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Effects of Energy Restriction during Gilt Development on Milk Nutrient Profile, Progeny Biomarkers, and Growth Performance

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By

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Two Experiments were conducted at separate times to evaluate the effects of sow dietary treatment on piglet growth performance. One experiment solely focused on the growth performance of parity 1 piglets from gilt batches 5 through 13. The other experiment focused on growth performance of piglets from batch 14, parity 1, as well as, analysis of growth biomarkers, GLP-2 and Insulin. Milk samples from sows of each dietary treatment were collected and analyzed for oligosaccharide composition, nutrient composition, and insulin levels.

The first experiment utilized 733 sows that were fed either a restricted (RESTR) of ad libitum (ADLIB) diet during the gilt development stage of days 123-240. Piglets weaned from gilts that were fed a RESTR diet during the development stage had a greater weaning weight compared to those piglets weaned from gilts that were on an ADLIB diet during the development stage. Growth performance of piglets may be correlated with a sows diet before she is pregnant and body score during early gestation.

In the second experiment sows were on three dietary treatments during their development stage, which consisted of 1) Control diet formulated to NRC (2012) specifications (CTL); 2) Restricted (20% energy restriction via addition of 40% soy hulls; RESTR); and 3) Control diet plus addition of crystalline amino acids equivalent to the SID Lys:ME of the RESTR diet (CTL+). Once again we saw that

sows that were on the RESTR diet weaned larger piglets and the trend continued for these piglets into the end of grower phase. Furthermore, milk samples were obtained from sows on d 0 and 14 post-farrowing for analysis of N, DM, GE and milk insulin, and piglet blood samples were obtained on d 1 and 15 for quantification of GLP-2 and insulin. In conclusion, different nutritional diets of the developing gilt may impact piglet serum biomarkers during lactation and overall growth performance of the piglet.

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ABBREVIATIONS

AA: Amino Acids

BF: Back Fat

BCS: Body Condition Score

BW: Body Weight

CAA: Crystalline Amino Acids

CLA: Conjugate Linoleic Acid

CP: Crude Protein

CTL: Control

CTL+: Control Plus

DM: Dry Matter

EFA: Essential Fatty Acids

GE: Gross Energy

GIT: Gastrointestinal Tract

GLP-2: Glucagon-Like Peptide 2

IGF-1: Insulin-Like Growth Factor 1

ME: Metabolizable Energy

N: Nitrogen

OS: oligosaccharide

RESTR: Restricted

SCFA: Short Chain Fatty Acid

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CHAPTER 1.

Literature Review: Effects on Progeny Growth Performance

INTRODUCTION

The pre- and postnatal environment can have long lasting effects on piglet growth performance. Pork producers continue to focus on increasing piglet weight while keeping the price of production down. Dietary interventions of the sow have effects on milk and immune biomarkers that may contribute to health and weight development of offspring post weaning.

Commercial pigs spend nearly half of their life in utero and during this time the sow's nutrition can greatly influence birth weight and postnatal growth (Amdi et al., 2012). As improvements in sow productivity continue to increase, there has to be a greater focus on nutrient requirements for enhanced lactation and piglet growth. Understanding why sows wean larger piglets has been attributed to sow diet, genetics, and sow weight. With genetics aside, research today is looking at how to increase piglet weight through diets fed to the sow. Sow diets have been shown to have specific effects on the composition and nutrient availability in milk. Pigs are said to have similar digestive tracts to humans, thus another reason why porcine milk has received increased attention in recent years. Research on milk oligosaccharide profiles has evolved and is more thorough today in the animal and human environment. Varying oligosaccharides have a specific niche and have been shown to have a great impact on the development of intestinal health.

Milk composition and nutritive value is reflective of both the diet and body score of the sow. Each component of milk has a direct effect on the piglets' immune system and growth performance. Specific attributes in a sow diet will have carry over affects in its mammary gland, and furthermore, when the neonate suckles from

the sow. Stress that the sow endures during gestation, whether it be from environment or poor nutrition will play a large role in offspring development. Growth retardation, specifically due to high cortisol levels inhibiting IGF-1 has been documented in various studies (Mullan & Williams, 1989; Clemmons, 2007; Amdi et al., 2012;). Furthermore, increased insulin concentrations in a neonate can increase muscle protein synthesis and GLP-2 concentration will increase intestinal mass. The availability of colostrum and milk to the neonate is prerequisite for optimal health and growth of piglets, and, due to the fact that that piglets largely rely on passive immune protection, composition of colostrum and milk is extremely important (Alizadeh, 2015).

In conclusion, nutritional management of gilts may impact the piglet's serum biomarkers and weight during lactation through milk composition. The goal of this review is to focus on the nutritional demand of a sow and how it can have a great impact on its offspring.

MILK

The first study on the composition of porcine milk was over 150 years ago by Von Gohren (1865). Today, as technology becomes more advanced and the industry is able to isolate certain components, more precise information is available relative to milk nutrient profile. While milk is thought of as being a great nutritional factor for neonates, it does much more than just that. Milk contains nutrients including protein, fat, vitamins, hormones, and carbohydrates and contains other components such as antimicrobial peptides, growth factors, and brain-derived

neurotrophic factors (Yang et al., 2014). The mammary gland is an excretory gland. Thus, most items found in the blood of the dam will also be present in the mammary gland (Hurley, 2010). Sow milk is still being studied today as there are not many experiments that have been conducted on all the components of the milk.

Milk fat greatly increases the neonate's adipose tissue depots. While fat is primarily in the triglyceride form it can vary among species with respect to composition and concentration (Hurley, 2010). Lactose is a major carbohydrate within milk and it is a readily digestible energy source for the neonate. It is a disaccharide made up of glucose and galactose. Other carbohydrates are also found in milk, but at much lower concentrations. For example, free glucose and free galactose are found in cow milk and other mammalian species as well as sugar phosphates, amino sugars, nucleotide sugars, and oligosaccharides (Hurley, 2010).

The composition of sow milk changes through lactation to meet the needs of its offspring (Jennes, 1974). Milk has numerous protein factors that are specific to only milk. The function of some of the milk proteins target development of tissues, while others target the immune system and contain growth factors. Specific proteins in milk are several types of whey and casein. Casein is more for growth and development where as whey has antibody and growth factors (Hurley, 2010). Sow milk has a high ratio of casein to whey protein throughout lactation. Furthermore colostrum has high protein, low fat, and low lactose composition and through lactation there will be a rise in fat and lactose concentration (Csapo et al., 1994). Protein in colostrum has been found to be around three times higher than that in late lactation. Immunoglobulins are proteins in milk that are found in the

whey protein fraction. Milk globulins are most important in colostrum, where α, β, and γ globulins are present (Csapo et al., 1994). While immunoglobulins are present in all stages of lactation to help with the passive immunity of the piglet (Csapo et al., 1994), IgG is dominant in colostrum and IgA is highest in milk (Bourne, 1977).

Major growth factors present in milk are epidermal growth factor (EGF) and insulin-like growth (IGF-I) and are seen to be at much higher concentrations in colostrum (Oguchi et al., 1995). Cytokines also present in milk help the neonate through their antimicrobial and anti-inflammatory properties. Cytokines present in human milk are interleukins (IL) -1, -6, -10 and tumor necrosis factor (TNF) (Garfalo, 1999). Each cytokine plays an important role in the development of the neonate from immunoglobulins to neonate homeostasis. Chemokines present in human milk are CXC and CC, which play an important role in host anti-bacterial defense (Garfalo, 1999). Water within milk is the only source of water for the neonate and plays a key role in the texture of milk. For example, cow's milk is up to 87% water (Hurley, 2010).

Human milk is similar to that of a sow, yet the concentration of some components varies greatly. The composition of human milk has a fat % of 4.5; protein % of 1.1; lactose % of 6.8; ash % of 0.2; and total solids % of 12.6 compared to that of a sow which is 8.2, 5.8 4.8, 0.63, and 19.9, respectively (Jensen 1995). Sow's milk, when compared to cow's milk, contains a much higher concentration of unsaturated fatty acids, specifically linoleic acid, also sows milk fat contained no $C_{4:0}$ and much fewer $C_{6:0}$ and $C_{8:0}$ fatty acids (Elliot et al., 1971). Also in the ratio of Ca:P,

it is slightly higher in sow's milk compared to that of the cow (Gurr, 1981). Minerals present in milk are necessary for cofactors for enzymes; furthermore, sow's milk contains greater ash, calcium, phosphorus, zinc, iron, and copper compared to a cow, but less potassium, sodium, and magnesium (Csapo et al., 1994). Also, sow's milk contains almost 3 times as much vitamin A, and 5 times as much Vitamin C as a cow's milk does (Elliot et al., 1971). When comparing sow milk to human milk, human milk has less glutamic acid, methionine, tyrosine, lysine and Histidine, yet it has more cysteine and tryptophan (Gurr, 1981). Some milk components will be further discussed in this review relative to their effect on the neonate and their variability based on diet of sow.

Effects of the sow diet on milk composition

As pork producers continue to focus on longevity and increased litter size, nutrition of the sow is becoming more important. Diet of the sow has been shown to greatly impact the nutrient composition of milk. Throughout lactation there are varying nutrients that all have specific effects on the piglet. The early environment (i.e., gestation, lactation, and sanitation) of a piglet has drastic effects on its health that can carryover and have long-term effects on both health and growth performance. In a study conducted by Amdi et al. (2013), it was concluded that fatty acid and fat composition change based on dietary interventions during the gestation and lactation period. There have been numerous studies focusing on the direct effect of sow diet on milk output and nutrient profile. In a study conducted with decreasing crude protein (CP) and increasing crystalline amino acids (CAA), it showed a positive correlation in milk protein composition (Huber et al., 2015).

Sows that consumed a diet which was targeting limiting amino acids (AA) and thus lower CP showed an increase in mammary milk protein as well as having increased nitrogen retention and utilization in the milk protein during peak lactation periods (Huber et al., 2015). Through targeting limiting AA, the nutrition requirements of the sow are more closely met and less of the diet is fed to excess. When limiting AA are targeted in the diet, CP can be reduced and there is an increase in feed intake (Huber et al., 2015). Also, a diet with additional CAA and reduced CP will increase absorption of limiting AA by the mammary gland, increasing milk protein (Guan et al., 2004). Consuming a diet that more closely matches the AA requirements of the gestating and lactating animal allows for a decrease in N excretion due to increased utilization.

With the high interest in increasing weaning weight while simultaneously keeping litter size high, nutritionists continue to supplement sow diets based on the demands of the progeny. Feed intake of the sow not only affects the number born alive, but also birth weight (Amdi et al., 2013). Research at the University of Nebraska investigating the effects of energy restriction on gilt development (including 14 batches with data collected over 4 parities per batch) has led to the observation that this approach increases sow longevity. However, this practice may also provide beneficial effects to first parity progeny with respect to health and growth. Specifically, parity one progeny derived from sows that were developed on an energy restricted diet may have increased weaning weight compared to progeny derived from gilts fed an ad libitum control diet (Barnett et al., 2017)

As fat percentage in the milk increases, it supplies more energy to the neonate. Sow diet and body condition score correlate with milk composition (Strathe et al., 2016). Varying ideas come from how a sow restricted in energy intake, assimilates fat within the mammary gland. Some researchers have concluded that when a sow is energy restricted, its total body fat is decreased and fat reserves are used to add nutrients to the secreted milk (Amdi et al., 2013). A sow lacking in nutrients will pull from endogenous sources, such as tissues, to mobilize fats for the mammary gland (Rosero et al., 2015). Other research shows that fat from the diet, an exogenous source, which the sow is receiving, is the primary contributor of fat for milk composition (Amdi et al., 2013). In a study conducted by Amdi et al. (2013), it was concluded that gilts with a greater percentage of fat in the diet had a greater amount of unsaturated fat when compared to saturated fat in late lactation. In contrast, sows on restricted feed had a higher percentage of saturated fat in the milk they produced (Amdi et al., 2013). Unsaturated fats are considered to be healthier fats as they are more readily digestible and in liquid form.

While lipid supplementation is important for energy of the sow, it is also a primary source of linoleic acid and alpha–linolenic acid, or essential fatty acids (EFA) that are present in milk (Farmer et al., 2010). Through increased EFA in milk, neonates can greatly benefit. Essential fatty acids have been shown to increase piglet neural development and immune system development, improve protective response of intestines and increase bone mass and strength (Rooke et al., 2001). Piglets that ingest milk with a higher content of fat will have increased growth and fat (Amdi et al., 2013). However, when measuring immunoglobulins in the piglets,

there was no difference. Furthermore, supplementation of conjugated linoleic acid (CLA) alters milk fatty acids in a negative way. Conjugated linoleic acid is a bioactive fatty acid that reduces milk fat (Krogh et al., 2012). Currently there isn't a complete understanding of why CLA reduces milk fat, but it is thought to be because it causes a decrease in de novo synthesis. Oleic acid is another type of fatty acid, which is produced naturally in animal fats and has been shown to have positive effects and was observed to have a greater concentration in milk of fat gilts. Due to it being produced naturally in the animal, higher levels of oleic acid are thought to improve the health value of the milk. Oleic acid has the ability to provide an indication when there is an overflow of nutrients to switch energy sources from carbohydrates to lipids because lipids are higher in energy (Boyd & Kensinger, 1998). Milk is very sensitive to the feeding levels of sows. Feed levels of sows and nutrients available will reflect on the nutritive value of the milk and can have positive and negative effects on the neonate.

Colostrum

Colostrum is typically the first substance ingested by the neonatal pig. However, ingestion of colostrum depends on the piglet's ability to suckle within hours after birth and the sow's ability to produce enough for the entire litter. A piglet's gut begins to close approximately 6 hours after birth, and it is important for the piglet to ingest the colostrum before this time in order to receive the full effect of colostrum, such as antibodies and growth factors that are present in it. Colostrum is high in protein and full of many nutrients and factors to help the newborn fight

against pathogens. Colostrum has been correlated to support neonate metabolic needs and is essential for organ growth and development (Burrin et al., 1997).

Within colostrum, numerous researchers have observed multiple peptide growth factors. Insulin-like Growth Factor -I (IGF-I) and Epidermal Growth Factor (EGF) are said to be more highly concentrated in colostrum when compared to mature milk (Simmen et al., 1988). Insulin-like Growth Factor -I is known to stimulate protein synthesis and in piglets consuming colostrum compared to those fed mature milk; the neonates with colostrum had 50% more skeletal muscle protein synthesis (Burrin et al., 1995). Colostrum enhances the neonates' protein anabolic rate, which stimulates the body to synthesize proteins for the tissues, especially in the liver and gastrointestinal tract (GIT) (Burrin et al., 1992). In a study conducted by Burrin et al. (1992) where piglets were either fed colostrum, formula, or mature milk, researchers concluded that colostrum fed piglets had a significantly higher rate of protein synthesis in the longissimus and gastrocnemius muscle as well as in the jejunum compared to those that did not receive colostrum. Greater protein synthesis will increase the weight and health status of the piglet due to increased bone mass and increased GIT development. Protein synthesis rates were greater in brain, lung, kidney, and spleen in colostrum fed pigs compared to formula and mature milk fed neonates. As seen in Figure 1, vital organ protein synthetic capacity also varied, except for in the brain.

Colostrum has also been discovered to have a greater concentration of insulin (Burrin et al, 1992). Insulin, like IGF-I, is considered a growth factor. Yet conflicting results show that insulin may not be the primary cause for muscle

growth stimulation. Due to proteolytic digestion, insulin absorption may be limited in the intestine. When comparing piglets fed colostrum to those fed formula or mature milk, piglets consuming colostrum had levels of circulating blood insulin that was equal or less than the formula and mature-milk fed piglets; however, colostrum fed pigs had significantly higher protein synthesis development in the brain and heart (Burrin et al., 1995). Colostrum is shown to play key roles in piglet growth/health far beyond weaning due to high circulating immunoglobulins and growth factors present, as well as protein synthesis initiated in the piglet through colostrum intake.

Oligosaccharides

Oligosaccharides (OS) are complex carbohydrates said to be a bioactive component of milk, which are mostly resistant to digestion (Newsburg et al., 2005; Bode, 2006). A crucial part of developing the gut has been attributed to specific OS in the sow's milk (Mudd et al., 2016). Oligosaccharides have two important functions: 1) to stimulate growth of good bacteria, and 2) to prevent pathogen binding to the epithelial wall (Newsburg et al., 2005). Oligosaccharides have prebiotic attributes that enhance the piglet's immune system. A prebiotic is known as a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit upon host health (Newsburg et al., 2005). Oligosaccharide composition does slightly vary with diet, health, stage of lactation and genetics (Bode, 2006). Milk OS are usually comprised of 3 to 10 repeating monosaccharaides including glucose, galactose, n-acetyl glucosamine, n-acetyl galactosamine, fucose, and sialic acid

(i.e.,NeuAc or NeuGc). There are numerous benefits to OS; thus, in recent years there has been a drive to characterize and understand the function of these prebiotic milk oligosaccharides.

Oligosaccharides are gaining attention in the human world, and much of the research is from porcine milk. Infant formula companies are beginning to add specific OS to their formulations for their prebiotic and growth stimulation effects it has on the neonates. Through mass spectrometry, researchers today are able to characterize the OS in mammalian milk. Although OS composition varies in diversity among mammals, they are shown to have similar functions across different species (Mudd et al., 2016). Porcine milk oligosaccharides, although not well researched, have been shown to be less diverse than human and bovine milk. In a study conducted by Salcedo et al. (2016), it was determined that when considering OS, bovine milk is more closely related to porcine milk and porcine milk is more closely related to human milk when compared to bovine milk, especially when looking at the components of fucosylated-oligosaccharides. Mudd et al. (2016) has quantified 60 different milk OS and while 60 different milk OS is much more than previously characterized in other studies, over 100 human milk OS have been identified to date (Ninonuevo et al., 2008).

Oligosaccharides promote beneficial bacteria that contribute to increasing the innate immune response of neonates. Depending on structure and function, each OS has direct reflection on its ability to decrease infection in the neonate. Porcine milk OS are used for preventing pathogens from binding to intestinal walls, while also encouraging healthy bacterial growth (Salcedo et al, 2016).

Bifidobacteria found in neonatal piglets is shown to reduce the inflammatory response while increasing anti-inflammatory cytokines (Chichlowski et al., 2012). Oligosaccharides have the ability to decrease enteric infections by decreasing the ability of pathogens to attach to epithelial walls.

Through the stages of lactation, there is a constant shift in abundance of OS. Not only do the specific types of OS change, but so does the diversity. Oligosaccharides are said to decrease in diversity throughout the lactation period. Colostrum has the most abundant levels of OS due to greater needs of the piglet when it is first born (Hamer et al., 2008; Mudd et al., 2016).

The composition of the OS is shown as a set of 5 monomers making up the OS. The abbreviations that follow are indicative of know OS species: 1) hex: glucose or galactose, 2) HexNAc: N-acetylhexososamine, 3) Fuc: Fucosamine, 4) Neu5Ac: N-acetylneuramic acid, and 5) Neu5Gc: N-glycolylneuramic acid (Mudd et al., 2016). Furthermore, when analyzed by Nano-LC Chip Quadrupole Time of Flight mass spectrometry (QTOF MS) is a program that divides oligosaccharides up to the 5 monomers that make its structure (e.g., 3_1_0_0_0 or 3 Hex, 1HexNAc) (Mudd et al., 2016). In porcine milk, when quantifying and characterizing the OS it was found that 6 OS species (2 hex-1 Neu5Ac, 3hex-1 NAc, 3 hex, 4 hex-1 hexNAc, 4 hex-2 hexNAc, 4 hex-2 hexNAc-1 Neu5Ac4 hex-2 hexNAc) made up 60% of the OS and 4 hex-2 hexNAc was the most prominent OS at early, peak, and late lactation (Mudd et al., 2016).

There are numerous types of OS that can either be classified as neutral or acidic. Neutral are shown to be most abundant through all stages of lactation (Mudd

et al., 2016). Examples of neutral OS are galacto-oligosaccharides (GOS), Lacto-N-neotetraose (LnNt), and fucosylated-oligosaccharides (FOS), and non-fucosylated oligosaccharides (Ten et al., 2014). Acidic OS, such as Sialyllactose, have functional roles in the milk and are thought to increase learning and memory (Pond et al., 2000).

Galacto-oligosaccharides have prebiotic components that play a key role in the immune system and growth of intestinal microbiota (Alizadeh et al., 2015). Galacto-oligosaccharides stimulate an increase in maltase, which has been associated with better adaptation post-weaning. Galacto-oligosaccharides may prevent villi shortening post-weaning and result in less post-weaning diarrhea and improved utilization of nutrients (Pluske et al., 1996). However, GOS is dose-dependent if added into a diet, and too much can cause diarrhea (Lackeyram et al., 2013). In piglets supplemented with GOS, there was a drop in cecal pH and an increase in butyrate (Alizadeh et al., 2016). Butyrate is an energy source for colonocytes, which in turn inhibit inflammation and carcinogenesis in the intestine (Difilippo et al., 2016). Also, an increase in butyrate is shown to prevent pathogens, especially *E. Coli*, and improve the function of the gastrointestinal barrier (Hamer et al., 2008).

Fucosylated-oligosaccharides have been observed to decrease or increase during lactation, but either way, they are found at very low levels in porcine milk (Salcedo et al., 2016; Tao et al., 2009). Fucosylated-oligosaccharides make up 1 to 4% of OS in sow milk; this is extremely low when comparing to humans in which it is around 70% (Tao et al., 2009). Fucosylated-oligosaccharides have prebiotic

factors that inhibit diarrhea caused by *E. Coli, Campylobacter Jejune,* Calicivirus, and other heat stable toxins (Newsburg et al., 2004). Higher levels correlate with better protection (Newsburg et al., 2004).

Lacto-N-neotetraose (LNnT) is a major human milk oligosaccharide that is also abundant in porcine and bovine milk (Tao et al., 2010). Lacto-N-neotetraose is shown to be a prebiotic that stimulates growth of bifidobacterium and is one of the few OS that increases with prolonged lactation.

Sialylated oligosaccharides (SOS) are the prominent acidic milk OS that decrease through late lactation. Sialylated oligosaccharides are abundant in porcine milk, but are highest in colostrum. Sialic acid is a monosaccharide that is a key component in making up SOS. Sialic acids are found on the non-reducing end of the OS and are important in biological functions. Approximately 31 to 42% of sialic acid is conjugated into SOS (Jahan et al., 2016).

Sialylated oligosaccharides enhance the prebiotic functions of milk because they cannot be digested, thus upon reaching the large intestine they can be used by beneficial bacteria (Tao et al., 2008). Sialylated oligosaccharides also play an important role in neural development and neural protection and are seen to decrease after d 4 post-farrowing, as seen in Figure 2 (Tao et al., 2008). Through competing for the adhesion sites on epithelial surfaces, SOS are able to inhibit pathogens. High concentrations of SOS in sow's milk protects the neonate from health challenges such as Rotavirus (Difilippo et al., 2016). 3' Sialyllactose is an abundant SOS that down regulates sialic acid, fucose, and galactose and inhibits pathogen adhesion to the epithelial cell wall (Difilippo et al., 2016). Most Rotavirus

diagnosed in piglets are dependent on sialic acid; therefore, having high SOS helps for protection from these diseases (Tao et al., 2010). Interestingly, Parity 1 sows have been found to have higher sialic acid concentration because they tend to produce less milk compared to multiparous sows, it is thought that the increase in SOS is due to the parity 1 sow compensating for its lower milk yield (Jahan et al., 2016).

Oligosaccharides have various attributes pertaining to a neonate's health and neurologic development. More research is required to characterize and fully understand the functions of specific OS. Research has attained that OS promote beneficial bacteria and can reduce the chances of a health challenge for a neonate. Oligosaccharides are present in milk, but can also be supplemented in the diet and have prebiotic effects on the neonate when consumed.

