

**QUANTITATIVE EVALUATION OF HUNGER IN PREGNANT SOWS:  
PHYSIOLOGICAL, BEHAVIORAL AND PERFORMANCE RESPONSES TO  
FEEDING TIME AND FREQUENCY UNDER LIMIT-FED REGIME**

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## **Dedication**

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## **Chapter 1. Literature review**

### **Introduction**

Excess caloric intake over requirement in gestation sows led to reduction in lactation feed intake (Kasanen et al., 2010; Tummaruk, 2013), extensive fat accumulation leading to health and welfare issues (Baker et al., 1969; Tolkamp and Eath, 2016), breeding difficulty after weaning (Frobish et al., 1973), and early embryonic losses (Bas Kemp et al., 2011). Dyck et al. (1980) demonstrated that embryonic survival decreased from 82.8% to 71.9% when feed intake increased above the daily requirement from 1.5 to 3.0 kg daily. Therefore, developing appropriate feeding strategy during gestation is of paramount importance. Under normal intensive swine production systems, a sow's feed is restricted to 40 to 60 % of their ad-libitum feed intake during gestation but it is fed to satiation during lactation (Meunier-Salaün et al., 2001; Peltoniemi et al., 2016). Such a feeding strategy demands a minimal restitution of weight in the next pregnancy, which is advantageous, because the greater the feed intake and weight gain during pregnancy, the greater the weight loss in lactation (Tummaruk, 2013). However, this feeding practice may allow gestation sows to experience hunger during most of the day, as evidenced by increased activity (foraging behavior), increased motivation for food and eventually negatively affecting a sows' welfare (Meunier-Salaün et al., 2001; D'Eath et al., 2009). These signs of hunger attracted the interest of legislators (EC Council, 2001; Parliament U.K, 2006), Farm Animal Welfare Council (FAWC, 1998), and animal welfare pressure groups (Defra, 2003).

Attempts to improve animal welfare by reducing hunger in gestation sows are geared toward dietary manipulations such as qualitative diet restriction and rationed

alternative diet. In qualitative diet restriction, low quality diet (feed dilution with fiber) is offered to animals *ad-libitum* (Sandilands et al., 2006) whereas in rationed alternative diet a low quality diet is offered restrictively (Danielsen and Vestergaard, 2001; de Jong et al., 2005). The former feeding strategy is an effective means to increase the time taken to consume a ration (D'Eath et al., 2009). Also, dilution with fiber may reduce hunger of restricted-fed pregnant sows by minimizing energy derived from starch in favor of greater amounts of energy derived from short-chain fatty acid (SCFA) through microbial fermentation of non-starch polysaccharides (Serena et al., 2009). However, the benefits of alternative diets to domesticated mammals are controversial. Proponents of the practices argue that the practice may lead to normal feeding behavior, enhance satiety and improve animal welfare; but their results are conflicting (Day et al., 1996; Meunier-Salaün et al., 2001; Mench, 2002; de Jong et al., 2005). Opponents argue that there is no evidence of welfare benefits because indicators of hunger such as total oral behaviors and activity levels are not different between alternative diets and quantitative restriction. If energy needs and nutrient requirements are not met, 'metabolic hunger' is unavoidable irrespective of the level of restriction of energy intake and weight gain that are achieved through fiber dilution (Lawrence et al., 1989; Owen, 1992; Savory et al. 1996; Dailey and McGlone, 1997; Savory and Lariviere, 2000; Mcglone and Fullwood, 2001; Jensen et al., 2012).

Feeding automation is a practice that helps feed management. It allows feeding with high frequency, thus smaller portions of feed more times during the daytime, besides allowing night time feeding and efficiently controlling the quantity of ration provided (de Castro et al., 2014). The utilization of high feeding frequencies provides good growth and feed conversion ratio in bullfrog production (Oliveira et al., 2009; Castro et al., 2012), and efficiency in fish production (Sousa et al., 2012). Increased meal

frequency is touted as a dietary strategy to enhance satiety, reduce hunger, improve metabolic health and increase energy expenditure in humans (Speechly and Speechly, 1999; Kulovitz et al., 2014). Feeding frequency may improve the welfare of gestating sows (Robert et al., 2002), enhance nutrient utilization (Holt et al., 2006), and improve sow reproductive performance (Wittman, 1986; Holt et al., 2006). Conversely, other researchers did not elicit a significant response of feeding frequency on sow performance traits (Douglas et al., 1998; Holt et al., 2006; Jensen et al., 2012).

An energy restricted diet for pregnant sows is generally provided once daily. Provision of feed within a narrow time window each day leads to significant changes in physiology and behavior (Johnston, 2014). Nutritional outcomes such as (total growth, fattening, risk of obesity, and blood insulin concentration) differed by the timing of food intake in many mammalian models under iso-caloric conditions (Noeske-Hallin et al., 1985; Arble et al., 2009; Nikkhah, 2012; Wang et al., 2014). Group housed pigs with an electronic feed station queue for their ration and feed at different times of the day. Although electronic sow feeders prevent excessive competition amongst sows, this may induce stress in sows, as they prefer to synchronize their feeding rather than queuing for food (Peltoniemi et al., 2016). Knowing the most appropriate feeding period and feeding frequency is important to determine the best strategy of feed management in order to more appropriately use ration to reduce stress and achieve the best production efficiency.

Restrictively fed gestation sows can experience stress that can impact their behavior (Heo et al., 2005). Evidence of a stress responses manifest as significant physiological and behavioral changes (Nayanatara, 2013). Increased and sustained stress are associated with compromised welfare. The hypothalamic–pituitary–adrenal (HPA)

axis is one of the physiological systems that almost always is activated by stress (Ralph and Tilbrook, 2016). Food availability stimulated the rhythmicity of the HPA axis such that severe food restriction or starvation increases the HPA axis activity in humans and rats, with an increase in glucocorticoids levels (Garcia-Belenguier et al., 1993; Kenny et al., 2014). Also, severe feed restriction in gilts during gestation elicited higher salivary cortisol concentrations than the control group fed higher feed levels (Amdi et al., 2013).

It was suggested that increasing meal frequency may directly affect hormones, gastric stretch, and gastric emptying, which can impact hunger and satiety (Speechly and Buffenstein, 1999). Two hormones extensively studied and known to have opposing effects on appetite are ghrelin and peptide YY (PYY) (Odunsi et al., 2009; Schwarz et al., 2011). Ghrelin is a hormone secreted primarily in the gastrointestinal tract (GIT), but it's mostly secreted from the stomach to trigger the hypothalamus to sense feelings of hunger and initiate feeding (Odunsi et al., 2009). Conversely, PYY is released after feeding, from the endocrine cells lining the mucosa of the distal ileum and colon, to suppress appetite. Postprandial suppression of ghrelin is a determinant of insulin secretion since ghrelin and insulin concentrations are inversely related (Flanagan et al., 2003). In turn, glucose is a major regulator of insulin secretion (Aronoff et al., 2004). Therefore, glucose and insulin have suppressive effects on ghrelin and are therefore important for ghrelin regulation (Flanagan et al., 2003; Vatansever-Ozen, 2011).

The objectives of this dissertation were first to determine the effects of feeding time and frequency on sow and reproductive performance under iso-caloric intake. Secondly, use feeding time and frequency to indirectly quantify stress and sow

behavioral activities under iso-caloric intake. Additionally, determine the effects of feeding time and frequency on plasma insulin, ghrelin and peptide YY and glucose patterns, and indirectly quantify hunger in pregnant sows. Finally, to use the ghrelin/PYY ratio as an index of hormonal exposure to predict at what times hunger in pregnant sows or desire to feed is greatest.

### **Physiological mechanisms controlling food intake**

From the 'Dual Centre Hypothesis' the hypothalamus has long been recognized to regulate appetite (Murphy and Bloom, 2004). Specifically, stimulation of the lateral hypothalamus (feeding center) initiates feeding, whether the animal is hungry or not and damage to the region results in starvation. On the other hand, the ventromedial hypothalamus (satiety center) signals satiation and its destruction led to uncontrollable appetite (Sartin et al., 2011). Therefore it was thought that the activities of the lateral hypothalamus were kept in check by the ventromedial hypothalamus to control feeding behavior (Vettor et al., 2002). Feeding initiation and termination are mediated through hormonal signals, neural signals and nutrient signals (Kaivala et al., 1995). The hypothalamic arcuate nucleus (ARC), located at the base of the hypothalamus, receives and integrates these signals with the aid of their neuronal receptors (Murphy and Bloom, 2004) and is considered the 'master hypothalamic center' controlling feeding. The two distinct populations of neurons control feeding in the ARC: the pro-opiomelanocortin (POMC) appetite-inhibiting neurons and the neuropeptide Y (NPY) and agouti-related proteins (AgRP) appetite-stimulating co-expressing neurons (Sohn, 2015). These neurons transmit orexigenic and anorexigenic neurotransmitters. The NPY and AgRP are orexigenic neurotransmitters while  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH), a cleavage product of the POMC gene is anorexigenic



neurotransmitter. The NPY binds Y<sub>1</sub> to Y<sub>6</sub> receptors but its orexigenic effect is via binding to only Y<sub>1</sub> and Y<sub>5</sub> receptors. The AgRP binds to MC3-R and MC4-R, antagonizing anorectic  $\alpha$ -MSH that binds to MC3-R and MC4-R to increase feed intake (Fan et al., 2008). Melanocortins are produced from POMC and they bind to melanocortin receptors 1 to 4 (MC1-R to MC4-R) to exhibit their anorectic effects. The predominant melanocortin in the hypothalamus is  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH). Peripheral signals determine which of these two neuronal sub-populations are stimulated to influence feeding behavior (Chaudhri et al., 2008). Therefore, NPY / AGRP and POMC neurons in the ARC reciprocally regulate feed initiation and termination. High activity of arcuate NPY neurons reduces energy expenditure, stimulate appetite and promote weight gain. On the contrary, arcuate neurons containing POMC increase energy expenditure and inhibit feeding, leading to a decline in body mass (Schwartz et al., 2000; Cone et al., 2001).

Many short-term nutrients (glucose, amino acids, fat), gastrointestinal hormones (CCK, GLP-1, PYY, Ghrelin) and long-term (insulin, leptin) peripheral signals contribute to feeding behavior, body weight regulation, and energy homeostasis (Havel, 2001; Carreiro et al., 2016). The long-term signals are activated in proportion to body adipose stores and the amount of calories consumed over long periods of time. This ensures that energy homeostasis is maintained, and adiposity and body weight remain relatively constant.

### **Short term regulation of food intake**

#### **(i) Glucose**

Glucose concentrations are tightly regulated since the brain depends heavily on glucose as a source of energy (Benelam, 2009). Impaired glucose metabolism, induced using the glucose analog (2-deoxy-D-glucose), increases food intake in animals (Smith

and Epstein, 1969), and increases hunger sensations and food intake in humans (Thompson and Campbell, 1977). Conversely, infusion of glucose increases sensations of satiety in humans (Novin et al., 1985) and decreases food intake in baboons (Woods et al., 1984). It was explained that glucose sensitive or responsive neurons in the ventromedial and lateral hypothalamus increase their firing rates in response to infusion of glucose, causes insulin release, and eventually induce satiety signals to reduce feed intake (Benelam, 2009). During glucoprivation the glucose responsive neurons are down regulated to induce feeding through increased expression of neuropeptide Y (NPY) or agouti-related peptide in the arcuate nucleus (Oomura et al., 1969). Others have also explained that larger arteriovenous differences of glucose correlated with satiety whereas smaller arteriovenous differences were associated with increased hunger. This suggests that arteriovenous glucose gradients were more important than absolute blood glucose concentrations in regulating feeding behavior.

**(ii) Amino acids**

Studies have shown that increased concentration of circulating amino acids or greater dietary protein intake regulate food intake. In the short term, protein intake induces satiety (Porrini et al., 1997) and consumption of protein-deficient diets increases appetite in human (Apolzan et al., 2007). Administration of phenylalanine and tryptophan, which are precursors to monoamine neurotransmitters, inhibits food intake in humans (Rogers and Blundell, 1994). Also, the ratio of plasma tryptophan to dietary amino acids may influence brain serotonin concentrations, which are known to suppress food intake (Fernstrom and Wurtman, 1971). Additionally, deficiencies of certain amino acids in the diet of rats resulted in rapid reduction of food intake. The mechanism through which proteins and amino acids regulates feed intake is poorly understood. However, Westtererp-Plantenga et al. (2007) explained a that high-protein

diet stimulates diet-induced thermogenesis. The associated increase in body temperature and oxygen consumption when on the high-protein diet may enhance satiety and reduce food intake.

### **(iii) Fat**

Increased concentration of lipids reduced feeding. The hydrolysis of triglyceride to fatty acids and glycerol by lipoprotein lipase reduced food intake in baboons (Woods et al., 1984). Conversely, inhibiting fat oxidation with methyl palmoxirate or mercaptoacetate increases food intake in animals, a process called lipoprivic feeding (Ritter et al., 1999). The increase in food intake is through increased expression of the orexigenic neuropeptide melanin concentrating hormone in the lateral hypothalamus. Production of apolipoprotein (Apo) A-IV in the intestine is stimulated by fat absorption. The hypothalamus also produces Apo A-IV. Administration of Apo A-IV suppresses food intake (Tso et al., 1999). Dietary fat reduces food intake by slowing gastric emptying, stimulating the release of cholecystokinin and peptide YY, and suppressing the release of ghrelin (Little et al. 2007; Carreiro et al., 2016). Surprisingly, intake of diets high in energy from fat leads to increased energy intake, weight gain, and obesity in animals and humans (Hill et al., 1992; Tremblay et al., 1998).

### **(iv) Other metabolites**

Metabolic products such as lactate, pyruvate (Nagase et al., 1996), and ketones (Fisler et al., 1995) have anorectic effects in animals. Lactate levels increase in proportion to the carbohydrate content of meals consumed and may contribute to short-term reduction in food intake (Havel et al., 1999). Production of ketone bodies correlates with moderate or short-term energy restriction and do not appear to affect food intake

or appetite. In contrast, during starvation or prolonged energy restriction, severe ketonemia occurs, which may inhibit hunger.

**(v) Gastrointestinal tract**

The gastrointestinal (GI) tract receives food, digests, absorbs, and senses nutrients. Nutrient sensing is critical to sustenance of life, since mice lacking enteroendocrine cells die within a week after birth (Mellitzer et al., 2010). Enteroendocrine cells, goblet cells, paneth cells, and absorptive enterocytes are the primary epithelial cell types originating from the crypt stem cells (Liou, 2013). Enteroendocrine cells represent less than 1% of all intestinal epithelial cells but constitute the largest mass of endocrine cells in the body and contribute most to nutrient sensing. Fasting or feeding stimulates intestinal receptors to chemically and / or mechanically sense and release peptide hormones from the GIT. Over fifty GIT hormones with specific biological activities are known (Celi et al., 2017). Some of these hormones, such as cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), oxyntomodulin, peptide YY (PYY), Apo A-IV, gastrin releasing peptide, neuromedin B, and leptin) are anorectic whereas ghrelin is orexigenic. They therefore act to regulate feed intake in mammals through neural and endocrine actions (Roura et al., 2016). The neural mechanism involves coupling of GIT hormones to their receptors and ensuing signal transduction through afferent fibers of the abdominal vagal nerve to the brain. The endocrine action involves systemic transport of GIT hormones to bind their receptors in the area postrema of the brain or by crossing the blood–brain barrier, and eventually coupling to receptors in specific brain regions (Roura et al. 2016).

The anorectic effect of CCK is confirmed in animals and humans (Steinert et al., 2013). Dietary fat, amino acids, and small peptides stimulate the release of CCK from

intestinal I cells in the proximal small intestine (Havel, 2001). The anorectic effect of CCK is through activation of the CCKA receptor subtype because antagonists of CCKA receptor signaling increase meal size in monkeys (Moran et al., 1993). Also, GLP-1 is co-secreted from the endocrine L cells in the ileum together with PYY in response to nutrients. PYY and GLP-1 induce satiety and reduces energy intake in healthy humans (Steinert et al., 2013). GLP-1 reduce food intake by binding to GLP-1 receptors on afferent nerves in the GI tract and/or liver and activating vagal afferent nerves to the CNS.

Conversely, ghrelin is an orexigenic hormone. The ability of ghrelin to stimulate short term feeding is more potent than any known peptide except NPY, which has an equal rating (Wren et al., 2001; Asakawa et al., 2001). Administration of ghrelin increases appetite, body weight, and feelings of hunger in human (Kirchner et al., 2012). The orexigenic effect of ghrelin is mediated through the central nervous system in the arcuate nucleus of the hypothalamus (Kilian et al., 2015). Apart from acting through Arc neurons in the hypothalamus, ghrelin binds to its receptors in the brainstem vagal afferent fibers and nucleus of the solitary tract (NTS). Ghrelin also down-regulates the firing of POMC neurons by increasing the frequency of spontaneous synaptic  $\gamma$ -aminobutyric acid (GABA) release (Cowley et al., 2003; Andrews et al., 2008).

(vi) *Other regulators of food intake*

**Cytokines**

Administration of cytokines such as IL-6 and TNF- $\alpha$  centrally or peripherally inhibits food intake (Plata-Salarnan, 1995), although their role in regulating food intake under physiological conditions is poorly understood. It was suggested that cytokines may

indirectly regulate food intake through leptin production (Finck and Johnson, 2000) or insulin sensitivity (Hotamisligil and Spiegelman, 1994).

### **Glucocorticoids**

Glucocorticoids are catabolic in the periphery but have anabolic effects in the CNS to increase food intake (Tempel et al., 1992). The central orexigenic effect of glucocorticoid is suggested to be mediated through the opposition of hypothalamic neuropeptide systems that inhibit food intake in response to insulin and leptin signals (Strack et al., 1995).

### **Thyroid Hormones**

Endogenous hyperthyroidism or thyroid administration increases food intake (Lin et al., 1983). The mechanisms by which thyroid hormones increase feed intake are not known. It was proposed that thyroid hormone increases the basal metabolic rate, leading to a state of negative energy balance, loss of body fat and reduction in circulating insulin and leptin levels, which would culminate in increase energy intake (Lin et al., 1983).

### **Growth Hormone and IGF axis**

Exogenous administration of growth hormone is associated with increased food intake whereas central administration of insulin-like growth factor IGF-I but not IGF-II, inhibits feeding (Foster et al., 1991). Both IGFs and growth hormone have a negative feedback inhibition on the somatotrophic axis via growth hormone releasing hormone (GHRH) in the hypothalamus. A possible mechanism through which IGF and peripheral growth hormone modulate feeding behavior could be through hypothalamic growth hormone releasing hormone (GHRH), since injection of GHRH into the

hypothalamic ventromedial nucleus (Tanaka et al., 1991) and intraventricular administration of GHRH agonists (Okada et al., 1996) increases food intake in rats.

### **Long term regulation of food intake**

#### **(i) Insulin**

Insulin secretion from the  $\beta$  cells of the pancreas following meal ingestion is mediated by activation of the parasympathetic nerves innervating the pancreas, by nutrients such as glucose and amino acids, and by the stimulatory effects of incretin hormones GIP and GLP-1 (D' Alessio et al., 2001). Dietary fat does not stimulate insulin secretion (Havel et al., 1999). Insulin has receptors in the regions of the arcuate nucleus of the hypothalamus implicated in the regulation of feeding behavior (Baskin et al., 1988). Insulin's action to decrease food intake involve hypothalamic neuropeptides that are involved in the regulation of feeding behavior such as NPY and melanocortin ligands and their receptors (Schwartz et al., 2000). Insulin is also known to potentiate CCK action to induce satiety and was shown to inhibit feeding in baboons (Figlewicz et al., 1995). Infusion of insulin into the cerebral ventricles of baboons consistently reduced food intake over a period of 20 days (Lin et al., 1983). The reduction in food intake was accompanied by a persistent reduction in body weight. Additionally, mice with a neuron-specific genetic knockout of the insulin receptor had increased food intake and larger adipose stores (Bruning et al., 2000). Apart from a reduction in food intake, insulin increases sympathetic neural activity and energy expenditure (Diamond and LeBlanc, 1988). Therefore, insulin can modulate energy balance by reducing food intake and increasing energy expenditure.

#### **(ii) Leptin**

Leptin concentration is proportional to body fat stores in animals and in humans (Maffei et al., 1995). Administration of leptin acutely decreases food intake and

induces weight loss in rodents and in human (Havel, 2001). Similar to insulin, leptin acts in the CNS to reduce food intake and increase energy expenditure, resulting in body weight loss (Levin et al., 1996). Leptin and CCK act synergistically to reduce food intake in many studies (Matson et al., 1997; Emond et al., 1999). Integration of leptin and short-term signals have also been reported to regulate energy balance.

In summary, many factors stimulate a wide range of GI hormones to regulate food intake and energy balance. Leptin and insulin provide long-term signals and are produced in proportion to body fat stores and circulating energy. Short-term signals originate from the GI tract to either induce sensations of satiety or hunger leading to meal termination or initiation. Both insulin and leptin activate the sympathetic nervous system (SNS) to reduce food intake and to increase energy expenditure.

### **Concept of hunger**

Feeding is a common and natural behavior of all animals since it determines survival and reproductive success of species (Tolkamp and D'Eath, 2016). Unlimited access to food reveals normal feeding behavior as feeding bouts (meals) followed by non-feeding intervals (Tolkamp et al. 2011). It follows that animals stop feeding when they are satiated and the likelihood of starting another meal shortly after feeding is very low (Forbes 1995; Zorrilla et al. 2005). Thus, feeding occurs when the animal experiences hunger as a (short-term feeding behavior) motivational state. In the long term, hunger is defined as 'negative subjective state of an animal that is chronically undernourished' (D'Eath et al. 2009). The 'undernourished' in the definition refers to energy and nutrient needs. Usually, restricted feeding regimes are aimed at restricting energy intake. The animal's subjective state in the definition is an essential animal welfare component even though it may be difficult to measure (Dawkins 1990). Chronic



restriction stimulates higher feeding motivation than short term feed deprivation in poultry (Savory et al. 1993).

### **Methodology to quantify hunger associated with feed restricted animals**

Visual analog scales applicable in human studies cannot be applied in animal studies since animals cannot express their degree of feeling (Krishnan et al., 2016). Methodology for assessment of hunger as a measure of animal welfare has been extensively discussed in the literature (Broom and Johnson 1993; Mason and Mendl, 1993; Botreau et al., 2007). Activation of the hypothalamic–pituitary–adrenal (HPA) axis, feed motivation (operant conditioning), feeding rate, compensatory feeding, stereotypy, ratio of glucose to nonesterified fatty acids (NEFA), and insulin levels in animal welfare assessment (D'Eath et al. 2009) have all been examined as methods of hunger assessment. It was suggested that indicators that change after some threshold level of food restriction is reached are likely to indicate hunger (Hocking et al., 1996). Various levels of quantitative restriction are applied to validate indicators of hunger. Indicators that have a linear association with the degree of restriction are identified as indicators of hunger (D'Eath et al., 2009). The approach is laudable but may have practical difficulties. For instance, with high level of quantitative restriction, plasma levels of NEFA (energy substrate) decreased, suggesting reduced adiposity, while glucose levels remained unchanged, resulting in an elevated glucose/NEFA ratio (de Jong et al., 2003). Two years later, in a similar study comparing quantitative restriction with three alternative diets (low quality diets) in growing broiler breeders, the glucose/NEFA ratio determined at similar time points of the day in all treatments was lower under quantitative restriction, suggesting that these birds experienced less hunger relative to the group on the alternative diets (de Jong et al., 2005). The authors

explained that a higher postprandial glucose peak with birds under quantitative restriction may have affected the result and lack of a 24 h profile makes it difficult to compare the alternative diets with quantitative restriction. Another challenge of such studies is that peripheral indicators that vary with the level of restriction may just measure the state of nutrition, which may not reflect an animal's negative emotional state.

Additionally, different levels of feed restriction will result in changes in live weight due to diet quantity and quality on gut fill (Day et al. 1996) rendering it difficult to compare hunger amongst treatments. "A comparison of empty body weight might be more appropriate, correcting (at least) for differences in the weight of gut contents, but this has never been reported in the studies reviewed" (D'Eath et al., 2009). Many sow studies do not report weight or backfat change associated with diet restriction. To overcome the issue of differences in body weight, adjustments could be made in statistical analyses by fitting body weight as a covariate (Savory and Lariviere, 2000). Finally, it is very common in animal welfare studies to integrate different measures and interpret them together; thus, using behavior to aid interpretation of physiology, (Broom and Johnson, 1993; Mason and Mendl, 1993). However, measurements from feed restriction studies often points to different conclusions. de Jong et al., (2005) concluded that alternative diets resulted in more feeding and more sitting, reduced object pecking, indicative of reduced hunger relative to quantitative restriction. Contrary to the behavior indicators, the physiological indicators of glucose/NEFA and the stress indicator heterophile/ lymphocyte (H/L) ratios were elevated in the birds on the alternative diets. The authors explained that the elevated H/L ratio was due to prolonged hunger emanating from delayed satiety as birds took much longer to finish

their food and concluded that a low-density diet might have a limited positive effect on welfare (de Jong et al., 2005).

In summary, many physiological and behavioral parameters of feed restriction or deprivation might not necessarily indicate hunger or an animal's negative subjective state. In addition, different levels of feed restriction results in differences in energy intake and live body weight changes that can be perceived to induce different levels of hunger. Comparisons in hunger studies on an empty body weight basis might be more appropriate.

