infants compared to low carbohydrate diets. The authors hypothesized that the carbohydrate was oxidized for energy which conserved protein.

The improved ADG observed in our trial may be due to the increased dietary starch inclusion. A high starch diet would result in an increased release of glucose to trigger insulin secretion for protein synthesis or slowing down protein degradation in the gut to reserve body protein. Furthermore, increasing availability of glucose from high starch diets might also aid the action of nutrients absorption and eventually lead to high ADG.

Average daily feed intake increased with increasing starch level in our trial and it could be one of the factors which led to increased ADG. As starch increased in the semi-purified diets the levels of cellulose decreased to maintain iso-caloric levels; hence, the improved ADFI and ADG may also be related to lower levels of fiber. Lower feed intake observed in current experiment which may be due to higher pure cellulose inclusion in low starch diets as reported by Owusu-Asiedu et al. (2006), where decreased feed intake was the direct result of higher NSP inclusion in growing pig diets. Non starch polysaccharides especially NDF leads to an increase in the bulkiness of the diet and lowers the appetite due to the physical limitation and gut capacity were reached (Cole and Chadd, 1989). The increased ADFI may be the result of increased dietary starch inclusion due to the high palatability from starch as well (Gunawardena et al., 2010).

Feed conversion was similar among five-semi-purified diets ($P > 0.05$). This is in accordance with Myrie et al. (2006) who found that feed conversion was not affected by different starch levels in isocaloric and isonitrogenous diets fed to weanling pigs. Also, Thacker (2006) pointed out that the level of wheat DDGS did not affect the feed conversion in a pig performance study. Although, his intention was not to look at starch content, the different starch level associated with different DDGS incorporation might be one of reasons (limited starch content in DDGS). However, feed efficiency improved with high fat diet compared with high starch diet at the same energy intake in weanling pigs (van Heugten et al. 1996). Different diet composition

and levels of fiber might explain the difference in feed conversion in our experiment and that of van Heugten et al.

In contrast to our results, Myer et al. (1992) observed that ADG and FC were improved and ADFI decreased linearly when canola oil was added at 0%, 5%, and 10% to diets for growing pigs. Although our trial had opposite results on performance with increasing canola oil content in diets, several factors could contribute to the differences between these two experiments. First, the diets used in the trial by Myer et al. (1992) were not iso-caloric or iso-nitrogenous. Second, Myer et al. (1992) used pigs that were 18 kg heavier (initial body weight 27 kg) than ours (initial body weight 9 kg) and presumably the pigs differ in gut capacity and maturity. Starting body weight of experimental pigs could influence the digestibility of nutrients and enhance the growth performance. Third, the fiber content in our diets was higher. High fiber in our experimental diets could trigger more endogenous protein and fat loss, increased satiety and interact with other nutrients and lead to low performance comparing to low dietary fiber in Myer and colleagues' (1992) trial.

Although five-semi purified diets were in the form of mash, and reference diet was in the form of pellet, the feed efficiency was similar among treatments. It could be more efficient with pellet form than mash form because less feed could be wasted on the pellet. The similar feed efficiency may be the limitation of the length of the trial (26 days).

The growth on top of maintenance from semi-purified diet calculated from the equation ((DE intake – DE maintenance)/ADG)) is 2.5, 2.5, 2.5, 2.8, and 2.7 kcal/g respectively. The values above implied that per unit gain required more energy involvement from higher dietary starch diets.

In summary, it is unlikely that the increased ADG was contributable to increased canola oil addition and difference in solka-floc levels due to the negative effect from adding canola oil

and no significant effect of solka-floc on ADG. Therefore, by deduction we conclude increased ADG and ADFI are directly related to starch level and this implies a starch requirement.