Milk has a direct effect on the growth and health of the neonate pig. Sow milk is full of nutrients to help offspring develop beneficial bacteria for better innate immune development as well as increase bone mass and organ development. While there isn't much research available on porcine milk, there is a growing interest in it due to the similarities the pig model has with humans. Although the nutrient composition of porcine milk plays a factor in piglet growth performance and health, genetics, diet, sow age, endogenous and exogenous factors also need to be taken into consideration.

PIGLET INTESTINAL BIOMARKERS

Glucagon-like Peptide 2

Glucagon-Like Peptide 2 (GLP-2) has a large impact on neonate piglets and their intestinal growth. Glucagon-like peptide 2 is a peptide released from post-translational processing of proglucagon in the enteroendocrine L cells on the small and large intestine (Petersen et al., 2001). As the piglet feeds on items such as carbohydrates and fat, there is an increase in circulating GLP-2, stimulating intestinal growth (Drucker, 1998). Additionally, nutrients in the lumen exert trophic effects on the intestine due to stimulation of growth factors such as GLP-2 (Drucker 2002).

Humans are said to have GLP-2 that is made up of 33 AA, where as porcine GLP-2 is a 35 AA peptide with Serine and Leucine at the C-terminal end of the peptide (Pedersen et al., 2008). In the final 20% of gestation, the prenatal piglet has a mucosal mass increase of up to 150% and continues to increase following parturition. The immediate postnatal period is an essential time for small intestine growth and is maintained by food intake in which directly increases GLP-2 function (Sangild et al., 2000). Furthermore, GLP-2 is also associated with gastric emptying and intestinal absorption (Kato et al., 2000).

The biological effects in the animal's intestine are mediated via activation of a G coupled protein receptor (GLP-2R) expressed mainly in the gastrointestinal tract and brain (Munroe et al., 1999). Receptors for GLP-2, primarily in the jejunum, play a specific role in intestinal physiology (Guan et al., 2006). Growth of mucosal epithelial cells stimulated by GLP-2 occurs via activation of GLP-2R. GLP-2R was

recently shown to be present in both enteroendocrine cells and enteric neurons in the piglet intestine. GLP-2R is not in most intestinal epithelial cells, but in a study with Caco-2 cells it showed that GLP-2 increases cell proliferation when c-adenylyl cyclase (cAMP) has decreased production (Sams et al., 2006). In a separate study conducted by Munroe et al. (2009), a in vitro experiment using cell culture showed GLP-2 increases cell survival through cAMP and cAMP protein kinase (PKA) dependent upon signaling mechanisms and it rapidly upregulates signaling pathways that mediate cell survival and proliferation (Burrin et al., 2006).

After secretion of GLP-2, it is degraded in the body at a fast rate by the enzyme dipeptidyl peptidase IV forming a truncated inactive peptide metabolite (Hansen et al 2007). The truncated form of GLP-2 has a longer half life, where as full GLP-2 in circulation has a half life of 7 to 8 min. Dipeptidyl peptidase IV has a higher intestinal activity in neonates than adults, possibly contributing to more mucosal growth at a young age; however, more research needs to be done in this area (Burrin et al., 2001).

GLP-2 stimulates epithelial cell proliferation thus increases SI mucosal mass, colon mass, villus height, and crypt death. Guan et al. (2006) showed the largest increase in small intestine (SI) blood flow happens within 30 min of infusion in neonates. GLP-2 infusion promptly activates SI intestinal blood flow, and increases mucosal and villi growth and mass when a piglet is total parenteral nutrient (TPN) fed, which is normally shown to cause mucosal atrophy within 48 h when not supplemented (Burrin et al., 2007). Due to GLP-2 stimulation through enteral nutrient intake, GLP-2 will decrease if an animal is fed through TPN because the

digestive system is surpassed in this process (Burrin et al., 2000). Understanding the beneficial effects of GLP-2, a study conducted by Burrin et al. in 2000 showed that to reverse gut atrophy from TPN feeding it can be done through supplementing GLP-2 intravenously. Supplementing GLP-2 in TPN fed neonates will cause increases in intestinal growth and adaption. Furthermore, exogenous GLP-2 is known to stimulate intestinal brush border enzyme expression and decrease apoptosis and proteolysis in preterm pigs while also stimulating intestinal blood flow in piglets (Burrin et al., 2006, Guan et al., 2006).

Interestingly, GLP-2 treatment could help reduce weaning associated diarrhea for it greatly affects intestinal function and adaptation in the growing pig. GLP-2 significantly reduces paracellular transport of ions and small molecules while inhibiting the endocytic uptake of macromolecules (Benjamin et al., 2000). It also rapidly activates divergent intracellular signaling involved in intestinal cell survival and proliferation in neonatal pigs (Burrin et al., 2007). Supplementation of GLP-2 helps the weaned piglet with intestinal adaption after weaning due to sudden change in diet. A study showed acylate GLP-2 supplementation, known to have a much longer half-life, improved intestinal function and score of colon luminal content in weanlings with bad sanitation. Also in that study, native GLP-2 has less effects, but showed increased density of goblet cells and reduced concentration of SCFA demonstrating that GLP-2 has beneficial effects on mucosal protection and nutrient absorption in TPN fed piglets (Thyman et al., 2014). GLP-2 has a large role in intestinal health and growth and is greatly affected by nutrient intake during the neonate period.

Serum Insulin

Insulin circulating in a neonate's blood plays a drastic role on muscle protein synthesis. Insulin is a hormone that is stimulated through feed intake and its sensitivity decreases as the mammal ages. Insulin regulates stimulation of protein synthesis in peripheral tissues, as well as whole body AA disposal, but it does not in visceral tissue, with the heart as an exception (Davis et al., 2001). Also, insulin plays a key role in regulating the absorption of nutrients. The rate of growth of a mammal is greatest at its neonate stage (Young, 1970) for the insulin receptor protein is two-fold higher in a newborn piglet than that of a weanling (Suryawan et al., 2001).

Insulin and the efficiency of it in its signaling pathways are essential determinants of efficient growth during development periods and will decrease with age. Intracellular signaling proteins are activated in a rise in insulin and have been correlated to increased muscle mass. Insulin signaling pathways lead to the regulation of protein synthesis as shown in Figure 3. Enhanced activation of intracellular signaling components of insulin in the neonate muscle contributes to the rapid rate of muscle protein synthesis and rapid gain in skeletal muscle mass of neonatal pigs (Davis et al., 2010).

There have been numerous studies on the effects of insulin on protein synthesis and which muscles insulin has the greatest effect on. A study by Davis et al. (2001) in which piglets were infused with insulin on d 7 and 26 of age, first

demonstrated that insulin lowered protein synthesis in the liver, intestine, pancreas, and kidney, but stimulated skeletal muscle, cardiac muscle, and skin. However, when the study was repeated again in 2001 they saw no decrease or stimulation in the organs that were previously reported as showing a decline (Davis et al., 2001). Furthermore, raising insulin in the neonate pig to those of a typical fed state and keeping AA (even EAA) and glucose at a fasted state increases the rate of skeletal muscle protein synthesis to that normal of a fed state.

Feed induced stimulation of protein synthesis occurs in most all tissues, but it is most prominent in skeletal muscle, particularly fast twitch glycolytic muscles (Davis et al., 2001). Insulin mediates the feeding induced stimulation of myofibrillar and sarcoplasmic protein synthesis, concluding that muscles of different fiber types in the neonate are effected by insulin and insulin increases the efficiency of translation in "wanted" muscle (Davis et al., 2001). Through the ingestion of colostrum, which is said to have the highest amount of insulin of all lactating periods, it further stimulates myofibrillar proteins and reinstates the importance of the neonate to suckle within few hours of being born (Fiorotto et al., 2000). In a study where the experimenter was able to maintain glucose and amino acid levels at a fasting state in weanling pigs, when infused with insulin, it increased the uptake and utilization of AA in the body and there were maximal results with minimal supplement ($30ng*kg^{-66}*min^{-1}$)(Wray-Cahen et al., 1997). When a neonate pig was infused with insulin during a fasting state, muscle protein synthesis was similar to that of pigs in a fed state (Wray-Cahen et al., 1998); thus, this is dose dependent and must be in the physiological range. Liechty et al. (1992) demonstrated that if a

weaned rat is fasted over night and infused with insulin, their body would show muscle protein synthesis; however; in studies conducted with insulin in grown animals and people there was little to no muscle synthesis with varying amount of insulin (Geflfand and Barrett, 1987; Bailie and Garlick, 1992). It was also shown that muscle protein synthesis through feeding can be blocked by an anti-insulin serum, emphasizing the impact insulin has on the developing mammal (Preedy and Garlick, 1986).

Insulin's response of causing muscle protein stimulation has to do with an increase in translational efficiency, not ribosomal number, which coincidentally also decreases with age and why it was originally thought to play a role. Insulin increases PKB activation and phosphorylation of mammalian Target of Rapamycin (mTOR) and decreases TSC2 activation in neonate muscle (Suryawan et al., 2007). Postprandial increase in efficiency of the translation process in a neonate is due to a noted increase in the activation of translation initiation factors involved in binding mRNA to the 43S preinitiation complex and relies on stimulation of a protein kinase mTOR (Kimball et al., 2000). Postprandial changes in protein synthesis in neonate pigs are correlated with changes in concentrations of circulating insulin (Davis et al., 1998). A postprandial rise in insulin, but not AA mediates the stimulation of protein synthesis through feeding in the cardiac, skin, and spleen. Insulin does not stimulate liver protein synthesis, but it will not decrease it either if the animal is infused with insulin (Ahlman et al., 2001).

MATERNAL EFFECTS

Energy

Optimal feeding strategies to maximize nutrient intake and supply feed that allows the sow to replenish body reserves while lactating are essential to offspring growth performance (Noblet et al., 1997). Altering specific nutrients and nutrition factors during different time points of a sow's reproductive cycle plays a role in embryonic development and survival (McNamara et al., 2011). Increased nutrition in sows during gestation can have a positive effect on offspring due to the fact that it influences the ratio of secondary to primary muscle fibers. In a study by Dwyer et al. (1994), it was observed that when a sow's feed was doubled during the d 25-50 of gestation, the offspring had an 8% increase in secondary muscles fibers, which are factors that can increase lifetime growth potential. Under nutrition of a sow can also cause a delay in estrus or even the possibility of not being able to get rebred. Interestingly, during the developmental period of gilts, d 123-230, when restricted in energy by 25% and no other nutrients based off NRC requirements it was found that they would have greater longevity (Miller et al., 2011).

Although sows are able to compensate their milk production with an inadequate diet and mobilize more tissues to meet lactation requirements, they cannot completely compensate for all the nutrients they are lacking and piglet litter performance will be lower in weight (O'Grady et al., 1973). When a lactating sow is restricted on energy it the body, for milk production, pulls from AA, resulting in

deamination and urea synthesis in the liver. Pulling from AA for energy can in turn cause a protein deficiency in the sow and negatively affect litter performance (Holden et al., 1968). Malnourished sows will produce offspring with lower weight due to a limited supply of essential nutrients and most likely reduced organ mass (Dwyer et al., 1995; Kind et al., 2005). There are varying results out there about the effect of energy levels in a sow and its affects on litter performance. In a study where low, medium, and high levels of lysine were tested in a sow's diet during lactation, low lysine intake tended to decrease litter growth performance and increase weight loss in the sow (Yang et al., 2008). The mobilized body reserves were most likely protein, as backfat in the sows did not decrease significantly, but there was greater protein degradation in the low lysine fed sows (Yang et al., 2008). These results agree with what was previously stated, that when a sow is low on nutrients it will move body reserves to uphold milk production (Tokach et al., 1992). In contrast to previous studies stated, Brendenmuhl et al. (1987), found that energy intake did not affect litter size and that varying protein levels can compensate for inadequate energy amounts.

Most energy retained in the uterus corresponds to protein. Energy that is retained in the uterine tissues depends on stage of pregnancy and litter size. Approximately 4.8 MJ of energy for each kg of neonate BW at birth is deposited in the sow uterus. Of that 4.8 MJ, 72% of the energy goes directly to the fetus while the other 28% goes to the placenta, fluids, and empty uterus (Noblet et al., 1995). Altering dietary energy could alter the metabolism of the pregnant sow and have an impact on fetal development during early gestation. The average piglet weight has

been shown to be correlated with sow energy intake, where as when the sow energy intake was greater, so was piglet BW.

Body Condition Score/Back Fat

Poor nutrition of the sow will have carry over affects on offspring due to the sow receiving a reduced nutrient supply. Feed level and maternal body condition score (BCS) can affect offspring growth and development. Opposite of what was stated on energy, it was shown that maternal BCS at time of gestation has a greater effect on offspring growth performance than maternal feed intake does. For example, lambs that are born to normal weight ewes have less adiposity compared to lambs born to obese ewes (Long et al., 2010). In a study by Amdi et al. (2012) it was shown that gestation feeding level affects the number of offspring born alive per litter and offspring birth weight, where as sow BCS affects weaning weight and growth of offspring. Piglets born to fat gilts had greater ADG between birth and weaning and were heavier at weaning than those born to lean gilts. In contrast to the importance of BCS, Howie et al. (2009), found that when a rat was switched to a high fat diet during pregnancy, offspring had greater fat adiposity compared to the control group; thus, stating maternal diet has a greater effect on offspring than BCS and the fetus will respond to dietary treatments. There is a positive correlation between sow body weight and piglet birth weight (Lewis & Bunter, 2011), but varying results on what plays the biggest nutritional part on piglet weight is still to be determined.

Developing fat reserves during gestation is a must for sows in order to have positive reproductive performance; however, excessive fat can have a negative

effect on the sow's reproductive performance (Dourmad et al., 1996). There is an optimal range for a sow's body fat in order to support positive reproductive performance. In a study where piglets were born from sows that had a backfat depth of around 19 mm at gestation those piglets had greater backfat fat depths, less lean tissue yield, and were heavier at slaughter than piglets born to sows with a backfat depth of about 12 mm at gestation (Amdi et al., 2013). Backfat is said to have three layers in the sow. It was concluded that these layers mobilize fat independently of each other due to weight loss from lactation. During lactation, the middle layer of backfat was shown to increase in thickness, the outer layer decreased, and lastly, the inner layer remained unchanged (Eggert et al., 1998). Sows will lose less weight and backfat when they are on a higher energy diet, yet all sows will catabolize tissue during early lactation even if fed ad libitum .

SOW INTESTINAL BIOMARKERS

Insulin

Insulin is an anabolic hormone that plays a large part in a lactating sow's mammary metabolism (Schrams et al., 1994). Insulin has been shown to increase cell division in vitro in the mammary tissue from lactating sows (Buttle and Lin, 1991). Insulin acts as an intermediary between nutrition and reproduction in the sow (Lucy, 2008) and it may play a key part in the restoration of reproduction during and after lactation (Tokach et al., 1992).

Energy source in feed also has an effect on insulin secretion. Insulin in sows regulates energy metabolism and milk production. Higher plasma insulin in sows

equals lower catabolic status and lower milk yield; supporting the fact that less nursing results in higher insulin levels, possibly to compensate nutrients to the neonates. Parity 1 sows commonly lose excessive body weight to meet energy maintenance and milk production needs during lactation. Furthermore, sows that have lost little weight during lactation vs. sows that have lost a lot of weight during lactation have higher insulin concentration. Due to the stress on the sow from a negative energy balance post-weaning reproductive performance can be a challenge (Chen et al., 2016) and other studies have shown that insulin concentration can be altered by feeding amount and dietary energy source during lactation. (Kemp et al., 1995; Koetsu etal., 1996).

Insulin concentration in a lactating sow can directly impact the neonate; ironically, the neonate can also directly effect the concentrations of insulin in a sow. In a study conducted by Spinka et al. (1988), sows that were nursed every 35 min vs. every 70 min had lower basal and maximal insulin concentrations. Nursing frequency of piglets and time suckling on the teat may be mediated by varying insulin concentrations, which a support catabolic or anabolic states of metabolism during lactation. The reason for this outcome may be due to the sow's body altering its nutrient content to keep up with the more frequent nursing (Rojkittikhun et al., 1992). In agreement with what was stated previously, the more the sow allows nursing the lower the insulin concentration is. Spink et al. (1999) stated a sow will have high insulin when avoiding udder massage or nursing and through this behavior prolactin concentration becomes low and insulin receptors begin to

decline in the mammary gland. Because of a decline in insulin receptors there is a higher insulin concentration in the blood.

Dietary treatments of varying proteins levels of lactating sows fed 7.8, 13, 18.2, and 23.5% CP had no effect on insulin; however, the studied showed that AA utilization in the sow's mammary gland seemed to be regulated by circulating insulin concentrations in the porcine mammary cells (Farmer et al., 2008). Some studies have shown that milk yield of a sow increased in correlation to insulin injections and that insulin concentrations were positively related to the major milk constituents. Mullan and Close (1991) reported the lactating sows on a restricted diet have restricted insulin production. In conclusion, insulin may control the transport of nutrients to the mammary gland and play a key role in progeny weight.

Cortisol

Maternal endocrine status can have an impact on the fetus and its development. Cortisol is a stress hormone that has the capability of crossing the placental barrier and excess exposure to it can cause a fetus to have reduced birth weight (Sekl, 2004; Kranedonk et al., 2006). Energy restricted gilts had the highest level of salivary cortisol. This is most likely attributed to being underfed and the stress of being pregnant. These high levels of cortisol may have been the reasons that lead to in utero growth restriction and a smaller birth weight. Pregnant sows that were administered hydrocortisone-acetate gave birth to piglets with a lower birth weight than sows that were used as a control (Kranedonk et al., 2006). Moreover, Kranedonk et al. (2006), showed in that same study in which

hydrocortisone-acetate was used, it caused lower birth weights, yet greater number born alive piglets with more mature organs.

11 beta-hydroxy steroid dehydrogenase type 2 enzyme is what converts cortisol to its inert form cortisone. Low Placental 11 beta-hydroxy steroid dehydrogenase type 2 enzyme is linked to lower birth weight. Also, an increase in placental 11 beta-hydroxy steroid dehydrogenase type 2 enzyme increases glucocorticoid steroid receptor mRNA expression of the amygdala and increases in the offspring all caused by under nutrition of the sow and causing her body the stress of not having the proper nutrients for pregnancy (Welberg et al., 2000). Mullan and Williams (1989) also found that restricted sows had offspring with lower birth weight, he suggests that this is because of low placental 11 beta-hydroxy steroid dehydrogenase type 2 enzyme which causes an increase in transplacental passage of active maternal glucocorticoids. Furthermore, cortisol inhibits IGF-I, which is a growth factor and in return causes growth retardation (Clemmons 2007). Cortisol may be a large factor in why under nourished sows give birth to lower weight piglets when compared to sows fed a proper diet.

Insulin-like Growth Factor – I

IGF-I is a growth regulator that can be reduced in utero in the neonate when the sow is malnourished during gestation and result in smaller birth weights. IGF-I can induce nitric oxide production in endothelial cells which results in greater blood flow to the placenta. With greater blood flow to the placenta this allows for higher nutrient availability to the fetus (Tsukahara et al., 1994; Reynolds et al., 2010). Pigs born from sows with greater fat grew faster until slaughter and had greater IGF-I

concentrations than pigs born from thin gilts. Once again it was shown that sow's BCS has a greater impact on offspring than feeding level during gestation (Amdi et al., 2012).

CONCLUSIONS

The growth performance of a piglet is dependent on many factors, both pre- and postatal. While genetics does play a part in the size of the piglet, nutrition also plays a key role. Adjusting diets that are ideal for a lactating sow have been shown to have a positive correlation with piglet growth performance and health. Furthermore, energy intake and body condition score of the sow mediate offspring weaning weight. When the diet of the sow is altered it affects the proteins and biomarkers transported to the mammary gland and later ingested by the neonate. There are numerous nutrients in a sow's milk, especially in colostrum, that enhance the growth of a neonate, as well as helping boost the immune system. It is vital to continue research on sows in gestation, and pre- and postnatal piglets to fully understand the key factors that effect neonate growth.

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Figure 1. Adapted by Burrin et al., 1997. Graphs represent vital organ protein synthetic capacities and are measured in microgram RNA·mg protein^{-1}. All bars not connected by the same letter are significantly different

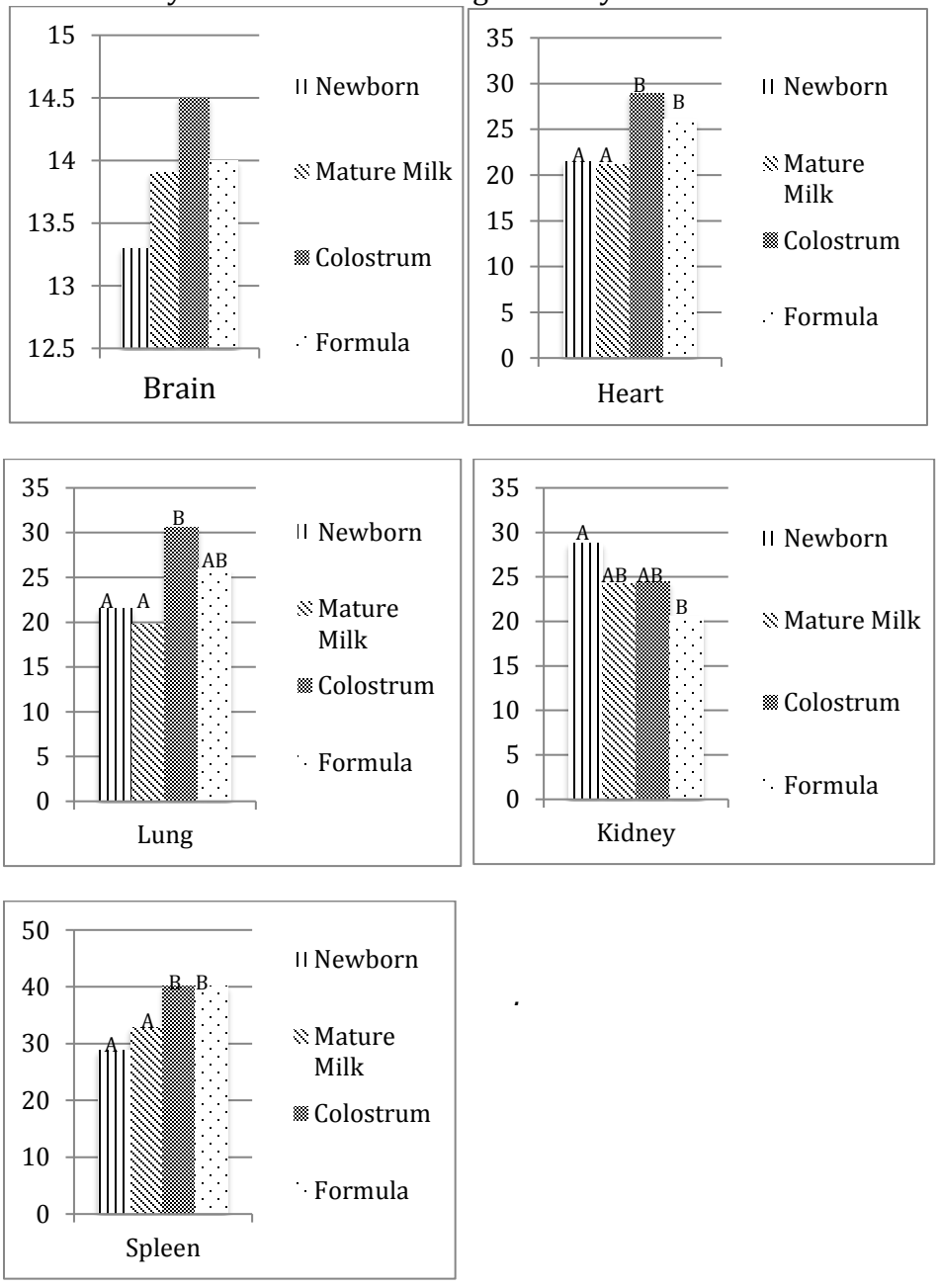


Figure 2. Adapted from Tao et al., (2010). Percent changes in abundance of siallylated oligosaccharides in colostrum and milk samples from day 4 and day 24.