### **Changes in behavior as an indicator of hunger in gestating sows**

Animals that are restrictively fed have high feeding motivation and consume their ration within a short period. They therefore exhibit behaviors suggesting that they have unsatisfied needs (D'Eath et al., 2009). Behavioral indicators of hunger offer indirect assessments of hunger by considering the animal's subjective perception of hunger. Hunger associated with quantitative restriction of feed to domesticated animals can be considered to have three indicators: (1) changes in observed behavior, (2) physiological indicators, and (3) specific behavioral tests (Tolkamp and Eath, 2016). The observed behaviors include an increase in foraging activity (Koubi et al., 1991; Weed et al., 1997) and an increase in overall activity signified by reduced resting (Savory and Lariviere, 2000; Hocking et al., 2004). The overall activity could be redirected oral behaviors, such as bar or chain chewing and rooting of substrate by sows (Appleby and Lawrence, 1987). The redirected oral behaviors lead to stereotypic behavior in pigs and broiler breeder chicken that are restrictively fed (Lawrence and Terlouw, 1993; De Jong et al., 2005). Stereotypic behaviors are behaviors that are regularly repeated and provide no benefit to the animal (Mason, 1991; Mason and

Latham, 2004). Stereotypic behavior increases with the severity of food restriction in pigs (Brouns et al., 1994). In poultry, severe feed restriction is associated with object pecking and hyperactivity (Savory et al., 1993; Hocking et al., 1996). Indications of poor welfare are stereotypic behavior, increased adrenal activity, stunted growth, and impaired reproduction (Holt et al., 2006). Examples of some stereotypic behaviors are bar biting, licking, or nosing the floor or the empty feeder, and sham chewing.

**(i) Sham chewing**

Sham chewing is chewing activity performed without feed in the oral cavity and that provides no benefit to the animal. Appleby and Lawrence, (1987) considered sham chewing as abnormal behavior that is associated with hunger. Experiment conducted by (Stewart et al., 2011) showed that a combination of fibrous feed and straw reduced sham feeding, bar biting, and hence a reduction in hunger in sows. They speculated that fibrous feed reduces hunger to some extent while the straw provides an opportunity for foraging. Several studies have reported that limit-fed, individually housed sows display stereotypic behaviors, which are performed repeatedly and without any apparent function (de Leeuw et al., 2008). Sham chewing; nosing stalls, feeder, or floor; and chewing bars or feeder are the most common stereotypes observed in pigs. Meunier-Salaün et al. (2001) have reported that limit-fed sows display more stereotypic behavior.

**(ii) Frequency of drinking bout**

The specific heat of water is 4.18 J/g °C compared with 2.40 J/g °C of alcohol. The relatively high specific heat of water enables sows to absorb heat derived either internally or externally and experience smaller changes in body temperature. When pigs have unlimited access to feed and water, the ratio of water to feed intake is 2.6:1

(Shaw et al., 2006). Higher ratios are reported when water disappearance is reported instead of actual consumption, the difference being wastage. Diet composition (Brooks et al., 1984), high ambient temperatures (Patience et al., 2005), high relative humidity (Huynh et al., 2005), and pig behavior (Fraser et al., 1990) drive water intake. Hunger, boredom, and heat stress induced polydipsia in gestation sows that are limited fed (Yang et al., 1981). Pigs under such conditions consume water far beyond their physiological need by taking frequent drinking bouts. Rushen et al. (2011) and Sandilands et al. (2006) reported excessive drinking or water spillage as indicators of hunger in feed-restricted animals.

### **(iii) Time spent standing or laying down**

The pig (*Sus Scrofa*) is an omnivorous and investigatory in nature. Domestication of pig has not changed this behavior. In the semi-natural environment, the sows spent approximately 30% of their time a day travelling, 15% rooting, drinking, or foraging and 50% sleeping or resting (Jensen, 2002). In the intensive system of production, sows fed on a high fiber diet ad-libitum spent on average 90 minutes day<sup>-1</sup> feeding (Brouns and Edwards, 1994). The level of activity depends on the time of day and temperature. Pigs are more active in the cooler months and less active during the warmer months. Most of the activities occur between sunrise and sunset (Blasetti et al., 1988).

### **(iv) Floor directed explorative behavior activity**

Pigs are exploratory in nature. In the natural setting, they exhibit their investigatory nature by rooting, sniffing, foraging, biting, and chewing on both digestible and indigestible substances (Studnitz et al., 2007). In confinement systems, it is not

possible to express these natural behaviors. Behaviors are directed towards pen-mates, stall fittings, and floor (Van Putten and Dammers, 1976).

Taken together, the behavioral changes associated with quantitative restriction are frequent drinking bouts, sham chewing, and oral redirected behavior (D'Eath et al., 2009). However, behavioral changes alone cannot provide enough information without considering additional data physiologically to permit objective assessment of animal welfare (Salas et al., 2018).

### **Specific behavioral tests to evaluate hunger**

A feeding motivation test is a valuable tool in the assessment of hunger since it reflects the animal's subjective state of hunger (Dawkins, 1990). Tests to measure feeding motivation as an indicator of hunger in animals are many. These include but are not limited to the amount of compensatory feed intake (Savory et al., 1993), operant conditioning (D'Eath et al., 2009), choice testing (conditioned place preference (CPP)) or conditioned place aversion testing (CPA) (Dixon et al., 2013).

Compensatory feed intake is the ad-libitum quantity of feed consumed after restricted feeding (Nielsen et al., 2011). Assessment of compensatory feed intake indicates that restricted fed animals have a very high motivation to feed (de Jong et al., 2003). Although a higher level of feed restriction increases short-term feeding rate, interpretation of such tests is difficult relative to when to test in relation to the feeding time (Nielsen, 1999). Operant conditioning is a technique where an animal is trained to perform an operant response (lever pressing) to obtain a food reward (Lawrence and Illius, 1989). The experimenter has the flexibility of altering the ratio of cost (lever pressing) to reward (food) to measure feeding motivation. The method was validated for quantitative restriction where a higher level of restriction resulted in increasing

feeding motivation (D'Eath et al., 2009). Also, chronic restriction resulted in higher feeding motivation than acute feed deprivation. Savory et al. (1993) and D'Eath et al. (2009) reported that broiler breeders restricted to 30% of ad libitum feed intake were over three times more feed motivated in an operant task as birds fed ad libitum and then fasted for 72 h. The methodology is used to measure motivation for access to social contact in pigs (Matthews and Ladewig, 1986) and for space and lighting in hens (Savory and Duncan, 1982). Operant conditioning test is, however, limited when comparing feeding motivation with access to different food qualities. Also, if animals are untrainable for a food reward, then their motivation cannot be measured (Beilharz and Zeeb, 1981).

Another methodology to quantify hunger associated with restricted feeding is by choice and conditioned place preference (CPP) or conditioned place aversion (CPA) testing (Tolkamp and D'Eath, 2016). The CPP methodologies stem from the principles of Pavlovian conditioning which state that: "an animal can be conditioned to prefer a previous neutral or un-preferred environment by pairing it with the presence of something that the animal finds rewarding" (Tzschentke, 1998). The pharmaceutical industry applies the methodology to investigate effects of pharmaceutical agents on the affective state of the animal (Bardo and Bevins, 2000; Bali et al., 2015). Briefly, the animal is injected with the test compound and placed in an environment to be conditioned. The procedure is repeated with injection of saline. If the animal experiences a positive affective state after the drug administration, it will prefer the former environment over the latter with the injection of saline (no affective state). However, CPP has been demonstrated with food as natural enrichment (Matsumura et al., 2010). Buckley et al. (2011) demonstrated the CPP with a Y-maze paradigm in which restricted-fed broilers had the option to choose between differently colored arms

of a Y-maze to obtain a large or smaller food reward. It was hypothesized that severe food restriction will induce more hunger and stimulate the birds to learn the task of obtaining food reward sooner. However, this was false. The most severely restricted birds could not complete the test successfully. The authors concluded that severe food restriction may limit the birds' ability to learn and the test therefore is limited to quantify hunger associated with different food restriction.

To sum up, there are no clear physiological indicators of hunger associated with quantitative energy restriction. This presents a limitation to quantify hunger associated with various quantitative energy restrictions in pregnant sows under normal production. Physiological and behavioral responses to quantitative energy restriction and hunger will provide a more thorough understanding of hunger associated with energy restriction under normal production conditions in pregnant sows.

### **Physiological indicators of hunger and associated stress in gestating sows**

Many conditions predispose swine to stressful conditions. These include but are not limited to heat and humidity, feed and water deprivation, social pressure, and mixing (Heo et al., 2005). Evidence of stress response manifests itself as significant physiological and behavioral changes (Nayanatara, 2013). Cortisol is a steroid hormone produced from the cortex of the adrenal glands. It is secreted in response to fear, pain, feeding, fasting, exercising, awakening, and psychosocial stressors (Vicennati et al., 2002; Wallerius et al., 2003). Therefore, cortisol release is responsive to a wide spectrum of stressors (Clark et al., 2011) and its concentrations can be used as a nonspecific biomarker of stress associated with chronic hunger in sows (Sutherland et al., 2007; D'Eath et al., 2009). Evaluation of plasma corticosterone



concentrations indicated that the level of diet restriction may induce chronic stress in broiler breeders (Hocking et al., 1996; Savory and Mann, 1997; Hocking et al., 2001).

Besides stress-induced cortisol secretion, there is diurnal variation in its plasma concentrations in pigs with high levels in the morning and low concentrations in the evening (Skarlandtová et al., 2011). Research conducted by Turner et al. (2002) has provided an insight into HPA axis responses to stress in pigs. Snout restraint for 5 minutes, exposure of a sow to a boar, 60 minutes confinement of gilt, mating, the back-pressure test, transportation of pigs, and mixing of unfamiliar pigs, induced peak plasma cortisol concentration of 108 ng/mL, 100 ng/mL, 94 ng/mL, 60 ng/mL, 37 ng/mL, 32 ng/mL, and 25 ng/mL respectively. It can be inferred from the above data that there is range of normal or appropriate cortisol responses to stressors and research is required to determine the impacts of these varying responses on the physiology and behavior of the animal. Cortisol concentrations in mammals is assessed from urine, plasma, or saliva by enzyme-linked immunosorbent assay (ELISA), gas chromatography-mass spectrophotometry, or radioimmunoassay (Clark et al., 2011).

However, the use of plasma glucocorticoid as an indicator of hunger and its associated stress has some interpretational problems (D'Eath et al., 2009). Birds with severe feed restriction have higher plasma glucocorticoid than those on ad libitum feeding regime; suggesting higher stress levels associated with restricted feeding regimes (Hocking et al., 1996; Kubíková et al., 2001; de Jong et al., 2003). But the differential feeding regimes resulted in differences in body weight gain of the birds of the same age with higher weights being associated with ad libitum fed birds. From these studies, there seems to be a strong relationship between plasma glucocorticoid concentration and body weight (Hocking et al., 1996; Kubíková et al., 2001; de Jong et al., 2003). Results

from these studies raise the question whether the differences in plasma glucocorticoid concentration was caused by the stress associated with feed restriction or by variation in body weight. Further studies in ad-libitum fed birds revealed that body weight alone has a considerable effect on plasma glucocorticoid in restricted fed and ad libitum-fed animals (Hocking et al., 1999; Yalcin et al., 2009; Kyriazakis and Tolkamp, 2011). Lower plasma glucocorticoid concentrations were associated with higher body weight in ad libitum-fed birds. The decline in plasma glucocorticoid concentrations cannot be attributed to a decrease in hunger with body weight since all birds were fed ad libitum throughout the study.

In addition to glucocorticoid concentrations, plasma metabolites, nutrients, and hormones (nonesterified fatty acids, beta-hydroxybutyrate, glucose, insulin) or their ratios have been reported as physiological indicators of chronic hunger in restricted fed animals (de Jong et al., 2003; Nielsen et al., 2011). The concentrations of metabolites, nutrients, and hormones vary throughout the day in response to feeding level or energy status of the animal. Also, the ratio of white blood cells (heterophils / lymphocytes ratio (HLR) was suggested as a physiological indicator of hunger, stress related to hunger, and a method of animal welfare assessment (Tolkamp and Eath, 2016). However, the use of HLR is not reliable indicator of hunger, as different studies find that HLR decreases, increases, or stays the same in response to different levels of quantitative restriction (D'Eath et al., 2009). Finally, a more recent attempt to quantify hunger is geared towards measurement of gene expression of neuropeptide Y or Agouti-related protein (AGRP). Although there is a strong association between the expression of such genes and levels of feed restriction, whether this indicates the animals' subjective state is not very clear (Boswell et al., 1999).

In conclusion, cortisol can be used for welfare assessment but may present some interpretational problems. It varies with level of feed restriction and body weight. Therefore, feeding regimes that result in changes in body weight cannot be used to assess cortisol responses. Our understanding of how feeding frequency or feeding time under iso-caloric intake in pregnant sows affects cortisol and sow behavior remains unknown. It is hypothesis that given similar amount of energy per  $BW^{0.75}$ , split and fed multiple times will reduce the activation of HPA axis and improve sow welfare compare to single feeding regime.

### **Reducing hunger in farm animals – challenges of dietary fiber**

Excess caloric intake over requirement in gestation results in obese sows at term (Baker et al., 1969), reduction in lactation feed intake (Noblet et al., 1998; Whittemore, 1998), breeding difficulty after weaning (Frobish et al., 1973), and early embryonic losses (Bas Kemp et al., 2011). As such, feed is restricted 50 to 60% of ad-libitum feed intake in gestation sows (Peltoniemi et al., 2016) and 25 to 50% in broiler breeders (Dixon et al., 2014) to maximize production. Quantitative diet restriction presents welfare concerns for fast-growing broiler breeders (de Jong et al., 2003) and gestation sows as they experience hunger most of the day, as evidenced by increased activity (foraging behavior) and increased motivation for food (D'Eath et al., 2009). These signs of hunger attracted the interest of legislators (Farm Animal Welfare Council (FAWC 1998); EC Council, 2001; Parliament U. K., 2006), advisory bodies, and animal welfare pressure groups (Defra, 2003). In many studies, qualitative diet restriction was conducted to reduce hunger and improve animal welfare by dietary manipulations such as reducing the energy density through fiber dilution and offering the diet ad libitum (Morrissey et al., 2014). Example of these fiber sources are oat hulls

(Sandilands et al., 2005), wheat bran and cobs (Robert et al., 1997) or sugar beet pulp (Whittaker et al., 2000; Danielsen and Vestergaard, 2001). Also, appetite suppressants, such as monensin sodium (Savory et al. 1996), calcium propionate (Savory and Lariviere, 2000), and phenylpropanolamine (Oyawoye and Krueger, 1990) are added to the diets of monogastrics, or by a combination of both appetite suppressants and fiber to extend the feeding time (Nielsen et al., 2011; Sandilands et al., 2006; Hocking et al., 2004; Savory and Lariviere, 2000). These feeding strategies are an effective means to increase the time to consume a ration (D'Eath et al., 2009). However, the benefits of these feeding strategies to domesticated mammals are controversial. Proponents of the practices argue that the practice may lead to normal feeding behavior, enhance satiety, and improve in animal welfare, but their results are conflicting (Day et al., 1996; Meunier-Salaün et al., 2001; Mench, 2002; de Jong et al., 2005). Opponents argue that there is no evidence of welfare benefits because indicators of hunger such as total oral behaviors and activity levels are not different between feeding strategies and quantitative restriction. If energy needs and nutrient requirements are not met then 'metabolic hunger' is unavoidable, irrespective of the level of restriction of energy intake and weight gain that is achieved through fiber dilution (Lawrence et al., 1989; Owen, 1992; Savory et al. 1996; Dailey and McGlone, 1997; Savory and Lariviere, 2000; Mcglone and Fullwood, 2001). A sow gestation diet contains about 20 to 30 percent fiber (Mcglone and Fullwood, 2001). For every 1 percent increase in fiber content of the diet, the digestibility of dry matter, energy, and protein decreases by at least 1 percent. Soluble fiber elevates luminal viscosity, increase the water-binding capacity of digesta in the small intestine, (Canibe and Bach Knudsen, 2002), and reduces the digesta flow and the rate of glucose absorption in rats (Takano et al., 2013). Also, insoluble fiber entraps nutrients, preventing exposure of

nutrients to enzymatic degradation, resulting in reduction in nutrient digestibility (Jha and Berrocoso, 2015). A study at North Carolina State University has shown that a swine diet containing 5.5% NDF, reduced waste output by 35% and 58% compared with corn-soybean meal diet containing 10% NDF and a diet supplemented with 20% soybean hulls (18% NDF), respectively. In addition to reduced energy digestibility, high fiber diets contribute to methane emissions (Cao et al., 2016) and increase fecal output.

Taken together, inclusion of fiber in swine diet is an effective means to increase time to consume a ration, but what remains unclear is whether these diets also improve satiety (a positive affective state) in sows. In the light of these concerns, farmers need to justify inclusion of high-fiber feed ingredients to swine diets. High fiber diets may also increase fecal output, methane emissions, and reduce nutrient digestibility. Therefore, there is the need to understand hunger using quantitative diet restriction.

### **Effect of feeding frequency on appetite and hunger**

Weight management professionals recommend eating smaller meals more frequently in order to spread the daily caloric intake throughout the day (Schwarz et al., 2011; Jakubowicz et al., 2012). Eating smaller meals has the potential effect to manage hunger, satiety, regulation of appetite stimulating hormones (Bachman and Raynor, 2009; Jakubowicz et al., 2012), improve metabolic health (Rashidi et al., 2003), and increase energy expenditure (LeBlanc et al., 1986). Smaller meals are postulated to enhance glucose and insulin control, increase metabolic rate, reduce body fat, increase weight loss, and reduce food cravings and hunger (Zelman and Gelfand, 2010). Conversely, Cameron et al. (2010) did not observe any significant differences in study subjects fed restricted dietary energy of 2931 kJ/d for 8 weeks, as either 3 or 6 meals

per day, on the desire to eat, on fullness, or on hunger ratings. Further, evidence comparing “nibbling” vs. “gorging” eating patterns in energy balance shows limited benefit (Taylor et al., 2001; Shahraki et al., 2007; Cameron et al., 2010; Hutchison and Heilbronn, 2016), since increasing meal frequency with nutrient dense diets can lead to obesogenic condition. Modified meal frequency regimens has been adopted with prolong fasting periods between meals to improve health parameters such as glycemic control (Munsters and Haris, 2012), lipid profiles (Stote et al., 2007), oxidative stress and inflammation (Kroeger et al., 2012), and body composition (Dixit et al., 2011).

Increasing meal frequency reduced subsequent energy intake in lean men (Speechly and Buffenstein, 1999), although perceived hunger and satiety scores did not differ. This outcome was not obtained in obese men (Allirot et al., 2013), while others have observed reduced feelings of fullness (Leidy et al., 2010) and increased sensation of hunger with increased meal frequency (Munsters and Haris, 2012; Ohkawara et al., 2013). Stote et al. (2007) conducted a crossover study and fed human participants iso-caloric meals as 3 meals per day or as 1 large evening meal for two 8-wk phases. In both conditions, assessment of appetite ratings was performed once a day prior to meal ingestion in the evening. Hunger sensation was significantly higher with the group fed 1 meal per day compared to those on 3 meals per day (Table 1.1). This result is expected because at the time of hunger assessment, the group on multiple feeding regimes had consumed higher calories relative to those on low meal frequency. In a randomized crossover trial, Perrigue et al. (2016) evaluated the effect of meal frequency on self-reported appetite. Participants felt significantly stronger hunger sensation and desire to eat when they consumed equal amounts of food as 2 meals compared with 1 meal over a 4-h period. This suggests that consumption of a larger meal suppresses appetite to a greater extent than a smaller meal within the same period

of time, which is expected. In that study, 547 kcal was consumed as a single meal while 273 kcal as 2 meals 4-h apart, but hunger was evaluated when those on 2 meals had ingested only half of their dietary intake. Spreading of nutrient load (4-5 smaller portions), significantly reduced subsequent energy intake in lean men (Speechly and Buffenstein, 1999), but hunger and satiety scores were not different between meal conditions. On the contrary, others reported reduced feelings of fullness (Leidy et al., 2010) and increased sensation of hunger in response to increased meal frequency of iso-energetic diet from 3 to 6 times daily (Munsters and Saris, 2012; Ohkawara et al., 2013). Conversely, Alliot et al. (2013) and Martine et al. (2016) did not observe any difference in hunger scores in response to meal frequency in lean or in obese men.

Table 1. 1 Response of plasma metabolites to meal frequency, hunger and satiety scores

<b>Variable</b>	<b>Fasting level</b>	<b>Low Frequency</b>	<b>High Frequency</b>	<b>Reference</b>
	<b>0 meals</b>	<b>2 meals</b>	<b>12 meals</b>	
Glucose (AUC), mmol/L x 8h	2115 ± 143	3131 ± 177	3232 ± 130	Solomon et al. (2008)
Insulin (AUC), mmol/L x 8h	6.58 ± 4.1 x 10 <sup>3</sup>	17.9 ± 2.6 x 10 <sup>3</sup>	15.9 ± 3.3 x 10 <sup>3</sup>	
Ghrelin (pg/ml)	140 ± 5.0 x 10 <sup>3</sup>	113 ± 10.0 x 10 <sup>3</sup>	112 ± 9.0 x 10 <sup>3</sup>	
		<b>3 meals</b>	<b>6 meals</b>	
Glucose (AUC), mmol/L x 2h	n/a	522 ± 99.3	710 ± 251.0	Carlson et al. (2007)
Glucose (AUC), mmol/L x 2h	n/a	103,974 ± 32, 123	80, 807.9 ± 27, 266	
		<b>3 meals</b>	<b>6 meals</b>	
Glucose			Decrease AUC by 30% compared with 3 meals	Leidy et al. (2010)
Insulin			Decrease AUC by 20 % compared to 3 meals	Leidy et al. (2010)
		<b>1 meal</b>	<b>5 meals</b>	
Hunger score		Single meal induced more hunger than 5 meals		Speechly et al. (1999)
		<b>2 meals</b>	<b>3 meals</b>	
Satiety score (AUC)			3 meals was more satiating than 2meals	Smeets and Westerterp-Plantenga, 2008
		<b>1 meal</b>	<b>3 meals</b>	
Hunger score		Hunger and desire to eat was significantly higher in 1 meal than 3 meals		Stote et al. (2007)



Feeding the daily allowance in the morning and afternoon (two equal portions) rather than once a day in the morning reduces the feeding motivation in the afternoon in sows (Robert et al., 2002). Similarly, diets high in fiber in combination with twice daily feeding reduces the variation in energy supply due to modification of the energy substrate. High fiber diets reduce energy derived from starch in the small intestine in favor of energy obtained from short-chain fatty acid due to microbial fermentation of non-starch polysaccharides in the caecum and colon of sows (Serena et al., 2009). On the contrary, Holt et al. (2006) reported that feeding a high fiber diet, using soybean hulls, or increasing meal frequency did not reduce stereotypic behaviors to improve welfare of sows. Toscano et al. (2007) characterized the physiological and behavioral responses of hunger of finishing pigs subjected to 57 h of feed deprivation. However, this condition does not mimic that of pregnant sows under normal production conditions. Additionally, without holding total caloric intake constant, the usefulness of meal frequency and meal-timing data can be limited (Kulovitz et al., 2014).

Taken together, the effect of feeding frequency on hunger is inconsistent in human studies and there is dearth of sow studies under limit-fed conditions. The inconsistency in study outcomes may be due to differences in diet composition, experimental periods, and species involved. Meal frequency data, holding total caloric intake per kilogram live metabolic weight constant, may provide insight on effect feeding frequency on sows' welfare.

## **Interactions between time of feeding, feeding frequency and circadian impact on endocrine function and body weight regulation**

Circadian rhythms are 24-h repeating cycles dictating many physiological functions in mammals (Bass, 2012). Almost all eukaryotic cells express endogenous circadian *clock* conceptualized as oscillators which can be reset by external cues (light), and internal signals (hormones) (Bailey and Silver, 2014). Within the suprachiasmatic nuclei (SCN) resides the central *clock*, which is synchronized by the approximately 24-h light/dark cycle through retinal photoreceptors. The retinal photoreceptors perceive the photic information and transmit them directly to SCN of the hypothalamus, which then transfers circadian information to the other peripheral organs via the nervous systems or hormonal signals (Hastings et al., 2003). Peripheral clock systems also exist in gastrointestinal tract, adipose tissue, liver, and the pancreas, where they control metabolic processes such as hormone release, glucose and lipid homeostasis, gastrointestinal motility, the immune response, and digestive processes (Garautet and Madrid, 2010).

In the gastrointestinal tract, gastric ghrelin-secreting cell is part of the peripheral clock network controlling the circadian release of ghrelin. Therefore, apart from the energy status, the circadian system determines plasma ghrelin concentrations and fine-tunes the timing of ghrelin release in the course of day (Yildiz et al., 2004; Goel et al., 2009). Plasma ghrelin level varies throughout the day with peak levels at night, during fasting, prior to feeding, and falls within one hour of meal ingestion (Cummings et al., 2001; Martin et al., 2011) especially with consumption of high-carbohydrate or high-caloric meals (Nematy et al., 2007; Dardzinska et al., 2014). This pattern of ghrelin secretion was reported in sheep fed 2 or 4 times per day (Sugino et al., 2002), in human (Cummings et

al., 2001), and in sows during energy insufficiency (Bradford and Allen, 2008). Additionally, the peripheral clock systems of adipose tissue and the liver are entrained by other *zeitgebers* such as nutrients, food intake and meal timing, body temperature and physical activity, since they are insensitive to light/dark cues directly (Brown et al., 2002; Kolbe et al., 2015). For instance, rodents are active in the night and feeding them exclusively during daytime alters the phase of peripheral clock gene expression, realigning it with the rhythm of food availability (Stokkan et al., 2001). It was reported that nutritional outcomes differed in mice by the timing of food intake, although the same type of food and amount with iso-caloric content were consumed, due to interplay between circadian clocks and energy metabolism (Arble et al., 2009; Dibner and Schibler, 2015).