3.6.2 Digestibility

Increased DM and DE digestibility with increasing starch concentration has been observed by others (Noblet and Shi, 1992; Miguel and Pettigrew, 2005; Mateos et al., 2007; Vicente et al., 2008; Morsy et al., 2009; Cozannet et al., 2010). This may be the result of the replacement of the highly digestible starch for less digestible fiber. Higher fiber inclusion reduces DM and GE digestibility in pigs (Noblet and Le-Goff, 2001; Owusu-Asiedu et al. 2006; Bindelle et al., 2008; Nitrayova et al., 2009). Undigested fiber may reduce DM digestibility and increase energy loss by increasing passage rate and decreasing retention time (Urriola and Stein 2010). In agreement with Graham et al. (1986), crude protein digestibility was unaffected by increasing starch in our experiments. In their experiment, similar faecal CP digestibility between a wheat bran diet (15.6% starch) and a beet pulp diet (0.99% starch) was observed. Similar apparent CP digestibility in our study might be due to the formation of bacterial protein in large intestine contributing to additional protein in feces. Microbial activity wasn't suppressed at low cellulose level (13.2%) but at high cellulose level (23.4%) in a pig trial implies the possibility of fermentation process occurs to some extent (Longland et al., 1993). In the present study, pure cellulose additions varied from 7 to 21% in five semi-purified diets and it can be speculated that microbial activity in high cellulose inclusion diets may be suppressed according to Longland et al. (1993). The undigested dietary protein, endogenous nitrogen loss caused by high fiber diets, along with additional bacterial protein might contribute to a lack of difference in CP digestibility observed in the current trial. Graham et al. (1986) found that a high wheat bran diet had numerically higher CP digestibility (78.5%) than a beet pulp diet (62.3%). This is similar to our

findings that high starch diets had numerically higher CP digestibility although this was not significantly different. Higher apparent total tract CP and energy digestibility were observed when 14 % cornstarch addition was compared to 7 % or 0 % cornstarch addition (Owusu-Asiedu et al., 2006). However, the differences in CP and energy digestibility were interpreted as an effect of dietary NSP. Difference in starch content may be part of the difference in nutrient digestibility. Because it can be speculated that availability of glucose from digestion of starch may contribute energy to intestinal epithelial cells to aid nutrient absorption. However, increased CP digestibility was not observed in our trial and this could be explained by some reasons mentioned above.

Fat digestibility decreased linearly with increasing dietary starch content. To make the diets isocaloric, fat content of the diets increased as starch decreased. Fat digestibility was shown to decrease as the content of fat decreased from 14.1% to 6.6%. However, fat digestibility was not as high as was expected in this trial and this may be related to: a) the use of young pigs; and b) higher levels of endogenous fat loss associated with high NSP levels of the diets.

It has been reported that weanling pigs have limited ability to digest and absorb fat and the full ability to digest fat achieved by 40 kg of BW (Baidoo et al., 1996). Our experiment pigs were weighed approximately 20 kg of BW and it could be speculated that the digestibility in fat was not fully developed.

Relative to reference diets, our diets are high in NSP (solubles and insolubles fibers). High dietary NSP may increase intestinal viscosity and lead to a reduction in fat emulsification and absorption, and thus decreased fat digestibility (Adams, 2006). Fiber type may affect fat digestibility. Tuomilehto et al. (1980) and Walsh et al. (1984) reported that solubles fiber forms a gel when mixed with fat while insolubles fiber induces endogenous fat loss.

The effect of fiber on fat digestibility may explain the differences in fat digestibility between 18.6 % starch diet (fat content 6.6% and fiber content 34.6%) and the reference diet (fat content 6.8% and fiber content 12.8%). Dietary starch (18.6%) had 44.0% fat digestibility while the reference diet had an improved fat digestibility of 63.9%. This is an evidence that similar dietary fat level in iso-nitrogenous and iso-caloric diets can have different fat digestibility due to different fiber contents.

A quadratic effect on decreased EE digestibility with increasing starch concentration was observed. We could not explain why, but we speculated that fat level in a growing pigs' diet may not exceed 7% due to the inability to digest fat at such young age. Lower digestibility found in higher starch diets may have some connections with endogenous fat loss. The reference diet had higher digestibility of DM, GE, and CP relative to the five semi-purified diets which may imply the shortcoming of high fiber diets (five semi-purified diets).

In summary, due to the highly digestibility of starch and incomplete development of the ability to digest dietary fat, the increased DM and GE digestibility were the direct outcome of increased dietary starch inclusion. The decreased fat digestibility is more likely due to the combination of fiber interaction and endogenous fat loss. The higher the level of fat addition, the lesser effect of endogenous fat and would lead to higher fat digestibility.