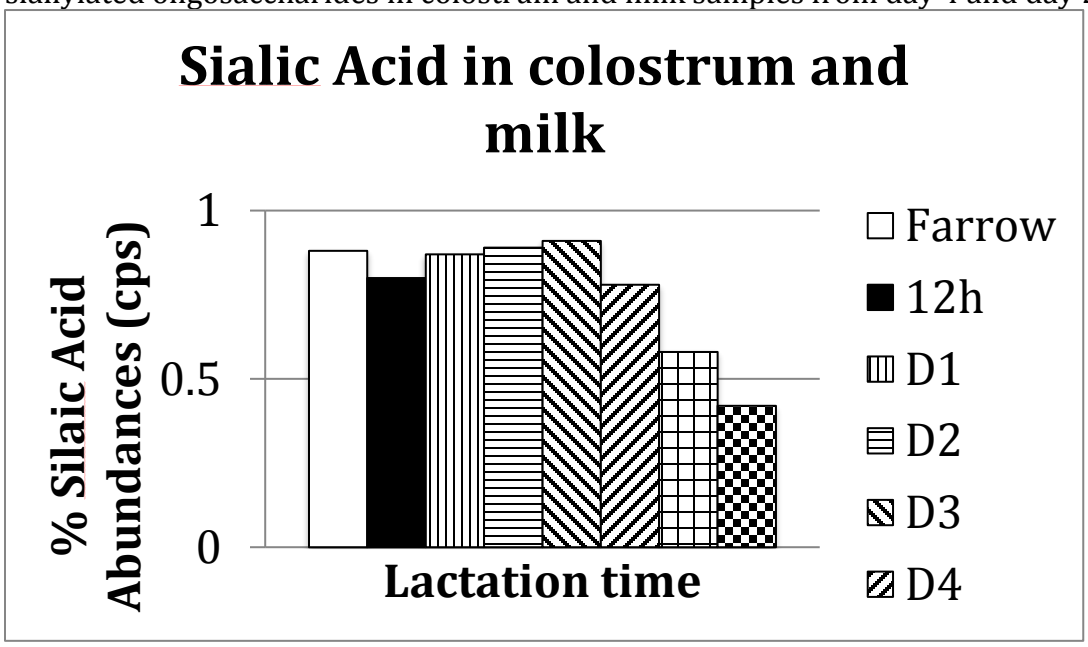
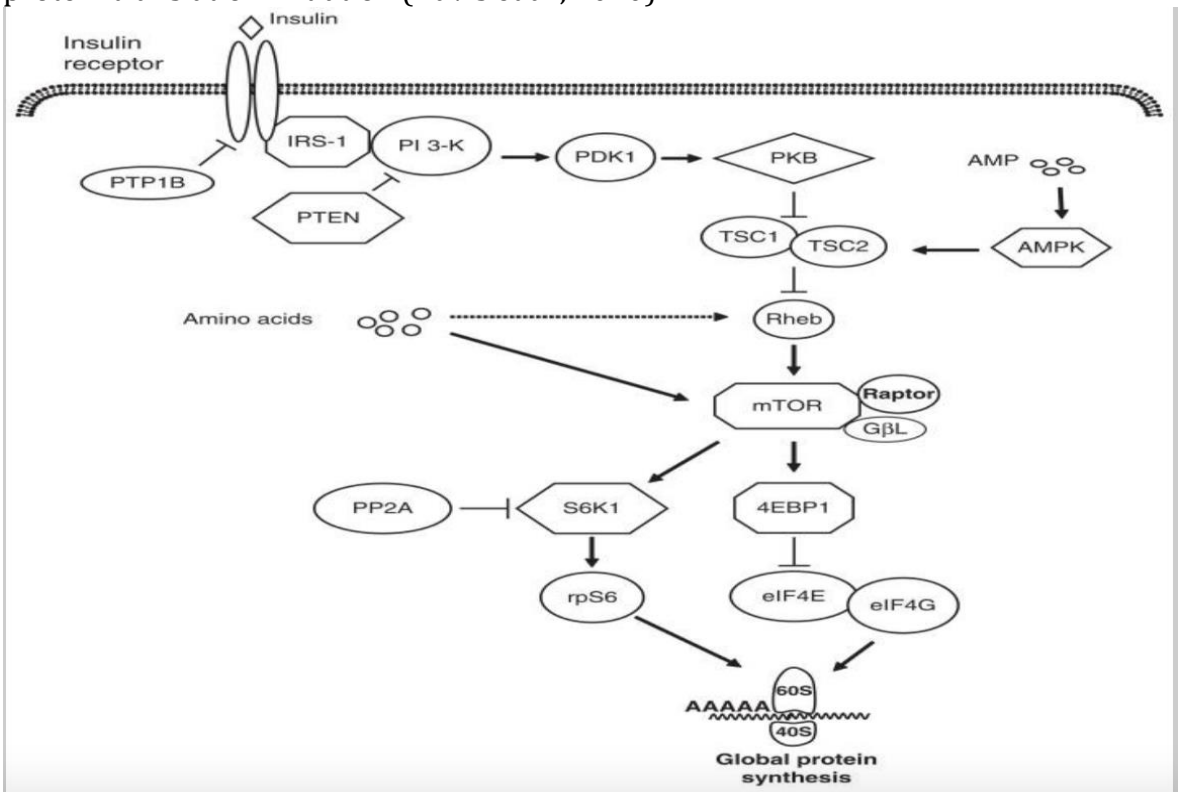


Figure 3. Insulin and amino acid signaling pathways that lead to the stimulation of protein translation initiation (Davis et al., 2010)



CHAPTER 2.

Effect of energy restriction on feed efficiency, nutrient digestibility, and immune biomarkers of growing/finishing pigs

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ABSTRACT: There are many factors that affect control of feed intake and regulation of energy balance in animals including external (e.g., environment, nutrients) and internal (e.g., hormones, metabolites) factors. The objective of this work was to evaluate the effects of energy restriction on feed efficiency, apparent total tract digestibility (ATTD), and an immune biomarker in growing-finishing pigs. Crossbred barrows and gilts (n = 36; initial BW = 52.3 kg) were randomly allotted to 36 individual pens with 2 dietary treatments in an 8 wk experiment. Treatments included a control (ADLIB; n = 18 pigs) diet formulated to meet or exceed 2012 NRC requirements and an energy restricted (RESTR; n = 18) diet. Pigs maintained on RESTR were provided an amount of feed representing a 50% (wk 1) or 25% (wk 2-8) reduction in the amount of feed relative to the ADLIB pigs. All diets were corn-soybean meal based, fed in 2 phases (wk 1 to 4 and 5 to 8) and contained 0.5% TiO_2 as an exogenous digestibility marker. Feed disappearance and individual BW were measured weekly for determination of ADG, ADFI, and G:F. At the end of each phase, fecal samples of each pig were collected twice daily for 3 consecutive days and pooled together by phase. Feces were analyzed for DM, TiO_2, and GE for both phases. Blood samples were collected from each pig (wk 0, 1, 2, 4, 6, and 8) and serum was analyzed for C-reactive protein (CRP) concentration. As expected, there were no differences in BW (P = 0.785) on d 0 and RESTR pigs had lower (P < 0.001) BW compared to ADLIB at all subsequent time points. Final mean BW was 100.53 and 112.01 kg, respectively for RESTR and ADLIB pigs. Overall, ADG (0.86 vs. 1.05 kg/d) and ADFI (2.65 vs. 3.44 kg/d) was decreased (P < 0.001) and G:F (0.37 vs. 0.34 kg/kg) was increased in RESTR compared to ADLIB pigs, respectively. With respect to ATTD, no differences were detected in phase 1; however, in phase 2, DM digestibility (83.45 vs. 81.62%) and GE digestibility (82.88 vs. 80.87%) was increased (P < 0.008) in

RESTR compared to ADLIB pigs, respectively. With respect to CRP, no overall differences were observed; however, CRP tended to decrease (P = 0.06) in RESTR compared to ADLIB pigs in wk 1. In conclusion, pigs raised under conditions where energy intake is restricted leads to greater feed efficiency and nutrient digestibility and severe energy restriction may compromise the pigs ability to synthesize acute phase proteins.

Key Words: digestibility, energy restriction, feed efficiency, grow-finish pigs

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Introduction

There are many factors that affect control of feed intake and regulation of energy balance in animals including external (e.g., environment, nutrients) and internal (e.g., hormones, metabolites) factors. Taking these factors into consideration, energy restriction may play a role on feed efficiency, nutrient digestibility, and immune biomarkers in growing/finishing pigs. Feed efficiency and immune response of market pigs can have a profound effect on the profitability of pork producers. These studies could result in new management practices as well as novel pharmacological agents to enhance feed efficiency in growing/finishing swine, increasing profitability of pork producers in Nebraska and the U.S. As of March 1, 2014, Nebraska had approximately 2.65 million market hogs on inventory, 1.8 million of which were in the growing/finishing phases (NASS, 2014).

Based on an average daily gain of 0.82 kg/d, each market pig will consume approximately 276.7 kg of feed during the growing/finishing phases. Even a 5% increase in feed efficiency, would decrease the amount of feed required to attain market weight by 13.83 kg or approximately $5/pig. This result would be substantial, saving pork producers in Nebraska about $9 million; however, reduction in feed intake is often associated with changes in immune stress biomarkers.

CRP is an acute phase protein and can be assessed as a biomarker of the immune response. It has been found that immune stimulation in the rearing environment results in the production of cytokines, which may antagonize anabolic growth factors that suppress growth (Spurlock, 1997; Johnson, 1997; Broussard et al., 2003). Collectively, the associated growth depression and diversion of nutrients away from tissue accretion ensures adequate energy and nutrients are available for high priority immunological and homeostatic

pathways.

Materials and Methods

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the University of Nebraska, Lincoln.

Animals, Experimental Design, and Dietary Treatments

In this experiment, growing/finishing pigs were provided either a feed-restricted (RESTR) or *ad libitum* (ADLIB)diet. Barrows and gilts derived from maternal white crossbred sows bred to terminal cross sires were transported from the UNL Agriculture Research and Development Center (ARDC) Swine Unit (Mead, NE) to the UNL Animal Science Building just prior to the growing phase with an initial BW of 52.3 kg. Pigs (n = 36) were individually housed, randomly selected for each treatment (n = 18), and provided ad libitum access to water. Based on an average daily gain of 0.82 kg/d, estimates for feed usage in *ad libitum* fed growing to finishing swine were: 0.6 kg/d for Grower 1 (20 - 36 kg); 2.30 kg/d for Grower 2 (36 – 61 kg); 2.9 kg/d for Finisher 1 (61 – 86 kg); and 3.40 kg/d for Finisher 2 (86 – 113 kg). Therefore, feed-restricted pigs (50% of *ad libitum for wk 1 and 25% for wk 2-8*) were fed 0.66 kg/d for Grower 1, 1.13 kg/day for Grower 2, 1.45 kg/d for Finisher 1, and 1.70 kg/d for Finisher 2.

Serum Analyses

Blood samples (n = 5) were collected at the beginning and end of each

grower/finisher phase and stored at 4°C overnight, centrifuged (12,000 × *g*) for 20 min and serum was isolated for storage at -20°C. Blood samples were collected from each pig (wk 0, 1, 2, 4, 6, and 8) and serum was analyzed via a porcine specific ELISA for C-reactive protein (CRP; R&D Systems, Minneapolis, MN)) concentration. Serum was diluted (1:50,000) prior to analysis. The intra- assay CV was 6.2 mg/L and inter-assay CV was 11.94 mg/L.

Digestibility and Growth Performance

Feed disappearance and individual BW were observed weekly for determination of ADG, ADFI, and G:F. Finally, these approaches were coupled with the assessment of apparent total tract digestibility (ATTD). Pigs were fed a standard multi-phase corn-soy based diet containing 0.5% titanium dioxide as an exogenous digestibility marker to estimate the ability of pigs to digest feed. At the start and end of each feeding phase, 3 fecal grab samples were collected from each pig and pooled within each pen. Chemical composition of the pooled homogenized feces was analyzed for DM, TiO_2, and GE in both phases. Titanium dioxide analysis was conducted using the protocol previously described by Kerr et al., (2010). Dry matter was recorded after fecal samples were dried in an oven for 48 h at 70° C. Next for gross energy (GE) analysis, the dried fecal matter was ground through a 1 mm screen. Fecal matter was weighed out to 0.50 g and compressed into a pellet and placed in metal crucibles for analysis in the bomb calorimeter (Parr 1281 Bomb Calorimeter). All samples were run in duplicates.

Statistics

The experiment was a completely randomized design. The model included

treatment as a fixed effect. Pen was the experimental unit and random effect. Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). The model for growth performance and digestibility was analyzed based on the two treatments.

Results

The effects of RESTR vs. ADLIB diets on growth performance are shown in Figure 1 and Table 1a. Final mean BW was 100.53 kg for RESTR and 112.01 kg for ADLIB pigs. Average daily gain (0.86 vs. 1.05 kg/d; Table 1a) and ADFI (2.65 vs. 3.44 kg/d) was decreased ($P < 0.001$) when comparing RESTR to ADLIB pigs as seen in Table 1b. G:F (0.37 vs. 0.34 kg/kg) was increased ($P < 0.001$)in RESTR compared to ADLIB pigs (Table 1b). In phase 1 and 2 of the experiment both G:F and ADFI had a significant difference ($P<0.001$; Table 1b). The G:F was greater in the RESTR pigs when comparing the ADLIB pigs in phase 1 ($P < 0.0001$), but this was reversed for phase 2 (Figure 1). The ADFI was greater in the ADLIB pigs in both phases 1 and 2 (Table 1b, Figure 1). Furthermore, in phase 1 the ADG was greater in phase 1, yet there was no significant difference in TRTs in phase 2 (FIgure 1).

Digestibility of the pigs in each treatment is presented by phases 1 and 2 in FIgure 2. In phase 1 there was no significant difference in ATTD. However, In Phase 2, DM digestibility (83.45 vs. 81.62 %) and GE digestibility (82.88 vs. 80.87 %) was increased ($P < 0.008$) in RESTR compared to ADLIB pigs (Table 1c, Figure 2). Figure 3 shows a significant difference in GE in phase 1, whereby GE was increased (P = 0.042) in RESTR compared to ADLIB pigs; however, there is no difference in GE in phase 2 as seen in Table 1c.

Concentrations of CRP at d 0, 7, 14, 28, and 42 are presented in Table 2. No overall differences were observed in CRP when comparing treatments except on d7 when ADLIB pigs tended to have greater (P = 0.061) CRP concentrations compared to RESTR pigs.

Discussion

The goal of the restricting feed intake in pigs during their growing and finishing stages is to positively impact pork producers and money spent on feeding pigs. Diet of a pig is a major factor that affects pork quality, as it can influence intramuscular fat content (Wiecek et al, 2010). Feed restriction of pigs will have a greater impact on BW during and after restriction when compared to pigs on an energy-restricted diet (Skiba et al., 2012). Through the feed restricted diet we saw less feed waste and greater nutrient utilization from the pigs. It has been widely studied that reducing crude protein (CP) and using crystalline amino acids (CAA) can more closely meet diet requirements, and thus increase gut health and nitrogen utilization with out adverse affects as long as AA requirements are met (Gloaguen et al., 2014). In a study by Stolzenbach et al., (2009) it was showed that a feeding regimen that incorporated compensatory gain can lead to meat tenderness and increased shear force.

A feeding strategy that includes compensatory growth will also influence the amount of fat deposited by the pig's body (Skiba et al., 2005) and fatty acid profile (Wieck et al., 2011). Furthermore, It has been documented in other studies that pigs in a growing stage have a great G:F as well as digestibility (Le Floc'h et al., 2014). Results of studies have varied however, due to genetics and external factors (i. e. diet composition, experimental design, length of restriction). In an experiment focusing on compensatory gain, Skiba et al.,

(2012) grouped his animals by age rather than weight due to age playing a crucial role in growth performance. While the pigs in our study were very similar in weight, they too were also in the same age group. Skiba et al., (2012) noted that pig's 30% feed restricted had the greatest compensatory gain and backfat thickness when put on ad libitum feed and compared to the control pigs; however the restricted still had an overall lower BW. The pigs in the current study were only 25% restricted and data past the restriction phase would need to be collected to see if our results line up with previous studies (Skiba et al., 2005, Skiba et al., 2012). Also, Pigs that are energy restricted continue to have a lower mass of the musculus longissimus dorsi after compensatory gain, possibly due to the quick growth of the animal focusing more on internal organs and less on muscle tissue (Skiba et al., 2005).

In a study done by Wiecek et al., (2011), a four phase restrictive study was constructed, the study showed that the ad libitum pigs had no significant difference in carcass lean contents when compared to those on the restrictive diet and that the restrictive pigs will have compensatory gain when fed the same ad lib diet as the control pigs. Furthermore, it has been demonstrated that feed restricted pigs (Iberian x Duroc) when fed a restricted diet during a certain growing stage (d 152 to 263) will have a greater primal cut without having any negative effects elsewhere (Serrano et al., 2009). This study agreed with Wiecek (2011), that when the feed restricted pigs were switched over to the ad libitum diet, the ADFI, ADG, and G:F increased when compared to the control pigs (Serrano et al., 2009). This agrees with our findings on the feed restricted pigs where, although we did not measure compensatory gain, we did see an increase in ADG and G:F when compared to the ADLIB however it is contrary to what was found by Le Floc'h in 2014. Le

Floc'h et al., (2014) concluded that RESTR pigs had lower feed efficiency and a higher protein deposition. The higher protein deposition correlates with decreased feed efficiency, as it is less energetically efficient compared to fat (Lovatto et al., 2006).

C- reactive protein (CRP) is a main acute phase protein in porcine. Acute phase proteins are part of the innate immune response and their concentration is altered when the animal is faced with an infection or health challenge (Murata et al., 2004). C-reactive protein is produced in the liver and is known to rise when there is inflammation through out the body. Furthermore, CRP plays a key role in clearing infectious agents and damaged cells through binding to phosphocholine (Black et al., 2004). As we saw in our results, CRP in the RESTR pigs were numerically lower when compared to the ADLIB pigs and on d 7 there was a tendency of RESTR having lower CRP concentration then ADLIB. The greater difference on d 7 between RESTR and ADLIB could be a result of the pig's body adapting to the new diet and the sudden change in caloric restriction from d 0 to 7 and due to the feed restriction being 50% during this time. Rats that were 40% feed restricted in their growing stage had clear decrease in CRP concentration when compared to control rats (Kalani et al., 2006). Furthermore, our results agreed with a study that tested pigs on a low fat/high fiber diet and a high fat/low fiber diet (Heinritz et al., 2016). The pigs on the low fat diet had a decreased CRP over time, as did our restricted pigs. Due to CRP being lower in the restricted pigs this follows the understanding of some studies that feeding pigs less during a time a sickness such as a restricted diet can deprive the immune system from overreacting and causing an inflammatory response (Le Floc'h et al., 2014). Similar to Kalani (2006), in a study conducted on rats, the results showed that with a short term caloric restriction there was a significant decrease in the CRP levels when compared to the

control group.

Through a focal point of light feed restriction a pork producer can increase a pigs ADG, ATTD, and G:F while possibly preparing pigs to recover from sicknesses quicker through reducing the impact of the disease on the animal. While optimizing feed intake and enhancing growth performance is a major focus in swine, ad libitum feed in growing animals can cause over consumption and digestive issues (Kil and Stein, 2010). Transient feed restriction has shown positive effects on the pig's ability to cope with inflammatory challenges (Le Floc'h et al., 2014). Feed costs represent about 60% of pig productive costs, by limiting feed costs and allowing animals a faster recovery time, the pork producer will be saving money in both areas. When an animal is sick it tends to display signs of anorexia and there are many changes in energy and nutrient metabolism (Gabler, 2017). For example, amino acids are redistributed from the muscle and go to the cells and tissues involved in the immune system, also a greater energy intake is usually required when an animal has a fever. There is an increasing need of around 13% more caloric energy per degree C increase in body temperature (Del Bene, 1990). Interestingly, data from Matsuzaki et al., (2001) show that feed restriction is beneficial for the digestive tract and may lessen the effects of systemic inflammatory response in growing animals, these results agreed with our results, for we saw a decrease in CRP in the RESTR pigs. In growing pigs on a short-term feed restricted diet, it was recorded that genes involved in the immune response were altered, suggesting a possible modification in the immune system (Lkhagvadori et al., 2010). However, feed restriction is mostly applied to diseases affecting the digestive tract (Rantzer et al., 1996). Furthermore, when the immune system in activated acute phase proteins will increase (Reeds et al., 1994) but this will not occur

when an animal is restricted as previously shown in the current experiment with CRP and by Matsuzaki et al., (2001) and Le Floc'h et al., (2014).

The objective of this study was to understand the effects of feed-restriction on growth, health, and digestibility of pigs. More research needs to be done on other immune biomarkers, such as IL-6, which is known to be a modulator of CRP; as well as the compensatory gain of the pigs to see how long it takes them to catch up to those on the control diet. An overall economic analysis would be needed to solidify the findings of how to save pork producers money.

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Figure 1. Growth performance of phase 1 (wk 1to 4) and phase 2 (wk 5 to 8). Restricted pigs (n=18) and ADLIB pigs (n=18) were evaluated in phase 1 (wk1-4) and phase 2 (wk 5-8). Each bar represents the least-square mean (±SEM) of 36 pigs for phase 1 and phase 2. Bars with corresponding letters (a,b) showed a significant difference ($P < 0.05$).

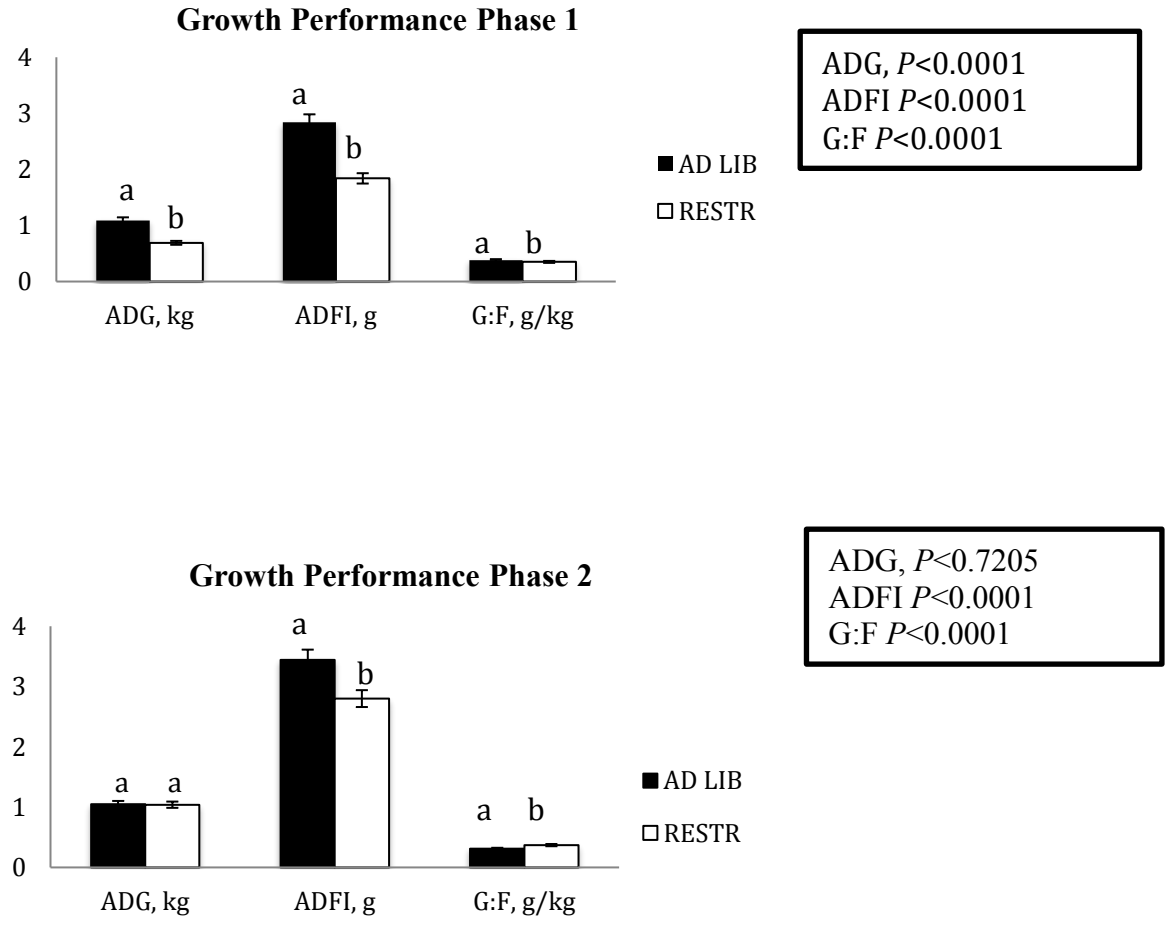

Figure 2: Digestibility of phase 1 (wk1-4) and phase 2 (wk 5-8). Restricted pigs (n=18) and ADLIB pigs (n=18) were evaluated in phase 1 and phase 2. Each bar represents the least-square mean (±SEM) of 36 pigs for phase 1 and phase 2. Bars with corresponding letters (a,b) showed a significant difference ($P < 0.05$).