Kuroda et al. (2012) conducted a study in mice consuming 2, 3, 4, or 6 equally spaced meals over 24-h. Increasing meal frequency with equal meal interval maintained the phase of the peripheral clock but feeding mice three discrete meals out of phase (i.e. eating when the animal would normally rest) induced significant alterations in peripheral clocks. Also, feeding mice 3 meals per day (7 am, 12 pm and 8 pm) with slightly increase in calories fed at lunch and dinner was sufficient for phase advancement of the peripheral clock. Similarly, rodents fed out of phase on iso-caloric diet become obese, even though energy expenditure was similar to group fed at normal feeding time. The authors explained that numerical reduction in activity and in energy intake may have additively resulted in body weight difference between groups (Arble et al., 2009). Epidemiological evidence confirmed that consuming more calories at night is likely to result in obesity relative to breakfast eaters (Ma et al., 2003). Spreading of the caloric load throughout the day may be beneficial to evening consumption (Seagle et al., 2009).

Presence of food therefore, can overrule the entraining signals from the SCN and become the dominant synchronizing signal, resulting in uncoupling of peripheral and master clocks. It is apparent that alterations in feeding time can uncouple the central and peripheral clocks to disrupt homeostasis and can cause disturbances in leptin release, insulin sensitivity as well as glucose and energy metabolism (Schibler et al., 2003). Under normal physiological conditions, metabolism and nutrient utilization oscillate at an optimal phase and amplitude in line with sleep/wake cycle, allowing for proper coordination of physiological functions (Marcheva et al., 2013). The amplitude and metabolic function are disrupted when circadian rhythms become misaligned. For instance, in human, there is clear time-of-day variation in glucose and insulin excursions with peak glucose concentrations in the morning, a trough in the evening and nadir at night (Saad et al., 2012; Cauter et al., 1989) which is independent of the sleep/wake and feeding/fasting cycles (Van Cauter et al., 1991; Cauter and Polonsky, 2017).

Provision of feed within a narrow time window each day leads to significant changes in physiology and behavior (Johnston, 2014). Behavioral activities preceding feed provision is termed 'food anticipatory activity' (FAA). Many vertebrate and invertebrate species exhibit FAA. In rodents, physiological changes accompany FAA are increased serum glucocorticoid concentration, locomotor activity, and core body temperature. However, FAA is not solely food-driven phenomenon because if the feeding time is abruptly delayed, the onset of FAA takes multiple 24h cycles to resynchronize to the new feeding time. If food is completely deprived, FAA are seen the same time every 24h until food deprivation is removed (Johnston, 2014). Therefore, the circadian basis of FAA allows scientists to postulate that animals contain a food-entrainable oscillator. When feed is

provided once a day, circadian rhythms are equally entrained, and circulating levels of metabolic hormones that regulate brain dopamine function differ pre- and postprandial. Circadian rhythms then uncouple from the light/dark cycle and become entrained by time of feeding (Escobar et al., 1998; Davidson and Stephan, 1999). Circadian rhythms may influence nutrient metabolism in humans (Ha and Park, 2005; Tüchsen et al., 2006) and some physiological processes affected by meal entrainment are levels of neuronal activity, brain monoaminergic activity, clock gene expression in nucleus accumbens, and corticosteroid secretion (Díaz-Muñoz et al., 2000; Ángeles-Castellanos et al., 2007). In addition, peak levels of corticosterone and ghrelin, nadir levels of insulin, precede scheduled feeding, with opposite extremes occurring postprandially (Díaz-Muñoz et al., 2000; Drazen et al., 2006). Therefore, eating late or at night disrupts circadian rhythms, and may negatively impact weight and health. Many studies in animal model have shown the importance of considering meal timing and modification of habitual meal patterns (Sato et al., 2006; Arble et al., 2009; Hatori et al., 2012).

Collectively, these studies provided evidence that nutritional outcomes differed by the timing of iso-caloric food intake. Therefore, integration of dietary formulation and nutritional management techniques to circadian clocks would significantly optimized food animal production. Whether feeding the same amount of feed at different time of day will significantly impact gestation sow body weight and backfat is not known, although increasing evidence in mice studies suggest a possibility.

### **Effect of housing and time of feeding on body composition**

Gestation sows are individually housed in many commercial swine facilities, but this may be short lived and give way to group housing due to animal welfare concerns. Group housing may alleviate chronic stress experienced by sows (Turner et al., 2002), speed the farrowing process (Ferket and Hacker, 1985), allow for social interaction and freedom of movement and therefore is perceived to be welfare-friendly relative to stalls (Trottier and Johnston, 2001). The down side of social interactions is aggressive behavior exhibited by dominant sows high on the social order and consumption of more feed at the expense of less dominant sows (Gonyou, 2001). Several strategies have been adapted to properly feed group housed sows. These include electronic sow feeders (ESF), feeding stalls within a pen, trickle feeding, and *ad libitum* feeding of high fiber diets (Trottier and Johnston, 2001). With the ESF, a group of about 60 sows is allocated to one feeder. On average, it takes about 15 to 25 min to feed one sow. Therefore, out of 24 hours about 20 hours is required to feed all the sows in a group within a day. Therefore, different sows are fed at different times of the day. Whether the sows access the electronic feed station in hierarchical order or at different times of the day is not known.

Feed restriction during the day constrains metabolism to alternate between storage and use of nutrients to ensure a continuous supply of energy to cells between meals (Le Naou et al., 2014). Supply of nutrients to cells may be coordinated with endogenous physiological rhythms such as glucose tolerance to optimize mammalian production and health (Nikkhah, 2012). Glucose tolerance in human dwindles as day comes into night signifying the importance of meal timing. Wang et al. (2014) reported that morning food intake was not related to obesogenic conditions in human but consumption of more than

33% of daily energy intake as evening meal increased the risk of obesity compared with consumption of more than 33% of energy intake at, or before 12:00 h. Also eating lunch after 15:00 h was associated with poorer weight loss during a 20-week dietary intervention (Garaulet et al., 2013). In another study, subjects who ate a larger proportion of their calories at 0700 h lost significantly more weight than the group who ate a major part of their calories at dinner (Jakubowicz et al., 2013). Similarly, in mice, nutritional outcomes differed by the timing of food intake under iso-caloric conditions when mice were fed either during the 12 hour circadian light phase or 12 hour circadian dark phase for 6 weeks (Arble et al., 2009). Mice fed during light phase gained significantly more weight than mice fed during the dark phase. Because mice are nocturnal, this suggesting that energy metabolism and circadian clocks interacted with each other to influence body weight. Furthermore, feeding time favorable for total growth (0730 h) differed from that auspicious to fattening (1600 h) in Channel catfish (Noeske-Hallin et al., 1985). On the contrary, evening and night feeding benefited ruminants through increased postprandial rumen volatile fatty acids (VFA), surges of blood insulin, lactate and beta-hydroxybutyrate secretions leading to increased milk fat synthesis and energy production (Nikkhah, 2012).

Taken together, feeding time seems to be a determinant of metabolic fate and an important regulator for energy homeostasis. These studies suggest that timing of meals is a major external cue during the wake cycle and likely affects body weight regulation differently in different species. Consumption of a larger proportion of total daily energy intake in the evening relative to the morning may lead to body weight gain. Nutritional intervention to control body composition of sows is utmost important. Feeding strategy to maximize body

condition during gestation is achieved through some form of limit feeding. Data on how timing of meal affects pregnant sows' body composition and performance under limit-fed condition is not known. It is hypothesized that feeding the same amount of energy per kilogram live BW<sup>0.75</sup> at different time of the day will result in different sow body composition changes and affect subsequent litter performance.

### **Effect of feeding frequency on energy expenditure, body composition, and body weight regulation**

Unlimited access to food (nibbling) and having access to food for a short daily period (meal-fed) is extensively studied in many species. Rats on meal-fed regime utilize their feed for BW gain more efficiently than rats with unlimited access to feed (Kekwick and Pawan, 1966; Anderson et al., 1988). It was speculated that the enhanced BW gain per unit of feed consumed was attributable to a reduced activity or a decreased metabolic rate, or both. Furthermore, it was explained that increase in glucose-6-phosphate dehydrogenase activity in meal fed rats resulted in an accelerated rate of lipogenesis (Allen, 1963). Further studies in rats by Leveille and O'Hea (1967) revealed that meal-feeding lowers the activity level but not the post absorptive metabolic rate and eventually reduce the energy expenditure. On the contrary, Spangler and Johnson (1981) reported that rats gained more weight when they had unlimited access to feed or fed 12 meals per day compared to similar group fed at 1.25× energy requirements in 2 meals per day. However, others observed that the lipogenic response associated with meal feeding occurs only in young but not in mature rats (Friend, 1967; Wardlaw et al., 1969). Contrary to rat, reduce feeding frequency resulted in reduced BF gains in growing pigs (Allen et al., 1963;



Friend and Cunningham, 1967) while both in vitro rate of lipogenesis in adipose tissue and the activity of the lipogenic enzymes remain unchanged (O'hea and Leveille, 1969). However, no differences in caloric efficiency were observed in growing pigs (55kg) fed once or five times daily (Friend and Cunningham, 1964). It can be inferred that young pigs and rats respond to frequency of feeding differently.

Weight management professionals suggest eating smaller meals more frequently to spread the daily caloric intake throughout the day (Schwarz et al., 2011; Jakubowicz et al., 2012). Plethora of studies in both animal and human subjects indicated that eating smaller meals can affect body weight (Yannakoulia et al., 2007; Duval et al., 2008; Kulovitz et al., 2014). Kulovitz et al. (2014) reported an inverse relationship between increase meal frequency and body weight in normal weight humans. Similarly, in a randomized controlled cross-over study, participants either consumed all of their calories required for weight maintenance over a 4 h period from 1700–2100 h, or as 3 meals per day for 8 weeks (Stote et al., 2007). Body weight and body fat mass were significantly reduced (measured by bioelectrical impedance analysis), by 1.4 and 2.1 kg respectively, with study subjects on time restricted feeding (Stote et al., 2007).

On the contrary, Li et al. (2005) and Noeske-Hallin et al. (1985) reported that feeding channel catfish (*Ictalurus punctatus*) either once daily in morning or afternoon as well as splitting the daily ration into two and fed morning and afternoon did not influence body weight gain or abdominal fat. It was hypothesized that increasing the daily feeding would promote energy expenditure through an increase in diet-induced thermogenesis (DIT). The DIT was doubled in dogs that were fed 4 small meals as compared with the group that received a single iso-calories meal (LeBlanc and Diamond, 1986). Similarly, DIT in

response to increased meal frequency was observed in human (Kinabo and Durnin, 1990; Tai et al., 1991). However, studies conducted in metabolic chambers over 24-h period did not find any difference in energy expenditure with subjects fed either 6 or 3 meals per day (Ohkawara et al, 2013) and 3-5 meals per day or 1- 2 meals per day (Taylor and Garrow, 2001; Smeets and Westerterp-Plantenga, 2008). In lean individuals altering meal frequency by consuming 1, 2, 3, or 6 meals per day over 5–8 weeks did not alter body weight (Finkelstein and Fryer, 1971; Hutchison and Heilbronn, 2016) .Similarly, reducing meal frequency from 4 to 3 meals per day or increasing meal frequency from 3 to 4 meals per day for 4 weeks, did not alter body weight in men but fat mass was increased by 360 g with reduced meal frequency (Chapelot et al., 2006). Increasing feeding frequency of 20% energy restricted diet in rats did not alter body weight, body composition or energy expenditure after 131 days on test diet (Hill et al., 1988). Also, obese women fed either 600 kcal per day or 1000 kcal per day energy restricted diet as either 2 or 3–5 meals daily for 4 and 26 weeks did not differ in terms of rate of weight loss, lean mass loss, and fat loss (Poston et al., 2005; Bachman and Raynor, 2012). On the contrary, increased meal frequency (6 versus 3 meals per day over 14 days) in obese women enhanced fat mass loss and preservation of lean mass under hypocaloric conditions (Jon et al., 2015).

To sum up, these studies suggest that increasing meal frequency under limit fed conditions or on hypocaloric diets has inconsistent results. Although there is no unanimous agreement in the literature concerning the effect of meal frequency on body composition; diet composition, experimental periods, lack of standardization of experimental conditions and species differences may account for the variation in experimental results. These studies provide an indication for the need for controlled feeding trials. Without holding

total caloric intake constant feeding frequency and time-restricted data can be limited. Gestation sows are limited fed at a level that allows them to gain body weight. Future research merits investigation to find out whether increase meal frequency based on live  $BW^{0.75}$  under limit fed conditions will reduce hunger and improve the welfare of sows.

### **Effects of frequency of feeding on sow and litter performance**

The subject of feeding frequency in gestational sows and its spillover effect in lactation are extensively studied using different approaches and methodologies to achieve objectives such as reduction in labor cost (Michel et al., 1980), improvement in animal welfare (Jensen et al., 2012), and enhancement of nutrient utilization (de Haer and de Vries, 1993). Michel et al. (1980) fed pregnant gilts and sows 1.9 kg corn-soybean meal diet with 12% crude-protein either once daily or as a bulk meal of 5.7 kg every third day in an intensive system of production to reduce labor needed for feeding and examined the impact of the feeding strategy on sow and litter performance. Gilts fed the accumulated meal, gained less in gestation, and farrowed fewer piglets which tended to have lower birth weight than those fed every day. Although, sows fed the accumulated meal tended to gain less during gestation, no adverse effect on reproductive performance was noticed. A plausible explanation for above result is that efficiency of nutrient absorption decreases with increase meal size (Parker and Clawson, 1967). In addition, the metabolic states of the pigs in the two feeding regimes were quite different. The half-time for particulate emptying from the stomach in mature pigs fed concentrate diet is about 10 hours (Clemens et al., 1975). This means the sows and gilts fed once daily may not have experienced post absorptive state, but similar group fed the accumulated meal achieved post absorptive state

for some hours during which tissue mobilization occurred to support metabolic homeostasis leading to a reduction in weight gained relative to pigs fed daily. In the same study by Michel et al. (1980), gilts weight loss during lactation, piglet weaning weights, and litter size at weaning did not change by feeding regime. Similarly, feeding pregnant sows 2× and 1× daily with barley based diet (84.5% barley and 12% soybean meal) did not affect piglet birth weight (Williams et al., 1979).

Lactating pigs are fed to appetite while growing pigs are fed ad-libitum. Growing pigs fed every second or third day showed enhanced lipogenic activity and stored excess energy as lipids through metabolic adaptations (Allee et al., 1972). This mechanism in pigs allows excess energy from large meals to be converted and stored as fat and mobilized during starvation periods to maintain reproductive performance (Romsos et al., 1978). Therefore, the fetal unit, energetically is homeostatic during maternal starvation (Knopp et al., 1970; Herrera and Freinkel, 1975). Herrera and Freinkel (1975), noted that 48 hours of starvation did not change both fetal liver acetyl-CoA and citrate levels in rats. Similarly, 48 hours of starvation resulted in enhanced gluconeogenic activity and increase rate of maternal fat metabolism in gravid rats (Knopp et al., 1970) as well as increase circulation of free fatty acids for energy (Robertson et al., 1971). It can be inferred from these studies that normal fetal development could occur during limited periods of maternal energy deprivation. Similar adaptation is reported in human (Wadhwa et al., 1973) and in the rat (Cohn and Joseph, 1960). Feeding gilts and sows every day increased backfat thickness measured either at the last rib or at the point shoulder during gestation compared to gilts and sows fed every third day but the reverse was the case at the end of lactation (Michel et al., 1980).

Increasing the feeding frequency from 2 to 6 times daily improved ADG and feed efficiency. The improvement is attributable to improve nutrient digestibility (de Haer and de Vries, 1993) and alteration in basal metabolism in response to meal frequency (Sharma et al., 1973; Ollivierre et al, 2017). Frequency of feeding stimulates frequent secretion of digestive enzymes in the small intestine (Leeuwen et al., 1997; Schneider et al., 2011). Hee et al. (1988) and de Haer and de Vries, (1993) suggested that increasing the number of meals increase pancreatic secretions which is positively correlated with nutrient digestibility. This finding is supported by Botermans et al. (2000) who demonstrated that increasing the number of meals (1 vs. 12) increased lipase activity, chymotrypsin, and protein output. In addition, the pancreatic secretions were independent of the caloric intake (Botermans and Pierzynowski, 1999). Another plausible reason for the enhanced performance is the second-meal phenomenon (Jenkins et al., 1980). The second-meal phenomenon is suggested to improve carbohydrate tolerance and reduce the insulin surges by spreading the nutrient load over a longer period. Time interval between meals determines the glycemic response. Shorter intervals eliminate extreme larger and smaller glycemic peaks leading to a smoother, more controlled response, which creates more efficient utilization. Sharma et al. (1973) demonstrated that frequency of feeding affects energy utilization. Pigs fed often had greater maintenance requirements but more efficient in utilization of available ME above the maintenance requirement for tissue accretion. Schneider et al. (2007) reported that gilts fed 6 times daily during the first trimester of gestation tended to have greater ADG than gilts fed 2× daily, but this response was not observed in sows. Improvements in ADG of the gilt could be due to availability of energy above the maintenance requirement.

On the contrary, studies conducted by van Leeuwen et al. (1997) and Friend and Cunningham (1964) did not elicit a response in performance when pigs were fed the same amount of feed once or multiple times. Similarly, Wittman, (1986) and Holt et al. (2006) did not observe any improvement on litter performance traits with meal frequency in sows. The conflicting results of increase meal frequency for sows could be attributed to lack of standardization of the experimental conditions between experiments. For instance, (Holt et al. 2006), fed 1.88 kg of standard corn soy bean diet to provide a daily energy intake of 6,200 kcal of ME/d. The daily amount of feed was increased for all treatment groups at d 40 and 80 of gestation if sow BCS was below 3. This suggests lack of standardization of the experimental conditions as well as the unknown factor that was used to increase the feed at d 40 and 80 of gestation. In gestation, placentation process is completed by d 30, mid-point of a period of high fetal death due to intrauterine competition (d 60), a period of rapid fetal demands for nutrients (d 90), and a point close to farrowing is (d 109) (Mesa et al., 2012). Utilizing kilogram live  $BW^{0.75}$  at d 30, 60, and 90 to adjust the amount of feed fed during gestation for the periods between d 30 – 60, d 61 – 90, and d 91 – 109 respectively enables the standardization of the ME intake. To standardize ME intake per kilogram live  $BW^{0.75}$ , the daily quantity of feed fed is scaled to the  $BW^{0.75}$  live weight (Le Naou et al., 2014) and fed at 1.25 times (Prunier and Quesnel, 2000) the maintenance requirements for sows ( $100 \text{ kcal} \times BW^{0.75}$ ) specified by NRC, (2012).

### **Effect of meal frequency on glucose and insulin secretions**

There is a plethora of research on the effect of increase meal frequency on metabolic markers of health such as glucose and insulin secretions in many species (Speechly and

Buffenstein, 1999; Poston et al., 2005). However, studies investigating responses of insulin and/or glucose to meal frequency in healthy, overweight or obese individuals are not consistent. Some researchers reported no significant differences in glucose concentration ( Bertelsen et al., 1993; Poston et al., 2005; Raynor, et al., 2015) whereas others observed significant differences between feeding regimes (Kanaley et al., 2013).

Meal frequency decreases the postprandial surge of glucose and insulin released (Poston et al., 2005). Additionally, stable blood sugar levels enhanced glucose uptake as fuel and may suppress free fatty acid release from the adipose tissue (Palmer et al., 2009). Research on obese human on iso-caloric diet fed either 2 or 6 times showed that the two-meal approach elicited 84% greater maximum amplitude in glucose concentrations and elevated circulating FFA relative to six-meal feeding regime (Bertelsen et al., 1993). Similarly, in healthy lean men fed iso-caloric ( $3450 \pm 466$  kJ) and identical macronutrient either as a single meal or split into five equal portions and served hourly; serum insulin concentration of single pre-load meal rose to a significantly higher level ( $123.04 \pm 61.51$   $\mu$ IU/ml vs.  $37.30 \pm 26.65$   $\mu$ IU/ml compared to the first of the multiple pre-load meals. Also serum insulin concentration was significantly elevated following the fifth and final of the multiple pre-load meals relative to the serum insulin concentrations in the single meal group at the same time into the trial ( $74.21 \pm 51.64$   $\mu$ IU/ml vs.  $24.98 \pm 13.46$   $\mu$ IU/ml (Speechly and Buffenstein, 1999). Additionally, low meal frequency (3 $\times$  daily) produced significantly lower troughs and higher peaks for insulin and glucose concentration than high feeding frequency (14 $\times$  daily) of equi-energetic diet in human study subjects (Munsters and Saris, 2012).

Conversely, the AUC of 24 h glucose secretion profile was significantly lower in the 3× daily feeding (7276.2 mmol/L) relative to 14× daily feeding regime (7664.7 mmol/L), although insulin AUC did not differ between feeding regimes (Munsters and Saris, 2012). Kanaley et al. (2013) reported significantly higher insulin incremental area under the curve (AUC) in healthy obese women fed iso-caloric diet on three eating occasions compared to those on six-meal condition. Feeding pregnant sows 2× and 1× daily with barley based diet (84.5% barley and 12% soybean meal) elicited mean glucose concentration of  $71.4 \pm 1.7$  mg/dl and  $67.4 \pm 1.9$  mg/dl, respectively, during second trimester of gestation (d 37 to 74) (Williams et al., 1979). However, glucose concentration was not different in gilts fed either 1× or 4× daily for 165 days (Romsos et al., 1978). Also, in a healthy human volunteers fed 2× daily (Low meal frequency) or 12× daily (high meal frequency) with equi-energetic diet, resulted in similar insulin AUC (Solomon et al., 2008).

Inconsistent results may be due to self-report with potential under reporting of caloric intake and lack of standardization of experimental conditions. The effects of low feeding frequency (1×, 2× or 3×) of equi-energetic diet based on live BW<sup>0.75</sup> of pregnant sows and its concomitant impact on glucose release and insulin secretion are presently not known.

### **Ghrelin as orexigenic hormone**

Ghrelin is a unique “hunger” hormone that accelerates gastric emptying, promotes food intake, weight gain and energy storage (Kilian et al., 2015) and exists in two forms: acylated ghrelin and des-acylated ghrelin (Granata, 2010). The acylation which is necessary for ghrelin biological activity is catalyzed by ghrelin *O*-acyltransferase



(GOAT) (Gutierrez et al., 2008; Yang et al., 2008). The GOAT is specifically expressed in the gastric mucosa and the amount and / or activity of the enzyme likely affects the level of acyl ghrelin. This enables ghrelin to bind to its receptor (GHS-R1a) to release growth hormone (GH) and exert its central orexigenic activities (Howard et al., 1996; Sun et al., 2004). The volume of circulating total ghrelin is more than 90% desacylated ghrelin while acylated ghrelin is less than 10% (Patterson et al., 2005) but desacylated ghrelin does not bind to GHS-R1a or release GH (van der Lely et al., 2004). As such desacylated ghrelin was initially thought to have no specific biological activities but later studies proved otherwise. Soares and Leite-Moreira, (2008), Inhoff et al., (2009), van der Lely, (2009) demonstrated that acylated and desacylated ghrelin recognize common binding sites, and exhibit biological activities in cells and tissues that do not express the GHS-R1a, suggesting other receptors for ghrelin isoforms are yet to be identified. Plasma esterases desacylate acyl-ghrelin whilst proteases break down circulating ghrelin (Nishi et al., 2005).

The orexigenic effect of acyl-ghrelin is mediated through the central nervous system in the arcuate nucleus of the hypothalamus (Nakazato et al., 2001; Kilian et al., 2015). It is thought that stimulation of the vagal nerve relays messages to the arcuate nucleus which contains agouti-related protein/ NPY neurons. Intracerebroventricular injections of the peptides increased feed intake in sheep (Whitlock et al., 2005) whilst disabling them reduce feed intake implying that the NPY are important in ghrelin orexigenic effect (Niemann et al., 2011). Apart from acting through Arc neurons in the hypothalamus, ghrelin binds to its receptors in the brainstem vagal afferent fibers and nucleus of the solitary tract (NTS). Ghrelin also down regulates the firing of POMC neurons by

increasing the frequency of spontaneous synaptic  $\gamma$ -aminobutyric acid (GABA) release (Cowley et al., 2003; Andrews et al., 2008). Efficacy of ghrelin to stimulate short term feeding is more potent than any known peptide except NPY with which they have equal rating (Asakawa et al., 2001; Wren et al., 2001). Administration of ghrelin increases appetite, BW, and feelings of hunger in human (Nakazato et al., 2001b). Ghrelin also stimulates gastric motility and acid secretions (Date et al., 2000; Masuda et al., 2000) further suggesting that ghrelin stimulates feed intake (Cummings, 2003). In addition, ghrelin through its autocrine and paracrine actions regulates cell proliferation and survival, apoptosis, inflammation, cardiovascular and gastric functions, metabolism, angiogenesis, development, and reproduction (Muccioli et al., 2007; Chanoine et al., 2009; van der Lely, 2009).