3.6.3 Blood metabolites

Insulin is secreted by pancreatic cells when blood glucose concentration rises (Henquin 2000) and blood levels of insulin parallel those of glucose (Rerat et al. 1985). Dietary starch levels were different across treatments, and we expected to observe an increased overall glucose concentration in the blood concurrent with increasing starch inclusion in the diets because increased glucose concentration with higher digestible starch level was reported by Martinez-

Puig et al. (2006). However, overall glucose concentration in the plasma and the cumulative glucose concentration (area under glucose curve) did not increase with increasing dietary starch in our experiment. Similar findings were reported by Owusu-Asiedu et al. (2006) when diets with increasing corn starch concentrations were fed to grower pigs. The lack of difference in glucose concentration among treatments may be due to the interference from the dietary solubles NSP, which improve glucose tolerance and insulin sensitivity by slowing glucose absorption rate (Owusu-Asiedu et al., 2006). Blood glucose concentration was not different when high carbohydrate (41%) and low carbohydrate (0%) diets were fed to 10 day weaning pigs (Oliver and Miles, 2010). Oliver and Miles (2010) did not explain why blood glucose concentration did not differ; it could be one of the reasons below.

In our trial, one explanation for why glucose concentrations were not significantly different among treatments is glucose may be quickly removed by the liver and converted to glycogen for storage in liver and muscle, in response to differences in insulin. If this is the case, more advanced surgical techniques (portal drain vein catheterization) may be required to find out if there is any difference in blood glucose concentration (Gunawardena et al. 2010). The second possibility is the form of the starch used in diets. Pure starch was added to five semi-purified diets which can be rapidly digested and release glucose to blood quickly. However, starch that is in a grain matrix and vary in amylose to amylopectin ratios may impact rate of starch hydrolysis and blood. The third possibility is the effect of cellulose, which was found to reduce glucose utilization to a certain extent in man (Jenkins et al., 1983). Reduced glucose utilization would lead to higher glucose appearance in blood and higher glucose in the blood could obscure the interpretation, which may cause the insignificant results in blood glucose concentration. The fourth possibility is high fat diets in our trial may exert the mechanism which oxidizes the fatty

acids to generate energy and inhibits the oxidization of blood glucose (Boden et al., 1994), therefore, it keeps the blood glucose concentration unchanged.

After feeding or in the fasted state, blood glucose is maintained and regulated by insulin and glucagon. In the fasted state, muscle protein are broken down to supply some amino acids for glucose production through gluconeogenesis in the liver (O'Donnell et al., 1976; Apatu and Barnes, 1991). In addition, the dietary glycerol (mainly coming from DDGS) in our trial will contribute to glucose through gluconeogenesis. Since the amount of glycerol was limited in diets (0.39%), it can be neglected because of the small quantity (data not shown).

Burke et al. (2000) and Helge et al. (2001) observed low carbohydrate / protein ratio could maintain blood glucose level in human diet. They also found that the postprandial insulin response was reduced with a decreased carbohydrate / protein ratio which is similar to our results. We found lower C-peptide concentration in lower starch inclusion diets compared to the higher starch diets. Because the protein concentration was the same among all diets, the ratio of carbohydrate / protein can be easily calculated.

C-peptide is released simultaneously with insulin secretion (Mestrez et al., 1992) and it is used to estimate insulin response because of its longer half life. Increasing C-peptide secretion in response to increasing starch level was observed in the five semi-purified diets. Although the reference diet had the highest starch content, this was not reflected in a higher C-peptide concentration. It could be the reason which was explained previously that slow digestion of grain starch (slow release of glucose to the circulating blood) does not trigger high insulin response in the reference diet. Insulin (C-peptide) level is highly sensitive to sudden blood glucose concentration changes (Yelich et al., 1995; De Leeuw et al., 2004). Pancreatic insulin production is mainly influenced by blood glucose concentration (De Leeuw et al., 2004).

Other minor factors could trigger insulin secretion apart from glucose. Higher insulin concentration associated with increasing starch level may be due to the numerical increase in protein digestibility and increased protein intake in current diets; some amino acids, such as leucine, stimulate insulin secretion. The availability of amino acids would increase with increased CP digestibility and it could contribute to a high insulin secretion (Viaplanna-Marin et al., 2006).