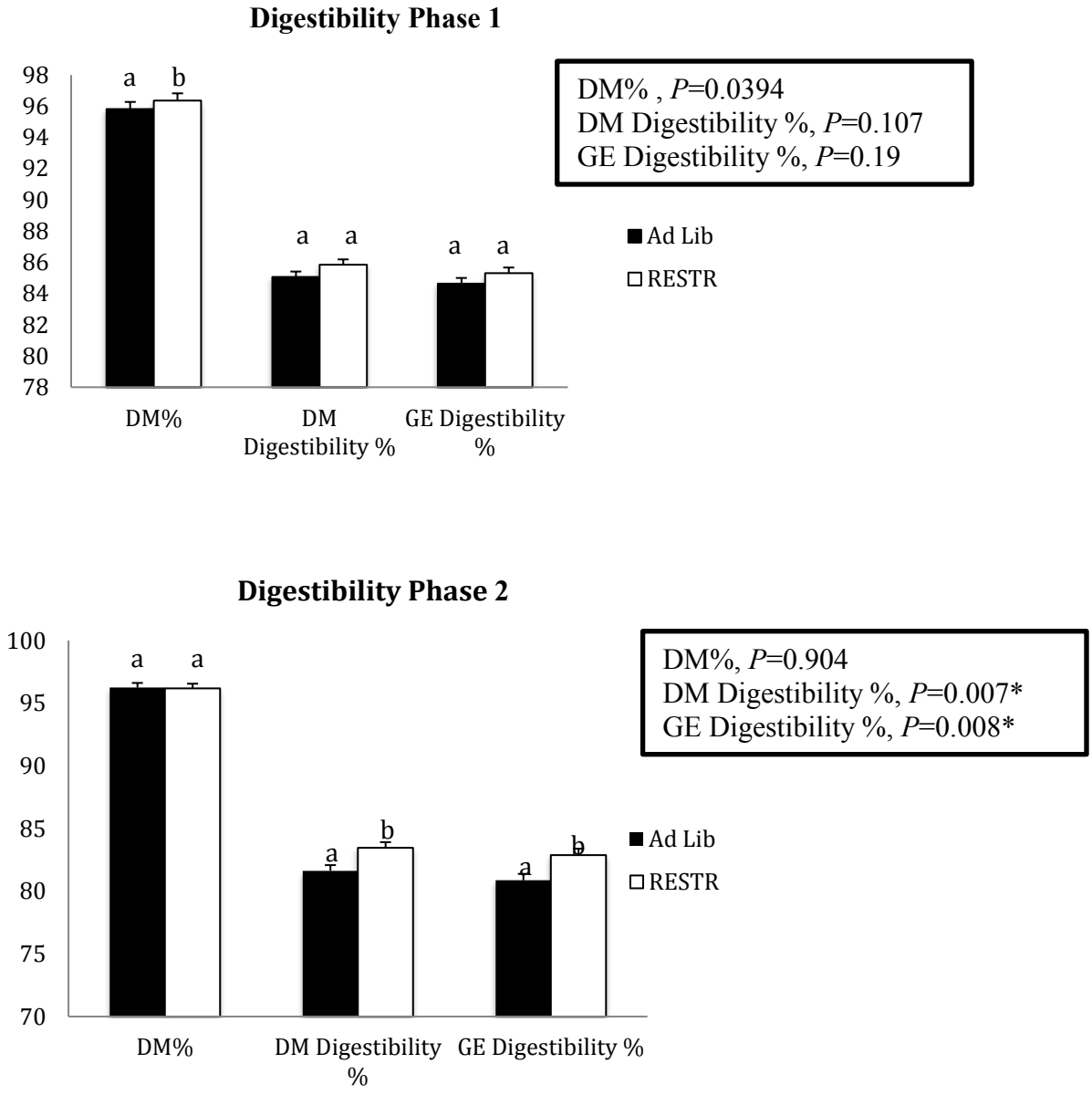

Figure 3. Gross energy is compared in ADLIB vs RESTR as well as divide up between phase 1 (wk1-4) and phase 2 (wk5-8). Restricted pigs (n=18) and ADLIB pigs (n=18) were evaluated in phase 1 (wk1-4) and phase 2 (wk 5-8). Each bar represents the least-square mean (±SEM) of 36 pigs for phase 1 and phase 2. Bars with corresponding letters (a,b) showed a significant difference ($P < 0.05$).

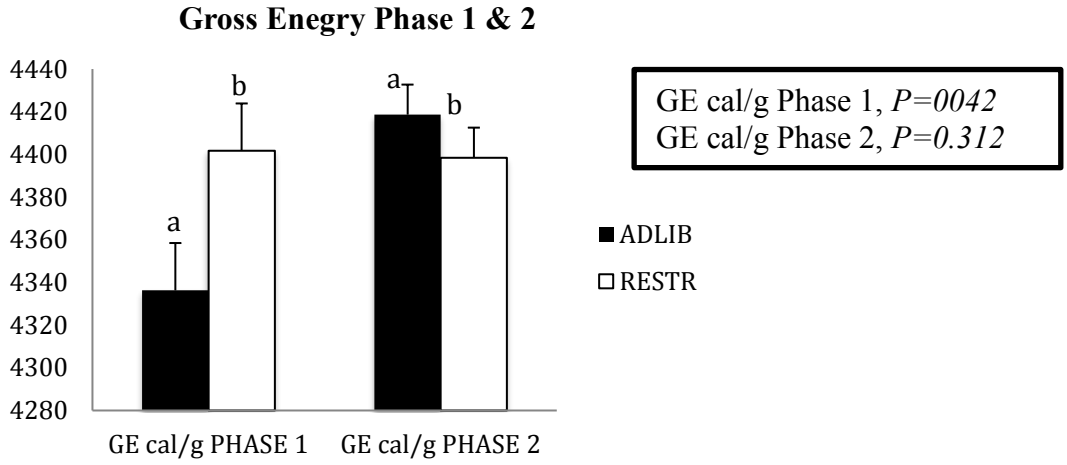

Table 1a. Effects of ADLIB vs RESTR diet on growth performance of grower-finisher pigs

Item	Treatment		SEM	*P*-value
	ADLIB	RESTR		
BW, kg				
d 0	52.5588	52.1737	0.8974	0.7571
d 7	59.3235	53.0263	0.8506	<0.0001
d 14	65.8	58.7737	0.8815	<0.0001
d 21	73.9471	64.5444	0.9518	<0.0001
d 28	82.9471	71.8611	1.0713	<0.0001
d 35	90.8353	78.8056	1.2373	<0.0001
d 42	98.147	87.6333	1.4563	<0.0001
d 49	105.64	95.022	1.666	<0.0001
d 56	112.52	100.68	1.65	<0.0001

Table 1b. Effects of ADLIB vs RESTR diet on growth performance of grower-finisher pigs

Item	Treatment		SEM	*P*-value
	ADLIB	RESTR		
Phase 1				
d 0 to 7				
ADG, kg	0.98	0.07	0.03	<0.0001
ADFI, g	2.55	1.3	0.02	<0.0001
G:F, g/kg	0.38	0.06	0.02	<0.0001
d 7 to 14				
ADG, kg	0.91	0.82	0.04	0.1121
ADFI, g	2.78	1.91	0.01	<0.0001
G:F, g/kg	0.33	0.43	0.02	<0.0001
d 14 to 21				
ADG, kg	1.17	0.83	0.05	<0.0001
ADFI, g	2.78	1.91	0.01	<0.0001
G:F, g/kg	0.42	0.43	0.02	0.5983
d 21 to 28				
ADG, kg	1.28	1.04	0.03	<0.0001
ADFI, g	3.25	2.24	0.02	<0.0001
G:F, g/kg	0.39	0.46	0.01	<0.0001
Phase 1 (Wk 1 to 4)				
ADG, kg	1.09	0.69	0.02	<0.0001
ADFI, g	2.84	1.84	0.01	<0.0001
G:F, g/kg	0.38	0.35	0.01	<0.0001
Phase 2				
d 28 to 35				
ADG, kg	1.13	0.98	0.05	0.0301
ADFI, g	3.39	2.45	0.06	<0.0001
G:F, g/kg	0.33	0.4	0.01	<0.0001
d 35 to 42				
ADG, kg	1.04	1.28	0.06	0.0032
ADFI, g	3.5	2.92	0.1	<0.0001
G:F, g/kg	0.29	0.44	0.01	<0.0001
d 42 to 49				
ADG, kg	1.05	1.04	0.08	0.8995
ADFI, g	3.38	2.91	0.08	<0.0001
G:F, g/kg	0.31	0.36	0.02	0.1876
d 49 to 56				
ADG, kg	0.99	0.84	0.08	0.1738
ADFI, g	3.47	2.92	0.07	<0.0001
G:F, g/kg	0.28	0.29	0.02	0.9578

Phase 2				
(wk 5 to 8)				
ADG, kg	1.05	1.04	0.03	0.7205
ADFI, g	3.44	2.8	0.07	<0.0001
G:F, g/kg	0.31	0.37	0.01	<0.0001
Wk 1 to 8				
ADG, kg	1.07	0.86	0.02	<0.0001
ADFI, g	3.14	2.32	0.04	<0.0001
G:F, g/kg	0.34	0.37	0	<0.0001

Table 1c. Effects of ADLIB vs RESTR diet on digestibility of grower-finisher pigs

Item	Treatment		SEM	*P*-value
	ADLIB	RESTR		
Phase 1				
DM%	95.82	96.37	0.46	0.394
DM Digestibility %	85.07	85.85	0.34	0.107
GE cal/g	4336.3	4401.7	22.17	0.042
GE Digestibility %	84.64	85.31	0.36	0.19
Phase 2				
DM%	96.24	96.18	0.37	0.904
DM Digestibility %	81.62	83.45	0.46	0.007
GE cal/g	4418.63	4398.47	14.07	0.312
GE Digestibility %	80.87	82.88	0.51	0.008

Table 2. Concentrations of C-reactive protein (CRP) mg/L (Ad lib vs RESTR) on d 0 to 42

CRP	Treatment		SEM	*P*-value
	ADLIB	RESTR		
d 0	322.96	293.16	42.02	0.609
d 7	256.43	172.21	31.16	0.061
d 14	163.51	151.34	19.83	0.667
d 28	299.88	262.84	43.93	0.353
d 42	121.51	122.95	9.19	0.911

CHAPTER 3.

Effects of Energy Restriction during Gilt Development on Parity 1 and 2 Progeny Growth Performance

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ABSTRACT: There are many factors (pre- and postnatal) that affect piglet health and growth performance including environmental, nutritional, and genetic effects. Ongoing sow reproductive longevity research at the University of Nebraska has generated data over 14 batches monitoring females up to parity 4. Gilts included in this work have been developed under different nutritional management strategies, including energy restriction, and it has been determined that energy restriction increases sow longevity. The objective of this work was to determine if nutritional management (i.e., energy restriction) during gilt development (123 to 240 d of age) effects parity 1 and 2 progeny growth performance (Batches 5 through 13; n = 733 gilts). Gilts included in the analysis were fed either a control diet (balanced according to the NRC, 1998; n = 333) or energy restricted diet (20% energy restricted accomplished via the addition of soy hulls; n = 400). At 240 d of age gilts were bred and fed a common ad libitum diet based on the 2012 NRC Requirements. At d 109 of gestation, sows were transferred to farrowing crates and pre-backfat (Pre-BF) and pre-BW were recorded. After farrowing, individual piglet birth weights and d 21 piglet weaning weights were recorded. In parity 1, gilts fed an energy-restricted diet tended ($P < 0.09$) to farrow piglets with greater birth weight (1.26 vs. 1.28 kg for control and restricted, respectively); however, in parity 2, treatment had no effect on birth weight (P = 0.30). Furthermore, in parity 1 energy-restricted sows weaned heavier pigs ($P < 0.05$; 5.20 vs. 5.34 kg for control and restricted, respectively) compared to control sows, but once again, in parity 2 there was no difference (P = 0.65\). This preliminary research indicates that energy restriction during gilt development may have a positive effect on parity 1 progeny growth

performance resulting in greater birth weights and weaning weights; however, effects of developmental diet on subsequent progeny growth performance may begin to disappear with increasing parity due to compensatory gain between parities.

KEY WORDS: Energy Restriction, Sow, Weaning

INTRODUCTION

Developing gilts that breed early and continue to be able to reproduce are major goals of pork producers. There is a substantial amount of work at the University of Nebraska that has been focused on improving sow longevity; however, until recently, performance of progeny resulting from different gilt development strategies has not been a focal point. Litter performance and subsequent growth performance has been attributed to sow diet, genetics, and sow weight. Given the fact that pigs spend nearly half of their life in utero, the nutritional management of the sow can greatly influence birth weight and postnatal growth (Amdi et al., 2013). The early environment (i.e., gestation, lactation, and sanitation) of a piglet has drastic effects on its health that can carryover and have long-term effects on both health and growth performance. A study conducted by Klindt et al. (2001) found that energy restricted gilts restricted during developmental period, consumed more feed than the control gilts when all sows were fed a common ad libitum diet during gestation. Due to the fact of greater intake during gestation, the restricted gilts had compensatory gain and nearly erased the negative BCS from the energy-restricted diet. Furthermore, increased feed intake after a restricted diet may result in increased metabolic rate and organ growth (Klindt et al., 2001). Body composition during time of breeding and gestation may play a key role in the weight of offspring (Amdi et al., 2013). Under-nutrition of a sow can cause a reduced supply of nutrients available to the progeny, but a low body condition score can cause greater catabolism in a sow. Sows will lose less weight and backfat when they are on a higher energy diet, yet all sows will catabolize tissue during early lactation even if

fed ad libitum. Maternal backfat depths also have been shown to play a role in fetal development from birth to slaughter (Amdi et al., 2013). There are conflicting ideas of what has a greater impact on offspring performance: maternal body condition score or maternal feed level intake; thus, both were analyzed in this experiment. The objective of this data analysis was to determine if energy restriction during gilt development confers advantages with respect to growth performance of their progeny.

MATERIALS AND METHODS

Animals and experimental design

The experimental protocol was reviewed and approved by the institutional Animal Care and Use Committee of the University of Nebraska, Lincoln. The analysis described below included seven hundred and thirty three gilts over the course of 9 batches with each batch consisting of data collected over two parities. Treatments were allotted randomly to gilts at d 123 of age (3 treatments; 8 gilts/pen). Dietary treatments (Table 1a) included 2 types of an energy restricted diet (P1, n=400; and P2, n=314; one energy restricted diet was 20% restricted in energy with increased fiber, the other energy restricted diet was 20% restricted energy with the same Lys:ME as the CTL diet) and a control diet (P1, n=333; and P2, n= 272). For the purpose of this analysis, data from the two restricted treatments were pooled and compared to the control treatment. All gilts were maintained on their respective dietary treatments for the duration of the gilt developmental period (d 123 to 240).

The number of sows in each parity varied due to normal culling of gilts from parity 1 to parity 2. During the entire experiment the pigs were given ad libitum access to water and feed. After breeding on d 240, sows were moved to gestation crates. At d 109 of gestation the sows were moved to farrowing crates and backfat (pre-BF) was measured. All sows were limit-fed during gestation (Table 1b) and given ad libitum access to feed during lactation (Table 1b). The study consisted of a feeding experiment during the developmental period of gilts that then followed the sow's progeny performance to weaning. Once piglets were weaned, sows were put back on the gestation diet until the farrowing of parity 2 progeny, where they were then put on the lactation diet.

Dietary Treatments

Diet ingredients and nutrient composition are presented in Table 1 and 2, respectively. Diets were all fed ad libitum, but 2 were restricted in energy. Dietary treatments included the following: 1) Control (CTL; formulated to 1998 NRC requirements) and 2) Restricted (RESTR; 2 restricted diets were used in batches 5-13, but all restricted diets were analyzed as 1). Each diet was fed as a 3 phase feeding regimens. For phase 1, 2, and 3 the control diet contained 3406, 3408, and 3410 kcal/kg, respectively. The two restricted diets contained 2706-2713 kcal/kg for phase 1, 2707-2715 kcal/kg for phase 2, and 2708-2717 kcal/kg for phase 3. Furthermore, both restricted diets contained higher amounts of fiber and lower crude protein than the control diet.

Data and Sample Collection

When the gilts were moved to farrowing crates (d 109 of gestation), pre-BF

was recorded using Aloka 500V real-time ultrasound instrument equipped with a 3.5-MHz, 17-cm linear transducer (Corometrics Medical System, Inc.) and pre-BW was recorded. On the day that pigs were weaned, backfat (post-BW) and BW (post-BW) measurements were recorded for all sows by the same methods used previously. Piglets were weaned at d 21 post-farrowing. Number born alive, total number born, and number weaned were also recorded for each sow. Piglet birth weight and piglet weaning weight were recorded to measure progeny performance based on sow diet.

Statistical Analysis

Data was analyzed in JMP 12 (SAS, Cary, NC). $P < 0.05$ was considered significant, non-significant factors were dropped and the model was run again. For birth weight (BiW): sow pre-weight (pre-BW) , sow pre-backfat (pre-BF), treatment (TRT), total number born (TNB) and batch number (REP) were included in the model as fixed effects, sire and litter nested in sire were random effects contrast statements were analyzed through LSM Tukey-HSD . When analyzing weaning weight (WW), sow pre-BF, sow post-back fat (post-BF), number nursed (NN), number weaned (NW), average BiW of litter, TRT, and REP were included in the model as fixed effects, sire and litter nested in sire were random effects contrast statements were analyzed through LSM Tukey-HSD. Average sow pre-BW, was analyzed with TRT, REP and TRT by REP as a fixed effect contrast statements were analyzed through tukey HSD and LSM Dunnett. Average Pre-BF was analyzed with trt and rep. Contrast statements were analyzed through LSM Dunnett. Continuing on, to analyze Avg post-BF, TRT, pre-BW, pre-BF, NW, post-BW, REP by TRT and

REP were included in the model as fixed effects contrast statements were analyzed through tukey HSD and LSM Dunnett. For the analysis of Avg post-BW; TRT, pre-BW, NW, TRT by REP and REP were included in the model as fixed effects contrast statements were analyzed through Tukey HSD and LSM Dunnett. ADFI was analyzed with TRT and REP as a fixed and contrast statements were analyzed through LSM Dunnett. All means are presented as least-squares means (SEM).

RESULTS

Parity 1 Sow performance

Sows allotted to an energy-restricted diet had a significantly lower pre-BF depth when compared to CTL sows (2.00 vs 2.31 mm respectively; $P < 0.0001$, Fig. 1). However, when backfat was measured at day of weaning, RESTR sows had lost the same amount of backfat as the CTL sows (-0.34 cm). Irrespective of dietary treatment, all sows had less ($P < 0.0001$) backfat at weaning (post-BF) compared to pre-BF. Interestingly, sows on both dietary treatments lost a significant amount of backfat during lactation ($P < 0.0001$). Interestingly, RESTR sows at had similar post-BF depth as CTL sows pre-BF depth ($P = 0.7292$). Furthermore, as shown in Figure 3, RESTR sows had lower ($P < 0.0001$) pre-BW (212.49 kg) compared to CTL sows (223.42 kg). Sow post-BW was not affected by developmental dietary treatment on the day of weaning ($P = 0.7469$). Lastly, sows were limit fed during gestation so there was no significant difference in ADFI, but during lactation sows were fed ad libitum and RESTR gilts consumed more ($P < 0.0001$) feed compared to CTL sows (4.17 vs. 3.90 kg/d, respectively;, Figure 4).

Parity 1 Progeny performance

Progeny derived from RESTR sows tended (1.26 Kg vs 1.29 Kg, respectively; *P* = 0.09, Fig. 2) to have greater BiW when compared to progeny derived from CTL sows. Piglets derived from RESTR sows had greater (5.2 kg vs 5.34 kg respectively; *P* = 0.018, Fig. 2) WW than those from CTL sows.

Parity 2 Sow performance

Sows that were included in the experiment and analysis of parity 1 data were followed through in subsequent parities. Here we describe results from Parity 2. Some sows were culled due to lameness or not returning to estrus. Sows allotted to an energy-restricted diet continued to have a significantly lower pre-BF depth in parity 2 when compared to control sows (1.83cm vs 1.99 cm respectively; *P* < 0.0001, Fig. 6). However, similar to parity 1, when post-bf was measured there was no difference in bf depth (1.71cm, P = 0.9990, Fig. 6). Overall CTL sows had lost more BF than RESTR (-0.28 vs -0.12 cm, respectively). Sows on an energy-restricted diet had an average pre BW of 237.57 kg while control diet sows had a weight of 242.49 (*P* < 0.0006, Fig. 7). Sows post BW was not significantly different among diets (*P* = 0.4623, Fig. 7). Lastly, in the analysis of ADFI for parity 2 sows, it was shown that energy-restricted sows ate more than CTL sows, as was also seen in parity 1 (P = .0539, Fig 8).

Parity 2 Progeny performance

Piglets farrowed from sows on a restricted diet did not have a large difference in BiW (1.46 kg vs 1.45 kg respectively; P = 0.3047) or WW (6.29 kg vs 6.31 kg respectively; P = .6525) from piglets farrowed from CTL sows.

DISCUSSION

While data on these sows was analyzed for breeding longevity in previous experiments (Miller et al., 2010), and it was discovered that RESTR gilts have longer breeding longevity, little was known on how or if the diets affected progeny performance. Furthermore, we wanted to see if RESTR diets had a positive effect not only on the sows breeding ability, but also its progeny. The aim of this analysis was to investigate the effect of energy-restricted diets on developing gilts and if there is carry over effects to their offspring's growth performance. Parity 1 progeny derived from energy-restricted gilts had a tendency to weigh more at birth and they had a significantly greater weaning weight when compared to piglets farrowed from CTL sows. These birth weight results suggest that the gilts body condition may have had an effect on offspring growth, due to the fact that all gilts were limit-fed during gestation and on a common diet. However, our results vary from Lewis and Bunter (2011) where it was observed that sows with greater BW farrow piglets with greater BW. Other studies have stated that maternal diet is more important than maternal body condition and the fetal response responds more to dietary changes (Howie et al., 2009).

Howie et al. (2009) found that when a rat was switched to a high fat diet during pregnancy, offspring had greater fat adiposity compared to the control group; thus, maternal diet has a greater effect on offspring. In the current work, while RESTR sow were not switched to a high fat diet, they did receive a diet with more energy than their development period and consumed more feed during

lactation when compared to CTL sows which may indicate that they consumed more fat than CTL sows. Furthermore, Vadmand et al. (2015) found that milk production in sows is positively correlated with feed intake; this may explain greater weaning weights for piglets from RESTR sows due to their increased ADFI during lactation.