The amount of ghrelin in circulation is determined by a balance among its secretion rate, degradation rate, and clearance rate. Clearance of ghrelin is mediated by its receptor and excreted in urine (Nishi et al., 2005). In pigs, ghrelin is more concentrated in the oxyntic and cardiac glands than the pyloric glands (Govoni et al., 2005). However, about 66% of plasma ghrelin in circulation is derived from the stomach and the rest originating from the small intestine (Peeters, 2005). Ghrelin is ubiquitous and found in many organs and cells of the body such as pancreas, lungs, testis, placenta, hypothalamus and pituitary, kidneys, immune cells, and the intestines. It decreases in concentration from the duodenum to the colon (Peeters, 2005). Ghrelin concentration in blood increases pre-prandially, in fasting conditions, and before meal initiation and rapidly falls post-prandially (Govoni et al., 2005). Feed deprivation increased plasma ghrelin concentration from 46 – 114 to 227 pg/ml in prepubertal gilts and weaned pigs (Govoni et al., 2005; Zhang et al., 2007). Also,

fasting resulted in postprandial plasma ghrelin concentrations increase from 6 pmol/l to 10 pmol/l in pigs (Barretero-Hernandez et al., 2010). Scrimgeour et al. (2008) showed that the secretion pattern of plasma ghrelin concentrations in pig is determined by duration of fasting and changed from 15 pmol/l (feeding) to 100 pmol/l (fasting). Huber et al. (2006), reported that ghrelin may play a role in appetite regulation, meal frequency, energy intake, control of food intake, and hunger. Ghrelin therefore function as survival kit of nature to maintain energy homeostasis (Xuefeng et al., 2009). However, Guillory et al. (2017) reported that Ghrelin knock-out mice had both decreased food intake and energy expenditure suggesting that there is redundancy in the hormonal system to control feeding. Besides the animal's nutritional status, the type of micronutrient consumed influence ghrelin concentration (Ariyasu et al., 2001; Otto et al., 2001; Erdmann et al, 2003). Carbohydrates rich meals have greater suppressive effect on ghrelin concentration than meals rich in fats in human studies (Erdmann et al, 2003) but not in rat (Gomez et al., 2004). It can therefore be inferred that the carbohydrates suppressive effect on ghrelin is species dependent.

Ghrelin is also known to exhibit a diurnal pattern with two peaks: one at 1500h (during the light period) and the other at 0600h (at the end of the dark period) in rodents fed *ad libitum*. Although, the two peaks coincide with the timing of the lowest and the greatest gastric emptying and filling activity, when gastric acid secretion and gastric motility are most apparent, the physiological significance of the two peaks remains elusive (Masuda et al., 2000). Similarly, nocturnal rise in ghrelin was reported in human with a nadir concentration of 640 pg/ml observed between 1900 and 2000 and a peak between 0100 and 0200 of 705 pg/ml but the biological significant is not clear (Koutkia et al., 2004).

Cummings et al. (2001) fed human subjects at three specific time points during the day and observed that plasma ghrelin rises and fall before and after feeding. They observed 1–2h pre-prandial plasma ghrelin concentrations increase and a decrease within 1 h of feeding. A similar finding has also been reported in sheep (Sugino et al., 2002) and rodents (Lee et al., 2002). From these studies it was proposed that ghrelin functions as a neuropeptide signal that initiates the onset of feeding. The human and the ovine subjects however were trained to receive meals at specific time point. Drazen et al. (2006) therefore, speculated that the pre-prandial rise in plasma ghrelin in those models was due to anticipatory response but not meal initiation. Because the study subjects were expectant of feeding, ghrelin levels rose to regulate processes that initiate feeding, absorption, and metabolism of caloric load. Similarly, Schussler et al. (2012) demonstrated that human study participant had increased ghrelin concentrations after observing photographs of hedonic food. More recently, sheep fitted with muzzle and exposed to feed (sham feeding) had highest levels of ghrelin concentration compared to fed and feed deprived groups outside the scheduled meal times (Stockwell-Goering et al., 2015). The sham fed animals could see, smell and hear the feed being placed in the feed buckets but cannot consume it. The authors concluded that visual, olfactory and auditory cues collectively contributed to elevated ghrelin levels in the sham fed group and suggested that ghrelin secretion by the autonomic nervous system is affected by the central nervous system. On the contrary, the feed anticipatory response to ghrelin hypothesis could not be supported in a study where both fasted rats not anticipating in a meal and *ad libitum* fed group showed no pre-prandial rise in ghrelin (Drazen, 2006).

Taken together, the orexigenic effect of ghrelin is mediated through the central nervous system in the arcuate nucleus of the hypothalamus and the brainstem. Stimulation of the vagal nerve of the brain stem relays messages to the arcuate nucleus containing agouti-related protein/ NPY neurons. In the process, firing of POMC neurons is down regulated to stimulate appetite and initiate feeding. Ghrelin increases with fasting and decreases in response to feeding in many mammals, but the reduction depends on calories ingested. It is hypothesis that more frequent meals under iso-caloric conditions will reduce hunger in pregnant sows. Feeding similar quantity of energy as 1×, 2× or 3× daily or feeding the same quantity of energy at different time of the day will elicit different response in ghrelin concentration. Ghrelin responses to feeding time and frequency in pregnant sows based on live metabolic weight  $BW^{0.75}$  is yet to be studied.

### **PYY as anorectic hormone**

Peptide YY (PYY) is a 36-amino-acid and a putative satiety hormone secreted by the L cells of the distal small intestine and the colon where they are highly concentrated (Xie et al., 2018). It belongs to pancreatic polypeptide fold family of peptides. It was first isolated from porcine intestine in 1980 and biologically related to NPY (Tatemoto and Mutt, 1980). The predominant form in the blood is PYY<sub>3-36</sub> hydrolyzed by dipeptidyl peptidase IV from PYY<sub>1-36</sub> precursor. PYY is able to cross the blood–brain barrier (Nonaka et al., 2003) and its biological effect is mediated through binding to Y family receptors (Y1R–Y6R) (Xie et al., 2018). In mice and rats, PYY<sub>3-36</sub> anorexigenic effect is through inhibition NPY/AgRP neurons by binding to Y2 receptors of the ARC of the hypothalamus. Inhibition of NPY/AgRP neurons then activates proopiomelanocortin /cocaine- and

amphetamine regulated transcript neurons in the arcuate nucleus to reduce feed intake. Additionally, PYY anorexigenic function is mediated through inhibition of gastric acid and pepsin secretion, inhibition of pancreatic exocrine secretion, delay of gastric emptying, and inhibition of jejunal and colonic motility (Dalton et al., 2016). It therefore serves as an “ileal brake” to slow gastric emptying and increase intestinal nutrient transit time for nutrient absorption (Xiao, et al., 1998). Besides mice and rats, PYY reduction in food intake had been shown in rhesus monkeys (Moran et al., 2004), normal human (Batterham et al., 2002), and obese and lean humans (Batterham et al., 2003).

PYY secretion is proportional to macronutrient or caloric content of diet (Essah, 2007; Batterham et al., 2003). In humans and pigs, PYY is secreted in the distal small and large intestine and the concentration increases after meal intake (Albrechtsen, 2016). Following meals, plasma PYY levels increase within 15 minutes, peak at around 60 minutes, and remain elevated for up to 6 hours in human (Batterham et al., 2003; Hill et al., 2012). Neural or endocrine mechanism may be responsible for the initial increase that occurs before nutrients arrive at the L cells where PYY is secreted. The sustained release was attributed to direct effects of the digesta on the L cells (Imamura, 2002). However, this sustained release was not observed by (Hill et al., 2012). Sustained secretion of PYY has been linked to its anorectic effect. Peptide YY response is meal driven and expected that more frequent meals throughout the day may enhance maintenance of higher circulating PYY and perhaps greater 24-h satiation (Hill et al., 2011; Paoli et al., 2015 ). Therefore, many authors have suggested that frequent meals evenly spread may have beneficial metabolic effects and improve satiety (Bellisle et al., 1997; Farshchi et al., 2005; Smeets and Westerterp-Plantenga, 2008).

In summary, postprandial concentration of total PYY is proportional to the calories ingested. Variations in total PYY concentration induced by feeding time and frequency may elicit anorectic effect in pregnant sows. Diurnal rhythm of total PYY is yet to be characterized in pregnant sows fed based on their live BW<sup>0.75</sup> under normal production conditions in relation to feeding time and frequency. It was hypothesized that increase meal frequency in sows would increase total PYY AUC relative to sows' on single feeding regime, suggesting enhance satiety. Sows fed once daily at different times would have similar AUC.

### **Adaptation and function of ghrelin and PYY during pregnancy**

During pregnancy several metabolic changes occur to enhance adipose tissue accretion to meet increased metabolic demand. This results in changes in orexigenic and anorexigenic hormone signals (Tovar et al., 2004). During pregnancy, in addition to the fundic portion of the stomach, significant amount of acylated ghrelin is produced by the placenta (Sloth, 2007). Ghrelin concentration is higher in non-pregnant than pregnant mammals (Leidy, 2007). Although significantly lower plasma ghrelin concentration was observed on days 10 and 15 of pregnant rats relative to non-pregnant rats; the levels were comparable at day 20 of pregnancy. Also, Ghrelin peptide concentration in the stomach of pregnant rat was similar to non-pregnant rats on day 20 of pregnancy (Shibata et al., 2004). In the same study, hypothalamic ghrelin mRNA expression of pregnant rat was significantly lower on day 15 of pregnancy compared to non-pregnant rats. Pregnant women have the highest and lowest levels of maternal ghrelin at 18<sup>th</sup> week and third trimester of gestation respectively (Batterham, 2002).

The down regulation of maternal ghrelin expression in the third trimester may be an adaptation to meet the increased energy requirements during late pregnancy. This suggests positive energy balance through metabolic adaptations or increased dietary intakes (Hill et al., 2011). Also, fetuses with intrauterine growth retardation have high levels of ghrelin suggesting a significant biological role to enable the fetus to adapt to the uterine environment (Leidy and Williams, 2006) and development of the embryo (Hill et al., 2011; Amir, 2012). Higher ghrelin concentration in placental blood relative to maternal blood indicates that ghrelin is easily transferred to the embryonic circulation for embryonic growth through stimulation of cell proliferation. Human fetuses therefore have higher total ghrelin concentration in placental blood which is not correlated with either maternal ghrelin levels or gestational age (Kitamura et al., 2003). Ghrelin play a pivotal role in regulation of onset of puberty (Tena-Sempere, 2007), gonadotropin secretion (Chouzouris et al., 2016), oocyte maturation acting through activation of Akt1 and ERK1/2 pathways (Chouzouris et al., 2017), pre-implantation embryos (Kawamura et al., 2003a) and fetal development during pregnancy (Dovolou et al., 2014). However, it was reported that leptin may enhance the development of early stage embryos and implantation but ghrelin may inhibit the development of preimplantation embryos and implantation in a paracrine and autocrine manner (Kawamura et al., 2003; Cervero et al., 2004). These regulatory mechanisms may act as an adaptation to maintain adequate embryo development and pregnancy outcome or to avoid excess metabolic demands during pregnancy under nutritional insufficiency (Budak et al., 2006).

Exogenous chronic treatment of pregnant rat with ghrelin increased fetal body weight at birth whereas mothers immunized against ghrelin produced fetuses with lower body



weight (Nakahara et al., 2006). In sows, peak plasma ghrelin concentrations occurred at 30 days of pregnancy and decrease thereafter until late lactation (Govoni et al., 2007). Regardless of current knowledge about the functional role of ghrelin its mode of secretion during pregnancy is not known (Govoni et al., 2007; Chouzouris et al., 2018).

Similar to ghrelin, PYY<sub>1-36</sub> and PYY<sub>3-36</sub> are found in human placenta and fetal membranes from 9.5 weeks of pregnancy to term (Xiao et al., 1989). Physiological function of PYY<sub>3-36</sub> includes but not limited to inhibition and secretion of gastric acid, pancreatic enzyme, electrolyte, intestinal fluid and, suppresses gastrointestinal motility to delay digesta transport. It therefore functions as ileal brake, delaying gastric emptying rate and digesta transit to improve nutrients absorption in GIT (Rolls, 2009). Tovar et al. (2004) reported pregnancy-associated increase in circulating PYY concentration when food is increased but this increase was absent in food restricted pregnant rat. The role of PYY in modulation reproductive function is not clear. Wade et al. (1996) speculated that PYY may enhance reproductive function by activation of hypothalamic-pituitary-gonadal axis. PYY may signal through to the hypothalamus which contains centers for energy balance and reproduction to suppress reproduction during energy deficiency to preserve energy for vital life processes. This action is mediated through suppression of gonadotropin-releasing hormone (GnRH) by metabolic cues yet to be identified. The GnRH produced from the hypothalamus stimulates the release of luteinizing hormone (LH). Fernandez-Fernandez et al. (2005) demonstrated that in both male and female rats, Y2 and Y5 receptors of PYY bind to their receptors in the pituitary cells to stimulate the release of LH in a dose-dependent manner. Furthermore, NPY which has opposing effect on PYY in the hypothalamus is known to inhibit estrous behavior in Syrian hamsters (Keene et al., 2003).

Pregnant women in their second trimester of gestation were fed once a day with 527 kcal meal containing (24.1% fat, 54.4% CHO, 21.5% protein) of nutrients. Fasting plasma ghrelin levels, PYY, insulin, and glucose were measure at (time 0 min) and postprandial (30, 60 and 120 min). Insulin and glucose were measured because they participate in the regulation ghrelin and PYY release. Values are presented in (Table 1.2).

Table 1. 2 Comparison of the fasting hormone levels in pregnant women with various body mass index

	CP	OLWG	OHWG	MOP
Acylated Ghrelin (pg/mL)	229.4 ± 57.	185.8 ± 56.8	210.3 ± 68.9	338.3 ± 88.9
PYY <sub>3-36</sub> (pg/mL)	40.0 ± 2.2	39.1 ± 3.7	40.7 ± 3.5	36.5 ± 0.1
Insulin (µU/mL)	10.8 ± 1.3	32.0 ± 5.3	25.0 ± 2.6	34.0 ± 5.7
Glucose (mg/dL)	96 ± 2.0	101.0 ± 4.0	106.0 ± 5.0	119.0 ± 5.0

Control pregnant (CP); overweight low weight gain <1kg/week (OLWG); overweight high weight gain >1kg/week; OHWG; morbidly obese pregnant – MOP. Data are presented as mean ± SEM, adapted from Sodowski et al. (2007)

Fasting acylated ghrelin concentration of 229.4 ± 57.3 pg/mL was reported in pregnant women (Sodowski et al., 2007). Postprandial decline of ghrelin occurred about 30 min (Sodowski et al., 2007) or 35 min in human fed mixed meal and attributable to glucose and insulin secretions both of which inhibit ghrelin secretion (Chabot et al., 2014). Although, ghrelin concentration falls with feeding, during pregnancy the decline is not abrupt but relatively stable in human (Sodowski et al., 2007).

In conclusion, ghrelin concentration is lower in pregnant than non-pregnant mammals. Ghrelin concentration declines with advancement of pregnancy possibly as an adaptation to meet the increased energy requirements during late pregnancy. Because ghrelin plays a role in implantation and fetal development its concentration in placental blood is higher relative to maternal blood. The role of PYY during gestation is not clearly defined but

chronic feed or acute feed deprivation leads to decrease concentrations. Ghrelin and PYY have significant effects on reproduction. These hormones may serve as links between gut and the reproductive system to supply and regulate energy needs during pregnancy by acting through the brain. Each time feed is fed ghrelin concentration declines and by comparing the AUCs of ghrelin, it was hypothesized that increase meal frequency would to reduce ghrelin AUC relative to single feeding regime suggesting minimization of hunger. Opposite effect would be found for PYY.

## Chapter 2. Effects of time of feeding during gestation on sow's performance

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## SUMMARY

The aim of this study was to investigate the effect of different feeding time regimes given similar energy intake per kilogram live BW<sup>0.75</sup> during gestation on sow's performance. One hundred and seventy-four sows [Topigs TN 70 (Landrace × Large White, Topigs USA); parity  $3.81 \pm 0.16$ ; initial BW =  $211.57 \pm 3.34$  kg; backfat (BF)  $13.70 \pm 0.42$  mm] were blocked by parity, farrowing date, balanced for BW and randomly assigned to 1 of 3 treatments in a randomized complete block design. Treatments included sows fed corn-soybean meal-based diet once at [0730 (control, T1), 1130 (T2), or 1530 h (T3)], with daily feed quantity kept at  $1.25 \times$  maintenance energy intake [ $100 \times (\text{BW})^{0.75}$ ] kcal ME/d. Sows received 6,758, 7,434, and 8,110 kcal ME/d from days 30 to 60, days 61 to 90, days 91 to 109 of gestation, respectively. The gestation diet was formulated to contain 3,379 kcal of ME/kg, 0.70% Ca, 0.61% total P, 0.58% SID Lys, 0.26% SID Met, 0.45% SID Thr, 0.12% SID Trp, and 0.48% SID Met+Cys. Body weight and BF were recorded on days 30, 60, 90, and 109 of gestation, 24 h after farrowing and at weaning. Results showed that feeding times evaluated did not alter BW changes from day 30 to day 109 of gestation ( $P = 0.81$ ) or from day 30 to weaning ( $P = 0.87$ ). Similarly, feeding sows daily at 1130 h did not influence BF gains and sow reproductive performance relative to the control sows ( $P > 0.10$ ). Sows fed once daily at 1530 h gained more BF compared with the control ( $3.69 \pm 0.47$  vs.  $2.12 \pm 0.50$  mm,  $P = 0.04$ ) from day 30 to day 109 of gestation. From day 30 of gestation to weaning, treatments did not influence BF gain ( $P = 0.24$ ). Feeding sows daily meal at 1530 h had propensity to increase ( $P = 0.09$ ) the number of piglets weaned by 0.54 piglets compared with the control sows. In conclusion, the present study demonstrated that feeding pregnant sows at 1530 h altered energy and nutrient metabolism

improving their BF gain and exhibited a potential to increase the number of weaned piglets compared with conventional feeding regime.

**Key words:** feeding time, iso-caloric, pregnant sow, reproductive performance

## INTRODUCTION

Nutritional intervention to control body composition of sows is of utmost importance. Feed restriction during the day constrains metabolism to alternate between storage and use of nutrients to ensure a continuous supply of energy to cells between meals (Le Naou et al., 2014). Supply of nutrients to cells may be coordinated with endogenous physiological rhythms such as glucose tolerance to optimize mammalian production and health (Nikkhah, 2012). Glucose tolerance in human dwindles as the day comes into night signifying the importance of meal timing. Wang et al. (2014) reported that consumption of more than 33% of daily energy intake as evening meal increased the risk of obesity compared with consumption of more than 33% of energy intake at or before 12:00 h. Also, nutritional outcomes differed by the timing of food intake under isocaloric conditions in mice (Arble et al., 2009). Furthermore, feeding time favorable for total growth (0730 h) differed from that auspicious to fattening (1600 h) in channel catfish (Noeske-Hallin et al., 1985). Conversely, evening and night feeding benefited ruminants through increased postprandial rumen VFA, surges of blood insulin, lactate, and beta-hydroxybutyrate secretions leading to increased milk fat synthesis and energy production (Nikkhah, 2012). Taken together, these studies suggest that timing of meals is a major external cue during the wake cycle and probably affects body weight regulation and production differently in different species. Data on how timing of meal affects pregnant sows under limit-fed condition are not known. We hypothesized that feeding the same amount of energy per

kilogram live  $BW^{0.75}$  at different time of day could alter energy and nutrient metabolism of gestation sows to influence their performance. The objective of this study was to investigate the effect of feeding time based on similar energy intake per kilogram live  $BW^{0.75}$  during gestation on sow's performance.

## **MATERIALS AND METHODS**

### ***Animals, Housing, and Management***

The study was carried out at the swine unit of University of Minnesota Southern Research and Outreach Center, Waseca, MN. University of Minnesota Institutional Animal Care and Use Committee (IACUC) approved all protocols used in the study (IACUC number 171011961). Five cohort group of sows of (TN 70; Landrace  $\times$  Large White; Topigs Norsvin USA, Burnsville, MN); total  $N = 174$ ; initial average BW  $211.55 \pm 3.42$  kg; and average parity  $3.8 \pm 0.16$  (with a range of 0 to 9) were artificially inseminated twice, 24 h apart during estrus using fresh diluted semen from Duroc boar (Compart Boar Store, Nicollet, MN).

Multiparous and nulliparous sows constituted 5 separate cohort groups: (cohort 1;  $n = 35$ ), (cohort 2;  $n = 35$ ), (cohort 3;  $n = 35$ ), (cohort 4;  $n = 34$ ), and (cohort 5;  $n = 35$ ) derived from 2 farrowing herds out of the station's 10 breeding groups. One of the 2 herds were sampled thrice (cohort groups 1, 3, and 5), whereas the other was sampled twice (cohort groups 2 and 4) from March 2016 to February 2017. Sows were kept in individual conventional gestation stalls with fully slatted floor measuring (2.1 m  $\times$  0.59 m  $\times$  0.97 m) under temperature-controlled environment (21 to 23 °C) on a 9 h of light and 15 h of dark schedule, with the light on at 0730 h and turned off at 1630 h. On the day 30 of gestation,

sows were weighed individually before feeding and backfat (BF) thickness was measured using an ultrasound machine (Lean-Meater, Renco Corp., Minneapolis, MN). Measurements were taken at the last rib about 5 cm lateral from the dorsal midline on both left and right sides using soybean oil as coupling fluid, and the 2 readings were averaged. Sows that returned to estrus were excluded from the experiment after pregnancy check on day 35 postcoitum with the aid of ultrasonic pregnancy checker (Classic medical supply, Inc., FL).

The conception rate was 88.3% for the 5 cohort groups sampled during the study. Throughout the experiment, sows had ad libitum access to water through nipple drinkers fitted to each pen or crate. On day 109 of gestation, sows were washed and transferred from gestation stalls to environmentally controlled farrowing rooms and placed in individual farrowing crates (213 cm × 97 cm × 66 cm) where they received a common lactation ration. Sows received 2.27-kg diet regardless of treatments from day 109 of gestation until parturition. After parturition, feeders were checked daily in the morning. Sows were fed twice daily at about 0800 and 1430 h daily. Feed offered was weighed and marked on individual sow card during feeding and the amount of feed offered increased gradually to allow for ad libitum feed intake without accumulation of feed in the feeder. Surplus feed was weighed and recorded once for each sow on the day of weaning.

Neonatal pigs were cross-fostered within treatment to equalize litter size of about 12 across sows within and limited to 24 h post-partum if it was required. Standard piglet management processing procedures of the farm includes intramuscular injection of 1-mL iron dextran shots, tail docking, cutting, and disinfection of naval cords within 72 h post-



farrow. Supplemental heat lamp was provided to maintain a constant temperature for piglets after birth for 48 h while they had access to floor heat pads until weaning. The farrowing barn was maintained at  $24 \pm 2$  °C during lactation. Piglets did not receive creep feed but had access to the dam's feed. Surgical castration of the male piglets was accomplished between 5 and 12 d of age. Piglets were vaccinated 4 d prior to weaning. Weaning occurred between at 0800 and 1000 h on  $18.9 \pm 0.37$  d post-partum. At weaning, sow BW and ultrasound BF thickness were recorded before transferring them to stalls in an environmentally controlled breeding facility where they were checked daily for signs of estrus using a mature boar housed in Contact-O-Max boar cart (Hog Slat Inc., Newton Grove, NC). Estrus was recorded when sows exhibited standing heat on exposure to the mature boar and estrous interval from weaning recorded. Removal of sows from the batch included failure to conceive after second mating, anestrous exceeding 8 d post-weaning, consecutive abortions, and difficulty to carry body weight on limbs. Feed offered to sows was restricted to 2 kg from weaning through breeding to 30 d, which is considered optimal for sows and gilt during that phase

### ***Experimental Design, Dietary Treatments, and Feed Line Calibration***

Sows were blocked by parity, breeding date, and balanced for body weight and randomly allocated to 1 of 3 treatments with 11 to 12 replicates per batch in a randomized complete block design. All 5 cohort groups of sows received a common corn-soybean meal-based diet during gestation and lactation. Nutrients met or exceeded NRC (2012) nutrient requirements for gestation and lactation sows. The chemical compositions of diets are presented in (Table 1). Experimental treatments were imposed from 30 d of gestation until day 109 of gestation. Body weights on days 30, 60, and 90 were used to adjust the amount

of feed fed between days 30 to 60, days 61 to 90, and days 91 to 109 of gestation, respectively. To standardize ME intake per kilogram live BW<sup>0.75</sup>, the daily quantity of feed fed was scaled to the BW<sup>0.75</sup> live weight (Le Naou et al., 2014) and fed at 1.25 times (Prunier and Quesnel, 2000) the maintenance requirements for sows ( $100 \times \text{BW}^{0.75}$  kcal ME/d; NRC, 2012). On average, sows received 6,834; 7,434; and 8,110 kcal ME/d during the days 30 to 60, days 61 to 90, and days 91 to 109 of gestation, respectively. To provide a daily energy intake, sows received on average 2.0, 2.2, and 2.4 kg, from days 30 to 60, days 61 to 90, and days 91 to 109 of gestation, respectively. Sows were fed individually by raising the feeder ball valve of an Accu-Drop Feed Dispenser (AP Systems, Assumption, IL) to drop the required amounts of feed into the feeding troughs. The Accu-Drop feed dispensers were calibrated at the days 30, 61, and 91 at various set points and related the volume of feed dispenser ( $Y$ , cm<sup>3</sup>) to kilogram weight of feed ( $x$ ) delivered as:  $Y = 5.4864x + 1.9087$ ;  $R^2 = 0.9892$ . The required daily feed allowance was provided once daily at 0730 h (control, T1), 1130 h (T2), or 1530 h (T3).