The fiber content of a diet may influence insulin secretion. Ludwig et al. (1999) argued that people could benefit from consuming high fiber diets because they lower insulin concentrations and help protect against obesity and cardiovascular disease. Fiber induces satiety and causes a reduction in feed intake and lower carbohydrate (starch) intake. Our results are in agreement with their finding that increasing dietary fiber is concomitant with decreasing insulin (C-peptide) concentration. Others reported that specific fibers, such as phenolic acids and tannins, induce insulin secretion (Dunn and Faulds, 2000; Nattrass and Lauritzen, 2000). In order to balance energy to be equal among experimental diets, increasing amounts of canola oil were added as starch decreased. The increased insulin secretion with decreased fiber content could be explained by the interaction between fat and fiber. High dietary fat may bind with fiber which could create a layer and prevent fiber from being exposed to digestive tract. This layer lowers the availability of phenolic acids and tannins to the digestive tract so that insulin secretion can not be higher. An increased insulin secretion was seen with decreased fat in semi-purified diets may be due to a lack of layer created in current trial. An effect of type of fibers on insulin secretion may explain why higher starch content (low fiber) in the reference diet had lower plasma insulin compared with the variable levels of starch and fiber in the purified diets. Phenolic acids and tannin in 14.2% and 18.6% dietary starch diets may stimulate insulin secretion and contribute

extra insulin concentration in the blood compared to the reference diet because of lower fat and fiber interaction. Another explanation may be due to the degree of viscosity of digestive tract. Higher degree of viscosity increases the insulin response (Jenkines et al., 1978; Hooda et al., 2010). Those authors reported that ß-glucan can increase the viscosity of digestive tract and induce insulin secretion. In a similar manner fat can interact with ß-glucan and make it less or unavailable to stimulate insulin release from pancreatic cells. However, this was not the case in our trial because ß-glucan is not high in DDGS and other ingredients we used in the diets. Since phenolic acids, tannin and ß-glucan contents were not analyzed in our study, the interpretations is only speculated by the author.

High blood urea nitrogen concentration is an indicator of dietary and lean muscle protein catabolism (Kohn et al., 2005). Oxidation of amino acids for energy is the cause of variation of blood urea concentration (Campanile et al., 1998). In the present study, BUN was not affected by the increasing starch level in the diets. This is in agreement with others. For example, Camp et al. (2003) reported that there was no increase in plasma urea nitrogen with additional dietary sucrose fed to growing-finishing pigs. Furthermore, Mersmann et al. (1984) found that there was no difference in BUN concentration due to high starch or fat diets in growing pigs. De Leeuw et al. (2004), however, found that higher starch led to the higher BUN concentration in sows when they compared a reference diet (55.1% starch) and an experimental diet (34.4% starch). They speculated that higher microbial growth in the hindgut led to higher absorption of urea in the 55.1% starch diet. This is not the case here because growing pigs in our study have less fermentation capacity when compared to sows as we speculated. However, Deutz et al. (1995) reported that additional carbohydrate reduced the urea production measured from portal drained viscera in growing pigs. Moreover, Oliver and Miles (2010) found the blood urea nitrogen was

reduced in a low fat diet (high starch) relative to a high fat diet (low starch) fed to pigs. The reason for the difference in these reports may be the high fat diet inhibits the oxidization process from dietary or body protein for energy in our trial. The reduced utilization of protein certainly would keep blood urea nitrogen concentration at comparable level to high starch diets. This explanation challenges Cera and his coworkers' idea in which young weanling pigs oxidized amino acids from either diet or body protein and led to high urea production in blood when a high fat diet was fed (Cera et al., 1988).

Total cumulative area under the curve (AUC) of blood metabolites were used instead of the "incremental area", which is calculated by subtracting baseline measures from all subsequent numbers before AUC (Allison et al., 1995) and the "positive incremental area", which is calculated from the values above the baseline and neglects the values below the baseline (Wolever et al., 1991). "Incremental area" method was not used due to the concerns of negative values being obtained if post baseline values are lower than baseline values. The issue with the "positive incremental area" method is it ignores the areas below baseline and may reduce important variation. Total area calculated the whole AUC and minimized the issues or concerns associated with other two methods (Potteiger et al., 2002). The total areas of glucose and BUN did not differ either within the five semi-purified diets alone or the five semi-purified diets compared to the reference diet reflecting the insignificant difference between two metabolites levels in blood.