Feeding level and maternal body condition score (BCS) can affect offspring growth and development. Average daily gain of piglets is correlated with the ADFI of sows as well as BW loss during lactation. Considering our results, specifically where RESTR sows had greater ADFI during lactation and had similar BF loss, this may be a reason for RESTR sows having piglets with greater WW. Increasing the ADFI by 100g/day could increase litter weaning weight from 0.5 to 1.0 kg (Strathe et al., 2016). Our results indicated that RESTR sows had an ADFI of about 270 g/d more than CTL sows and weaned significantly heavier piglets, agreeing with the results of Strathe et al. (2016). When a sow has increased ADFI more energy and nutrients are available for milk production, which is correlated with piglet growth (Eissen et al., 2003).

Improving efficiency of a sow's protein utilization when feed-restricted plays a large role in the growth of the fetuses as well as in milk production (Kim et al., 2008). When a lactating sow is energy-restricted it pulls from AA, resulting in deamination and urea synthesis of the liver. Fetal and mammary tissue growth is rapid during late gestation, and calls for an even greater demand of AA during late gestation (McPherson et al., 2004, Ji et al., 2006). Pulling from AA for energy can cause a protein deficiency in the sow and negatively affect litter performance (Holden et al., 1971). Kim et al (2008) stated feed restriction may also limit protein

intake, thus directly leading to a protein deficiency, specifically during late gestation and lactation and thus why it is important to feed a diet with high efficiency of protein utilization. Continuing, sow AA requirements change through out gestation and phase feeding it as good way to compensate for the changing nutrient requirements of a sow, similar to what was done in the current experiment (Moehn et al., 2012).

Due to RESTR sow eating more than CTL sows during lactation the increased energy consumption could counteract deamination in the body to a lesser degree of the CTL sows. Furthermore, Bettio et al., (2016) found that restricted-fed lactating sows are more efficient, thus producing more milk per g of feed intake. While the current study did not analyze milk output, our result of heavier pigs from RESTR sows could agree with this data due to data on the growth performance of progeny. Furthermore, in our study we saw that RESTR sows were able to have compensatory gain to improve litter performance or have it be even with CTL sows as was shown in both parity 1 and 2 litter performance. These results of greater intake and compensatory gain agree with Bikker et al., (1996) where restricted gilts gained on average 140 g/d faster. Also, as we saw in our data that RESTR sows had a greater ADFI when put an a ADLIB diet during lactation. Similar results were recorded when Wiecek (2011) and Serrano et al (2009), that when the feed restricted pigs were switched over to the ad libitum diet, the ADFI, ADG, and G:F increased when compared to the control pigs.

In conclusion, parity 1 sows that were energy-restricted during the developmental period had increased piglet BiW/WW that was likely caused by

increased ADFI, allowing for greater milk production. Parity 2 saw no significant differences among diets in post-BW, or post-BF suggesting why there were no growth performance differences in the piglets. Future research should try to focus on milk out put in correlation to feed intake and nutrient composition of milk.

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Table 1. Composition and nutrient contents of gestation and lactation diet.

	Parity 1 and Parity 2	
Item:	Gestation[1]	Lactation[2]
Ingredient, %		
Corn	77.25	65.68
Soybean Meal	16	27.5
Tallow	3	3
Dicalcium Phosphate	1.9	2.33
Limestone	0.925	0.6
Salt	0.5	0.5
Vitamin Premix[1]	0.25	0.25
Trace Mineral	0.15	0.15
Phytase	0.02	-
Nutrient Content:		
ME (kcal/kg)	2605	2536
Crude Protein	11.74	15.75
Lys	0.56	0.85
Total P %	0.67	0.8
Ca %	0.87	0.9

[1]Gestation diet was fed d 0 of breeding to d 0 post farrowing

[2]Lactation diet was fed d 0 post farrowing until d 21 post farrowing, sows were put immediately back on gestation diet at d 21 post farrowing

[3]Provided per kilogram of diet for phase 3: 6,600 IU of Vitamin A, 600 IU of Vitamin D_3, 66 IU of Vitamin E, 4.40 IU of Vitamin K, 33.00 mg of Niacin, 22.05 mg of Panothenic Acid, 11.00 mg of Riboflavin, and 22.05 μg of Vitamin B_{12}, 550 mg of Choline Chloride, 1.65 mg of Folic Acid, 0.22 mg of Biotin

Table 2. Composition and nutrient contents of experimental diets fed to developing gilts d 123 – 240*. Phase 1 and 2 were each 6 weeks long and phase 3 was 4 weeks long.

	Phase 1			Phase 2			Phase 3		
Item	**CTL[1]**	**RESTR 1[2]**	**RESTR 2[3]**	**CTL**	**RESTR 1**	**RESTR 2**	**CTL**	**RESTR 1**	**RESTR 2**
Ingredient, %:									
Corn	72.52	39.95	45.25	76.32	43.73	48.2	80.13	47.5	51.28
Soybean Meal	21.53	17.43	12.2	17.66	13.57	9.2	13.79	9.71	6.1
Soybean Hulls	-	40	40	-	40	40	-	40	40
Beef Tallow	3	-	-	3	-	-	3	-	-
Dicalcium phosphate	1.37	1.72	1.65	1.46	1.8	1.7	1.54	1.89	1.72
Limestone	0.68	-	-	0.66	-	-	0.64	-	-
Sodium Chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin Premix[4, 5]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix[6]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Lysine	-	-	-	-	-	-	-	-	-
Methionine	-	-	-	-	-	-	-	-	-
Threonine	-	-	-	-	-	-	-	-	-
Tryptophan	-	-	-	-	-	-	-	-	-
Nutrient Content:									
ME (kcal/kg)	3406	2706	2713	3408	2707	2715	3410	2708	2717
Lys, g/kg	0.7	0.69	0.56	0.61	0.59	0.48	0.51	0.5	0.41
Crude Protein %	16.25	15.72	13.66	14.72	14.19	12.47	13.18	12.66	11.25
P %	0.6	0.6	0.56	0.6	0.6	0.56	0.6	0.6	0.55
Ca %	0.67	0.71	0.68	0.67	0.72	0.68	0.67	0.73	0.67
Lys/ME* (g/Mcal)	2.059	2.536	2.057	1.78	2.185	1.785	1.5	1.835	1.504

* Data from RESTR HF and RESTR LOW AA were analyzed together for growth performance and here on out considered RESTR

[1] Control diet (CTL) was formulated to meet NRC requirements for developing gilts.

[2]Energy restricted diet (RESTR 1) was 20% restricted energy with the same Lys:ME as the CTL diet.

[3]Energy restricted diet (RESTR 2) was 20% restricted in energy with increased fiber.

[4] Provided per kilogram of diet for phase 1 and 2: 5,500 IU of Vitamin A, 550 IU of Vitamin D_3, 30 IU of Vitamin E, 4.40 IU of Vitamin K, 33.00 mg of Niacin, 22.05 mg of Panothenic Acid, 11.00 mg of Riboflavin, and 33.00 μg of Vitamin B_{12}

[5]Provided per kilogram of diet for phase 3: 6,600 IU of Vitamin A, 600 IU of Vitamin D_3, 66 IU of Vitamin E, 4.40 IU of Vitamin K, 33.00 mg of Niacin, 22.05 mg of Panothenic Acid, 11.00 mg of Riboflavin, and 22.05 μg of Vitamin B_{12}, 550 mg of Choline Chloride, 1.65 mg of Folic Acid, 0.22 mg of Biotin

[6] Provided per kilogram of diet: 10.50 mg of Copper Sulfate Pentahydrate, 0.26 mg of Calcium Iodate, 127.50 mg of Ferrous Sulfate, 30.00 mg of Manganese Oxide, 0.30 mg of Sodium Selenite, 127.50 mg of Zinc Sulfate, 226.03 mg of Calcium Carbonate.

Table 3. Sow reproductive performance, feed intake, and growth performance across parity 1 and 2[1]

	Parity 1			Parity 2		
	Treatment			Treatment		
Item	CTL	RESTR	POOLED SEM	CTL	RESTR	POOLEDSEM
[2]PRE-BF, cm	2.33[a]	2[b]	0.02	1.99[a]	1.83[b]	0.07
[3]POST-BF, cm	1.84[a]	1.78[b]	0.03	1.71[a]	1.71[a]	0.01
[4]BiW, Kg	1.26[a]	1.29[a]	0.01	1.46[a]	1.45[a]	0.01
[5]WW, Kg	5.2[a]	5.34[b]	0.05	6.29[a]	6.31[a]	0.05
[2]PRE-BW, Kg	223.42[a]	212.49[b]	0.85	242.49	237.57	1.02
[3]POST-BW, Kg	184.96[a]	185.25[a]	0.64	217.57[a]	216.89[a]	0.67
[6]ADFI, kg/d	3.89[a]	4.14[b]	0.11	5.55	5.69	0.12

[a,b] Within row and experiment, rows without a common superscript differ ($P < 0.05$). Parity 1 and 2 were analyzed separately

[1]Data are means, parity 1 n=733; parity 2 n= 586.

[2]Pre-backfat (Pre-BF) and Pre-body weight are measured at d 109 of gestation

[3]Post-backfat (Post-BF) and Post- body weight (Post-BW) are measured at d 21 post farrowing

[4]Birth weight (BiW) is measured on d 0 of piglet being born and is the average of the litter

[5]Weaning weight (WW) is measured on d 21 of post-farrowing and is the average of the litter

[6]Average daily feed intake (ADFI) was measured d 0 to d 21 of lactation

Figure 1. Effects of diet on backfat in parity 1 sows. Each bar represents the least-squares mean of 733 sows before and after farrowing. Bars with different subscript differ at $P < 0.05$. Pre-backfat represents the backfat of sows at d 109 of gestation. Post-backfat represents backfat of sows at d 21 post farrowing.

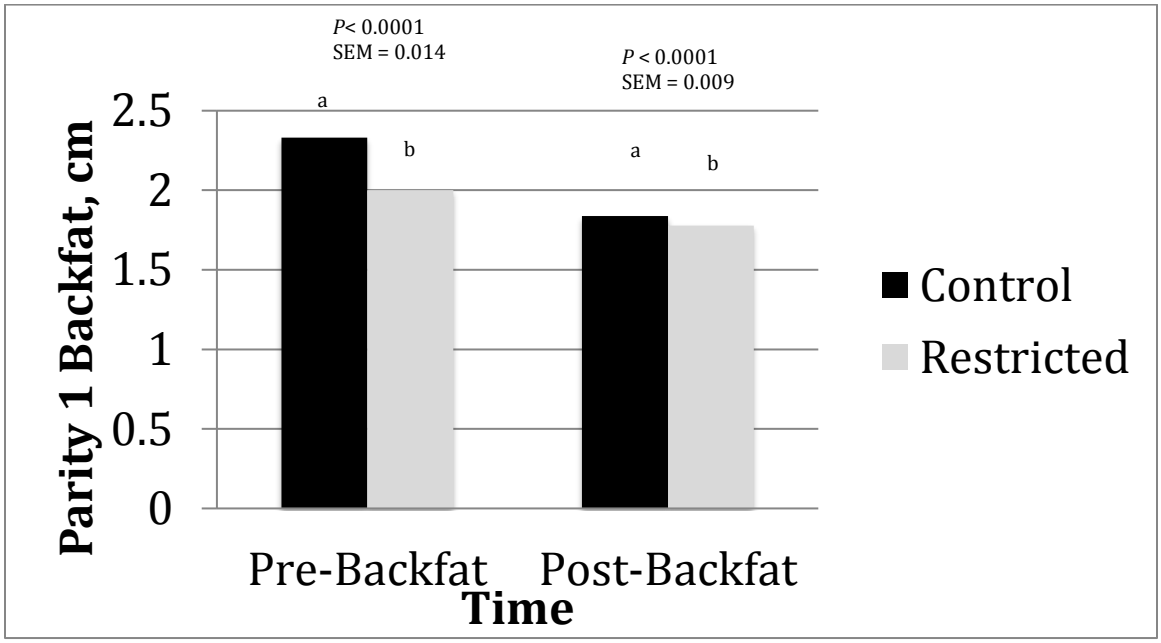


Figure 2. Effects of dietary treatments (CTL or RESTR diet) during gilt development on Parity 1 piglet growth performance at birth (BiW) and at weaning (WW). Each bar represents the least-squares mean of the offspring of gilts that were on a restricted or control diet. Bars with different subscript differ at $P < 0.05$.

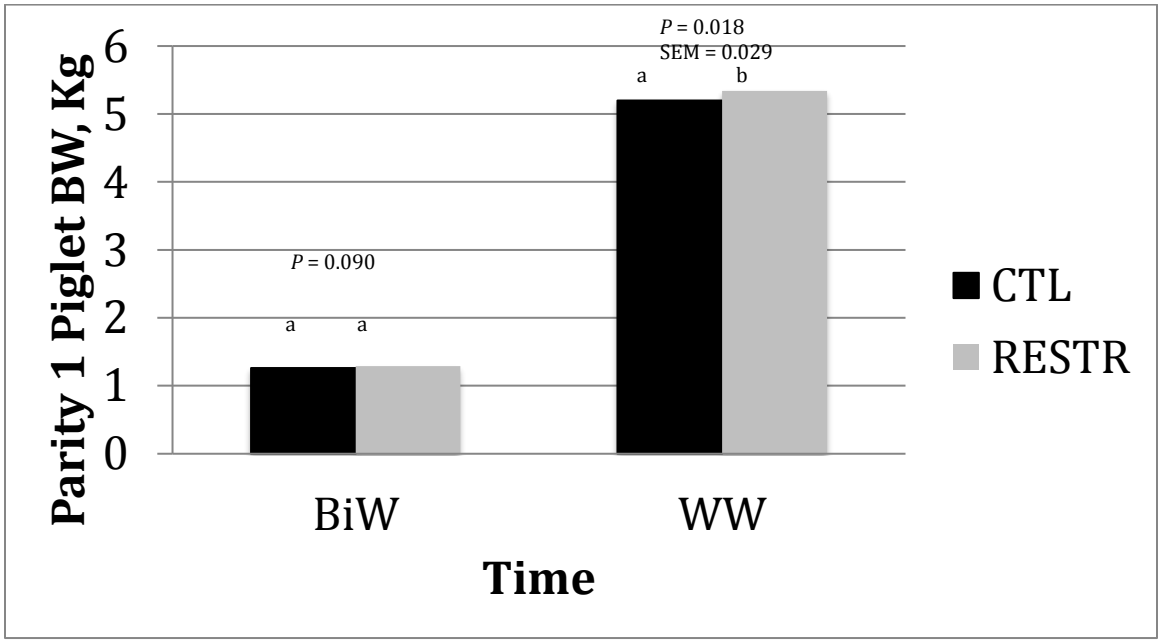
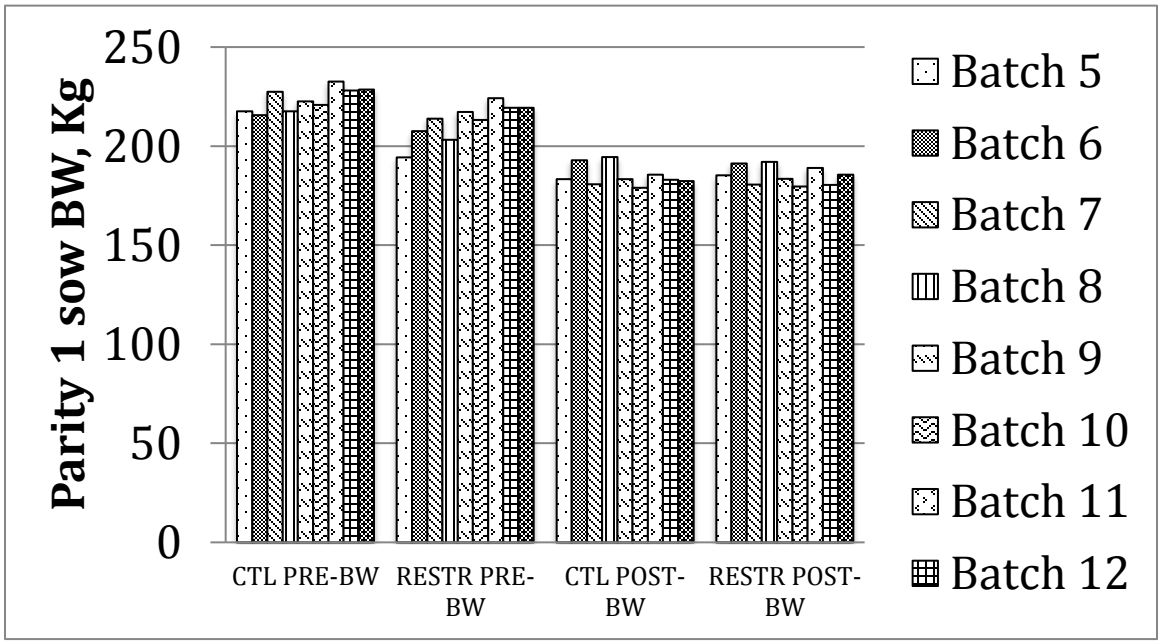


Figure 3. A. Effects of RESTR vs CTL diet on Parity 1 sow BW across all batches. B. Effects of RESTR vs CTL diet on sow BW averaged by diet through out all batches. Each bar represents the least-squares mean of gilts BW. Pre-weight represents the weight of sows at d 109 of gestation. Post-weight represents weight of sows at d 21 post farrowing.

A.



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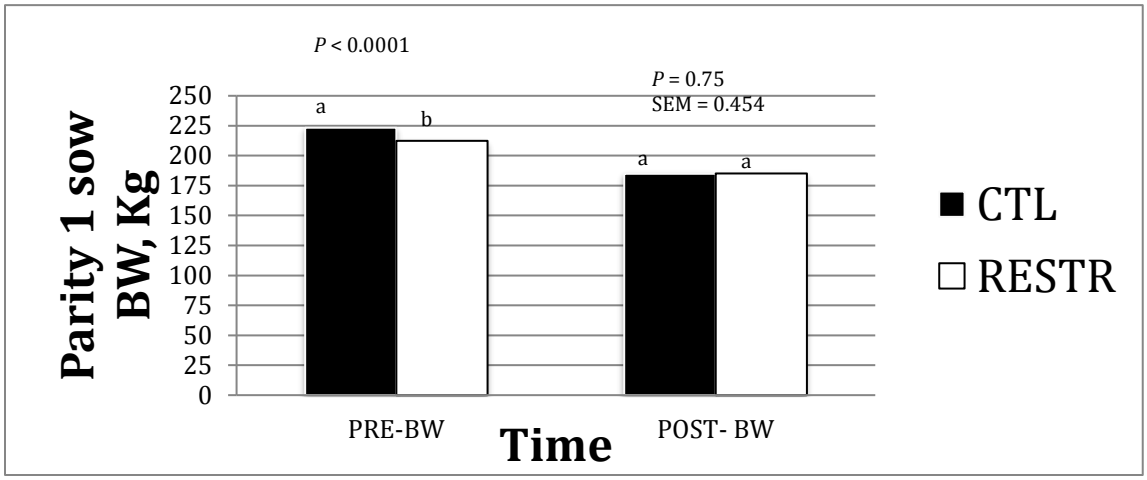


Figure 4. Effects of RESTR vs CTL diet on parity 1 sow ADFI during lactation averaged across all batches. Each bar represents the least-squares mean of sow ADFI.

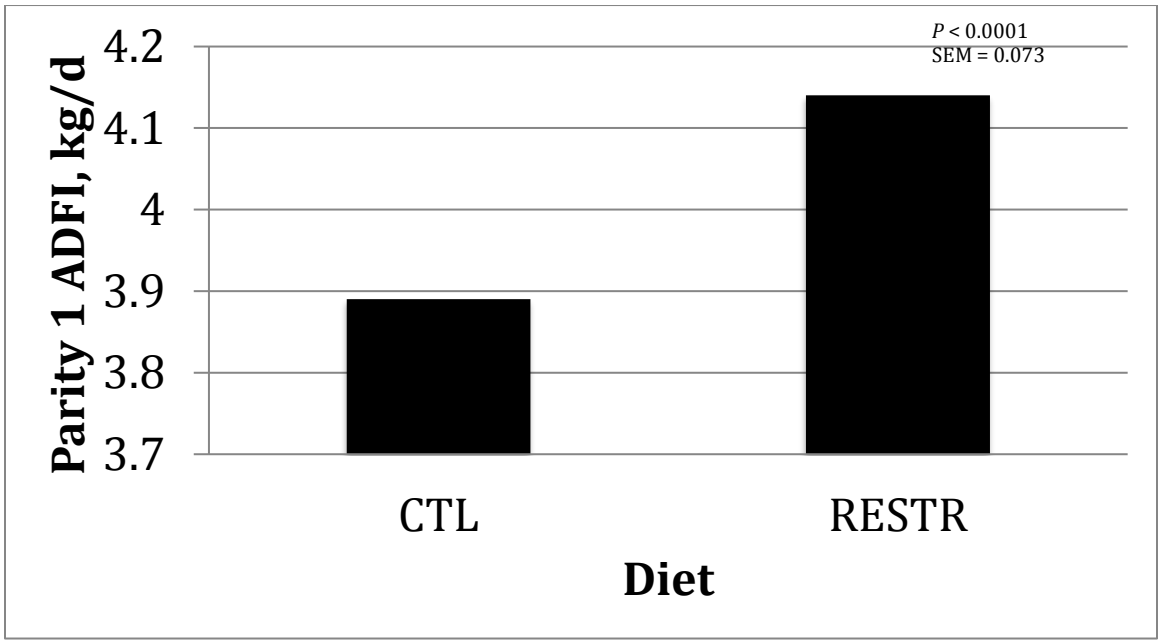


Figure 5. Effects of dietary treatments (CTL or RESTR diet) during gilt development on Parity 2 piglet growth performance at birth (BiW) and at weaning (WW). Each bar represents the least-squares mean of the offspring of gilts that were on a restricted or control diet. Bars with different subscript differ at $P < 0.05$.

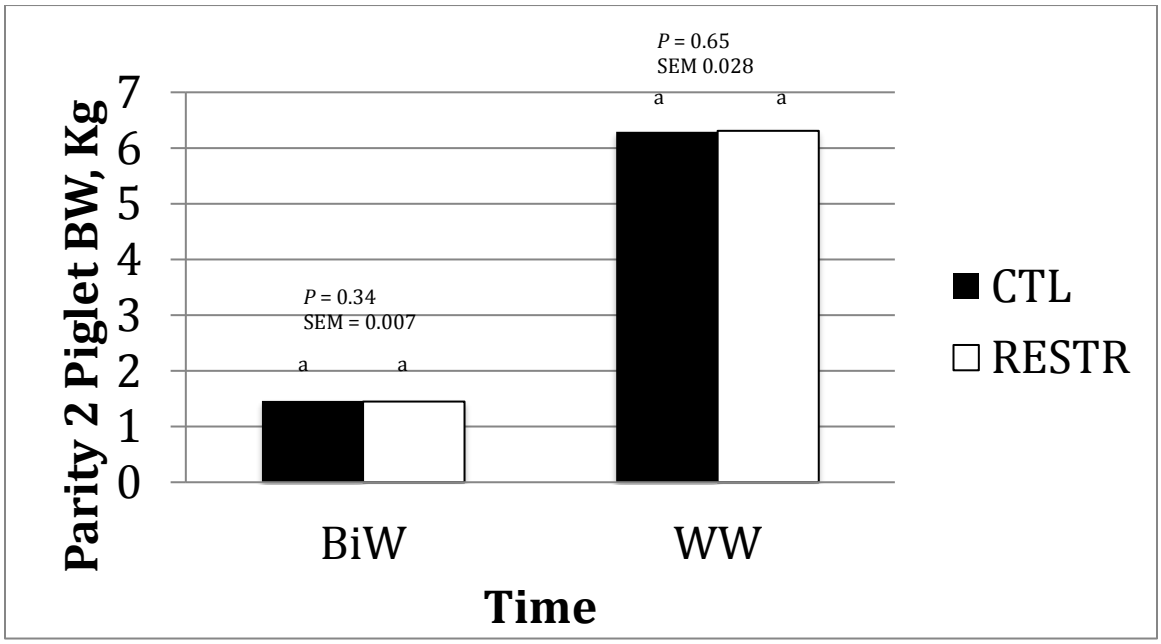


Figure 6. Effects of diet on backfat in parity 2 sows. Each bar represents the least-squares mean of 733 sows before and after farrowing. Bars with different subscript differ at $P < 0.05$. Pre-backfat represents the backfat of sows at d 109 of gestation. Post-backfat represents backfat of sows at d 21 post farrowing.