### ***Collection of Sow Performance and Reproductive Data***

Sow performance and reproductive data were recorded during gestation and after farrowing, respectively. This includes total born, piglets born alive, number of stillbirths, count of mummified fetuses, birth weight, number weaned, and weaning weight. Return to estrous interval was recorded for each sow. Sow BW and BF thickness were recorded on days 30, 60, 90, and 109 of gestation, within 24 h of farrowing, and at weaning. These gestational stages were chosen as they represent completion of placentation process (day 30), midpoint of a period of high fetal death due to intrauterine competition (day 60), a period of rapid fetal demands for nutrients (day 90), and a point close to farrowing (day

109; Mesa et al., 2012). Approximately, 35 sows farrowed per batch and neonates from these sows were weighed within 24 h after farrowing. Three birth weight categories were used. Piglets weighing less than 1.25 kg (the minimum BW was 870 g), from 1.26 to 1.59 kg, and from 1.6 to 2.01 kg were classified as low birth weight, intermediate birth weight, and high birth weight, respectively (Douglas et al., 2014). Feed not consumed on the day of weaning was subtracted from the total feed offered to determine lactation feed disappearance. Sow lactation feed efficiency was measured as sum of sow BW loss (or gain) and litter weight gain relative to feed disappearance (Rosero et al., 2012). Sow and piglet mortality events were recorded daily.

### ***Chemical Analysis***

Duplicate samples of basal diets were analyzed for DM, GE, CP, NDF, and ADF and maintained a laboratory coefficient of variation of less than 5%. Moisture content of feeds was determined by the oven-drying method (method 934.01) as indicated by AOAC (2006). Gross energy was determined by bomb calorimeter using IKA WERKE c2000 basic bomb calorimeter (IKA Werke GmbH and Co. KG, Staufen, Germany) with benzoic acid in the samples for a standard. The CP content ( $N \times 6.25$ ) in the basal diet was determined using the Kjeldahl method (method 984.13, AOAC, 2006; Kjeltec 2300 Analyzer, Foss, Höganäs, Sweden). Determination of crude fat was by ether extract (method 920.39; AOAC, 2006) using an ANKOM XT15 extraction system (ANKOM Technology, Macedon, NY). Analysis for NDF and ADF was carried out using filter bag technique (ANKOM 2000 fiber analyzer, methods 12 and 13; ANKOM Technology, Macedon, NY; methods 973.18 and 973.19; AOAC, 2006). To determine the total ash content, samples of basal diet were weighed before and after ashing in a high-temperature

muffle furnace at 600 °C for 6 h (Isotemp Muffle Furnace, Thermo Fisher Scientific Inc., Hampton, NH).

### ***Statistical Analysis***

Normality of the data sets was checked using PROC Univariate procedure of SAS 9.4 (SAS Inst., Inc., Cary, NC). Number of mummified fetuses, number of stillborn, 72-h mortality, and preweaning mortality data had excessive zeros and exhibited overdispersion. The data sets were analyzed using PROC GENMOD procedure of SAS with negative binomial distribution. The model provided deviance values of 0.73, 1.14, 0.87, and 0.81, respectively, for goodness-of-fit criteria. Other count and continuous sow reproductive data were analyzed as a randomized complete block design using PROC GLIMMIX with Poisson distribution and PROC MIXED procedures, respectively. The models included treatment group (time of feeding) as a fixed effect and block (batch of sows) as a random effect. Sow was the experimental unit in all analysis. Data collected repeatedly throughout sow's gestation and lactation (BW, BF, BW changes, and BF changes) were analyzed as repeated measures ANOVA using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included fixed effects of treatment, gestation day, and treatment  $\times$  gestation day interaction while block was considered as random effects. Sow BW and BF at day 30 were used as covariate in the repeated measures model. Autoregressive process of first order was used to model repeated observation within sow as covariate structure (Littell et al., 1998). Least squares means of fixed effects with their corresponding SE were calculated using the LSMEANS statement of SAS. The estimation method was based on residual maximum likelihood. A chi-square test of homogeneity was used to determine the proportion of birth weight

category amongst the treatments using the FREQ procedure of SAS. Data are presented as least squares means  $\pm$  SEM. Differences between least squares means were requested using PDIFF option of SAS, and significant differences were declared at  $P \leq 0.05$ , whereas a trend considered between  $0.05 < P \leq 0.10$ . The  $P$  values were adjusted for multiplicity based on the Tukey–Kramer method.

## RESULTS

Treatment did not influence either sow BW or sow BW change in gestation or lactation ( $P > 0.05$ ; Table 2). Sows fed once daily at 1130 and 1530 h had similar BF thickness at day 109 of gestation, farrowing, and weaning compared with the control group ( $P > 0.05$ ). Sows that received their daily ration at 1130 h did not differ in terms of BF gain relative to the control sows during either gestation or lactation ( $P > 0.05$ ). Conversely, sows fed once a day at 1530 h gain BF from day 30 to day 109 of gestation ( $P = 0.04$ ), but not at the complete reproductive cycle (day 30 to wean;  $P = 0.24$ ) compared with the control (0730 h) sows. Sow gestation ADG, lactation ADFI, wean to estrous interval, and efficiency of feed utilization during lactation were not affected by treatment and averaged 0.462 kg/d, 5.65 kg/d, 5.5 d, and 0.466, respectively ( $P > 0.05$ ; Table 3). No differences in total piglet born, live piglet born, average piglet birth weight, number of piglets with low viability, number of mummified fetuses, number of stillborn, average weaning weight, piglets ADG, and adjusted litter weight gain were observed among treatment groups ( $P > 0.10$ ). Number of piglets weaned tended to be 0.54 more piglets in sows fed once daily at 1530 h compared with the control group (10.87 vs. 10.33;  $P = 0.09$ ). Treatment did not influence the proportion of birth weight category evaluated ( $\chi^2 = 2.49$ ;  $P < 0.64$ ).

## DISCUSSION

Nutritional outcomes differed by the timing of feed intake in many animal models under iso-caloric conditions. The present study reports the impact of feeding pregnant sows at different time of the day under limit-fed conditions on sow's performance.

### *Effect of Time of Feeding on Sow BW and BF changes*

The effect of time of feeding in pregnant sows and nutrient utilization by the gravid pig has not been studied. Under iso-caloric conditions per kilogram live metabolic weight, feeding time did not influence sow BW and BW gain during either gestation or lactation. Excessive BW loss during lactation adversely impairs subsequent reproductive performance by extending the weaning to estrous interval (De Bettio et al., 2016). Consumption of total daily energy intake per kilogram live metabolic weight ( $BW^{0.75}$ ) during gestation at different times of the day resulted in similar BW gain during lactation. Subsequently, wean to estrous interval did not differ with reference to feeding time ostensibly due to similar lactation feed intake observed. It was reported that BW loss greater than 12% during lactation extends the weaning-to-service interval in pigs (Eissen et al., 2003; Thaker and Bilkei, 2005). It can be inferred that the nutritional needs of the sows for energy were not compromised at the various feeding times in this study.

Sows fed once a day at 1530 h had increased BF gain during gestation. Feed restriction during the day constrains metabolism to alternate between storage and use of nutrients to ensure a continuous supply of energy to cells between meals (Le Naou et al., 2014). Supply of nutrients to cells may be coordinated with endogenous physiological rhythms such as glucose tolerance to optimize mammalian production and health (Nikkhah, 2012).

Glucose tolerance in human dwindles as the day comes into night signifying the importance of meal timing. Backfat gained in sows fed at 1530 h could be attributable to time of feeding with its associated regulation of plasma triacylglycerols (TAG). Animal fats, including lard, are primarily composed of TAG, diacylglycerols, FFA, phospholipids, sterols, tocopherols, carotenoids, and fat-soluble vitamins, with the TAG being the main components (Rohman et al., 2012). In human studies, eating at night resulted in increased plasma TAG that remains elevated for longer period than response to the same meal given during the day (Sopowski et al., 2001; Johnston, 2014). Similarly, postprandial response to lunch provided approximately 50% less change in plasma TAG concentration than breakfast, although the plasma TAG fraction did not differ in palmitic acid concentration which was included in each meal (Burdge et al., 2003). Therefore, Burdge et al. (2003) concluded that the physiological basis for differences in postprandial TAG response could be independent of absorption or mobilization from the gut but rather to meal timing. It was reported that meal timing alters lipid profile postprandial and regulates adiposity by synchronizing local circadian rhythms in metabolically active tissues (Johnston, 2014). If this phenomenon is applicable to swine, we speculate that the increase BF thickness observed in sows fed at 1530 h could be attributed to elevated levels of plasma TAG concentration, but the physiological mechanism for this observation remains to be elucidated and warrants further study.

Although this study was conducted under limit-fed regime, our result resonates with a study reported by Wang et al. (2014) who stated that morning food intake was not related to obesogenic conditions in human but consumption of more than 33% of daily energy intake as evening meal increased the risk of obesity compared with consumption of more

than 33% of energy intake at or before 12:00 h. However, for ethical reasons, direct quantification of adipose tissues mass gain associated with such feeding time is not usually undertaken in human studies making a direct comparison with our result difficult.

### ***Effect of Time of Feeding on Sow Reproductive Performance***

Neonatal piglet survival rate has declined and remains a major economic and welfare concern in modern pig production (Theil et al., 2014; Baxter and Edwards, 2018). One of the major findings in the current study was that sows fed once daily at 1530 h, tended to wean 0.54 more piglets per litter than sows fed once daily at 0730 h, although the total number of piglets born and litter size after cross-fostering were similar. This seems to be the first study to report the effects of feeding time on sow reproductive performance and definitive explanation for the tendency to increase the number of piglets wean are not clear to us. However, more than 50% of piglet preweaning mortality occurs during the first 3 d after farrowing (Hales et al., 2014; Phillips et al., 2014) due to inadequate colostrum intake, starvation and hypothermia (Kirkden et al., 2013). In addition, neonates have low reserves of glucose and fat at birth and therefore rely exclusively on colostrum and milk intake for their survival (Koketsu et al., 2017). An interesting observation made in this study was that sows fed once at 1530 h had increased BF thickness compared with the other sows that received the same amount of energy at other feeding times. It was also documented that besides dietary effects, the chemical composition of the colostrum and milk of sows depends on the body condition of the sows (Jin et al., 2018). Sows in good condition produced more milk, energy, and protein than thin sows (Klaver et al., 1981). It is therefore speculated that sows fed once daily at 1530 h exhibited high energy reserves to support colostrum and milk synthesis to enhance the piglet's survivability during the



first 3 d of life. Healthy piglets in the postnatal period are paramount to preweaning outcomes and likely to offset the negative impact associated with super-prolific breeding programs to achieve production targets of 35 to 40 piglets per sow per year (Baxter, 2018).

The present study demonstrates that time of feed consumption is a critical regulator of body condition. The literature concerning the effects of time of feeding in pregnant sows to the best of our knowledge is absent, but our data suggest beneficial effect of BF gains in gestation sows under limit-fed condition. Similarly, evening and night feeding benefited ruminants through increased secretions of VFA, surges of blood insulin, lactate, and beta-hydroxybutyrate leading to increased energy production and milk fat synthesis (Nikkhah, 2012). Conversely, feeding at “inappropriate” time entrains rhythms in liver triglycerides and proteins into a phase opposite to the phase of physiological rhythms dictated by the master biological clock. Misalignment of the circadian rhythms resulted in reduction in glucose tolerance, downregulation of satiety hormone leptin in human (Scheer et al., 2009), inefficiency in energy expenditure, hepatic fat accumulation, physical inactivity, and adiposity in rat (Adamovich et al., 2014; Yasumoto et al., 2016). Therefore, further studies are required to evaluate potential welfare implications of such feeding times, metabolic profile, and its underlying circadian mechanisms in pregnant sows.

## **CONCLUSION**

Under limit-fed conditions, feeding pregnant sows once daily at 1130 h did not change sow BW, BF, and reproductive parameters relative to sows fed at 0730 h. Consumption of total daily energy intake once daily at 1530 h was more beneficial for BF gained during gestation, tended to improve the number of piglets weaned, and supported normal reproductive

performance in sows compared with conventional feeding time. Therefore, linking nutritional management techniques to time of feeding may enhance BF gains and number of piglets weaned during gestation and lactation, respectively.

Table 2. 1 Composition and nutrient analysis of gestation and lactation diets, as fed basis

Ingredients, %	Gestation diet	Lactation diet
Corn, Yellow Dent	65.35	61.28
Soybean Meal, Dehull, Sol Extr	10.00	17.20
Corn DDGS <sup>2</sup> , >6 and <% oil	20.00	15.00
Choice White Grease	1.50	3.00
Di-calcium phosphate	1.20	1.15
Limestone, ground	1.00	0.88
Sodium chloride	0.35	0.35
L-Lys-HCL	0.10	0.46
L-Thr	0.00	0.13
DL-Met	0.00	0.01
L-Trp	0.00	0.04
Swine breeder premix (EB Plus <sup>1</sup> )	0.50	0.50
<b>Total</b>	<b>100.00</b>	<b>100.00</b>
<b>Analyzed composition</b>		
DM, %	89.34	89.60
GE, kcal/g	4,431.00	4,576.00
CP, %	15.70	17.90
NDF, %	13.30	12.00
ADF, %	4.80	4.50
<b>Calculated nutrient composition</b>		
SID Metabolizable energy (ME), kcal/kg	3,379.00	3,456.20
SID Lys, %	0.58	1.03
SID Met, %	0.26	0.28
SID Met+Cys, %	0.48	0.53
SID Thr, %	0.45	0.65
SID Trp, %	0.12	0.19
Total available P	0.61	0.61
Ca, %	0.70	0.66
Crude fat, %	6.30	7.40
SID Lys/ME, g/Mcal	1.71	2.96

<sup>1</sup>Mineral and vitamin mixture supplied per kilogram of diets: 15 mg of Cu (as CuSO<sub>4</sub>); 124 gm of Fe (as FeSO<sub>4</sub>·7H<sub>2</sub>O); 40 mg of Mn (as MnO); 124.7 mg of Zn (as ZnO); 2.2 mg of I (as Ca(IO<sub>3</sub>)<sub>2</sub>); 0.30 mg of Se (as Na<sub>2</sub>SeO<sub>3</sub>); 11000 IU of vitamin A; 2750 IU of vitamin D<sub>3</sub>; 55 IU of vitamin E; 4.4 mg of vitamin K<sub>3</sub>; 1.1 mg of thiamine; 9.9 mg of riboflavin; 55 mg of nicotinic acid; 33 mg of d-pantothenic acid; 2.2 mg of pyroxidine; 0.06 mg of vitamin B<sub>12</sub>; 2.6 mg of folic acid; and 0.22 mg of biotin, and 1.92 mg of Na.

<sup>1</sup>Swine breeder premix was supplied by Agric-Nutrition Services, INC. Shakopee, MN.

<sup>2</sup>Dried distiller's grains with solubles.

Table 2. 2 Main effect of treatment during gestation on sow performance (least squares means)<sup>1</sup>

Item	Treatment			P-value
	T1 <sup>2</sup>	T2 <sup>3</sup>	T3 <sup>4</sup>	
Number of sows	58	57	59	-
Body weight, kg				
Initial d 30	211.97 ± 3.44 <sup>6</sup>	211.29 ± 3.42	211.40 ± 3.39	0.85
At d 109	247.97 ± 3.34	248.95 ± 3.42	247.35 ± 3.39	0.78
Farrowing	224.13 ± 3.34	227.42 ± 3.43	224.56 ± 3.39	0.56
Weaning	232.80 ± 3.34	230.86 ± 3.42	231.55 ± 3.37	0.86
Body weight gain <sup>5</sup> , kg				
d 30 to 109	37.03 ± 3.86	37.38 ± 3.83	35.88 ± 3.80	0.81
During lactation	7.97 ± 3.76	3.55 ± 3.74	7.19 ± 3.68	0.42
d 30 to wean	20.79 ± 4.66	19.21 ± 4.64	19.92 ± 4.61	0.87
Sow BF, mm				
Initial d 30	13.69 ± 0.42	13.64 ± 0.41	13.46 ± 0.41	0.66
At d 109	15.81 ± 0.42	16.18 ± 0.41	17.16 ± 0.40	0.19
At farrowing	13.70 ± 0.58	14.67 ± 0.54	15.35 ± 0.52	0.83
At Weaning	13.81 ± 0.42	13.68 ± 0.41	14.64 ± 0.40	0.81
BF gain or loss <sup>5</sup>				
d 30 to 109	2.12 ± 0.50 <sup>a</sup>	2.58 ± 0.50 <sup>ab</sup>	3.69 ± 0.47 <sup>b</sup>	0.04
During lactation	0.17 ± 0.47	-0.66 ± 0.45	-0.73 ± 0.45	0.17
d 30 to weaning	0.11 ± 0.52	0.07 ± 0.52	1.02 ± 0.51	0.24

<sup>1</sup>Feed drops were adjusted on d 30, 61 and d 91 based on the BW of pigs on d 30, 60 and 90 d of gestation, respectively.

<sup>2</sup>Received full daily ration of feed at 0730 h.

<sup>3</sup>Received full daily ration of feed at 1130 h.

<sup>4</sup>Received full daily ration of feed at 1530 h.

<sup>5</sup>BW and BF gain or loss was calculated as the difference between the final and the initial value.

<sup>6</sup>Least squares means ± standard error.

<sup>a-b</sup>Means within a row of treatment with different superscripts differ ( $P \leq 0.05$ ).

Table 2. 3 Main effects of treatment on sow and reproductive performance during gestation and lactation (least squares means)<sup>1</sup>

Item	Treatment			P-value
	T1 <sup>2</sup>	T2 <sup>3</sup>	T3 <sup>4</sup>	
Number of sows	58	57	59	-
Initial BW, d 30	211.97 ± 3.33 <sup>7</sup>	211.58 ± 3.32	211.16 ± 3.37	0.85
Lactation length, d	19.70 ± 0.36	20.15 ± 0.36	19.78 ± 0.35	0.26
ADFI <sup>5</sup> (d 1 - 18), kg	5.59 ± 0.31	5.58 ± 0.31	5.77 ± 0.31	0.71
ADG (d 30 - 109), kg	0.459 ± 0.05	0.474 ± 0.05	0.454 ± 0.05	0.81
Wean to estrus I., d	5.47 ± 0.35	5.56 ± 0.35	5.54 ± 0.35	0.77
Sow lactation G:F	0.479 ± 0.03	0.441 ± 0.03	0.479 ± 0.03	0.20
Total pigs born	14.62 ± 0.61	14.08 ± 0.60	15.04 ± 0.58	0.36
No. born alive	12.02 ± 0.62	12.09 ± 0.61	13.00 ± 0.59	0.25
Mummified fetuses	0.32 ± 0.10	0.39 ± 0.12	0.34 ± 0.10	0.85
No. of stillborn	2.30 ± 0.36	1.59 ± 0.27	1.91 ± 0.29	0.40
Average birth wt., kg	1.39 ± 0.03	1.37 ± 0.04	1.38 ± 0.03	0.85
Litter size after cross fostering	12.42 ± 0.41	12.49 ± 0.41	12.60 ± 0.40	0.91
72 h Mortality, %	4.78 ± 1.66	5.58 ± 1.88	3.81 ± 1.21	0.63
Pre-weaning mortality, %	8.04 ± 1.98	7.99 ± 1.91	6.06 ± 1.37	0.40
Low viability piglets	1.04 ± 0.26	0.94 ± 0.26	0.83 ± 0.25	0.65
Pigs weaned <sup>6</sup> , d 18	10.33 ± 0.23 <sup>x</sup>	10.78 ± 0.23 <sup>xy</sup>	10.87 ± 0.22 <sup>y</sup>	0.09
Av. weaning Wt., kg	6.00 ± 0.13	5.93 ± 0.13	5.99 ± 0.13	0.90
Adjusted litter Wt. gained	44.88 ± 1.75	46.71 ± 1.76	48.02 ± 1.68	0.32
Piglet ADG <sup>5</sup> , kg	0.233 ± 0.01	0.229 ± 0.01	0.233 ± 0.01	0.81

<sup>1</sup>Feed drops were adjusted on d 30, 61 and d 91 based on the BW of pigs on d 30, 60 and 90 d of gestation, respectively.

<sup>2</sup>Received full daily ration of feed at 0730 h.

<sup>3</sup>Received full daily ration of feed at 1130 h.

<sup>4</sup>Received full daily ration of feed at 1530 h.

<sup>5</sup>Lactation length was used as covariate for average lactation feed intake and piglet ADG

<sup>6</sup>Litter size after cross fostering was used as covariate for number of piglets wean

<sup>7</sup>Least squares means ± standard error.

<sup>x-z</sup>Means within a row of treatment with different superscripts tend to differ ( $P \leq 0.10$ ).

**Chapter 3. Effect of feeding frequency and sow parity based on iso-caloric intake  
during gestation on sow performance**

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## SUMMARY

The aim of this study was to investigate the effect of feeding frequency and sow parity based on same rate of maintenance energy intake during gestation on sow performance. One hundred and seventy-seven sows [Topigs Norsvin 70, Landrace × Large White, Topigs Norsvin USA, Burnsville, MN; parity  $3.80 \pm 0.16$ ; initial BW =  $211.34 \pm 3.37$  kg; backfat (BF)  $13.57 \pm 0.54$  mm] were blocked by parity, balanced for BW, and randomly assigned to 1 of 3 treatments in a randomized complete block design. Treatments included sows fed corn–soybean meal-based diet 1× daily at 0730 h (control, T1), 2× daily [half ration at 0730 and 1530 h (T2)], or 3× daily [a third portion at 0730, 1130, and 1530 h (T3)], with daily feed quantity kept at  $1.25 \times$  maintenance energy intake [ $100 \times (\text{BW})^{0.75}$ ] kcal ME/d. Treatments were imposed from day 30 of gestation. Sows received on average 6,921, 7,129, and 7,399 kcal ME/d from days 30 to 60, days 61 to 90, days 91 to 109 of gestation, respectively. Feeding frequency during gestation had no effect on lactation ADFI ( $P > 0.10$ ). Sows fed 3× daily during gestation had improved lactation G:F ( $P = 0.040$ ) compared with sows fed 2× but similar to control sows ( $P = 0.338$ ). Treatment did not alter BW or BW variations during gestation, lactation, or from days 30 to wean ( $P > 0.10$ ). Sows fed 2× daily had tendency to gain BF from day 30 to day 109 of gestation ( $P = 0.053$ ) but tended to lose BF during lactation ( $P = 0.091$ ) relative to the control sows. Feeding frequency (2× and 3× daily) tended to increase the number of piglets weaned by 0.40 ( $P = 0.056$ ) and 0.53 ( $P = 0.098$ ) piglets, respectively, compared with control sows. Sows fed 2× daily during gestation had reduced number of stillborn relative to control sows ( $P = 0.035$ ). From day 30 to wean, gilts had propensity to lose BF relative to P1+ ( $P = 0.094$ ), but lost BF compared with P3+ and P6+ sows ( $P = 0.003$ ). Parity P6+ sows

had highest percentage of both 72 h and pre-weaning piglet mortality than P0, P1+, and P3+ sows ( $P < 0.05$ ). In conclusion, parity (P6+) had greater lactation BW gain but higher mortalities relative to lower parity sows. Sows fed 2× daily tended to gain BF from days 30 to 109 of gestation and had reduced number of stillborn relative to control sows. It appears that increasing gestation sow feeding frequency from 1× daily to 2× and 3× daily may reduce the number of stillborn and increase litter size at weaning although most of the reproductive traits were not affected.

**Key words:** Feeding frequency, iso-caloric intake, pregnant sow, sow parity, sow performance

## INTRODUCTION

The practice of feeding frequency in pregnant sows and its carryover effect in lactation are extensively studied using different approaches and methodologies to evaluate objectives such as increasing fetal fatty acids (Williams et al., 1979), reduction in labor cost of feeding (Michel et al., 1980), improving sow reproductive performance (Wittman, 1986), enhancement of nutrient utilization (Holt et al., 2006), and improvement in animal welfare (Jensen et al., 2012). However, feeding frequency results on performance have been contradictory in pregnant gilts and sows. Schneider et al. (2007) reported that gilts fed 6 times daily during gestation tended to have greater ADG and higher born alive piglets than gilts fed 2 times daily, but this response was not observed in sows. Similarly, Michel et al. (1980) and Wittman (1986) indicated that increasing the feeding frequency of gilts and sows resulted in greater number of born alive piglets. On the contrary, others did not elicit a response of feeding frequency on sow performance traits (Douglas et al., 1998; Holt et al., 2006; Jensen et al., 2012). Lack of standardization of



energy intake among sow feeding frequency studies has resulted in conflicting reports. For instance, Michel et al. (1980), Holt et al. (2006), Schneider et al. (2007), and Jensen et al. (2012); fed 1.9, 1.88, 2.5, and 2.6 kg corn–soybean or barley–soybean meal-based diets to provide daily energy intake of 6,371, 6,200, 8,140, and 8,362 kcal of ME/d, respectively, during gestation. A procedure is required to standardize the experimental dietary level of the energy intake among experiments. Utilizing kilogram live BW<sup>0.75</sup> at days 30, 60, and 90 to adjust the level of energy intake during gestation enables the standardization of the ME intake. Therefore, the objective of this study was to investigate the effect of feeding frequency and sow parity given similar energy intake per kilogram live BW<sup>0.75</sup> during gestation on sow performance.

## **MATERIALS AND METHODS**

### ***Animals, Housing, and Management***

The study was conducted at the sow unit of University of Minnesota - Southern Research and Outreach Center, Waseca, MN. University of Minnesota Institutional Animal Care and Use Committee approved the protocol used in the study (IACUC number 171011961). Five contemporary groups of Topigs Norsvin sows (Landrace × Large White, Topigs Norsvin USA, Burnsville, MN); total  $N = 177$ ; initial average BW  $210.12 \pm 3.93$  kg; and average parity of  $3.8 \pm 0.13$  (range 0 to 9).