In summary, the increased C-peptide concentration was the result of increased dietary starch in current trial. The numerical reduction in total area under the curve of blood urea nitrogen may be the indication of better utilization of amino acids with increasing starch level in the diets.

3.6.4 Carcass composition and nutrient accretion

Carcass lipid, crude protein, ash and moisture content increased with increasing dietary starch level in the current experiment. Average daily starch intake was 25, 83, 137, 210, and 275 g respectively for five semi-purified diets. Lipid accretion increased with increasing dietary starch which is in agreement with Kashyap et al. (2001) who reported fat deposition increased with high carbohydrate diet in infant. Although, Sevette et al. (2001) found higher carbohydrate diet did not induce higher carcass fat deposition comparing with higher fat diet in male rats, the experimental diets were not iso-nitrogenous or iso-caloric. It is not surprising that fat deposition increased with increased starch level because DE intake increased along with increased dietary starch level and fat accretion was mainly regulated by energy intake. The second possible reason for higher fat deposition in our trial might be due to the shift to fatty acids oxidation because of lower starch level in high fat diets. Feeding a high fat low starch diet to pigs would lead to increased fat oxidation which caused less fatty acid available for being stored as triglyceride (McCargar et al., 1989). The third possible reason may be the effect of high carbohydrate diet which resulted in increased lipogenesis (Acheson et al., 1984). Lipid storage from carbohydrate energy appears to be reflected in the reference diet which has the highest starch content and results in highest lipid deposition compared to semi-purified diets. Higher fat deposition in the reference diet also could be due to higher lipid digestibility comparing with 18.6% dietary starch (6.6% fat) and lead to more absorption and storage of fat.

Crude protein deposition is closely associated with the circulating insulin level (Lee and Pilch, 1994; White and Kahn, 1994; Ueki et al., 1998). High insulin secretion facilitates glucose uptake by muscle and other tissues (Hirshman et al., 1990) and protein accretion (Levenhagen et

al., 2001). Macfic et al. (1981) pointed out that the combination of glucose and fat as energy sources were more effective in gaining protein than either glucose or fat alone in human requiring parenteral nutrition. In addition, water and fat gain were observed if glucose was the sole source of energy in a diet (Macfie et al., 1981). However, Baker et al. (1984) compared non-protein energy from different glucose: lipid ratios and found the whole body protein synthesis and breakdown among treatments were similar in patient.

In our trial, protein deposition increased linearly with increasing starch content in the diet ($P<0.05$). Feed intake may be one of the factors that contributed to higher protein accretion. Some researchers observed that higher feed intake led to higher growth in growing pigs which may imply potential higher carcass protein deposition (Avelar et al., 2010). Efficient nutrient utilization is closely associated with synchronization of starch and protein intake which highlighted the important of starch (Drew et al., 2012). In our experiment, the lower starch diets may not synchronize the utilization of available AA and led to lower protein accretion according to Drew's research. Schrama et al. (1996) reported that higher starch content in the diet led to higher protein accretion when they compared starch inclusion levels of 38.7% and 27.7% in growing pigs. The reason for higher protein retention was explained by lower physical activity of pigs fed high starch diets and more energy was conserved for protein retention (Schrama et al., 1996). Possible reason for increased protein deposition with increased starch content observed in our study could be a) increased feed (energy) intake; b) high glucose releasing from dietary starch facilitates high insulin (C-peptide) secretion to the blood stream which decreases the degradation of protein and increases the utilization of blood nitrogen for protein synthesis (i.e., protein sparing effect of carbohydrate); c) the young pigs could not utilize fat efficiently and there is an energy deficit in the high fat low starch diets. In order to compensate for an energy

deficiency, young pigs may mobilize the dietary protein or body protein as an energy source. This could explain the decreased nitrogen deposition in high fat and low starch diets because less dietary protein would be available for the protein deposition or more body protein was utilized for energy production and resulted less body protein in the carcass.