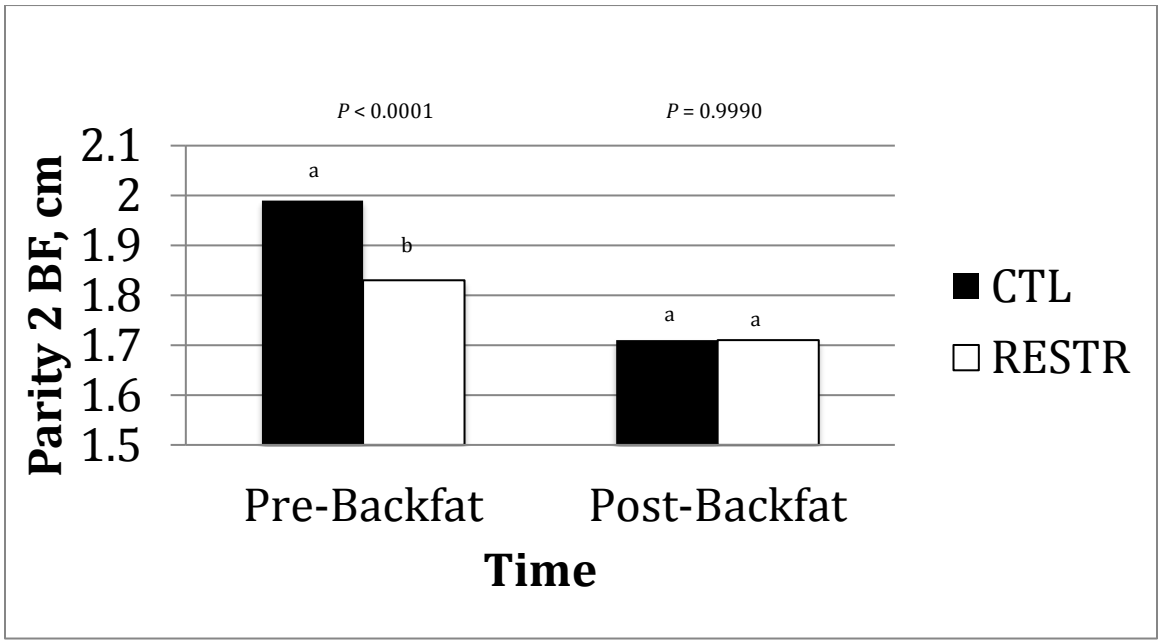
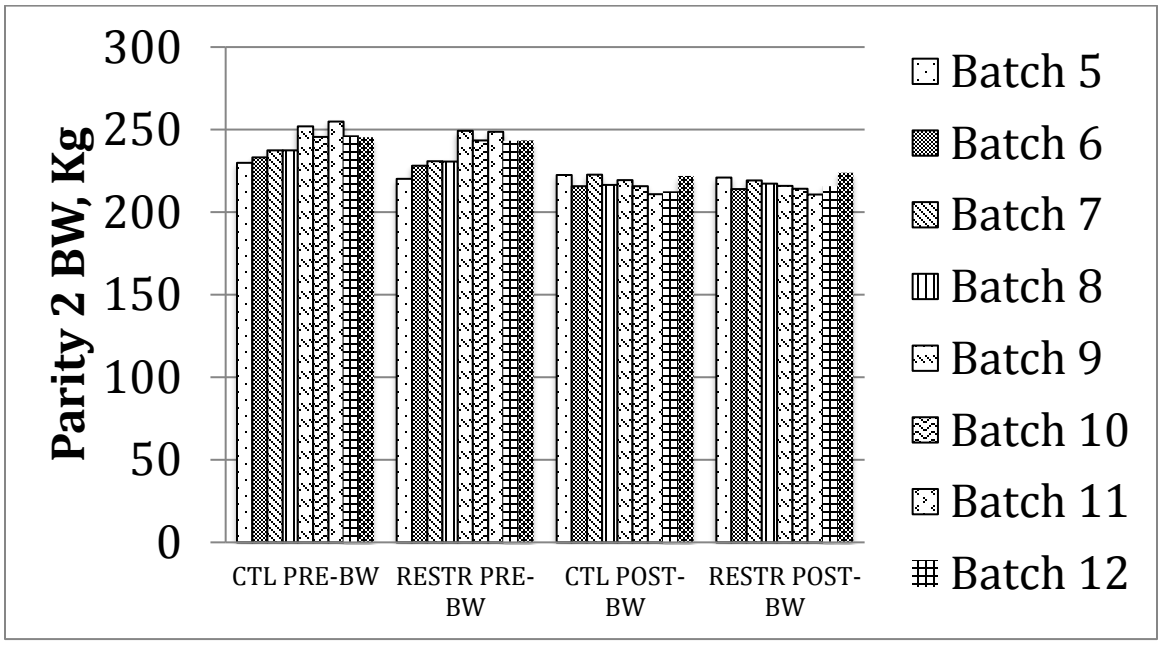


Figure 7AB. A. Effects of RESTR vs CTL diet on Parity 2 sow BW across all batches. B. Effects of RESTR vs CTL diet on sow BW averaged by diet through out all batches. Each bar represents the least-squares mean of gilts BW. Pre-weight represents the weight of sows at d 109 of gestation. Post-weight represents weight of sows at d 21 post farrowing.

A



b

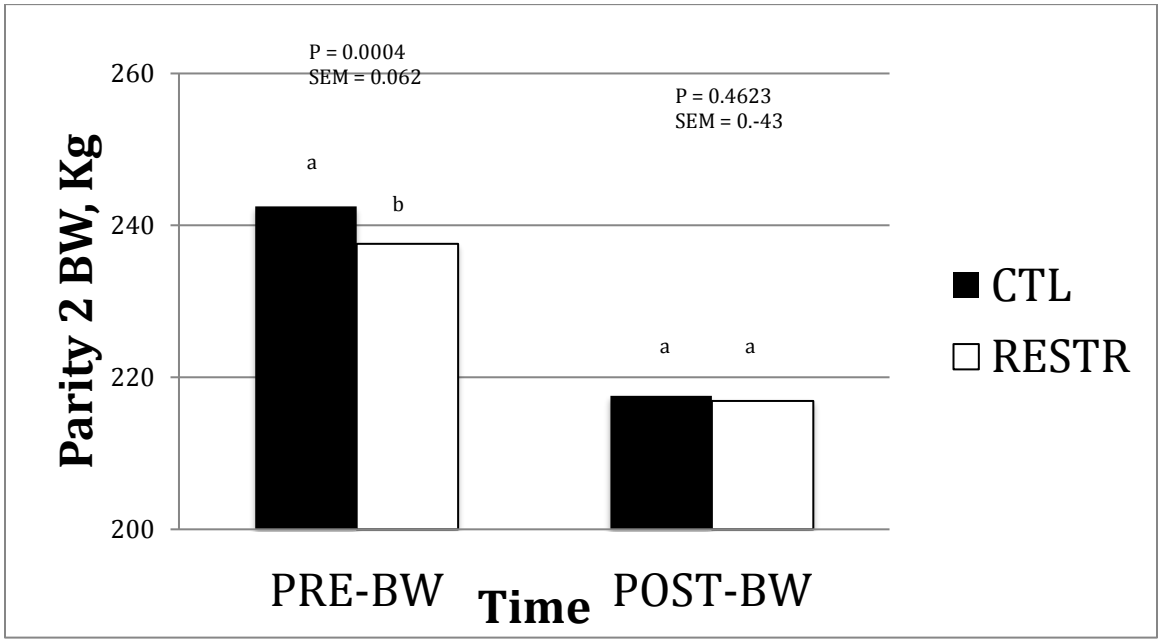
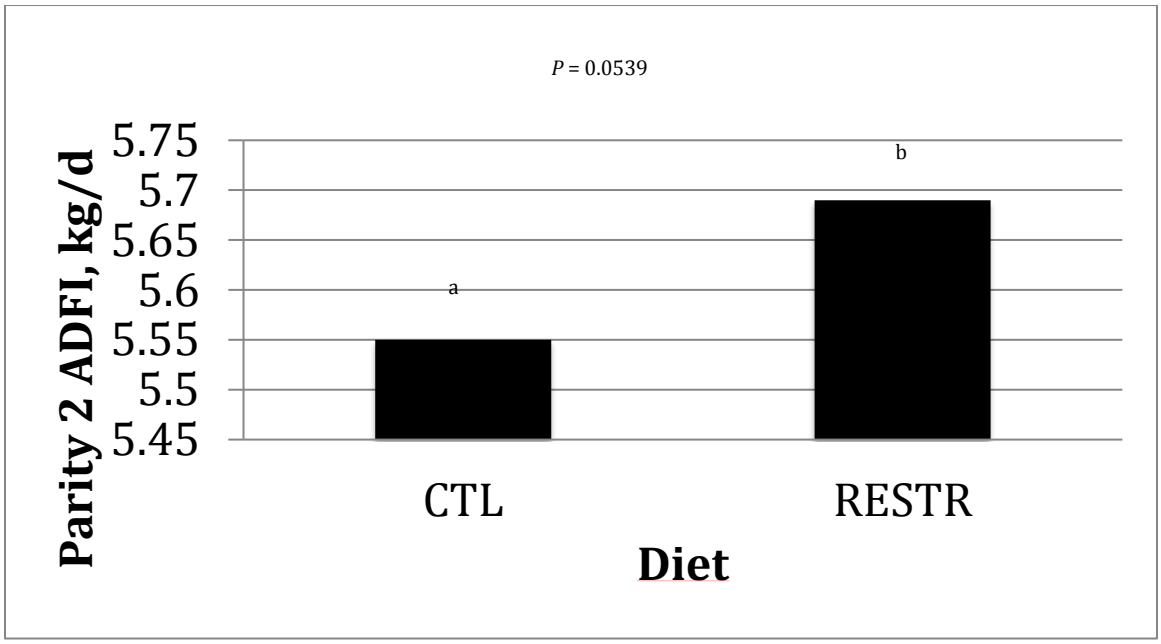


Figure 8. Effects of RESTR vs CTL diet on parity 2 sow ADFI during lactation averaged across all batches. Each bar represents the least-squares mean of sow ADFI.



CHAPTER 4

Effects of Energy Restriction during Gilt Development on Milk Nutrient Profile, Milk Oligosaccharides, and Progeny Biomarkers

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ABSTRACT: Increasing sow longevity has been the focus of many studies to help pork producers maximize profits. An ongoing study at the University of Nebraska-Lincoln (which included 14 batches of gilts) has resulted in the observation that energy restriction during the developmental period of a gilt will increase longevity, but may also have beneficial effects on offspring; particularly, parity 1 progeny. This study focuses on the effects of energy restriction during gilt development on milk nutrient profile and post-natal progeny biomarkers. During the development period, gilts (n = 128, 8 gilts/pen) were fed three dietary treatments including: 1) Control diet formulated to NRC (2012) specifications (CTL); 2) Restricted (20% energy restriction via addition of 40% soy hulls; RESTR); and, 3) CTL diet plus addition of crystalline amino acids equivalent to the SID Lys:ME of the RESTR diet (CTL+). All diets were fed ad libitum and applied in a 3 phase feeding regimen during gilt development (d 123 to 230 of age). Average daily feed intake was used to estimate daily ME intake (Mcal/d) during each phase (Phase 1: 10.13, 6.97, 9.95; Phase 2: 11.25, 8.05, 10.94; and Phase 3: 9.47, 7.95,11.07) for CTL, RESTR, and CTL+, respectively. At 240 d of age gilts were bred and fed a common diet. For this analysis, milk samples were collected from batch 14 gilts (n = 7/treatment) on d 0 and 14 post-farrowing for analysis of N, CP, DM, GE, oligosaccharide (OS) composition and milk insulin. Piglet blood samples (n = 6 piglets/sow) were obtained on d 1 and 15 post-farrowing for quantification of glucagon-like peptide-2 (GLP-2) and insulin. No effects of developmental diet were observed for milk N, CP, DM, or GE; however, N, CP, DM, and insulin were increased ($P < 0.05$) on d 1 compared to d 14. Milk OS profile was significantly different for neutral and acidic

OS ($P < 0.05$) on d 0 but there were no significant differences on d 14. For piglet GLP-2, a treatment by time interaction was observed ($P < 0.009$); specifically, GLP concentrations were greater ($P < 0.001$) in CTL+ compared to RESTR (6.73 vs. 1.21 ng/mL). For serum insulin, a treatment by time interaction was observed ($P < 0.01$); specifically, insulin in RESTR progeny was greater ($P < 0.03$) than CTL on d 1. In conclusion, nutritional management of the developing gilt may impact piglet serum biomarkers, milk nutrient composition, and OS profile during lactation and growth performance.

KEY WORDS: Energy Restriction, Insulin, Protein, Sows

INTRODUCTION

Research at the University of Nebraska investigating the effects of energy restriction on gilt development (which included 14 batches of gilts, n = 90 gilts/batch) has lead to the observation that this approach increases sow longevity, but may also provide beneficial effects to first parity progeny with respect to health and growth. Specifically, parity 1 progeny may have increased weaning weight compared to progeny derived from gilts fed an ad libitum control diet (Miller et al., 2010; Barnett et al., 2017). Developing gilts fed an energy-restricted diet that is adequate in all other nutrients will cause a restriction in fat deposition, but should not have a significant affect on gilt muscle development (Miller et al., 2010). While most studies focus on diet alteration during gestation/lactation rather than prior to breeding, there is evidence that backfat and body weight will effect milk nutrient profile and offspring development (Chen et al., 2009). Furthermore, maternal backfat depth during gestation has a greater effect on offspring development and health than maternal feed intake does (Amdi et al., 2013). Growth biomarkers such as GLP-2 and insulin are maintained through enteral food intake and nutrients ingested (Sangild et al., 2000). Insulin and GLP-2 have been shown to help increase protein synthesis of the neonate. While insulin has its greatest impact on fast-twitch muscles, GLP-2 is related to intestinal adaptation (Davis et al., 2001; Burrin et al., 2007). Energy from the diet is the primary contributor of fat for milk synthesis in the mammary gland (Amdi et al., 2013). Energy source in feed also has an effect on insulin secretion. Insulin in sows regulates energy metabolism and milk production. Sows with insufficient body weight or feed intake will mobilize body tissue to try to

maintain adequate milk levels for offspring (Harrell et al., 2000). Sows with greater fat have been observed to have milk with a greater fat content (Amdi et al., 2013). The milk nutrient profile will vary based on ingredients in the diet. Huber et al. (2015) showed that the use of crystalline amino acids (CAA) to target limiting amino acids and lower CP improved N retention and utilization efficiency in milk protein production. Milk OS vary slightly with diet, but drastically throughout lactation. Oligosaccharides have many prebiotic and growth factors and are resistant to digestion (Boehm et al, 2005). Oligosaccharides are generally divided into three groups – acidic, neutral, and fucosyl, in which each has a niche to enhance neonate health and development.

Therefore, the objective of this experiment was to evaluate how management strategies in gilt development may impact progeny performance and milk nutrient profile. We hypothesized that the growth performance of progeny derived from gilts developed on energy-restricted diets may vary dependent on milk composition.

MATERIALS AND METHODS

The University of Nebraska, Lincoln Animal Care and Use Committee approved all animal care and handling procedures used in this experiment. The Experiment was carried out at the University of Nebraska Swine Research Center.

Animals and Experimental Design

Batch 14, parity 1 gilts (n = 128) were randomly allotted to a dietary treatment (3 treatments 8 gilts/pen) during their developmental period (d 123 to

230 of age). Gilts were housed in a temperature-controlled room and were given ad libitum access to water. Gilts were fed in a 3 phase feeding regimen in which phase 1 and 2 were 42 days, and phase 3 was 28 days. At 230 d of age gilts were bred and moved to individual gestation crates where they were all fed a common diet to meet the requirements of a gestating sow (NRC 2010). At d 109 of gestation the sows were moved to farrowing crates.

Dietary Treatments

Diet ingredients and nutrient composition are presented in table 1 and 2. Diets were fed ad libitum and varied based on energy content. Dietary treatments included the following: 1) Control (CTL, formulated to 2010 NRC requirements) 2) restricted (RESTR, containing 40% soy hulls and 20% energy restricted) and 3) Control Plus (CTL+, containing an addition of crystalline amino acids equivalent to the SID Lys:ME of the RESTR diet).

Data and Sample Collection

Feed disappearance was measured every 2 wks from d 123 to 240 during the gilt development period to calculate ADFI and ME per gilt. When the gilts were moved to farrowing crates (d 109 of gestation), backfat (pre-BF) was measured using Aloka 500V real-time ultrasound instrument equipped with a 3.5-MHz, 17-cm linear transducer (Corometrics Medical System, Inc.) and BW (pre-BW) weight was recorded. After farrowing at the time progeny were weaned (d 21 post-farrowing), sow backfat (post-BF) and BW (post-BW) were observed and recorded as described previously. Piglets were weaned at d 21 post-farrowing. Piglets that were cross-fostered were moved to a farrowing crate with a sow on the same treatment as that

from which it was derived. All piglets' birth weight (BiW) and weaning weight (WW) were recorded to measure progeny performance based on developmental diet. Milk samples were collected on d 0 and 14 post farrowing from 21 sows (7 sows/treatment). Oxytocin (1 to 2 mL was administered in the neck via *IM* injection to facilitate milk letdown. Piglets (n = 6/litter) from the sows selected for milk sampling were randomly selected and blood samples were collected on d1 and 15 post-farrowing. All blood samples were collected via the jugular vein. Serum was harvested following centrifugation (20 min at 2,5000 × *g*). Serum and milk samples were frozen at -20C for later analyses.

Serum biomarker measures

A porcine specific enzyme-linked immunosorbent assay (ELISA) was used to quantify circulating insulin (Mercodia; Uppsala, Sweden) using manufacturers instructions with an intra-assay and inter- assay CV percent of 3.47 and 3.23, respectively. Glucagon-like peptide 2 (GLP-2) concentrations were measured by ELISA (AssayPro, St. Charles, MO, USA) using manufacturers instructions and an intra-assay and inter-assay CV percent of 5.03 and 9.1, respectively.

Milk Composition Analysis

Dry matter content of the milk samples were measured by drying 1 g of sample overnight in a 100-degree oven and then calculating the difference. Dry matter was then used in the bomb calorimetry to calculate GE. 1 mL of oil was added to each sample before analyzing the content in the bomb calorimeter. A porcine specific ELISA was used to quantify circulating insulin in the milk (Mercodia; Uppsala, Sweden). To analyze milk N% and CP% The LECO TruSpec N-

Nitrogen/Protein Analyzer was used. It is a microprocessor based, software-controlled instrument that determines the nitrogen content in a variety of materials. Samples were ground thoroughly through a 2 mm screen and next into a 1 mm screen. Next the milk sample was weighed to approximately 0.2500 g and analyzed. All samples were analyzed in duplicates. The machine has three phases during an analysis cycle and results are shown as % N and % CP where the duplicates were than averaged.

Oligosaccharide Analysis

Chemicals and reagents

Acetonitrile (ACN), chloroform, formic acid (FA), methanol (MeOH), ethanol (EtOH), trifluoroacetic acid (TFA) and sodium hydroxide (NaOH) were obtained from Thermo Fisher Scientific (Waltham, MA); sodium acetate (NaAc) was from Sigma-Aldrich (St Louis, MO). Oligosaccharide standards Lacto-N-difucohexaose (LNDFH), Lacto-N-fucopentaose I (LNFP-I), Lacto-N-tetraose (LNT), Lacto-N-neotetraose (LNnT), Lacto-N-hexaose (LNH), Lacto-N-neohexaose (LNnH), N-acetylgalactosaminyllactose, **α**1-3,**β**1-4-D-galactotriose (3-Hex), 3'-Sialyllactose (3'SL), 6´-Sialyllactose (6'SL), 3´-Sialyl-N-acetyllactosamine (3'SLN) and 6´-Sialyl-N-acetyllactosamine (6'SLN) were purchased from V-Labs Inc. (Covington, LA), while LNH and LDFT standards were purchased from Prozyme Inc. (Hayward, Ca). All solvents were MS grade, and the water used was nanopure (18.2 ohms).

Oligosaccharide Isolation and Purification

Milk OS were isolated and purified as previously described, with minor modification (Barile et al., 2010). Briefly, frozen milk samples were completely

thawed, and a 0.5-mL aliquot of each sample was mixed with an equal volume of nanopure water and centrifuged at 14,000 × g in a microfuge for 30 min at 4°C to remove lipids. The top fat layer was removed, and 4 volumes of chloroform/methanol (2:1, vol/vol) were added, vigorously mixed and the resulting emulsion was centrifuged at 4,000 × g for 30 min at 4°C. The upper methanol layer containing OS was transferred to a tube, two volumes of cold ethanol were added and the solution was frozen for 1 h at –30°C, followed by centrifugation for 30 min at 4,000 × g and 4°C to precipitate the denatured protein. The supernatant (OS-rich fraction) was collected and freeze-dried using a speed vacuum centrifuge.

For OS characterization by Nano LC Chip QToF-MS (Agilent Technologies, Santa Clara, CA), extracts were purified from the mixture by solid-phase extraction using nonporous graphitized carbon cartridges (GCC-SPE). Prior to use, each GCC-SPE cartridge was activated with 3 column volumes (**cv**) of 80% acetonitrile, 0.1% trifluoroacetic acid (v/v) and equilibrated with 3 CV of nanopure water. The carbohydrate-rich solution was loaded onto the cartridge, and salts and mono/disaccharides were removed by washing with 6 CV of nanopure water. The OS were eluted with a solution of 40% ACN with 0.1% TFA (v/v) in water and dried in speed vacuum centrifuge at 35°C overnight.

Characterization by Nano LC Chip QTOF MS

Prior to MS analysis, dried OS samples were reconstituted in 100 µL of nanopure water. MS analysis was performed with an Agilent 6520 accurate-mass Quadrupole-Time-of-Flight (**Q-TOF**) liquid chromatography/mass spectroscopy (**LC/MS**) with a micro-fluidic nano-electrospray chip (Agilent Technologies, Santa

Clara, CA) as previously described (Wu et al., 2011). The micro-fluidic chip contained one enrichment and one analytical column, both packed with graphitized carbon. Chromatographic elution was performed with a binary gradient of 3% ACN/0.1% formic acid in water (solvent A) and 90% ACN/0.1% formic acid in water (solvent B). The column was initially equilibrated with a flow rate of 0.3 μL/min for the nanopump and 4 μL/min for the capillary pump. The 65-min gradient was programmed as follows: 0–2.5 min, 0% B; 2.5–20 min, 0–16% B; 20–30 min, 16–44% B; 30–35 min, 44–100% B; 35–45 min, 100% B; and 45–65 min, 0% B. Data were acquired in the positive ionization mode with a 450–2500 mass/charge (m/z) range. The electrospray capillary voltage was 1700–1900 V. The acquisition rate was 0.63 spectra/s for both MS and MS/MS modes. Automated precursor selection was employed based on ion abundance, performing up to 6 MS/MS spectra per individual MS when precursor was above ion abundance threshold. The precursor isolation window was selected to be narrow (1.3 m/z) to improve accuracy. Fragmentation energy was set at 1.8 V/100 Da with an offset of –2.4 V. Internal calibration was continuously performed by infusing two reference masses: *m/z* 922.009 *and* 1221.991 (ESI-TOF Tuning Mix G1969–85000, Agilent Technologies).