A total of 177 multiparous and nulliparous sows from 5 contemporary groups were used in the study. Sows were kept in gestation stalls with fully slatted floors measuring (2.1 m × 0.59 m × 0.97 m) under temperature-controlled environment ( $22 \pm 1$  °C). Sow BW and backfat (BF) depth were recorded on day 30 of gestation prior to feeding. Backfat depth

was measured at the last rib and 0.55 m from the dorsal midline using an ultrasound scanner (Lean-meater, Renco Corp., Minneapolis, MN) on both the left and right side and averaged. Gilts and sows were artificially inseminated at onset of standing estrus and repeated 24 h later using fresh diluted semen from Duroc boar (Compart Boar Store, Nicollet, MN).

Sows pregnancy status was confirmed on day 35 after inseminations with the aid of ultrasonic pregnancy checker (Classic Medical Supply, Inc., Florida). The conception rate was 88.9% for the 5 contemporary groups. Water was available on an ad libitum basis to sows through nipple drinker in stalls. On day 109 of gestation, sows were washed, moved to environmentally controlled farrowing rooms, and placed in individual farrowing stalls (2.13 m × 0.97 m × 0.66 m). Sows were fed the lactation diet of 2.27 kg (32.84 MJ ME/d) once per day irrespective of treatments until farrowing. After parturition, sows were fed twice daily at about 0800 and 1430 h. Lactation feed offered was weighed and marked on individual sow card during feeding and the amount of feed offered increased gradually to allow for ad libitum feed intake but preventing feed accumulation in feeders. Surplus feed from each crate was weighed and recorded at weaning. Litter size was equalized within 24 h postpartum through cross-fostering to approximately 12 pigs per litter within treatment.

The farm standard management processing procedures of piglets include intramuscular injection of 1 mL iron dextran shots, tail docking, cutting, and disinfection of naval cords within 24 h post-farrow. Room temperature was maintained at  $24 \pm 2$  °C except at farrowing when supplemental heat lamp provided source of heat for piglets after birth for

48 h while they had access to floor heat pads. No creep feed was provided but piglets had access to the dam's feed. Male piglets surgically castrated between 5 and 8 d of age and all piglets vaccinated against porcine circovirus disease 4 d prior to weaning. Weaning occurred between 0800 and 1000 h on day  $19 \pm 0.25$  postpartum. At weaning, sow BW and ultrasound BF thickness were recorded before transferring them to stalls for daily estrus detection using a mature boar housed in Contact-O-Max boar cart (Hog Slat Inc., Newton Grove, NC). Estrus was recorded when sows exhibited standing heat on exposure to the mature boar and estrous interval from weaning calculated. Culling reason of sows included failure to conceive after second mating, anestrus exceeding 8 d postweaning, consecutive abortions, and difficulty to carry body weight on limbs. An amount of 2 kg (28.3 MJ ME/d) of gestation feed was offered once a day in stalls to sows and gilts from weaning through breeding to 30 d, which is considered optimal during that phase.

### ***Experimental Design, Dietary Treatments, and Feed Line Calibration***

Sows were blocked by parity, breeding date, and balanced for body weight, and randomly allocated to 1 of 3 treatments of feeding frequency with 11 to 12 replicates per batch in completely randomized block design. All contemporary groups of sows received a common corn–soybean meal-based diet during gestation and lactation. Nutrient specifications (Table 1) met or exceeded NRC (2012) nutrient requirements for gestating and lactating pigs. Dietary treatments were imposed from 30 d of gestation until day 109 of gestation following the procedures of Michel et al. (1980). Amount of feed fed from days 30 to 60, days 61 to 90, and days 91 to 109 of gestation was adjusted using body weights on days 30, 60, and 90, respectively. Daily ME intake was standardized using live metabolic BW ( $BW^{0.75}$ ) based on recommendations of Le Naou et al. (2014) and fed at

1.25 times (Prunier and Quesnel, 2000) the maintenance requirements for sows ( $100 \times \text{BW}^{0.75}$  kcal ME/d; NRC, 2012). On average, sows received 28.96, 29.83, and 30.96 MJ ME/d during the first, second, and third trimester of gestation, respectively. To provide the desired daily energy intake, sows received on average 2.17 kg (28.96 MJ ME/d), 2.24 kg (29.89 MJ ME/d), and 2.32 kg (30.96 MJ ME/d), from days 30 to 60, days 61 to 90, and days 91 to 109 of gestation, respectively. Sows were fed individually by raising the feeder ball valve of an Accu-Drop feed dispenser (AP Systems, Assumption, IL) to drop the required amounts of feed into the feeding troughs. The Accu-Drop feed dispensers were calibrated on days 30, 61, and 91 at various set points and related the volume of feed dispenser ( $Y$ ,  $\text{cm}^3$ ), to kilogram weight of feed ( $x$ ) delivered as  $Y = 5.4864x + 1.9087$ ;  $R^2 = 0.9892$  (Fig. 1). The required daily full allowance of feed was provided once ( $1\times$ ) daily at: 0730 h (control, T1), twice ( $2\times$ ) daily (half ration each at 0730 and 1530 h, T2), or thrice ( $3\times$ ) daily (a third portion of ration allotment at 0730, 1130, and 1530 h, T3).

### ***Collection of Sow Performance and Reproductive Data***

Data about sow performance and reproductive parameters were recorded during gestation and lactation, respectively. This includes total born piglets, live born piglets, birth weight, number of stillbirths, count of mummified fetuses, number weaned, weaning weight, and return to estrous interval. Sow BW and BF depth were recorded on days 30, 60, 90, and 109 of gestation, within 24 h of farrowing, and at weaning. These gestational stages were selected as they represent completion of placentation process (day 30), midpoint of a period of high fetal death due to intrauterine competition (day 60), a period of rapid fetal demands for nutrients (day 90), and a point close to farrowing (day 109) (Mesa et al., 2012). Approximately 35 sows farrowed per batch from 5 contemporary groups and

piglets from these sows were weighed within 24 h of farrowing. To study the effect of parity, sows were grouped into 1 of 4 categories as of day 109 of gestation. Parity categories were defined as parity 0 = pregnant gilts that had not produced a litter ( $n = 25$ ), parity 1+ = first and second parity sows ( $n = 46$ ), parity 3+ = third to fifth parity sows ( $n = 54$ ), and parity 6+ = sixth to ninth parity sows ( $n = 52$ ), modified from Rootwelt et al. (2013). Lactation feed disappearance was calculated as the difference between the total feed offered and surplus feed not consumed on the day of weaning. Sow lactation feed efficiency was calculated as summation of sow BW loss (or gain) and litter weight gain relative to feed disappearance (Rosero et al., 2012). Sow and piglet preweaning mortality events were recorded daily.

### ***Chemical Analysis***

Feed samples were analyzed for DM, GE, CP, NDF, and ADF. Dry matter content of feeds was determined by the oven-drying method (method 934.01; AOAC, 2006). Gross energy was determined by basic bomb calorimeter (IKA Werke GmbH and Co. KG, Staufen, Germany) with benzoic acid in the samples being used as calibration. The CP content ( $N \times 6.25$ ) in the basal diet was determined using Kjeldahl method (method 984.13, AOAC, 2006; Kjeltex 2300 Analyzer, Foss, Höganäs, Sweden). Determination of crude fat was by ether extract (method 920.39; AOAC, 2006) using an ANKOM XT15 extraction system (ANKOM Technology, Macedon, NY). The NDF and ADF content were determined using filter bag technique (ANKOM 2000 fiber analyzer, method 12 and 13; ANKOM Technology, Macedon, NY) (methods 973.18 and 973.19; AOAC, 2006). To determine the total ash content, samples of basal diet were ashed in a high temperature

muffle furnace at 600 °C for 6 h (Isotemp Muffle Furnace, Thermo Fisher Scientific Inc., Hampton, NH).

### ***Statistical Analysis***

All data were checked for normality using PROC UNIVARIATE procedure of SAS (SAS Inst., Inc., Cary, NC). The data sets (born alive piglets, mummified fetuses, and number of stillborn) were analyzed as discrete binomial proportions using PROC GENMOD procedure of SAS. The ILINK option of SAS was used to determine least squares means and SE of the proportions. Other count and continuous data were analyzed as a randomized complete block design using PROC GLIMMIX with Poisson distribution and PROC MIXED procedures, respectively. When interactions are not significant, they were removed from the model and the final statistical model included fixed effects of treatment and parity; block was random effect with sow as the experimental unit in all analyses.

The repeated measures model for sow BW, BF, BW changes, and BF changes included fixed effects of treatment (feeding frequency), parity, gestation day, treatment × gestation day interaction, parity × gestation day interaction, and treatment × parity × gestation day interaction, whereas block was considered a random effect. Sow BW and BF at day 30 were used as covariate in the repeated measures model. Autoregressive process of first order was used to model repeated observation within sow as covariate structure (Littell et al., 1998). Least squares means of fixed effects with their corresponding SE were calculated using the LSMEANS statement of SAS. The estimation method was based on residual maximum likelihood (REML). Data are presented as means ± SEM. Difference between least squares means was requested using PDIFF of SAS and significant

differences were declared at  $P \leq 0.05$  while a trend was considered between  $0.05 < P \leq 0.10$ . The Tukey–Kramer’s adjustment method for multiple comparisons was used for means separation.

## RESULTS

No two- or three-way significant interactions were observed for any sow performance traits measured ( $P > 0.10$ ) except that gilts fed 3× daily during gestation had improved lactation feed efficiency compared with those fed 2× daily (0.321 vs. 0.524;  $P = 0.014$ ).

### *Effects of Feeding Frequency and Parity on Sow BW and BF under Iso-caloric*

#### *Condition per Kilogram Live BW<sup>0.75</sup>*

Feeding frequency (either 2× or 3×) did not influence sow BW and BW variations in gestation, lactation, or from day 30 to wean compared with the control sows ( $P > 0.10$ ; Table 2). Sow parity affected BW at day 109 with P0 sows having greater BW over parity P3+ ( $P = 0.046$ ) and P6+ ( $P = 0.002$ ) sows but not different from P1+ sows ( $P = 0.452$ ). Consequently, gilts had greater BW gain during gestation than P3+ ( $P = 0.050$ ) and P6+ ( $P = 0.020$ ) sows but similar to P1+ sows ( $P = 0.694$ ; Table 2). Conversely, gilts lost the greatest weight during lactation followed by P1+, P3+, and P6+ ( $P = 0.001$ ). From day 30 to wean, BW gain was greatest for parity P6+ compared with P0 ( $P = 0.001$ ), P1+ ( $P = 0.001$ ) but similar to P3+ ( $P = 0.278$ ) sows. Sows fed 2× daily had tendency to gain BF from days 30 to 109 of gestation ( $P = 0.053$ ) but tended to lose BF during lactation ( $P = 0.091$ ) relative to the control sows. Feeding sows 3× daily resulted in similar BF gains during gestation compared with sows fed 2× ( $P = 0.288$ ) and control group ( $P = 0.697$ ). Lactation BF lost tended to be greater with sows fed 3× daily ( $P = 0.077$ ) and 2×

daily ( $P = 0.091$ ) relative to sows on single feeding regime. Sows on 2× feeding schedule had similar lactation BF lost compared with sows fed 3× daily ( $P = 0.124$ ). Sow parity did not affect gestation and lactation BF gain ( $P > 0.10$ ). From day 30 to wean, gilts lost more BF compared with P3+ ( $P = 0.003$ ) and P6+ ( $P = 0.003$ ) sows but had tendency to lose BF relative to P1+ ( $P = 0.094$ ). Parity P1+, P3+, and P6+ sow had similar BF gain from day 30 to wean ( $P > 0.10$ ).

***Effects of Feeding Frequency and Parity on Sow and Reproductive Performance under Iso-caloric Condition per Kilogram Live BW<sup>0.75</sup>***

Feeding frequency did not affect ( $P > 0.10$ ) gestation ADG, sow lactation ADFI, lactation length, and wean-to-estrus interval relative to the conventional feeding regime (Table 3). Sows fed 3× daily during gestation had improved lactation feed efficiency compared with sows fed 2× daily ( $P = 0.040$ ) but similar to sows fed 1× daily ( $P = 0.338$ ). Compared with the conventional feeding regime, feeding sows 2× ( $P = 0.056$ ) or 3× daily ( $P = 0.098$ ) tended to have larger litter size at weaning. Sows fed 2× daily had lower number of stillborn relative to the control sows ( $P = 0.035$ ) but similar to sows fed 3× daily ( $P = 0.180$ ). Sows fed 2× and 3× daily had similar number of stillborn ( $P = 0.782$ ) and piglets weaned ( $P = 0.997$ ). Feeding frequency did not change ( $P > 0.10$ ) total number of piglets born, live piglet born, mummified fetuses, average piglet birth weight, 72 h mortality, pre-weaning mortality, number of piglets with low viability, average weaning weight, piglet ADG, and adjusted litter weight gain. Parity P6+ ( $P = 0.007$ ) and P3+ ( $P = 0.005$ ) had greater number of stillborn than P1+ sows.



Younger sows (group P0) had lower lactation ADFI than sows of P1+, P3+, and P6+ ( $P = 0.001$ ; Table 3). Sow parities P1+ ( $P = 0.031$ ) and P3+ ( $P = 0.014$ ) had improved lactation feed efficiency than parity group P0 sows but did not differ from P6+ sows ( $P = 0.122$  and  $P = 0.954$ ), respectively. Wean-to-estrus interval was not different among sow parities ( $P > 0.10$ ) or treatment ( $P > 0.10$ ). Older sows (P6+) had shorter ( $P = 0.033$ ) lactation length relative to parity P3+ sows. When parity increased from parity 0 to 5, there were increases in total piglets born and piglet born alive, but these parameters declined after parity 5 ( $P < 0.05$ ). Compared with P3+ ( $P = 0.027$  and  $P = 0.028$ ) and P6+ ( $P = 0.008$  and  $P = 0.038$ ), P1+ sows had greater piglet birth weight and reduced number of low viable piglets, respectively. No difference in number of mummified piglets was detected among sow parity ( $P > 0.10$ ). Parity P6+ sows had higher percentage of 72 h piglet mortality compared with P1+, P3+, and P0 sows ( $P < 0.05$ ). Similarly, pre-weaning piglet mortality was greater in parity P6+ relative to P0, P1+, and P3+ sows ( $P < 0.05$ ).

## **DISCUSSION**

Feeding frequency results in pregnant sows has been contradictory in experiments (Williams et al., 1979; Michel et al., 1980; Wittman, 1986; Holt et al., 2006; Schneider et al., 2007). The discrepancies could be partly due to differences in methods of equalizing energy intake between different feeding regimes in the experiments. For the first time we fed nulliparous and multiparous sows at 125% of their maintenance requirement as recommended by NRC (2012) to arrive at feed restriction levels similar to their normal gestation energy intake to throw more light on effects of feeding frequency and parity on sow performance. It is common to restrict breeding dams to 10% to 65% of their ad libitum feed intake which is above, below, or equal to energy requirements for maintenance. Such

level of feed restriction results in retarded growth in prepubertal, mature, and early pregnant gilts (Prunier and Quesnel, 2000), suppressed growth (Booth et al., 1996), or enhanced growth (Amdi et al., 2013). In the current study, feeding nulliparous and multiparous pregnant pigs 25% above their maintenance requirements from day 30 of gestation increased body weight of breeding dams throughout gestation, suggesting that their requirement for energy was not compromised.

### ***Effects of Feeding Frequency under Iso-caloric Intake per live BW<sup>0.75</sup> on Sow***

#### ***Performance***

Under iso-caloric conditions per kilogram live BW<sup>0.75</sup>, meal frequency did not influence sow BW changes either during gestation or lactation. This result resonates with the findings of other researchers under pair feeding regime (Romsos et al., 1978; Schneider et al., 2007) but contradicts the results of Holt et al. (2006). Holt et al. (2006) reported that sows fed 2× daily during gestation consistently gained BW in gestation and throughout lactation relative to sows fed 1× daily. The difference in results is attributable to differences in feeding levels. While sows in the current study received similar energy per kilogram live BW<sup>0.75</sup>, Holt and others fed their sows to a target BCS of 3 and some sows received additional feed when desirable. Our results and others (Romsos et al., 1978; Schneider et al., 2007) suggest that an alteration in meal frequency does not influence BW, provided the sows are pair-fed or fed based on kilogram live BW<sup>0.75</sup>.

Sow fed 2× daily tended to have increased BF thickness during gestation compared to the control sows but this observation was not evident in sows fed 3× daily. Our initial assumption was that under iso-caloric conditions, consumption of multiples meals within

the day could be a metabolic advantage. Our data suggest that under limit-fed regime, the threshold of 2× daily feeding is more desirable than 3× daily feeding regime. At the whole animal level, Sharma et al. (1973) reported that pigs fed thrice daily had greater maintenance requirements, greater energy needs for digestion due to the thermic effect of feed and increase in activity and energy expenditure. Similarly, rats (Leveille and O’Hea, 1967) and pigs (Friend and Cunningham, 1964) fed 2× or 1× daily were generally less sensitive to the excitation associated with the distribution of feed than animals receiving multiple small meals. Also, LeBlanc and Diamond (1986) reported that diet-induced thermogenesis was doubled in dogs that were fed 4 small meals compared to single meal of iso-caloric diet. Therefore, it could be speculated that nulliparous and multiparous sows fed 2× in our study had lower energy expenditure than the group fed 3× daily and this probably explains the greater BF in sows fed 2× vs. 3×.

However, the greater gestational BF gain of sows fed 2× daily relative to sows fed 1× daily is puzzling since they received the same amount of dietary energy per kilogram  $BW^{0.75}$ . It is documented that given similar quantity of feed, sows fed 2× daily spent more time feeding than similar group of sows receiving their daily ration 1× (Holt et al., 2006) while heat production (HP) per minute of standing activity was 14.9 kJ in sows (Noblet et al., 1993). We therefore expected sows fed 2× daily to have increased activity, greater maintenance energy requirement, and reduced BF compared to sows fed 1× daily. We theorized that sows fed 2× daily utilize dietary energy more efficiently than sows fed 1× daily. Therefore, we speculate that the marginal energy derived from 2× feeding is far more than is needed to compensate for the marginal energy required for increased activity and feeding. Botermans et al. (2000) noticed a greater output of enzymes from the

exocrine pancreas when pigs were fed 12 small meals in comparison with 1 large meal per day. However, it was reported that energy digestibility of diets was not influenced by feeding frequency in sows (Holt et al., 2006) and with young pigs (Sharma et al., 1973). The proper explanation for this disparity in sow BF gain requires further investigation. Although feeding frequency did not influence BW changes in sows, it tended to affect BF thickness. Therefore, BW change alone, without assessing BF change, can be misleading when evaluating sows during gestation and lactation.

The number of pigs weaned per sow per year (PWSY) is a short-term good measurement of sow herd productivity (Koketsu et al., 2017). One of the significant findings in the current study was that sows fed 2× and 3× daily during gestation tended to wean 0.40 and 0.53 more piglets, respectively, than sows on the conventional feeding regime. Previously, Holt et al. (2006) and Williams et al. (1979) reported that feeding frequency had no effect on any litter performance traits. A plausible explanation for differences in results is the method of feeding between experiments. To standardize our experimental conditions in terms of ME intake, we fed our sows based on their live  $BW^{0.75}$  and adjusted the feed with advancing of pregnancy. On the other hand, Holt et al. (2006) fed 1.88 kg of feed and increased the feed at day 40 and 80 of gestation if sow BCS was below 3. Although Holt et al. (2006) feeding regime resulted in a 0.5 mm gain in BF from day 40 to 109 of gestation, our feeding regime resulted in 3.38 mm gain in BF thickness from day 30 to 109 of gestation. We speculate that higher BF gains in our study provided additional energy reserves that was mobilized to support colostrum and milk synthesis enabling piglet survival and improved the number of piglets weaned since sows in good condition produce milk with a higher fat and energy content relative to thin sows (O'Grady et al.,

1973; Klaver et al., 1981; Amdi et al., 2013). Additionally, the amount of body fat reserves stored during gestation partly determines the performance during lactation (Amdi et al., 2013).

Feeding frequency elicited a response on the number of stillborn. Sows fed 2× daily had lower number of stillborn compared to sows fed 1× daily during gestation. Pigs utilize glucose as the main energetic substrate for fetal growth and the rate of glucose utilization decreases in sows during late pregnancy to spare glucose for the gravid uterus utilization (Raguvaran et al., 2017); which also occurs in pregnant rat (Leturque et al., 1984). Physiological reduction in maternal glucose tolerance during late gestation resulted in higher number of stillborn piglets (Schaefer et al., 1991). We speculate that increasing meal frequency during gestation enhanced glucose tolerance of pregnant sows by spreading the nutrients load during the day to reduce the number of stillborn at farrowing.

#### ***Effects of Sow Parity under Iso-caloric Intake per live $BW^{0.75}$ on Sow Performance***

With respect to parity, BW gain and ADG during gestation was greater in gilts than older sows. Kraeling and Webel (2015) explained that while mature sows have decreased growth rate, gilts are still growing and therefore have a higher growth rate. Furthermore, maintenance during late gestation represents approximately 80% and 60% of energy requirement for sows and gilts, respectively (NRC, 2012). Hence, sows preferentially partition more energy toward maintenance than gilt while gilts partition more energy toward growth than sows (Gonçalves et al., 2016). As such, parity of sow BW gain during gestation followed a pattern with younger sows gaining more than older sows. Parity

category P0, P1+, P3+, and P6+ gained 44.83, 39.71, 35.69, 33.76 kg, respectively, during gestation.

In this study, gilts' feed intake during lactation was lower than other sow parities. This resonates with a body of scientific literature (Gonçalves et al., 2016; Pouloupoulou et al., 2018). Lower lactation feed intake of primiparous sows was attributable to lower feed intake capacity which led to significant BW losses in lactation. The amplitude and the nature of the nutritional deficit are determinants of the degree of body reserve mobilization (Quesnel et al., 2005). It was reported that BW loss greater than 12% during lactation extends the weaning-to-service interval in primiparous sows (Eissen et al., 2003; Thaker and Bilkei, 2005). Lactation weight loss of 6.1% in our primiparous sows did not prolong the wean-to-service interval relative to older parity category sows. Wean-to-service intervals appeared to be minimized at lactation weight losses of about 5% (Thaker and Bilkei, 2005).

Both 72 h and pre-weaning piglet's mortality was greater in older sows (P6+) than younger parity sows and gilts. The finding is consistent with data presented by Gonçalves et al. (2016). The number and positioning of functional teats are both important for suckling piglets. Piglets are inclined to suckle first from teats that are close to the abdominal midline with longer inter-teat distances (Balzani et al., 2016). With younger parity sows, proximity to the midline renders teats more accessible. However, with older parities sows, larger udders may make teat access for piglets more difficult (Vasdal and Andersen, 2012; Alexopoulos et al., 2018). In addition, older sows are more susceptible to lameness (Li and Gonyou, 2013) and increase the possibility of piglet crushing which will culminate

in higher mortality rates (Pluym et al., 2013). The magnitude of this impact on production was observed at weaning where older sows wean fewer piglets than younger parity sows even though litter size was equalized 24 h postpartum through cross-fostering. Cross-fostering is widely adopted swine industry practice to reduce the risk of high piglet mortality when piglet numbers exceed available functional teats or sow's rearing capacity (Baxter et al., 2013). To improve production efficiency, our data suggest that cross-fostering to balance the number of piglets nursed by a sow may work with younger parities but not older or advanced parities or litter standardization practiced instead of litter size equalization as fostering strategy.

Parity groups 3+ and 6+ sows produced higher number of stillborn. The occurrence of stillbirth is usually greatest in high parity sows, which could be attributed to poor uterine muscle tone, longer farrowing duration, and dystocia caused by fatness (Kirkden et al., 2013). In addition, Maes et al. (2004) reported a significant association between low BF at the end of gestation with a high incidence of stillborn piglets. They reported that sows with 14 to 15.5 mm BF or more had a lower percentage of stillborn piglets. Our data suggest that sows with 17.1 mm BF or more had reduced number of stillborn piglets at the end of gestation which was observed in parity categories 0 and 1+ sows.

## **CONCLUSION**

Under iso-caloric intake per kilogram live metabolic weight, feeding frequency had no effect on pregnant sows' BW changes during gestation, lactation feed intake, litter characteristics at farrowing, piglet' BW and gain at the end of the suckling period. Feeding gestation sows 2× daily tended to increase BF depth during gestation and reduced the

number of stillborn compared with control sows. Sows fed 2× and 3× daily during gestation exhibited a potential to improve the number of piglets weaned compared with sows on once a day feeding regime. Under iso-caloric conditions per live BW<sup>0.75</sup>, sow parity did not affect gestation BF change although gilts had greatest BW gain. Parity (P6+) sows had greatest BW gain during lactation but greater number of stillborn, 72 h, and pre-weaning mortalities relative to younger sows.