Digestible energy and CP intake increased with increasing dietary starch. The efficiency of ingested protein utilization for protein accretion showed a tendency to increase with increasing starch level ($P=0.07$). The crude protein accretion to crude protein intake ratio increased with increasing starch level ($P < 0.05$) which implies that protein utilization is more efficient with high starch inclusion in diets. Both Reeds et al. (1981) and Roth et al. (1999) pointed out that excessive protein in diet increases body protein turnover. Substantial energy expenditure was involved in protein breakdown (van Milgen et al., 2001). In the current study, higher CP content (29.2%) in semi-purified diets compared to reference diet (CP 22.4%) may imply more energy expenditure from semi-purified diets. If this is the case, then it could lead to less dietary protein available for protein accretion in semi-purified diets compared to the reference diet. Furthermore, high CP diets would lead to inefficient utilization of CP for energy production. In addition, more lean muscle protein had to be break down to provide glutamine to eliminate ammonia for urea production and this could be the outcome of less carcass protein deposition comparing with the reference diet.

Increasing carcass moisture with protein deposition reflects the close relationship of protein holding capacity of water (Halas and Babinszky, 2010). Whittemore et al. (1981) clearly mentioned the increased water content in the carcass is due to the lean tissue increase but not fat.

3.7 Summary and conclusion

This study explored the influence of dietary starch on carcass protein deposition in growing pigs, particularly in light of increased utilization of DDGS in feed. It provides information required by the feed and swine industries regarding the incorporation of DDGS into swine formulations. This trial provides information on performance, nutrient digestibility, blood metabolites, and carcass protein deposition.

Average daily feed intake and average daily gain increased with increasing dietary starch levels. The increase in average daily gain could be due to the decreased dietary canola oil and solka-floc levels in the diet, increased average daily feed intake or increased dietary starch levels per se. Due to the negative impact of increasing dietary canola oil inclusion and the lack of effect from solka-floc on performance, these factors are not likely the reasons for improved performance. Increased average daily feed intake could be due to higher palatability from higher inclusion of starch. Considering all the possible factors, we conclude the increased average daily gain and average daily feed intake are more likely attributable to the increased dietary starch inclusion per se.

Increasing the apparent digestibility of dry matter and energy in response to increased starch inclusion is most likely a direct effect of starch because starch is highly digestible compared with canola oil for weanling piglets. Decreasing fat digestibility implies energy supply from starch increased with increasing starch level and therefore, more fat remained unutilized for energy purposes or the decreased fat inclusion with potential increase in endogenous fat loss associating with high fiber inclusion.

Blood glucose concentration did not show any significant difference among treatments. Similar glucose concentration might imply that blood glucose is quickly removed by the liver and other tissues and animals have the ability to oxidize fat for energy requirements and preserve

blood glucose or generate glucose (through gluconeogenesis) to maintain normal blood glucose levels. C-peptide showed a linear increase among treatments. This may imply rapid removal of glucose by the liver and quick insulin secretion in response to sudden glucose concentration changes by the pancreatic beta cells or some other factors could contribute to the C-peptide (insulin) secretion instead of just glucose alone as discussed previously. In addition, in our study, blood urea nitrogen concentration did not differ among the pigs fed the five semi-purified diets which imply that pigs are capable of utilizing dietary fat for energy purposes and prevent dietary or body protein from being utilized for energy production. The numerical decrease (not significant) in blood urea nitrogen concentration may imply an effect from increasing starch level because higher insulin (C-peptide) measured could facilitate the absorption of amino acids for protein synthesis and cause the lower blood urea nitrogen concentration observed in higher dietary starch diets.

Carcass crude protein, lipid, ash and moisture content increased with increasing dietary starch. Protein accretion to ingested protein ratio ($P<0.05$) increased and there was a tendency for increased efficiency of utilization of energy for protein accretion ($P=0.07$). The increased protein accretion with increasing dietary starch in the growing pigs' carcass is the proof of "protein sparing effect of carbohydrate".

In combination with growth, digestibility, blood metabolites (C-peptide) and more efficient utilization of ingested protein, it could be concluded a) increasing carcass protein deposition is the outcome of combined effect of increasing starch concentration and feed intake. b) starch isn't an essential nutrient for growing pigs but it does require level of dietary starch for optimal protein deposition in growing pigs.