QTOF Data Analysis

The Molecular Feature Extraction function of Mass Hunter Qualitative Analysis Version B.06.00 (Agilent Technologies) was used to generate a list of deconvoluted masses selected to be in a range of 450–1500 m/z with a ≥1000 height count and a typical isotopic distribution of small biological molecules. Charge states allowed were restricted to single and double species. OS compositions were

determined from the deconvoluted mass list with in-house software, and all OS compositions were confirmed by tandem MS (MS/MS) analysis. Following MS/MS identity validation and assessment of reproducible retention times (**RT**), individual peaks for each OS were automatically integrated using the Targeted Feature Extractor from MassHunter Profinder Version B.06.00 (Agilent Technologies). The RT window allowed for compound matching was restricted to ± 0.5 min and ± 0.25% of the RT at each time point. Each sample was analyzed in triplicate, 2-fucosyllactose (2'-FL) added as internal standard to minimize instrumental variation.

Oligosaccharide quantification by High Performance Anion Exchange Chromatography –Pulsed Amperometric Detection (HPAEC-PAD)

The quantification of 9 neutral oligosaccharide standards (LNDFH, LDFT, LNFP-I, LNT, LNnT, N-acetylgalactosaminyllactose, 3-Hex, LNH, LNnH) and 4 acidic oligosaccharide standards (6'-SLN, 3'-SLN, 6'-SL, 3'-SL) was carried out with a high-performance anion-exchange chromatography with pulsed amperometric detection system (Thermo Scientific HPAE-PAD ICS-5000), equipped with a detector/chromatography module including a pulsed amperometry electrochemical detector, an electrochemical cell with a disposable gold working electrode, a pH-Ag/AgCl reference electrode, an auto-sampler, and a single pump. Samples were diluted and filtered through a 0.22-µm membrane (Pall, Port Washington, NY) before analysis. A 25-µL sample was injected into the CarboPacPA200 analytical column (3 × 250 mm, Dionex, Sunnyvale, CA) and a CarboPacPA200 Guard Column (3 × 50 mm, Dionex) for oligosaccharide analysis, eluting with a 0.5 ml/min and a

non-isocratic gradient: 0-10 min 50% B, 10-50 min 45% B – 10% C. The column was equilibrated for 5 min with 10% B followed by 10 min with 50% B. Solvent A was deionized water, solvent B 200 mM NaOH and solvent C was 100 mM NaAc in 100mM NaOH.

Quantification was assessed by external calibration using a mixture of all oligosaccharide standards ranging from 0.0001 to 0.03 g/L (coefficient of determination > 0.999).

Statistical Analyses

Data was analyzed in JMP 12 and used LSMEANS Differences with Tukey-HSD Adjustment. $P < 0.05$ was considered significant, non-significant factors were dropped and the model was run again. For AVG BiW, sow pre-wt, sow pre-BF, Diet, and TNB were included in the model as fixed effects, sire and litter nested in sire were random effects. When analyzing AVG WW, sow pre-BF, sow post-BF, number nursed, number weaned, average birth weight of litter, and diet were included in the model as fixed effects, sire and litter nested in sire were random effects. For the analysis of Milk: CP, N, DM, GE, and Insulin were analyzed separately as the response variables with Diet as a fixed effect. Serum analysis for GLP-2 and Insulin were analyzed separately as the response variable with diet as a fixed effect. The model for milk and biomarker analysis included diet, diet x day, and day interactions. All means are presented as least-squares means (SEM). For the OS analysis the normal distribution of the data was evaluated using the Kolmogorov-Smirnov test ($P < 0.05$), while homoscedasticity and was checked using Levene´s test. A two-way analysis of variance (ANOVA) was carried out to evaluate the effect

of diet and/or time of lactation on Oligosaccharide abundances and concentrations. In all cases, the Tukey test was also used to assess differences between groups. R package "stats" (version 2.15.3) was used for all the analyses.

RESULTS

Growth Performance

Gilts fed a restricted diet had significantly less ME intake when compared to gilts on the other diets ($P < 0.0001$, Fig. 1). Gilts on the RESTR diet weighed less at day of breeding compared to the other two diets ($P < 0.05$). Restricted sows had the highest amount of total BF at weaning (1.59 mm) numerically, but there was no statistical difference as shown in Figure 2. Diet of sow had no effect on piglet BiW (P = 0.39) or piglet WW (P = 0.84).

Milk Composition

Dry matter of milk was not affected by diet, but had a significant day effect in which dry matter decreased over time (P = 0.003). There were no significant effects on the average GE of milk. Percent nitrogen and percent crude protein had a significant day effect in which % N (Fig. 3a) and % CP (Fig. 3b) decreased over time ($P < 0.0001$). Lastly, when milk insulin was analyzed there was a Diet x Day effect (P = 0.035) where the milk from RESTR sows had the highest insulin at d 0, but the lowest insulin concentration at d 14.

Oligosaccharide Profile

Across the two diets (RESTR and CTL) 63 OS were identified. Of the OS identified, 58.73% were neutral, 15.873% were fucosyl and 25.397% were acidic (Table 3). At d 0, CTL had more neutral OS and less acidic OS ($P < 0.05$) when

compared to RESTR (Fig. 4). Of the neutral OS quantified, RESTR had more LNnT than CTL ($P < 0.05$). Also, both RESTR and CTL had a increase in fucosyl OS and decrease in acidic OS from d 0 to d 14 ($P<0.05$; Fig 5). Of the fucosyl OS quantified, samples from CTL had more LNDFH-I than RESTR ($P<0.05$) at d 0. Lastly, only the RESTR showed an increase in neutral OS over time (Fig. 5). Total OS quantification was lower in the RESTR when compared to CTL ($P < 0.05$; Fig. 6). Quantification of OS also decreased in both dietary treatments over time ($P < 0.0001$; Fig. 7).

Growth Biomarkers

For GLP-2, main effects of day ($P < 0.0001$), day × diet (P = 0.0087; Fig 8b), and diet (P = 0.0008) was observed. Across all treatments, concentrations of GLP-2 decreased *(P*=0.0087) with time. Across all time points, CTL+ had the greatest concentrations at each time point and RESTR had the lowest concentrations at each time point when compared to the other treatments. Insulin concentration saw a diet x day effect (P = 0.0149; Fig. 8a). Of all insulin concentration RESTR had the highest (0.044 mlU/L) and CTL had the lowest (0.019 miU/L; P = 0.032).

DISCUSSION

Growth Performance

Today there is a high drive for increased longevity of a sow, while maintaining or improving offspring health and growth performance. In a study conducted by Miller et al. (2010) it was observed that gilts on an energy-restricted diet have greater longevity and may result in offspring with a greater WW (Barnett et al., 2017). The idea of restricting energy during gilt development is based on the premise that restricting the ME should result in decreased fat deposition, but muscle

accumulation should not be affected (Miller et al., 2010) Although, results from the batch in the current experiment do not show significant differences in piglet WW, there is a numerical difference and with more statistical power a greater weight difference may be seen. Furthermore, it was concluded that restricted energy gilts tend to eat more once put on a control diet for gestation, thus their energy intake is greater than the gilts that were always on the control diet. Also, restricted sows lost less BF than control sows. In a study by Amdi et al. (2013) it was shown that gestation feeding level affects the number of offspring born alive per litter and offspring birth weight, where as sow BCS affects weaning weight and growth of offspring. A sow that loses excessive weight during lactation has higher cortisol levels. Cortisol is known to be a stress hormone that has the capability of crossing the placental barrier and excess exposure to it can cause a fetus to have reduced birth weight (Sekl, 2004; Kranedonk et al., 2006).

Milk Composition

This study showed no difference in milk nutrient composition based on diet. However due to sows being fed the different dietary treatments before gestation/lactation this was expected. To our knowledge no previous studies have analyzed milk samples based on diets fed during the developmental stage of gilts. However, many studies show that diet does affect maternal milk when fed during late gestation and lactation. In a study conducted by Amdi et al. (2013), it was concluded that fatty acid and fat composition change based on dietary interventions during the gestation and lactation period. While the sows in this study were not restricted during gestation/lactation they did weigh significantly less than CTL sows

at this time. According to Amdi et al. (2013), restricted sows pull from fat reserves and use these components to add nutrients to the secreted milk. Sows are able to produce adequate quality and quantity of milk in spite of the nutrient deficit that may be associated with an inadequate diet. However, in order to compensate they must mobilize more tissues to meet lactation requirements resulting in lower litter weight (O'Grady et al., 1973). Additionally, energy from the diet is the primary contributor of fat for milk composition (Amdi et al., 2013). After the developmental phase, when sows are switched from their respective treatment diets to a common diet, RESTR sows have greater feed intake; thus, a greater intake of energy. A diet which targets limiting amino acids (AA) showed an increase in mammary milk protein through increased AA absorption to the mammary gland, as well as having increased nitrogen retention and utilization in the milk protein during peak lactation periods (Huber et al., 2015). The current experiment was not in agreement with the study by Huber et al. (2015). The CLT+ diet which has added CAA showed no differences in milk N when compared to the other dietary treatments; however, a main cause of these varying results are most likely due to the time period difference of when the treatment diets were fed.

Insulin regulates energy metabolism and milk production. Furthermore, the energy source in feed will have an effect on insulin secretion. In this experiment we saw Insulin concentration in sows milk had a day by diet interaction ($P < 0.0349$). Interestingly, milk from sows developed on the RESTR diet had the highest insulin concentration in early lactation (d 1) but the lowest concentration during mid to late lactation (d 14). Sows that have greater weight loss during lactation tend to

have lower insulin levels (Spinka et al., 1999). Due to the fact that CTL sows lost significantly more BF than RESTR sows, this could explain the higher d 0 insulin concentrations in RESTR sow milk. Additionally, the more a neonate suckles, the lower the insulin levels become according to Spinka et al. (1999). Due to the fact that restricted sows may wean heavier piglets, lower insulin levels could correlate with RESTR piglets suckling more.

Milk Oligosaccharides

Milk oligosaccharide profiles have shown to vary depending on diet (Difilippo et al., (2016). Oligosaccharides are usually classified into 3 groups: Neutral, Acidic or Fucosyl in which each group has specific beneficial factors. Previous studies by Mudd et al. (2016) resulted in the characterization of 60 OS species; however, in the current study, 63 types of OS were quantified. Today there are over 100 human milk OS quantified (Ninonuevo et al., 2008). Oligosaccharide (OS) composition analyzed for this study showed a slight difference based on diet (CLT or RESTR). Total OS abundance decreased over time, which is also seen in human milk OS. Restricted gilts produced milk with significantly less neutral OS and significantly more acidic OS on d 0. Of the three subgroups of neutral OS identified, 2 Hex-1 HexNAc, Lacto-N-neotetraose, and Lacto-neotetraose – colostrum of RESTR gilts had greater amounts of Lacto-N-neotetraose (LNnt). LNnt is shown to be a prebiotic that stimulates growth of bifidobacterium (LoCasio et al., 2007). According to Tao et al. (2010), LNnt is one of the few OS that increases in mammals throughout lactation; however, our results showed the opposite effect. In general total neutral OS had a slight increase in abundance through lactation, unlike Acidic

and Fucosyl, agreeing with the results by Mudd et al. (2016). Additionally, there were no over significant differences in Fucosyl OS, but LNDFH-I (a type of Fucosyl OS) was significantly lower in RESTR sows on d 0. Currently there are not standards for all Fucosyl OS that have been quantified. Fucosyl OS normally remain the same or lower over the period of lactation, however this was not seen in our results. While Fucosyl OS can deflect pathogens through its prebiotic effects, it has been shown to cross feed pathogens such as *Bifidobacterium adolescentis and Firmicute bacteria* through the end products of Fucosylated-OS fermentation and from products during the partial breakdown of substrates (Belenguer et al., 2006). *Bacterias such as these* reduce the populations of non-utilizing bacteria through competition and promote gut health (Belenguer et al., 2006). Simple fucosyl OS are beginning to make their way into infant formula and are said to make formula more closely related to human breast milk and The microbial profile of infant fecal samples more similar to fecal samples obtained from infants that consumed breast milk (Steenhout et al., 2016). However, more complex fucosyl OS are said to have more beneficial effects. Fucosylated-oligosaccharides can inhibit diarrhea caused by *E. Coli, Campylobacter, Jejune* Calicivirus, and higher levels correlate with better protection (Newburg et al., 2004). Fucosylated-OS are found at very low levels in porcine milk (Tao et al., 2009; Salcedo et al., 2016) as were they in this experiment. Tao et al. (2010) found fucosylated-OS make up 1 to 4% of OS in sow milk; however, in humans, concentrations of fucosylated-OS can reach levels as high as 70%. In the current experiment Fucosyl OS were ranged between 0.76% and 2.8%; thus, even lower than previous studies reported.

Lastly, There was a significant difference in overall acidic OS abundance among diet on d 0, but no particular acidic OS was significantly higher among diets. A type of an acidic OS is Sialylated oligosaccharides (SOS), which plays an important role in neural development and neural protection (Tao et al., 2008). Through competing for the adhesion sites on epithelial surfaces, SOS are able to inhibit certain pathogens and possibly even help with post-weaning diarrhea. High levels of SOS in sow's milk protect the neonate from health challenges such as Rotavirus (Difilippo et al, 2016). 3' Sialyllactose is an abundant SOS that down regulates sialic acid, fucose, and galactose and inhibits pathogen adhesion to the epithelial cell wall (Difilippo et al., 2016). 3'SL was the most prominent Acidic OS in the milk analyzed, but was considerably less abundant on d 14 compared to d 0.

Growth Biomarkers

Both insulin and GLP-2 concentration were measured in piglets for insight on growth biomarkers. While both play a role in protein synthesis, insulin plays a key role in the development of fast-twitch muscles and GLP-2 stimulates intestinal growth (Drucker 1998, Davis et al., 2001). In this experiment, a main effect of diet and a day by diet interaction was observed in blood samples obtained from progeny for both insulin and GLP-2. Progeny from RESTR sows had the lowest GLP-2 concentrations at both time-points, but was only significantly different from CTL+ at d 0. Interestingly, RESTR was the only treatment that didn't have a great decrease in GLP-2 concentration from d 1 to d 15 Increased GLP-2 can help reduce weaning diarrhea and stimulate intestinal adaptation to new diets (Thyman et al., 2014). GLP-2 is stimulated by enteral intake of nutrients and piglets total parenteral

nutrition (TPN) fed will have significant decreases in GLP-2 circulation (Petersen et al., 2001).

GLP-2 has growth related effects on a neonate through epithelial cell proliferation resulting in increased intestine mucosal mass, colon mass, villus height and crypt death. However, low levels of GLP-2 do not correlate with increased weight. Our results did agree with Petersen et al. (2010) where there was a decrease in plasma GLP-2 through the postnatal period. Furthermore, Petersen et al. (2010) reported no difference in body weight based on a control group of neonate pigs compared to pigs infused with GLP-2 same as our results, however, Petersen et al, (2010) studied the piglet's intestines were there was a difference in small intestine and colon weight.

Insulin concentrations were significantly higher in progeny from RESTR sows when compared to CTL on d 0. The circulating insulin concentration coincided with milk insulin of RESTR because, as previously stated, RESTR numerically had the highest milk insulin concentration at d 0. The growth rate of a mammal is greatest at its neonate stage (Young, 1970) and the insulin receptor protein is two-fold higher in a newborn piglet than that of a weanling (Suryawan et al., 2001). The results of this current experiment agreed and disagreed with those of Suryawan et al. (2001). While the insulin concentration decreased with age in RESTR progeny, there was an increase in insulin concentration for both CTL+ and CTL. The results of varying insulin concentration may relate back to nursing frequency and maternal stress. Insulin and the efficiency of it in its signaling pathways are essential determinants of efficient growth during development periods and will decrease

with age as seen in RESTR piglets (Davis et al., 2010). Furthermore, insulin regulates stimulation of protein synthesis in peripheral tissues, as well as whole body AA disposal (Davis et al., 2001). Increased insulin may play a role in the increased BiW and WW of piglets from RESTR sows.

In Conclusion GLP-2 had a treatment effect in which progeny from RESTR sows had the lowest concentrations and progeny from CTL + sows had concentrations that were highest at both time points when compared across all treatments. Interestingly, insulin concentrations were increased in progeny from RESTR sows and much lower in progeny from CTL sows. Furthermore, progeny of RESTR sows saw a decrease in insulin over time where as progeny from CTL and CTL+ sows showed an increase. Similar to Petersen et al. (2010) our results did not show progeny body weight differences; however, future research looking at the structure of tissues would need to be conducted to see if the differences in GLP-2 concentrations affected the small intestine and colon. Lastly, recording nursing frequency could help determine why insulin concentrations varied among treatments. These growth factors are likely to play a key role in growth performance not only in the nursery, but through out the lifetime of the pig.

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Table 1. Diet Composition of experimental diets (As-Fed Basis, Kg) Composition and nutrient contents of experimental diets fed to developing gilts d 123 – 240*. Phase 1 and 2 were each 6 weeks long and phase 3 was 4 weeks long.

	Phase 1			Phase 2			Phase 3		
Item	**CTL[1]**	**RESTR[2]**	**CTL+[3]**	**CTL**	**RESTR**	**CTL+**	**CTL**	**RESTR**	**CTL+**
Ingredient, %:									
Corn	72.52	39.95	70.38	76.32	43.73	74.66	80.13	47.5	78.6
Soybean Meal	21.53	17.43	23.35	17.66	13.57	19	13.79	9.71	15
Soybean Hulls	-	40	-		40	-		40	-
Beef Tallow	3		3	3		3	3		3
Dicalcium phosphate	1.37	1.72	1.37	1.46	1.8	1.46	1.54	1.89	1.54
Limestone	0.68		.68	0.66		0.66	0.64		0.64
Sodium Chloride	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin Premix[4, 5]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix[6]	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
L-Lysine	-	-	.15	-	-	0.15	-	-	78.6
Methionine	-	-	.05	-	-	0.05	-	-	15
Threonine	-	-	.09	-	-	0.09	-	-	-
Tryptophan	-	-	.03	-	-	0.03	-	-	3
Nutrient Content:									
ME (kcal/kg)	3406	2706	3408	-	3408	2707	3410	-	3410
Lys, g/kg	0.7	0.69	0.86	-	0.61	0.59	0.76	-	0.51
Crude Protein %	16.25	15.72	17.21	-	14.72	14.19	15.48	-	13.18
P %	0.6	0.6	0.61	-	0.6	0.6	0.6	-	0.6
Ca %	0.67	0.71	0.67	-	0.67	0.72	0.68	-	0.67
Lys/ME* (g/Mcal)	2.059	2.536	2.057	1.78	2.185	1.785	1.5	1.835	1.504

* Data from RESTR 1 and RESTR 2 were analyzed together for growth performance and here on out considered RESTR
[1] Control diet (CTL) was formulated to meet 2010 NRC requirements for developing gilts.
[2]Energy restricted diet (RESTR) was 20% restricted in energy with increased fiber.
[3]Control Plus (CTL+) contained an addition of crystalline amino acids equivalent to the SID Lys:ME of the RESTR diet
[4] Provided per kilogram of diet for phase 1 and 2: 5,500 IU of Vitamin A, 550 IU of Vitamin D_3, 30 IU of Vitamin E, 4.40 IU of Vitamin K, 33.00 mg of Niacin, 22.05 mg of Panothenic Acid, 11.00 mg of Riboflavin, and 33.00 μg of Vitamin B_{12}
[5]Provided per kilogram of diet for phase 3: 6,600 IU of Vitamin A, 600 IU of Vitamin D_3, 66 IU of Vitamin E, 4.40 IU of Vitamin K, 33.00 mg of Niacin, 22.05 mg of Panothenic Acid, 11.00 mg of Riboflavin, and 22.05 μg of Vitamin B_{12}, 550 mg of Choline Chloride, 1.65 mg of Folic Acid, 0.22 mg of Biotin
[6] Provided per kilogram of diet: 10.50 mg of Copper Sulfate Pentahydrate, 0.26 mg of Calcium Iodate, 127.50 mg of Ferrous Sulfate, 30.00 mg of Manganese Oxide, 0.30 mg of Sodium Selenite, 127.50 mg of Zinc Sulfate, 226.03 mg of Calcium Carbonate.

Table 2. Composition and nutrient contents of gestation and lactation diet.

	Parity 1 and Parity 2	
Item:	Gestation[1]	Lactation[2]
Ingredient, %		
Corn	77.25	65.68
Soybean Meal	16	27.5
Tallow	3	3
Dicalcium Phosphate	1.9	2.33
Limestone	0.925	0.6
Salt	0.5	0.5
Vitamin Premix[1]	0.25	0.25
Trace Mineral	0.15	0.15
Phytase	0.02	-
Nutrient Content:		
ME (kcal/kg)	2605	2536
Crude Protein	11.74	15.75
Lys	0.56	0.85
Total P %	0.67	0.8
Ca %	0.87	0.9

[1]Gestation diet was fed d 0 of breeding to d 0 post farrowing

[2]Lactation diet was fed d 0 post farrowing until d 21 post farrowing, sows were put immediately back on gestation diet at d 21 post farrowing

[3]Provided per kilogram of diet for phase 3: 6,600 IU of Vitamin A, 600 IU of Vitamin D_3, 66 IU of Vitamin E, 4.40 IU of Vitamin K, 33.00 mg of Niacin, 22.05 mg of Panothenic Acid, 11.00 mg of Riboflavin, and 22.05 μg of Vitamin B_{12}, 550 mg of Choline Chloride, 1.65 mg of Folic Acid, 0.22 mg of Biotin

Table 3. Oligosaccharide Composition organized by mass. The composition of the OS is shown as a set of 5 monomers. The following order is as stated with their abbreviations hex: glucose or galactose; HexNAc: N-acetylhexososamine; Fuc: Fucosamine; Neu5Ac: N-acetylneuramic acid; and Neu5Gc: N-glycolylneuramic acid (i.e 3_1_0_0_0; or 3 Hex, 1HexNAc; Mudd et al., 2016. RT stands for retention time in which it is the time when the injection is made and elution occurs

Compound Name	Mass	RT	Compound Name	Mass	RT	Compound Name	Mass	RT
2_0_1_0_0	488.1708	12.65	1_1_0_1_0	674.2378	23.48	3_2_0_0_0	910.3267	22.66
2_0_1_0_0	488.1646	14.84	3_1_0_0_0	707.2477	15.82	4_0_0_1_0	957.3281	26.59
2_0_1_0_0	488.1723	16.35	3_1_0_0_0	707.2492	17.86	6_0_0_0_0	990.3279	16.59
3_0_0_0_0	504.1685	12.74	3_1_0_0_0	707.2478	20.44	3_1_0_1_0	998.3431	25.58
3_0_0_0_0	504.1683	13.38	3_1_0_0_0	707.2477	22.63	3_1_0_1_0	998.3446	29.05
3_0_0_0_0	504.1684	13.97	3_1_0_0_0	707.2481	28.36	4_1_1_0_0	1015.358	15.87
3_0_0_0_0	504.1685	15.02	2_2_0_0_0	748.2741	17.54	4_1_1_0_0	1015.359	26.8
3_0_0_0_0	504.1683	16.22	2_2_0_0_0	748.2744	15.96	4_1_1_0_0	1015.355	17.33
2_1_0_0_0	545.1948	13.44	2_2_0_0_0	748.2747	19.45	4_2_0_0_0	1072.381	22.64
2_1_0_0_0	545.1949	15.77	3_0_0_1_0	795.2644	26.07	4_2_0_0_0	1072.378	25.03
2_1_0_0_0	545.1948	19.14	3_0_0_1_0	795.2648	27.28	4_2_0_0_0	1072.379	30.62
1_2_0_0_0	586.2208	13.42	3_0_0_1_0	795.2643	24.91	3_3_0_0_0	1113.406	20.77
1_2_0_0_0	586.2211	15.08	5_0_0_0_0	828.2748	15.4	3_3_0_0_0	1113.404	23.44
2_0_0_1_0	633.2116	19.03	2_1_2_0_0	837.3014	24.38	4_1_0_1_0	1160.397	28.27
2_0_0_1_0	633.2116	23.92	4_1_0_0_0	869.3016	20.43	3_2_0_1_0	1201.42	27.13
4_0_0_0_0	666.2209	15.69	4_1_0_0_0	869.3015	21.6	4_2_1_0_0	1218.437	18.95
4_0_0_0_0	666.2229	20.55	4_1_0_0_0	869.3007	28.28	4_2_1_0_0	1218.436	20.56
4_0_0_0_0	666.2217	21.47	2_2_1_0_0	894.3348	12.08	4_3_0_0_0	1275.456	22.28
4_0_0_0_0	666.2215	13.55	3_2_0_0_0	910.3268	18.43	4_2_0_1_0	1363.481	27.09
1_1_0_1_0	674.2345	17.2	3_2_0_0_0	910.3271	19.37	4_2_0_1_0	1363.478	30.63
1_1_0_1_0	674.2374	18.96	3_2_0_0_0	910.3271	20.19	4_3_0_1_0	1566.55	26.22