Table 3. 1 Composition and nutrient analysis of gestation and lactation diets, as fed basis

Ingredients, %	Gestation diet	Lactation diet
Corn, Yellow Dent	65.35	61.28
Soybean Meal, Dehulled, Solvent Extraction	10.00	17.20
Corn DDGS, > 6 and < 9% oil	20.00	15.00
Choice White Grease	1.50	3.00
Di-calcium phosphate	1.20	1.15
Limestone, ground	1.00	0.88
Sodium chloride	0.35	0.35
L-Lys-HCL	0.10	0.46
L-Thr	0.00	0.13
DL-Met	0.00	0.01
L-Trp	0.00	0.04
Swine breeder premix (EB Plus <sup>1</sup> )	0.50	0.50
<b>Total</b>	<b>100.00</b>	<b>100.00</b>
<b>Analyzed composition</b>		
DM, %	89.34	89.60
GE, kcal/g	4,431	4,576
CP, %	15.70	17.90
NDF, %	13.30	12.00
ADF, %	4.80	4.50
<b>Calculated nutrient composition</b>		
SID Metabolizable energy (ME), kcal/kg	3,379	3,456
SID Lys, %	0.58	1.03
SID Met, %	0.26	0.28
SID Met+Cys, %	0.48	0.53
SID Thr, %	0.45	0.65
SID Trp, %	0.12	0.19
Total available P	0.61	0.61
Ca, %	0.70	0.66
Crude fat, %	6.30	7.40
SID Lys/ME, g/Mcal	1.71	2.96

<sup>1</sup>Mineral and vitamin mixture supplied per kilogram of diets: 15 mg of Cu (as CuSO<sub>4</sub>); 124 gm of Fe (as FeSO<sub>4</sub>·7H<sub>2</sub>O); 40 mg of Mn (as MnO); 124.7 mg of Zn (as ZnO); 2.2 mg of I (as Ca(IO<sub>3</sub>)<sub>2</sub>); 0.30 mg of Se (as Na<sub>2</sub>SeO<sub>3</sub>); 11000 IU of vitamin A; 2750 IU of vitamin D<sub>3</sub>; 55 IU of vitamin E; 4.4 mg of vitamin K<sub>3</sub>; 1.1 mg of thiamine; 9.9 mg of riboflavin; 55 mg of nicotinic acid; 33 mg of d-pantothenic acid; 2.2 mg of pyridoxine; 0.06 mg of vitamin B<sub>12</sub>; 2.6 mg of folic acid; and 0.22 mg of biotin, and 1.92 mg of Na.

<sup>1</sup>Swine breeder premix was supplied by Agric-Nutrition Services, INC. Shakopee, MN.

<sup>2</sup>Dried distiller's grains with solubles.

Table 3. 2 Main effects of feeding frequency and parity on sow's performance during gestation and lactation under iso-caloric intake (least squares means)<sup>1</sup>

Item	Feeding frequency <sup>2</sup>			Parity <sup>2</sup>			
	T1(1×)	T2 (2×)	T3 (3×)	P0 <sup>3</sup>	P1+ <sup>4</sup>	P3+ <sup>5</sup>	P6+ <sup>6</sup>
Sow number	58	61	58	25	46	54	52
Sow BW, kg							
Initial d 30	207.79 ± 4.26 <sup>2</sup>	211.49 ± 3.36	211.09 ± 4.17	200.46 ± 5.29	206.45 ± 4.50	214.14 ± 4.23	219.44 ± 4.28
At d 109	245.15 ± 4.23	250.73 ± 4.11	248.59 ± 4.16	246.00 ± 5.67 <sup>a</sup>	244.35 ± 4.58 <sup>ab</sup>	249.49 ± 4.17 <sup>b</sup>	252.78 ± 4.25 <sup>b</sup>
Farrowing <sup>7</sup>	221.48 ± 5.24	228.32 ± 5.14	224.86 ± 5.19	221.00 ± 5.19	220.84 ± 5.53	224.51 ± 5.19	233.21 ± 5.25
Wean	226.96 ± 4.06	231.65 ± 3.97	228.72 ± 4.03	206.73 ± 5.18	221.97 ± 4.37	237.74 ± 4.02	250.05 ± 4.07
Sow BW Changed, kg							
d 30 to 109	37.95 ± 4.02	40.15 ± 4.00	37.39 ± 4.00	44.83 ± 4.32 <sup>a</sup>	39.71 ± 4.09 <sup>ab</sup>	35.69 ± 4.01 <sup>b</sup> <sup>c</sup>	33.76 ± 4.03 <sup>c</sup>
Lactation	6.74 ± 3.64	4.51 ± 3.61	4.83 ± 3.63	-14.27 ± 4.16 <sup>a</sup>	1.83 ± 3.74 <sup>b</sup>	13.21 ± 3.58 <sup>c</sup>	16.34 ± 3.60 <sup>d</sup>
d 30 to wean	19.53 ± 4.80	20.62 ± 4.78	18.01 ± 4.78	8.87 ± 5.29 <sup>a</sup>	16.74 ± 4.87 <sup>b</sup>	23.18 ± 4.79 <sup>c</sup>	28.85 ± 4.81 <sup>d</sup>
Sow BF, mm							
Initial d 30	14.72 ± 0.62	14.28 ± 0.60	14.03 ± 0.61	18.60 ± 0.82	14.01 ± 0.67	12.54 ± 0.61	13.57 ± 0.48
At d 109	16.91 ± 0.81	17.83 ± 0.79	17.25 ± 0.80	22.05 ± 1.09	17.42 ± 0.88	15.58 ± 0.80	14.28 ± 0.81
Farrowing	14.68 ± 1.82	16.59 ± 1.74	15.16 ± 1.75	18.98 ± 1.98	14.90 ± 1.83	14.89 ± 1.85	13.12 ± 1.77
Weaning	14.61 ± 0.70	15.17 ± 0.68	14.53 ± 0.69	17.63 ± 0.92	14.27 ± 0.76	13.76 ± 0.69	13.42 ± 0.70
Sow BF Changed, mm							
d 30 to 109	2.19 ± 0.64	3.56 ± 0.63	3.21 ± 0.54	3.37 ± 0.80	3.49 ± 0.68	3.06 ± 0.64	2.03 ± 0.65
Lactation	-0.24 ± 0.65	-0.85 ± 0.62	-0.88 ± 0.63	-1.62 ± 0.69	-0.97 ± 0.65	-1.23 ± 0.65	0.20 ± 0.63
d 30 to wean	-0.12 ± 0.60	0.87 ± 0.59	0.52 ± 0.59	-1.13 ± 0.72 <sup>a</sup>	0.41 ± 0.63 <sup>b</sup>	1.24 ± 0.60 <sup>b</sup>	1.17 ± 0.60 <sup>b</sup>

<sup>1</sup>BW and BF at d 30 was used as covariate for BW and BF evaluations at d 109, 24 h post-partum, and at weaning. <sup>2</sup>Least square means ± SE.

<sup>3</sup>Parity group 0 = pregnant gilts that had not produced a litter. <sup>4</sup>Parity group P1+ = first and second parity sows.

<sup>5</sup>P parity group P3+ = third to fifth parity sows. <sup>6</sup>P parity group P6+ = sixth to ninth parity sows.

<sup>7</sup>No apparent two-way significant interaction was noticed for any of the variables tested.

<sup>a-c</sup>Means within a row of treatment with uncommon superscripts differ ( $P \leq 0.05$ ).

<sup>a-d</sup>Means within a row of parity with uncommon superscripts indicate a trend ( $P \leq 0.05$ ).

Table 3. 3 Main effects of feeding frequency and parity on sow's reproductive performance during lactation (least squares means)<sup>1</sup>

Item	Feeding frequency			Parity			
	T1 (1×)	T2 (2×)	T3 (3×)	P0 <sup>2</sup>	P1+ <sup>3</sup>	P3+ <sup>4</sup>	P6+ <sup>5</sup>
Number of sows	58	61	58	25	46	54	52
Lactation length, d	19.70 ± 0.25 <sup>6</sup>	19.69 ± 0.24	19.82 ± 0.25	19.44 ± 0.33 <sup>ab</sup>	19.83 ± 0.27 <sup>ab</sup>	20.27 ± 0.25 <sup>a</sup>	19.41 ± 0.25 <sup>b</sup>
ADFI (d 1 - 19), kg	5.40 ± 0.24	5.55 ± 0.23	5.41 ± 0.24	3.94 ± 0.29 <sup>a</sup>	5.64 ± 0.25 <sup>b</sup>	6.01 ± 0.24 <sup>b</sup>	6.23 ± 0.24 <sup>b</sup>
ADG (d30 - d109), kg	0.474 ± 0.05	0.497 ± 0.05	0.473 ± 0.05	0.568 ± 0.06 <sup>a</sup>	0.487 ± 0.05 <sup>ab</sup>	0.453 ± 0.05 <sup>b</sup>	0.417 ± 0.05 <sup>b</sup>
Wean to estrus interval, d	5.49 ± 0.30	5.57 ± 0.29	5.46 ± 0.30	5.67 ± 0.30	5.40 ± 0.30	5.41 ± 0.30	5.54 ± 0.30
Sow lactation G:F <sup>7</sup>	0.472 ± 0.04 <sup>ab</sup>	0.451 ± 0.04 <sup>a</sup>	0.504 ± 0.04 <sup>b</sup>	0.421 ± 0.04 <sup>a</sup>	0.501 ± 0.04 <sup>b</sup>	0.506 ± 0.04 <sup>b</sup>	0.474 ± 0.04 <sup>ab</sup>
Litter performance <sup>8</sup>							
Total pigs born <sup>7</sup>	14.70 ± 0.51	14.99 ± 0.50	14.77 ± 0.50	14.79 ± 0.71 <sup>ab</sup>	15.30 ± 0.57 <sup>a</sup>	15.97 ± 0.51 <sup>a</sup>	13.23 ± 0.51 <sup>b</sup>
No. born alive	12.10 ± 0.26	12.90 ± 0.19	12.49 ± 0.20	12.83 ± 0.26	13.14 ± 0.22	13.35 ± 0.21	10.68 ± 0.21
No. of mummified piglets	0.27 ± 0.08	0.36 ± 0.10	0.51 ± 0.10	0.17 ± 0.09	0.58 ± 0.12	0.42 ± 0.09	0.42 ± 0.10
No. of stillborn	2.29 ± 0.20 <sup>b</sup>	1.67 ± 0.17 <sup>a</sup>	1.81 ± 0.19 <sup>ab</sup>	1.74 ± 0.25 <sup>ab</sup>	1.56 ± 0.19 <sup>a</sup>	2.19 ± 0.20 <sup>b</sup>	2.19 ± 0.20 <sup>b</sup>
Average birth wt., kg	1.39 ± 0.04	1.38 ± 0.04	1.40 ± 0.04	1.40 ± 0.05 <sup>ab</sup>	1.47 ± 0.04 <sup>a</sup>	1.35 ± 0.04 <sup>b</sup>	1.33 ± 0.04 <sup>b</sup>
Litter size after cross fostering <sup>9</sup>	12.48 ± 0.31	12.99 ± 0.30	12.58 ± 0.31	12.44 ± 0.41 <sup>a</sup>	12.83 ± 0.34 <sup>a</sup>	13.38 ± 0.31 <sup>a</sup>	12.08 ± 0.31 <sup>b</sup>
72 h Mortality, %	4.59 ± 1.25	5.13 ± 1.22	4.99 ± 1.24	3.85 ± 1.60 <sup>a</sup>	3.59 ± 1.34 <sup>a</sup>	4.12 ± 1.26 <sup>a</sup>	8.06 ± 1.27 <sup>b</sup>
Pre-weaning mortality, %	7.75 ± 1.14	7.05 ± 1.11	5.67 ± 1.12	4.56 ± 1.56 <sup>a</sup>	5.22 ± 1.25 <sup>a</sup>	6.83 ± 1.15 <sup>a</sup>	10.69 ± 1.17 <sup>b</sup>
Low viability piglets	1.03 ± 0.17	1.06 ± 0.17	0.92 ± 0.17	0.81 ± 0.23 <sup>ab</sup>	0.61 ± 0.19 <sup>a</sup>	1.30 ± 0.17 <sup>b</sup>	1.27 ± 0.17 <sup>b</sup>
Pigs weaned per litter, d 18	10.48 ± 0.18	10.88 ± 0.17	11.01 ± 0.18	11.12 ± 0.24 <sup>a</sup>	11.49 ± 0.20 <sup>a</sup>	10.92 ± 0.19 <sup>a</sup>	9.63 ± 0.19 <sup>b</sup>
Av. weaning Wt., kg	5.96 ± 0.11	5.93 ± 0.10	6.02 ± 0.11	5.69 ± 0.14 <sup>a</sup>	6.29 ± 0.12 <sup>b</sup>	6.00 ± 0.11 <sup>ab</sup>	5.89 ± 0.11 <sup>a</sup>
Adjusted litter Wt. gained	45.44 ± 1.15	47.64 ± 1.09	48.63 ± 1.12	46.59 ± 1.59 <sup>a</sup>	53.61 ± 1.26 <sup>b</sup>	48.71 ± 1.16 <sup>a</sup>	40.03 ± 1.15 <sup>c</sup>
Piglet ADG, kg	0.232 ± 0.01	0.231 ± 0.01	0.234 ± 0.01	0.218 ± 0.01 <sup>a</sup>	0.245 ± 0.01 <sup>b</sup>	0.235 ± 0.01 <sup>a</sup>	0.232 ± 0.01 <sup>a</sup>

<sup>1</sup>Lactation length was used as covariate for ADFI, piglet ADG, average weaning weight, and adjusted litter weight gain.

<sup>2</sup>Parity group 0 = pregnant gilts that had not produced a litter. <sup>3</sup>Parity group P1+ = first and second parity sows.

<sup>4</sup>Parity group P3+ = third to fifth parity sows. <sup>5</sup>Parity group P6+ = sixth to ninth parity sows. <sup>6</sup>Least square means ± SE.

<sup>7</sup>Sow lactation G:F was calculated as summation of sow BW loss (or gain) and litter weight gain relative to feed disappearance.

<sup>8</sup>No apparent two-way significant interaction was noticed except for sow lactation G:F. <sup>9</sup>Litter size after cross fostering was used as covariate for number of piglets weaned.

<sup>10</sup>Low viable piglets are weak piglets with no suckling reflex, lack vitality, and have no possibility of survival.

<sup>a-b</sup>Means within a row of treatment with uncommon superscripts differ ( $P \leq 0.05$ ).

<sup>a-d</sup>Means within a row of parity with uncommon superscripts indicate a trend ( $P \leq 0.10$ ).

$$Y = 5.4864x + 1.9087, R^2 = 0.9892$$

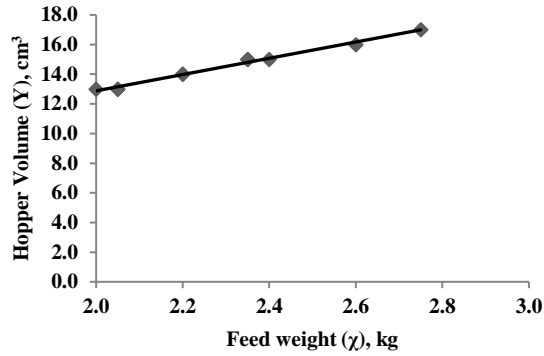


Figure 3. 1 Calibration of Accu-Drop feed dispenser (AP Systems, Assumption, IL). Relationship between hopper volume (y) of Accu-Drop feed dispenser and feed weight (x):  $y = 5.4864x + 1.9087, R^2 = 0.9892$ . Feed drops were adjusted on d 30, 61 and d 91 based on the BW of pigs on d 30, 60 and 90 d of gestation, respectively.

## **Chapter 4. Behavioral and cortisol responses to feeding time and frequency in pregnant sows under iso-caloric intake**

### **SUMMARY**

The study focused on behavioral and cortisol responses to feeding time and frequency in pregnant sows under iso-caloric intake. Forty sows [(Landrace × Yorkshire); BW  $216.87 \pm 4.17$  kg; parity  $3.09 \pm 0.54$ ] were blocked by parity and randomly assigned to 1 of 5 treatments in a randomized complete block design in stalls from days 30 to 60. Treatments include sows fed corn-soybean meal-based diet once at [0730 (Control, T1), 1130 (T2), or 1530 h (T3)], twice [half ration at 0730 and 1530 h (T4)], or thrice [one third portion at 0730, 1130, and 1530 h (T5)], with daily feed quantity kept at  $1.25 \times$  maintenance energy intake ( $100 \times (BW)^{0.75}$ ) kcal ME/d. Sows received 7058 kcal ME/d during gestation from 2.20 kg of diet formulated to contain SID Lys/ME of 1.71 g/Mcal from d 30 of gestation. The data were analyzed using PROC MIXED and PROC GLIMMIX procedures of SAS 9.4 for cortisol and behavior count data, respectively. Results were considered statistically significant when  $P \leq 0.05$  and were considered as trends when  $P \leq 0.10$ . Sow was the experimental unit. A 24 h area under the curve (AUC) was calculated by trapezoidal method. The *P*-values were adjusted for multiplicity based on Tukey Kramer's method. Sows fed  $2 \times$  daily tended to have lower ( $P = 0.024$ ) and reduced ( $P = 0.004$ ) 12-h cortisol AUC compared with control sows ( $1 \times$  daily) and sows on  $3 \times$  daily feeding regime, respectively. Sows on  $2 \times$  daily feeding regime had reduced feed anticipatory activity (FAA) ( $P < 0.01$ ), lower 24-h total ( $P < 0.001$ ) and feeding activities AUC ( $P < 0.001$ ) compared with both sows fed  $1 \times$  and  $3 \times$  daily, respectively. Sows on  $3 \times$  daily feeding regime had greater FAA ( $P < 0.001$ ) and 24-hour total activity

AUC ( $P = 0.010$ ) compared with control sows (1× daily). A 12-h cortisol total area under the curve (AUC) for sows fed once daily at 1130 h was reduced relative to sow group fed at 1530 h ( $P = 0.046$ ) but similar compared with the control sows ( $P = 0.323$ ). The control sows (0730 h) had reduced total ( $P < 0.001$ ) and feeding ( $P = 0.001$ ) activity AUC relative to sows on 1130 and 1530 h feeding schedules. Sows on 1130 h feeding schedule had greater FAA, 24-h total activity count, total ( $P < 0.001$ ) and feeding ( $P < 0.001$ ) activity AUC compared with sows fed daily at 1530 h. In conclusion, under iso-caloric condition per kilogram live metabolic weight, 2× daily feeding appears to be the threshold that reduces sows' total activity, feeding activity, and activation of the HPA axis and exhibit the potential to improve sow welfare in relation 1× and 3× daily feeding regimes. Also, feeding sows once daily at 0730 h appears to minimize sows' activity compared with sows fed daily at 1130 h but similar to sows on 1530 h feeding schedule. Sows on 1130 h feeding schedule had greater activities but reduced cortisol concentration, suggesting that elevated sow activity might not necessary indicate activation of hypothalamic-pituitary-adrenal axis.

**Key words:** Behavior, cortisol, feeding frequency, feeding time, iso-caloric intake, pregnant sows

## INTRODUCTION

Welfare of farm animals is important to producers, consumers and society at large (Cornish et al., 2016). Modern hyper-prolific sows are restrictively fed for efficient reproduction and to improve sow longevity. Restrictively fed gestation sows can experience stress and change their behavior. Increased and sustained stress is associated with compromised welfare. Hypothalamic–pituitary–adrenal (HPA) axis is one of the physiological systems almost always activated by stress (Ralph and Tilbrook, 2016). Food availability stimulates the rhythmicity of the HPA axis such that severe food restriction or starvation increases the HPA axis activity in humans and rat with an increase in mean glucocorticoids levels (Garcia-Belenguer et al., 1993; Kenny et al., 2014). Severe feed restriction in gilts during gestation elicited higher salivary cortisol concentrations than control gilts fed higher feed levels (Amdi et al., 2013). Increasing feeding frequency may enables the performance of natural behavior to improve welfare compared with sows fed less often (Verdon et al., 2018).

Nutritional outcome such as (total growth, fattening, risk of obesity, and blood insulin concentration) differed by the timing of food intake in many mammalian models under iso-caloric conditions (Noeske-Hallin et al., 1985; Arble et al., 2009; Nikkhah, 2012; Wang et al., 2014). Provision of feed within a narrow time window each day leads to significant changes in physiology and behavior (Johnston, 2014). Nevertheless, behavioral studies to evaluate animal welfare are characterized by scan sampling (Robert et al., 2002; Hwang et al., 2016; Basavaraju et al., 2017) and single time point cortisol measurements (Hemsworth et al., 2016; Li et al., 2017). Although acceptable, such procedures may not provide a complete evaluation leading to loss of information if

biological reasoning behind the scan sampling and single time cortisol measurement is not known. Sow welfare is a concern 24 hours. Our understanding of how feeding time and frequency under iso-caloric intake in pregnant sows affect cortisol and sow behavior remains unknown and warrant utilization of Sow Management Solution system.

One technology to assess behavior of sows is the Remote Insight Sow Management Solution system. Remote Insight's Sow Management Solution is designed to identify pig's events of interest 24 hours using wireless ear tag. The ear tag data is periodically sent to a gateway in the barn that forwards it to Google's Cloud Platform where the movement data is processed using machine learning models trained to identify when an animal is performing feeding related behaviors. The objective of this experiment was to determine the effects of feeding time and frequency on cortisol response, FAA, and 24-h feeding and total activities of pregnant sows under iso-caloric intake per kilogram live BW<sup>0.75</sup>. The hypothesis tested in this experiment was that, given same amount of energy per BW and feeding multiple times per day will reduce the activation of HPA axis and improve sow welfare compare to feeding once per day. Also, it was hypothesized that feeding the same amount of energy per kilogram live BW<sup>0.75</sup> at different time of day could alter energy and nutrient metabolism to influence 12-h cortisol response, feed anticipatory activity (FAA), and 24-h feeding and total activities of pregnant sows.



## MATERIALS AND METHODS

### *Animals, Housing, and Management*

The study was conducted at the swine unit of University of Minnesota Southern Research and Outreach Center, Waseca, MN. University of Minnesota Institutional Animal Care and Use Committee approved all protocols used in the study (IACUC number 171011961).

Sows were kept in individual stall housing system with fully slatted floor measuring (2.1 m × 0.59 m × 0.97 m) under temperature-controlled environment (21 – 23 °C) on a 9-h light and 15-h of dark schedule, with light on at 0730 h and turned off at 1630 h. On day 30 of gestation and before feeding, initial BW and BF thickness were recorded using an ultrasound machine (Lean-Meater, Renco Corp., Minneapolis, MN). Measurements were taken at the last rib about 5 cm lateral from the dorsal midline on both left and right sides using oil as coupling fluid and the two readings were averaged. Sows that returned to estrous were excluded from the experiment after pregnancy check on d 35 postcoitum with the aid of Ultrasonic pregnancy checker (Classic Medical Supply, Inc., Florida, FL). Throughout the experiment sows had unlimited access to water through nipple drinkers fitted to each stall. Feed offered to sows was restricted to 2 kg from weaning through breeding to day 30 which is considered optimal for sows and gilt during that phase.

### *Experimental Design, Dietary Treatments, and Feed Line Calibration*

Sows were blocked by parity, breeding date and balanced for BW and randomly allocated to 1 of 5 treatments with 6 to 8 sows per treatment in complete randomized design. All pregnant sows received a common corn-soybean meal-based diet from days 30 to 60 of

gestation. Nutrients met or exceeded NRC (2012) nutrient requirements for gestation sows. The chemical composition of the diet is presented in table 4.1.

Experimental treatments were imposed from days 30 to 60 of gestation. Body weights on day 30, was used to adjust the amount of feed fed between days 30 and 60 of gestation. To standardize ME intake per kilogram live  $BW^{0.75}$ , the daily quantity of feed fed was scaled to the individual  $BW^{0.75}$  live weight (Le Naou et al., 2014) and fed at 1.25 times (Prunier and Quesnel, 2000) the maintenance requirements for sows ( $100 \times BW^{0.75}$  kcal ME/d; NRC, 2012). On average, sows received 2.21 kg of feed per day (7055 kcal ME/d) from d 30 to 60 of gestation. Sows were fed individually. Feed amounts were adjusted by raising the feeder ball valve of an Accu-Drop Feed Dispenser (AP Systems, Assumption, IL) to drop the required amounts of feed into the feeding troughs. The Accu-Drop feed dispensers were calibrated at day 30 at various set points and related the volume of Feed Dispenser,  $Y$  ( $cm^3$ ) to kilogram weight of feed ( $x$ ) delivered as:  $Y = 5.4864x + 1.9087$ ;  $R^2 = 0.9892$ . The required daily feed allowance was provided once daily at: 0730 h (Control, T1), 1130 h (T2), 1530 h (T3), twice [half ration at 0730 and 1530 h (T4)], or thrice [a third portion at 0730, 1130, and 1530 h (T5)].

### ***Cortisol Measurements***

Multiparous and nulliparous pregnant focal sows of [TN 70 (Landrace x Large White, Topigs Norsvin USA, Burnsville, MN); total  $N = 40$ ; 8 sows per treatment; parity  $3.08 \pm 0.54$ ; and BW  $216.90 \pm 4.17$  kg] were sampled. Saliva cortisol concentrations were measured on day 52 of gestation. Five sows (from different treatments) adjacent to each other in stalls were sampled at a time. Focal sows were sampled 7 times during the day. Saliva samples were collected 1 h before and after each feeding time and 3 h after the last

feeding occasion (i.e. 0630, 0830, 1030, 1230, 1430, 1630, and 1830 h) using neutral synthetic swab Salivette® (Sarstedt, Aktiengesellschaft and Co, Numbrecht, Germany) attached to cotton string. The string was hung in the stall to allow sows to chew on the salivette until it became completely soaked with saliva. Maximum duration of 1 minute was allowed for each sow to chew on the salivette during sampling, modification of Greenwood et al. (2016). Saliva samples were collected on ice and centrifuged 2 h later at  $2,500 \times g$  for 10 min at 4°C. Approximately 0.5 mL saliva was obtained from each swab and frozen at -20°C until analyzed for cortisol concentration using enzyme-linked immunosorbent assay.