4 Implications

Producers and nutritionists are always interested in utilizing alternative feed ingredients to reduce feed cost and generate more income. To some extent, the ethanol industry competes for the usage of grains with livestock industries. Incorporating DDGS into swine ration is one of the outlets for increasing DDGS production. However, the low starch and high fiber, protein and fat content of DDGS create an obstacle to a large percent incorporation for swine feed formulation. Although DDGS can replace a certain amount of soybean meal as well as parent grain and phosphorus, the upper limit is still under investigation. Since DDGS loses most the starch during fermentation, formulating diet with high DDGS inclusion will result in low starch content. This trial indicated more protein deposition with increasing starch inclusion in the diets implying that maximizing the inclusion of DDGS or other low starch by-products in swine diets may require a consideration of the starch content of the diet to maintain optimal protein deposition.

5 Future research

It may be interesting to look at the carcass protein deposition response if protein and net energy intake is kept at the same level with/ without the addition of multiple fiber degrading enzymes in growing pigs at approximately 40 kg of body weight. Pigs at approximately 40 kg of body weight have developed full capacity to digest fat and other nutrients.

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APPENDIX A

The surgery procedures:

Jugular vein catheterization

1. Surgery items lists:

Drugs: (8 pigs(BW 20 kg) need)

1. stresnil ----------2.2x20/40x8=8.8 ml
2. astropine,----------0.04x20/0.5x8=12.8ml
3. katamine,----------11x20/100x8=17.6ml
4. ketoprophen,----------4x20/100x8=6.4ml
5. excenel,----------0.06x20x8=9.6ml
6. trivetren,---------- 0.06x20x8=9.6ml
7. lidocanine,
8. penicillin----------2x8=16ml

Materials:

1. 2.0 suture with needle and without needle----------4x8=32 packs
2. tubings----------10 pieces
3. shoulders----------15 pieces
4. alcohol----------500ml
5. saline ----------150ml

6. bottles,----------3

7. blade#10 and #11----------10 pieces each

8. adaptors----------10 pieces

9. plugs----------10 pieces

10. bags for containing tubings----------10 pieces

11. EDTA (2.5 mg/ml)----------250ml

12. heparinized saline----------250ml

13. halothane----------don’t know

14. oxygen tank----------don’t know

15. 10 cc ,3 cc and 1 cc syringers----------20 each

16. 12 G needles----------6 each

17. masks----------one box

18. hair covers----------one box

19. vet wraps----------10 pieces

20. heparin----------2 vials

21. 20 G needles----------30 needles

22. surgical gloves----------8 each

23. gown----------2 each

24. bowls----------3

25. gauze----------2 packs

26. brusher----------2 packs

27. surgical instrument----------6 packs

28. glue----------some

29. shaver(clipper)----------1

30. vacuum----------1

31. skin drapes----------6 sheets

32. Make EDTA±Heparin±disinfect tubings and other stuff

33. trocar

34. towel for pigs to lay on

35. betadine

36. hibitane

37. elastoplast----------1.5 rolls

38. gauze----------6 packs

2. Surgery Materials Preparation

Before surgery prepare catheters:

1. Cut tubing into 90-100cm long lengths and sterilize soaking in alcohol for at least 3 hours. Prepare a sterile catheter by making a v cut at one end. Mark the first 10-15cm of the tubing to make sure that the tubing will be insert deep enough.

2. Cut 1 cc syringes off at hub end, approximately the 0.3mm line. Glue and tape the plunger into the barrel of the syringe. These are to be used as plugs.

3. Blunt off 20 g needles to be used as adapter.

3. Surgery Procedures

Sedation

After an overnight fasting period, the pigs will be sedated and taken one by one to the surgery room. The catheterization will occur as follows: Animals will be sedated using 2.2 mg/kg bodyweight Stresnil, 11 mg/ kg bodyweight Ketamine and 0.04 mg/kg bodyweight of atropine sulfate.

Preparation for surgery:

1. Once the pig is outside the surgery room, the anaesthesia machine is rolled out near the door. The pig is masked and receives initially 4-5% halothane for about 1min. After approximately 15 minutes pigs will start receiving approximately 3.0% halothane at 1.5 L/min O2 flow.
2. The ventral left side of the neck will then be shaved. The pig is then moved to the surgery table.
3. Place the pig in dorsal recumbence. Pull the front legs slightly posterior.
4. Scrubbed and disinfected with Betadine solution followed by isopropyl alcohol (70%).