Figure 1. Metabolizable Energy intake during gilt developmental period organized by phase. Means in the same phase with different letters differ ($P < 0.05$)

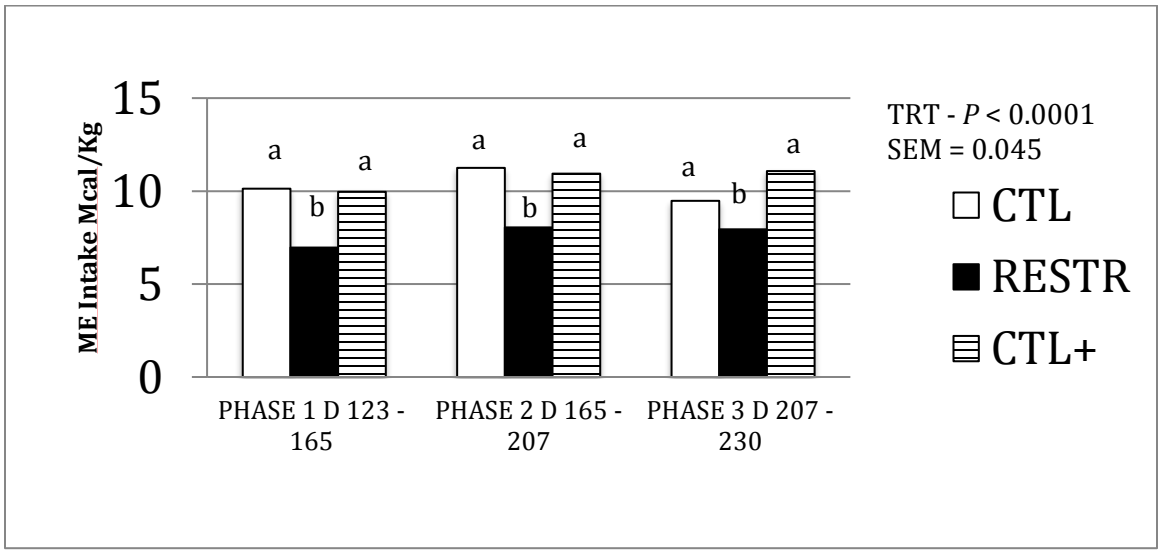


Figure 2. Effects of feeding gilts a RESTR, CTL, or CTL+ diet on Backfat depth at d 109 of gestation (Pre-BF) and d 21 post farrowing (Post-BF) using Aloka 500V real-time ultrasound instrument. Bars at same time point with different letters differ based on diet ($P < 0.05$)

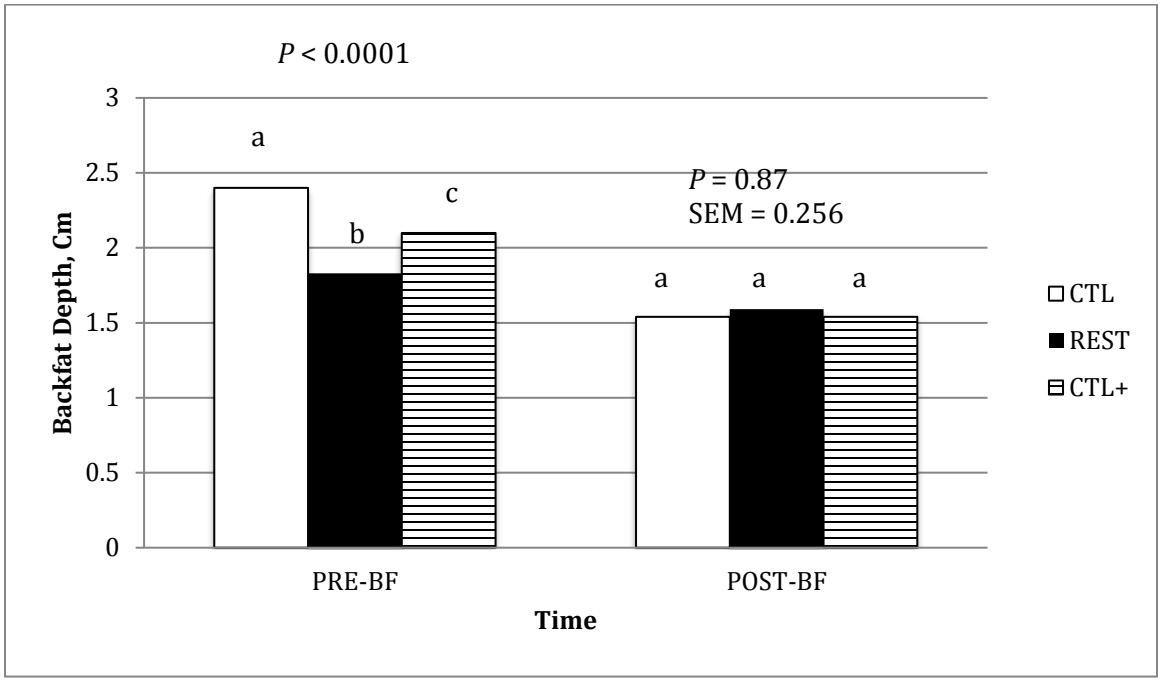


Figure 3. Effects of feeding gilts a RESTR, CTL, or CTL+ diet on milk composition. Each bar represents the LSM for %N of 7 sows/diet on d 0 and d 14. Bars of the same diet group with * differ based on day. * represents a significant difference of $P < 0.05$

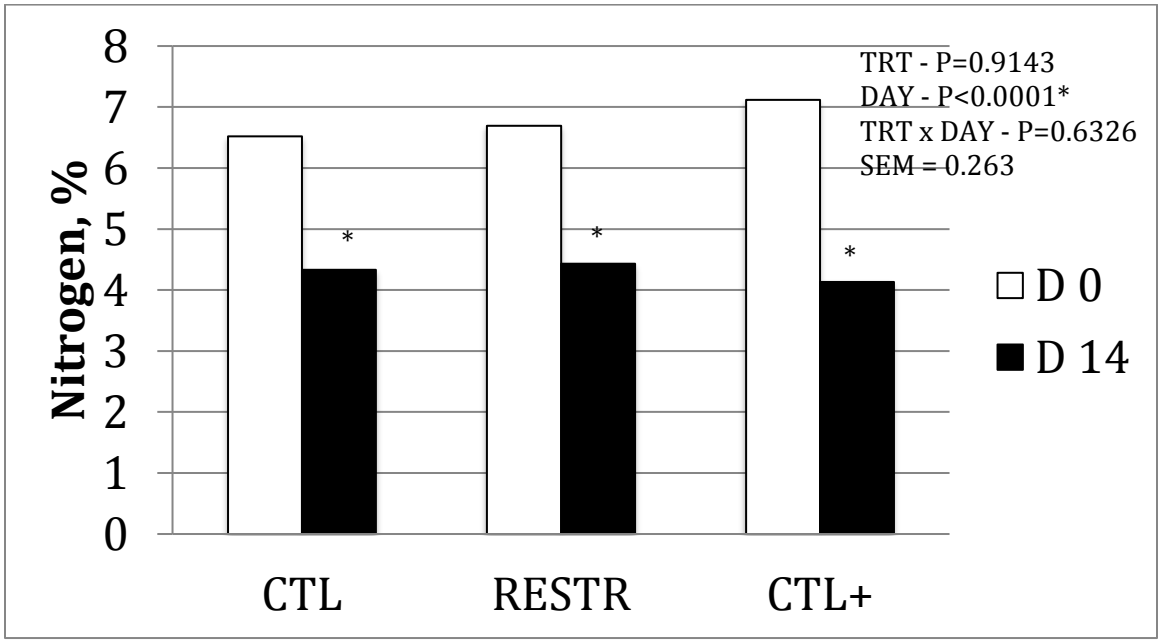
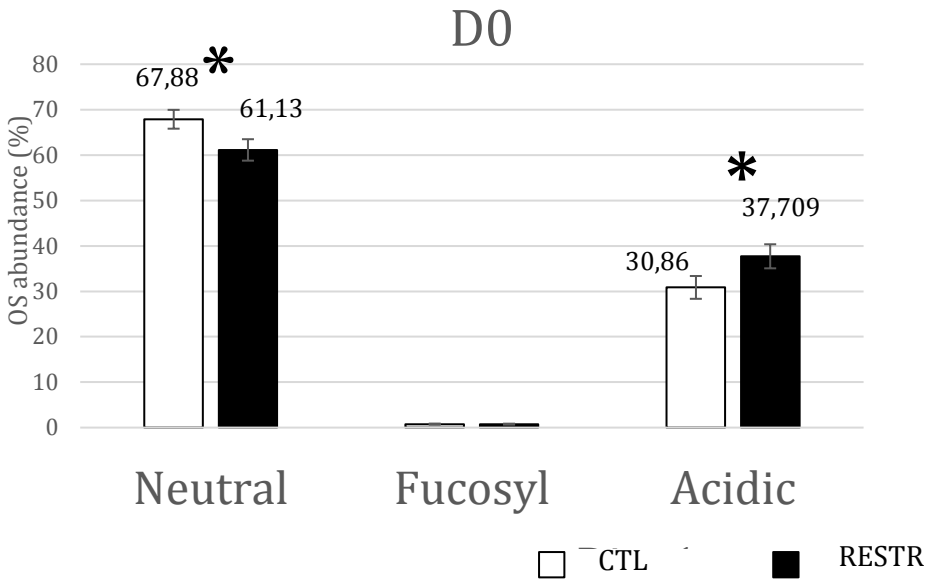


Figure 4. Effects of feeding gilts a RESTR or CTL diet on Oligosaccharide Profile. Each bar represents the LSM for OS abundance of 7 sows/diet on d 0 (a) and d 14 (b). Bars of the same OS group with * differ based on abundance. * represents a significant difference of $P < 0.05$



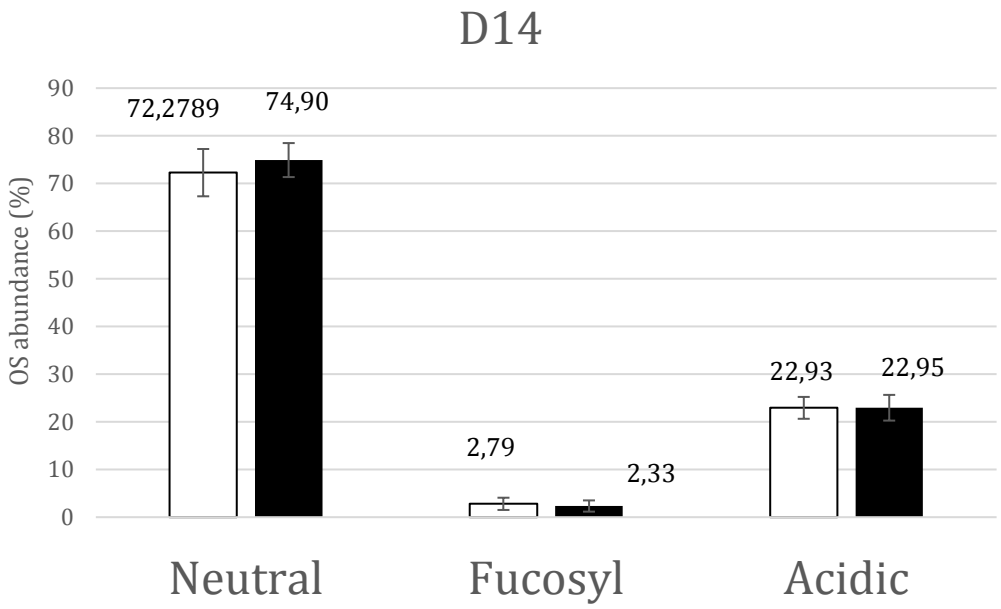
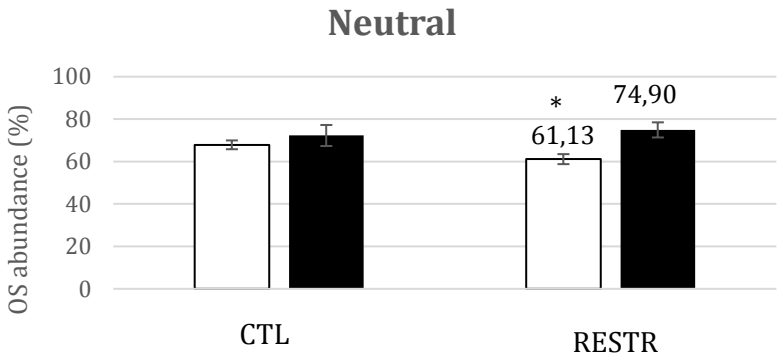


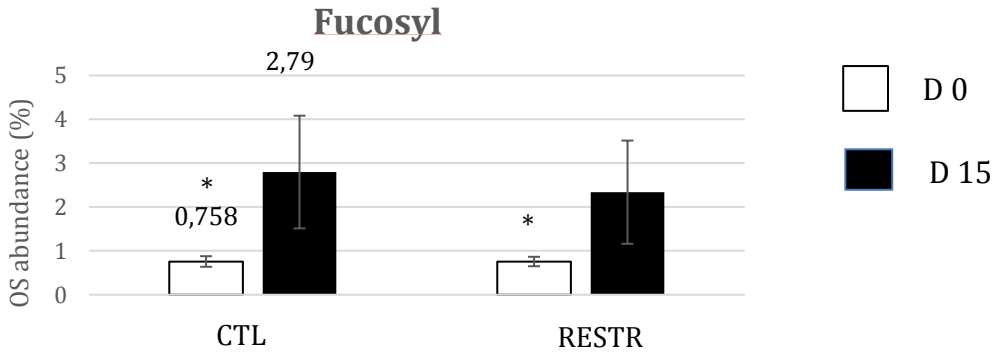
Figure 5. Effects of feeding gilts a RESTR or CTL diet on neutral (a) Fucosyl (b) or Acidic (c) OS based on time. Each bar represents the LSM for OS abundance of 7 sows/diet on d 0 and d 14. Bars of the same diet group with * differ based on abundance. * represents a significant difference of $P < 0.05$

A.



B.

C.



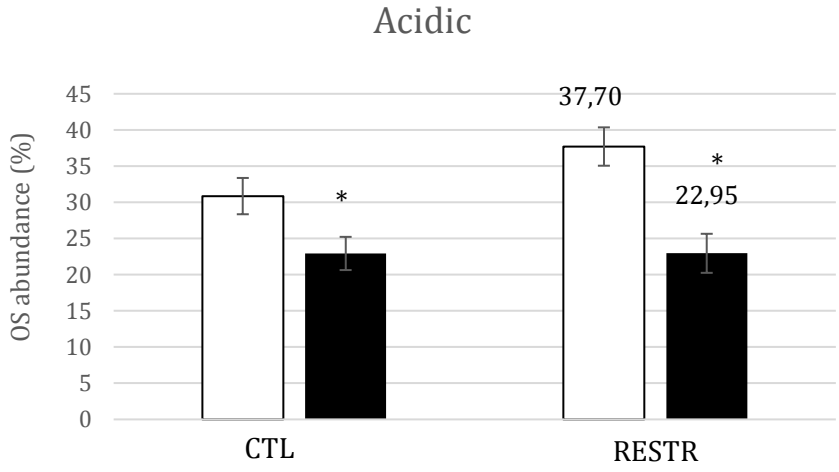


Figure 6. Effects of feeding gilts a RESTR or CTL diet on Oligosaccharide abundance. Each bar represents the LSM for OS abundance of 7 sows/diet on d 0 (a) and d 14 (b). Bars of the same diet group with * differ based on abundance. * represents a significant difference of $P < 0.05$. Oligosaccharide abundance decreased significantly over time ($P < 0.0001$)

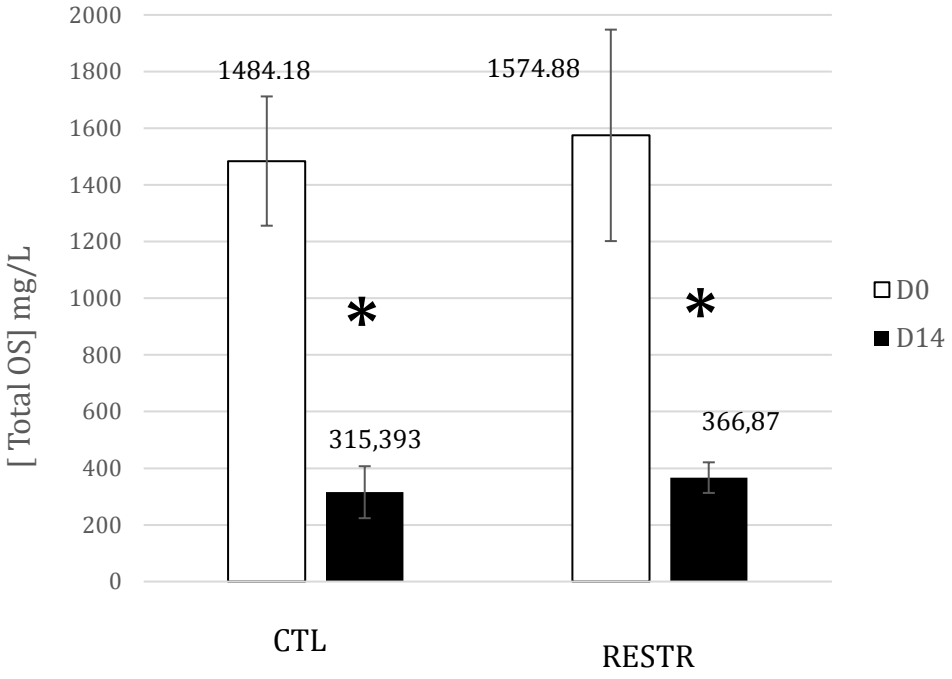


Figure 7. Effects of feeding gilts a RESTR or CTL diet on Oligosaccharide abundance. Each bar represents the LSM for OS abundance of 7 sows/diet on d 0 (a) and d 14 (b). Bars of the same time group with * differ based on abundance. * represents a significant difference of $P < 0.05$. Oligosaccharide abundance decreased significantly over time ($P < 0.0001$)

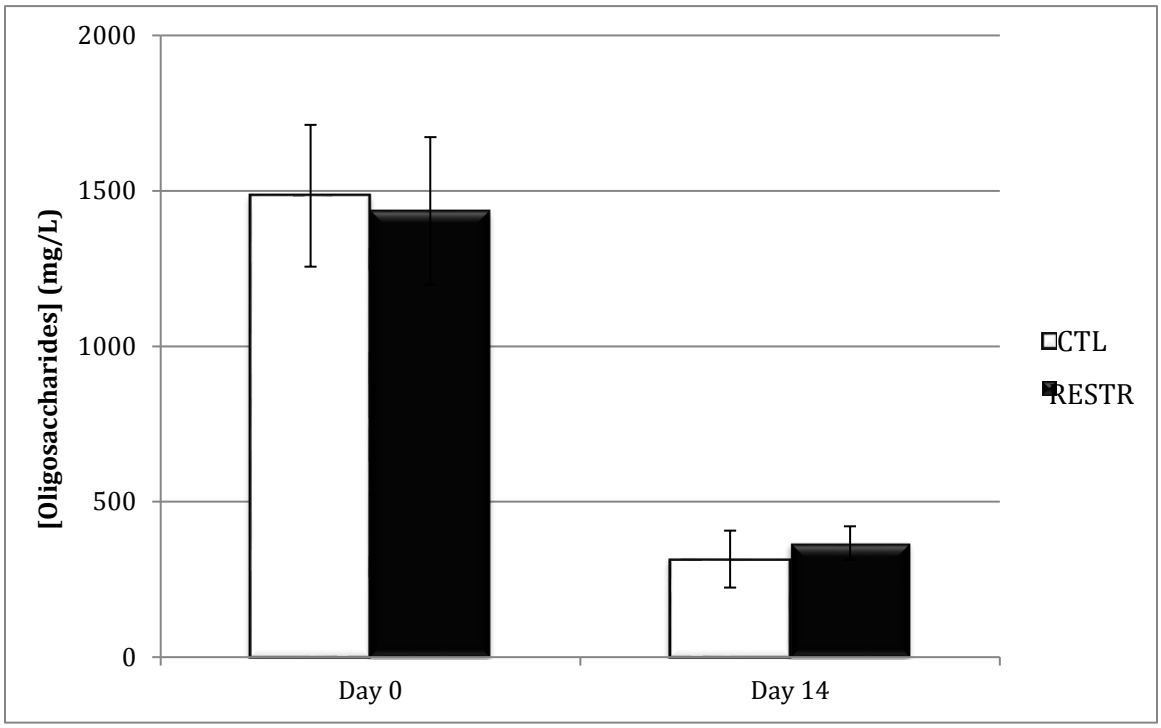
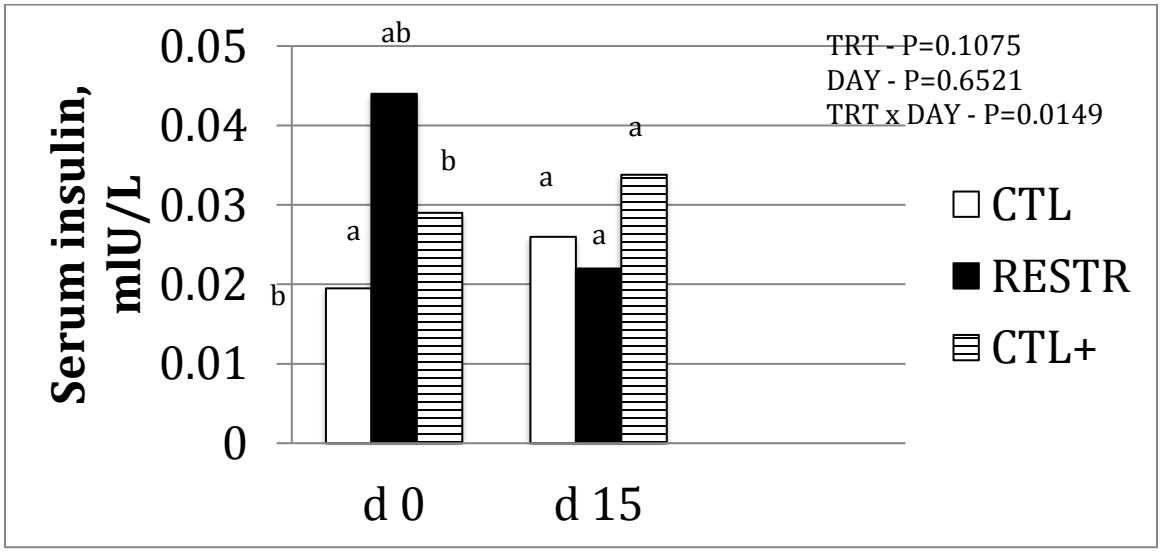


Figure 8. Effects of feeding gilts a RESTR, CTL, or CTL+ diet on growth biomarkers, insulin (a) and GLP-2 (B), in piglets on d 1 and d 15. Bars at same time point with different letters differ based on diet of the mother ($P < 0.05$)

A



B