Surgery:

1. On the left side of the neck, make a 5 – 7cm incision through the skin using a surgical blade.
2. Dissect and expose the external jugular using blunt dissection. Separated from adjacent tissue for approximately 3cm.
3. The isolated jugular vein section will then be pulled out and suspended over a scalpel handle. A 12 g needle will be inserted into the vein. A sterile 100cm catheter (Saint- Gobain Tygon Microbore Tubing Formula S-54-HL, 1.78mm OD, 1.02mm ID) will be inserted through the needle approximately 10 to 15 cm so that the v-shape tip is placed into the anterior vena cava.
4. The needle will be removed from the vein and the catheter.

5. A 20-gauge needle, ground square and dull, will be placed into the exposed end of the catheter as a syringe adapter. A plug for the adapter will be made by cutting off the hub end of a 1cc syringe at approximately the 0.3ml mark.

6. Blood will be withdrawn to test the patency of the catheter, which will be flushed with 5-10 ml of heparinized sterile saline.

7. The incision will be sutured closed and the tubing will be taped or sutured onto the pig's neck and around to its back. The extra tubing will be placed into a pouch on the animal's neck. The entire catheter and pouch will be held in place with a fabric wrap.

8. The Cronyxin (1 ml per 45kg bodyweight) will be administered at this time, into the neck muscle, but away from the catheterization site.

9. The animal will then be taken off anesthesia and monitored for recovery.

Recovery

1. After catheterization, which takes approximately 30min, the pig is relocated to its individual pen or crate. The pig's recovery is closely monitored by trained animal care Technicians.

2. Feed allowance is increased progressively at the discretion of the Study Technicians, starting 4 hours post catheterization. The goal is to return pigs to ad lib level by 24h post-catheterization. Water will be available freely throughout the study.

During the recovery period (two – three days), the individual pig's catheter is checked for patency and flushed usually twice daily (AM and PM). First time using around 10-15 cc physiological saline to flush tubing, then using 5ml of 2.5mg/ml EDTA saline to seal the tubing (prevent it from clotting).

Appendix B

Pearson correlations among dietary starch levels and other relative parameters using means.

	Starch level	ADFI	ADG	G:F	GE dig	DM dig	CP dig	EE dig	Glucose	C-peptide	BUN	CP body	EE body	CP intake	DE intake	CP:DE
Starch level	1.00															
ADFI	0.95	1.00														
ADG	0.93	0.89	1.00													
G:F	-0.20	-0.39	0.06	1.00												
[1]GE dig	0.99	0.91	0.96	-0.07	1.00											
DM dig	0.99	0.90	0.94	-0.09	1.00	1.00										
CP dig	0.54	0.25	0.54	0.50	0.62	0.64	1.00									
EE dig	-0.95	-0.91	-0.97	0.09	-0.97	-0.94	-0.53	1.00								
Glucose	0.53	0.62	0.62	0.04	0.54	0.52	-0.01	-0.45	1.00							
C-peptide	0.98	0.98	0.89	-0.32	0.95	0.95	0.39	-0.89	0.64	1.00						
BUN	-0.91	-0.76	-0.76	0.10	-0.90	-0.94	-0.71	0.77	-0.41	-0.88	1.00					
CP body	0.94	0.90	0.98	0.03	0.95	0.94	0.50	-0.91	0.75	0.93	-0.81	1.00				
EE body	0.82	0.90	0.91	-0.15	0.82	0.77	0.18	-0.91	0.62	0.82	-0.50	0.87	1.00			
CP intake	0.99	0.97	0.97	-0.19	0.98	0.97	0.47	-0.98	0.58	0.97	-0.83	0.95	0.90	1.00		
DE intake	0.92	0.97	0.95	-0.21	0.91	0.88	0.27	-0.93	0.69	0.93	-0.68	0.94	0.97	0.97	1.00	
[2]CP:DE	0.81	0.68	0.83	0.27	0.85	0.86	0.68	-0.73	0.72	0.79	-0.85	0.90	0.58	0.78	0.71	1.00

[1]dig means digestibility

[2]CP accretion to DE ingested ratio