### ***Collection of Sow Behavior Data***

A subset of multiparous and nulliparous pregnant sows [TN 70 (Landrace x Large White; Topigs Norsvin USA, Burnsville, MN); total  $N = 30$ ; 6 sows per treatment; initial average BW  $222.89 \pm 3.82$  kg and average parity  $3.61 \pm 0.65$ ] were studied for sow behavior from d 53 of gestation for 7 days without human interference. Data was collected by affixing a remote insights ear tag to each pregnant sow. The ear tag sent three axis accelerometer data in x, y, and z plane collected at 2Hz to a cloud database. The raw accelerometer data was then passed through a machine learning model which classified the activity of the sow every 5 seconds into one of three categories: "Active", "Feed" or "Dormant". This resulted in 120,960 data points per sow over the 7-day study period after 21 days adaptation to the feeding regime. The data was aggregated and reported every 15 minutes for 24-h. The results presented are average daily "Feed" and/or "Active" classifications per sow.

### ***Precision of the Machine Learning Model and Ethogram of Behavioral Activity***

The precision of the machine learning model is measured as a percent confidence. This was measured by training our model with 60% of the samples and testing with the remaining 40%. The precision of the model was approximately 95% confidence. Ethogram of behavioral activity is presented in table 4.2.

### ***Determination of Feed Anticipatory Activity in Sows***

Feed anticipatory activity (FAA) in all sows was recorded every 15 minutes as feeding activity up to 1-h prior to the scheduled feeding times. Total daily FAA was the sum of all feeding activity 1-h prior to each feeding time (de Godoy et al., 2015).

### ***Saliva Cortisol Analysis***

Cortisol concentrations in saliva samples were determined with a commercially available ELISA kit (Neogen Corp., Product #402710, Lexington, KY). Samples were analyzed in duplicate and according to the manufacturer's instructions. The ELISA was validated for recovery and parallelism with swine saliva as previously described (Y. Li et al., 2017). The minimum detectable concentration of cortisol was 0.04 ng/mL and the intra and inter-assay coefficient of variation were 8.8% and 12.9% respectively. To minimize inter-assay variations, samples from all treatments and same time points were analyzed within the same assay.

### ***Chemical Analysis***

Feed samples were analyzed for DM, GE, CP, NDF, and ADF. Dry matter content of feeds was determined by the oven-drying method (method 934.01; AOAC, 2006). Gross energy was determined by basic bomb calorimeter (IKA Werke GmbH and Co. KG,

Staufen, Germany) with benzoic acid in the samples being used as calibration. The CP content ( $N \times 6.25$ ) in the basal diet was determined using Kjeldahl method (method 984.13, AOAC, 2006; Kjeltec 2300 Analyzer, Foss, Höganäs, Sweden). Determination of crude fat was by ether extract (method 920.39; AOAC, 2006) using an ANKOM XT15 extraction system (ANKOM Technology, Macedon, NY). The NDF and ADF content were determined using filter bag technique (ANKOM 2000 fiber analyzer, method 12 and 13; ANKOM Technology, Macedon, NY) (methods 973.18 and 973.19; AOAC, 2006). To determine the total ash content, samples of basal diet were ashed in a high temperature muffle furnace at 600 °C for 6 h (Isotemp Muffle Furnace, Thermo Fisher Scientific Inc., Hampton, NH).

#### ***Calculation of Sow Activity and Cortisol Area Under the Curve (AUC)***

The area under the curve (AUC) of sow activity count ( $\text{ng}\cdot\text{mL}^{-1}\cdot\text{h}^{-1}$ ) and cortisol ( $\text{ng}\cdot\text{mL}^{-1}\cdot\text{min}^{-1}$ ) were calculated for 24 hours and 12 hours, respectively, using trapezoidal summation rule:  $\sum\{[(C_t + C_{t+1}) \times 0.5] \times \Delta I\}$  where  $C_t$  is either the behavior count of an animal or concentration of a saliva cortisol sample in nanograms per milliliter at time  $t$ , and for the next data  $C_{t+1}$ , with a time interval of  $\Delta I$  in hours between data points, and  $\sum$  is the sum of the responses from  $C_t$  to  $n-1$  total number of data time points (Veissier et al., 2001).

#### ***Statistical Analysis***

All statistical analyses were performed using SAS (version 9.4; SAS Inst., Inc., Cary, NC). Data normality was checked using PROC UNIVARIATE. Sow behavior count and AUC data showed lack of normality and heterogeneity of variance. Data were transformed using

the equation ( $X'3 = \log_{10}(X + 0.5) + 0.5$ ) to achieve variance homogeneity (Hwang et al., 2016). The log transformed behavior activity count, FFA, and AUC data were analyzed by fitting a logistic model using the GLIMMIX procedure.

Cortisol data collected repeatedly were analyzed as repeated measures ANOVA using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). The model included fixed effects of treatment, time, and treatment  $\times$  time interaction with sow as random effect. Autoregressive process of first order was used to model repeated observation within sow as covariate structure (Littell et al., 1998). Adjustment to the denominator of degree of freedom was determined by Kenward-Roger's method (Kenward and Roger, 1997). Differences in basal cortisol concentration at 0630 h and at 1830 h were compared using a 1-sided paired test with PROC T-Test in SAS. Cortisol AUC, basal, 1-, 3-, and 5-h postprandial cortisol concentration data were analyzed by PROC MIXED procedure of SAS. All pairwise differences of least squares means were evaluated with the PDIFF option of SAS and adjusted for multiplicity by the Tukey-Kramer procedure. Sow was the experimental unit in all analysis. Statistical significance and tendencies were set at  $P \leq 0.05$  and  $P < 0.10$  for all statistical tests, respectively.

## RESULT

### *A 12-h Cortisol Response to Feeding Time and Feeding Frequency under Iso-caloric Intake in Pregnant Sows*

The least squares means of 12-h salivary cortisol concentrations in pregnant sows with respect to feeding time are presented in Table 4.3. Cortisol concentration by time interaction was not significant ( $P = 0.202$ ). Peak cortisol concentrations of 0.66, 0.69, and 0.68 ng/ml occurred at baseline (0630 h) for treatment 1, 2, and 3 respectively, but there was no difference between treatments ( $P \geq 0.10$ ). Similarly, feeding time did not alter cortisol concentrations at 1030, 1230, 1430, 1630, and 1830 h ( $P > 0.10$ ). Mean cortisol level was affected by time with concentration at 0630 h being greater than 1830 h (0.677 vs. 0.449 ng/ml;  $P = 0.026$ ). The 12-h cortisol total area under the curve (AUC) for sows fed once daily at 1130 h was reduced relative to sow group fed at 1530 h ( $P = 0.046$ ) but similar compared with the control ( $P = 0.323$ ). Feeding sows once daily at 1530 h did not alter the 12-h cortisol AUC relative to the control sows fed daily at 0730 h ( $P = 0.479$ ).

The 12-h salivary cortisol concentrations in pregnant sows are presented in Table 4.4. Cortisol concentration by time interaction was not significant ( $P = 0.754$ ). Peak cortisol concentrations of 0.66, 0.52 and 0.74 ng/ml occurred at baseline (0630 h) for treatment 1, 2, and 3, respectively but there was no difference between treatments ( $P \geq 0.10$ ). Similarly, feeding frequency did not alter cortisol concentrations at 1030, 1230, 1430, 1630, and 1830 h ( $P > 0.10$ ). Mean cortisol level was affected by time with concentration at 0630 h being greater than 1030, 1230, 1430, and 1630 h for all treatments ( $P < 0.05$ ). Sows fed 2× daily had lower 12-h cortisol AUC compared with control sows fed 1× daily ( $P =$

0.024) and sows on 3× daily feeding regime ( $P = 0.004$ ). The 12-h cortisol AUC did not differ between the control sows and sows fed 3× daily ( $P = 0.622$ ).

### ***Effect of Feeding Time and Frequency on Basal, Pre- and Post-prandial Cortisol Concentrations and AUC***

Least squares means of basal, pre- and post-prandial cortisol concentrations and AUC with reference to feeding time are presented in Table 4.5. The 3-h post-prandial cortisol concentrations and 1-h pre-prandial cortisol levels were not affected by feeding time ( $P > 0.10$ ). Feeding time affected 1-h post-prandial cortisol concentration with sows fed at 1130 h having lower values ( $P = 0.014$ ) relative to the control sows but did not differ compared with sows fed at 1530 h ( $P = 0.458$ ). The control sows (0730 h) and sows receiving their daily ration at 1530 h did not differ in cortisol levels 1-h after feeding ( $P = 0.131$ ). The 5-h post-prandial cortisol concentration did not change for sow groups fed at 0730 and 1130 h ( $P = 0.744$ ). The 3-h ( $P = 0.030$ ) and 5-h ( $P = 0.020$ ) cortisol AUC was lower for sows receiving their daily ration once daily at 1130 compared with the control sows. The 3-h cortisol AUC was not different between the control sows and sows fed daily at 1530 h ( $P = 0.192$ ). Likewise, sows on 1130 and 1530 h feeding schedule had similar 3-h cortisol AUC ( $P = 0.527$ ).

Least squares means of basal, pre- and post-prandial cortisol concentrations and AUC with respect to feeding frequency are presented in Table 4.6. Sows on twice daily feeding regime tended to have lower cortisol levels 1-h post-prandial ( $P = 0.071$ ) compared with the control sows but similar to sows fed 3× daily ( $P = 0.644$ ). The control sows and sows fed 3× daily did not differ in cortisol levels 1-h post-prandial ( $P = 0.299$ ). Sows on 2×



daily feeding schedule had reduced cortisol concentration 3-h after feeding ( $P = 0.034$ ) relative to sows fed 3× daily but similar to sows fed 1× daily ( $P = 0.341$ ). Sows receiving their daily feed 1× and 3× did not differ in cortisol levels 3-h post-prandial ( $P = 0.440$ ). The 5-h post-prandial cortisol concentrations were not affected by feeding frequency ( $P > 0.10$ ). Feeding sows 2× daily reduced both the 3-h ( $P = 0.039$ ) and 5-h ( $P = 0.015$ ) cortisol AUC compared with control sows (1×). Similarly, sows on 2× daily feeding schedule tended to have lower ( $P = 0.072$ ) and reduced ( $P = 0.008$ ) 3- and 5-h cortisol AUC respectively, relative to sows fed 3× daily. The 3-h ( $P = 0.908$ ) and 5-h ( $P = 0.986$ ) cortisol AUC were similar for sows on 3× daily feeding regime relative to the control (1×) sows.

***Behavioral Activity of Pregnant Sows in Response to Feeding Time and Frequency under Iso-caloric Intake***

Behavioral activities in response to feeding time are presented in Table 4.7. Sows fed daily at 0730 h had lower 24-h total activity count compared with sows fed daily at 1130 h ( $P < 0.001$ ) and sows on 1530 h feeding schedule ( $P < 0.001$ ). Feeding sows at 1130 h daily resulted in greater 24-h total activity ( $P < 0.001$ ) and tendency to have greater total feeding activity ( $P = 0.051$ ) relative to sows fed daily at 1350 h. A 24-h total feeding activity was lower with sow fed at 0730 h than sows receiving their daily ration at 1130 h ( $P < 0.001$ ) but similar to 1530 h sow group ( $P = 0.265$ ). Sows on 0730 daily feeding schedule had lowest FAA compared with treatment group fed at 1130 and 1530 h ( $P < 0.001$ ). Also, the 1130 h treatment group had greater FAA relative to sows fed at 1530 h ( $P < 0.001$ ). The total and feeding activity AUC mirrored the FAA of pregnant sows. The control sows (0730 h) had reduced 24-h total ( $P < 0.001$ ) and feeding ( $P = 0.001$ ) activity AUC relative

to sows on 1130 and 1530 h feeding schedule, respectively. Sows on 1130 h feeding schedule had greater 24-h total ( $P < 0.001$ ) and feeding ( $P < 0.001$ ) activity AUC compared with sows fed daily at 1530 h.

Behavioral activities with respect to feeding frequency are presented in Table 4.8. A 24-h total sow activity and feeding activity were reduced with sows on 2× daily feeding regime compared with sows fed 1× and 3× daily ( $P < 0.001$ ). Sows fed 1× and 3× daily did not differ in 24-h total ( $P = 0.965$ ) and feeding activities ( $P = 0.999$ ). Feeding sows 2× daily reduced the feeding FAA) compared with the control sows (1× daily) and sows fed 3× daily ( $P < 0.001$ ). Sows on 3× daily feeding regime had greater FAA compared with sows fed at 1× daily ( $P < 0.001$ ). Likewise, sows on 2× daily feeding regime had lower 24-h total ( $P < 0.001$ ) and feeding ( $P < 0.001$ ) activity AUC compared with control sows and sows fed 3× daily. The 24-hour feeding ( $P = 0.428$ ) and total ( $P = 0.306$ ) activity AUC of sows on 3× daily feeding regime was similar to sows fed 1× daily.

Total activity of the sows started to increase from 0530 h and nadir around 1900 h. Total activity of sows on 1530 h schedule nadir at 2000 h. The control sows (0730 h) had 2 peaks of total activity at 0730 and 1530 h. Sows fed at 1130 h had highest total activity peak at each feeding time followed by sows fed daily at 1530 h. Sows on 1530 h feeding schedule had intermediate peak at each feeding time (Figure 4.1). Sows on 2× daily feeding regime had least total activity throughout the day relative to sows fed 1× and 3× daily. Sows on 1× daily feeding regime had two peaks of feeding activities at 0730 and 1530 h whereas those on 3× feeding schedule had peaks at all the feeding times (Figure 4.2). Feeding activity of the sows started to increase from 0530 h and nadir around 1900 h. Feeding activity of sows on 1530 h schedule nadir at 2000 h. All treatment groups

had peak feeding activity at their respective feeding times, but sow group fed at 1530 h had extended peak feeding activity which lasted for about 3 hours. Sows fed at 1130 and 1530 h had 3 peaks while the control sows had 2 peaks of feeding activity (Figure 4.3). Feeding sows 2× daily resulted in 3 lowest feeding activity peaks throughout the day. Control sows had 2 peaks of feeding activities. Sows on 3× daily feeding regime had elevated feeding activity at each feeding time (Figure 4.4).

## **DISCUSSION**

Increasing feeding frequency enables the performance of natural behavior to improve welfare compared with sows fed less often (Verdon et al., 2018). Also, provision of feed within a narrow time window each day leads to significant changes in physiology and behavior (Johnston, 2014). Therefore, this study focused on the effects of different feeding times and frequency regimes on cortisol and behavior of pregnant sows under iso-caloric conditions.

Cortisol is a steroid hormone secreted by the adrenal gland and has a circadian rhythmicity with greater concentration around 08:30 h which gradually declines to the lowest levels at around midnight (Chan and Debono, 2010; Sunaina et al., 2016). This secretory pattern was comparable to that observed in our study. Cortisol concentration at 0630 h was greater relative to 1830 h regardless of treatment which is in accord to earlier findings (de Jong et al., 2000; Hemmann et al., 2012; Amdi et al., 2013). We conjecture that our study design, feeding frequency regime and sampling protocol did not inhibit the circadian rhythmicity of cortisol in the sows. Furthermore, we speculate that feed intake in pregnant

sows is not antecedent to peak cortisol concentrations since all sows had peak levels before feeding. This confirmed an earlier report that the circadian clock in the adrenal gland sets specific time intervals during which the adrenal gland most effectively responds to adrenocorticotrophic hormones (Chan and Debono, 2010).

Gestation sows are fed about 2.5 kg feed per day which represents approximately 50% of their *ad libitum* feed intake (Meunier-Salaün et al., 2001). Splitting the limited feed further into two or three meals and fed multiple times within the day did not alter the basal (fasting) cortisol concentrations which is consistent with findings in other studies (Terpstra et al., 1978; Levay et al., 2010). Terpstra et al. (1978) reported that human study subjects on a fixed solid 65% carbohydrate diet under steady state conditions in a metabolic unit had basal cortisol levels that did not change on varying meal frequency. Additionally, the basal glucocorticoids concentrations and calorie restrictions are dose dependent, but the absolute differences in circulating corticosterone between 50% and 25% calorie restriction are small (Levay et al., 2010). Therefore, we did not expect to see any difference in the fasting cortisol levels when all treatments groups had similar energy intake per kilogram live metabolic weight.

Twice daily feeding reduced cortisol AUC compared with the control sows. Our result is in agreement with Farmer et al. (2002) but contradicts Holt et al. (2006). Farmer et al. (2002) reported that feeding pregnant sows with concentrate diet twice daily reduced the cortisol AUC compared to sows on single feeding regime. Conversely, Holt et al. (2006) did not elicit a response of feeding frequency (2× daily at 0730 and 1430h) on salivary cortisol concentration relative to sows fed 1× daily. The differences in results are

attributable to the extent of sampling and the method of evaluation. In the current study, we sampled our sows every 2 hours from 0630 to 1830 h and evaluated them using the AUC. Holt et al. (2006) evaluated their experimental animals using single time point measurements at 1300 h. Because of the diurnal rhythmicity of cortisol secretion pattern, single time point measurement might not be informative if the biological reasoning behind the sample is not known (Hänninen et al., 2006; Medica et al., 2011 ; Stewart et al., 2017).

Feeding frequency elicited an effect on the feeding activity which mirrored total sows' activity. Sow group on 1× daily feeding regime had increased feeding activity at 0730 h and 8 hours after feeding (1530 h) whereas a different pattern of reduced sow feeding activity was observed in sows on 2× daily feeding schedule. Overall, sows on 2× daily feeding regime had reduced feeding and total activity compared with sows fed 1× and 3× daily. The 0730 and 1530 h time points correspond to 24 and 8 h after the morning feeding for sows fed 1× daily and 8 and 16 h after the 1530 h feeding for sows fed 2× daily. Therefore, sows fed 1× daily were highly motivated to feed at 1530 h and the next morning (0730 h) than the sows fed 2× daily since they experienced post-absorptive state at 0730 h. To the contrary, Robert et al. (2002) reported that gilts fed two meals per day on concentrate diet had similar activity compared with gilts fed 1× daily. However, these researchers only evaluated sow welfare in 5 minutes prior to the morning ration at 08:00 h. Intuitively, increasing the feeding frequency for pregnant sows should improve their welfare because stereotypic behaviors could be directed to naturalness of eating and enhanced productivity.

Furthermore, sows on 3× daily feeding regime had similar total and feeding activity relative to sows fed 1× daily which contradicts observation in cats. Cats fed 4× daily had

increased (~18%) voluntary physical activity than similar group fed 1× daily (de Godoy et al., 2015). The differences in result could be due to species differences, housing systems, and the level of caloric restriction. While de Godoy et al. (2015) fed their cats to a targeted ideal body condition score in a group housing system, we fed our sows based on their live metabolic weight to standardized sow energy intake in individual stall system of production. Our data suggests that 2× daily feeding appears to be the threshold of feeding frequency under limit fed conditions as it reduces total and feeding activities of sows as well as activation of the HPA axis.

Sows on 3× daily feeding regime had elevated feeding activity at each feeding time. This observation is consistent with those of Lawrence and Illius (1989) who showed that pigs given a lower amount of their normal daily ration had a higher motivation to perform feeding behavior through operant conditioning test. Additionally, Douglas et al. (1998) reported that indicators of feeding motivation and arousal are strongly influenced by feeding regimen. In line with our findings, sows fed 3× daily had increased total and feeding activity compared with sows on fed 2× daily. The increased activity could be attributed to inadequate gut fill because of the smallest amount of energy and / or volume of feed received at each feeding time. In support of this theory, Lawrence et al. (1988) explained that the conventional North American sows' diet is concentrated in nutrients and although sufficient for good health and performance, it might not fulfill other needs (foraging activity) of the sow. Furthermore, the small amount of feed is unlikely to give a feeling of satiety (Verdon et al., 2018). The daily total activity and feeding activity pattern were similar among treatments but sows fed 2× had the lowest peaks at all feeding time. Although, the pattern of sows fed 1× and 3× daily was similar, sow fed 3× had 3 peaks

while sows on 1× daily feeding regime had only 2 peaks. Therefore, sows on 3×daily feeding regime had greater total activity AUC compared with the control sows and sows fed 2× daily. Previous studies have reported similar findings in cats. Deng et al. (2011) and Deng et al. (2014) reported greater average total daily activity for cats fed multiple meals in comparison with cats fed 1× daily. It was explained that when meal size is too small to induce satiety, non-feeding activities would persist in pigs (Terlouw et al., 1993; Robert et al., 2002).

Behavioral activities preceding feed provision is termed ‘food anticipatory activity’ (FAA) (Johnston, 2014). Sows on 3× daily feeding regime had greatest FAA compared with sows fed at 1× and 2× daily. This finding agrees with previous results in cats. Cats fed 4× daily had increased FAA compared with cats fed 1× daily (de Godoy et al., 2015). We hypothesized that increasing the daily meal could be metabolic advantage by spreading the nutrient load throughout the day or improve welfare of sows because stereotypic behaviors could be directed to a natural and productive activity of feeding. However, with increased FAA with 3× daily meals, we speculate that the feeding regime did not provide adequate gut fill or distension with the small volume of the concentrate diet at each feeding time. One mechanism by which meal termination occurs is through activation of gastric mechanoreceptors following the distension of the stomach. The mechanoreceptors transport their signal along the vagus nerve to communicate the digestive state to the nucleus of the solitary tract (NTS) which relays the signal to the feeding centers of the brain, such as the hypothalamus to influence initiation or termination of a meal (Hargrave and Kinzig, 2012). Furthermore, the distention of the gastric wall and subsequent activation of stretch receptors and mechanoreceptors led to a

lower threshold necessary for cholecystokinin and leptin to induce decreases in food intake (Hargrave and Kinzig, 2012).

Sows fed once daily at 1130 h had reduced cortisol AUC relative to sows fed daily at 1530 h but similar to the control sows. This observation is in agreement with the results of other investigators who studied cortisol rhythmicity in human volunteers in relation to meal time (Legler et al., 1982). Conversely, feeding sows once daily at 1130 h resulted in an increased sow total and feeding activity but lower cortisol AUC compared with other treatments groups. Our data provides evidence that feeding time influence the daily plasma cortisol pattern, but no clue was found as to why feeding the same amount of energy per kilogram live BW<sup>0.75</sup> at different time of day will affect the hypothalamic pituitary-adrenal axis differently. We theorized that the 1130 h meal may at least have a synchronizing role on plasma cortisol diurnal variations. Although actions of glucocorticoids may impact animal welfare, using peripheral measures alone is limited. For instance, Ralph and Tilbrook (2016) demonstrated that gluconeogenesis in laying hens was upregulated only when corticosterone in the liver was increased and glucose was depleted. Therefore, they concluded that a change in peripheral corticosterone was not an indicative of the effect of the stressor on the welfare of the hens. Additionally, Jensen et al. (1996) reported that the effects of stress on physiological and behavioral data can be contradictory. They explained that plasma cortisol concentrations of pigs submitted to unpredictable and unavoidable electric shocks for 30 days did not differ from control animals that experienced no shocks; although the behavior of the treatment group suggests that they were still stressed by the electric shocks.



Total activity and feeding activity of the sows in this study did not return to baseline from 0600 to 1830 h. This observation was expected. The Remote Insight's Sow Management solution detected increased sows' total and feeding activity between 0530 and 1900 h for sows fed at 0730 and 1130h. Feeding sows at 1530 extended this period by 2 hour. The pig is investigatory in nature and they explore their surroundings by rooting, sniffing or chewing on objects during their active time (Studnitz et al., 2007). Our observation is in agreement with the findings of Rijnen et al. (2003) who reported that pigs are most active from 0700 to 2200 h. Sows in the current study had restricted amount of concentrate diet for optimum production and sow longevity. However, considering the feeding activity pattern of the sows is possible that the feeling of fullness was not met during the day. For instance, sows on the 0730 h feeding schedule had increased feed activity at 0730 h and a gradual decline over time. During the second feeding time 1130 h (4 hours after feeding), the 0730 h fed sow had reduced feeding activity. But after 8 hours of feeding, sows on 0730 h feeding schedule had increased feeding activity probably due to the fact that they have entered into the post-absorptive state.

Sow groups fed at 0730 and 1130 h had peak feed activity at their respective feeding time which declined drastically 1-h post-prandial. This was not the case with sow group fed daily at 1530 h. The drop in their feeding activity from the peak was minimal and remained elevated for about 3 hours after feeding. Brouns et al. (1994) reported that sows that are restrictively fed searched for feed for at least one hour after they had consumed their feed. The reason why sows fed daily at 1530 h had about 2 hours of extended feeding activity is hard to explain and merits further investigation. It could be speculated that feeding sows once daily at 1530 h modulated their feeding and activity behavior around the time of

feeding. Treatments groups fed at 0730, 1130, and 1530 h had peak feeding activities at their respective feeding times relative to other times of the day. This suggests that the sows adapted well to their feeding schedule. Also, the daily total activity pattern was similar among feeding times and peaked around feeding period, with sows fed at 1130 h having activity counts above the 0730 and 1530 h fed groups for most of the 24-h period. Feeding activity pattern followed the same trend except that at 0730 h, the control sows had greatest feeding activity peak. Overall, the control sows had lower total and feeding activity pattern relative to treatment group fed at 1530 h.

Pregnant sows on 0730 h feeding schedule had lowest 1-h total FAA compared with other feeding times. Although all treatment groups had increased FAA prior to their schedule feeding times (data not shown), sows fed at 0730 had only one additional FAA prior to 1530 h feeding time whereas sows fed at 1130 and 1530 h had two additional increased in FAA at (0730, 1530 h) and (0730, 1130 h), respectively. This additional increase in FAA was more pronounced in sows on 1130 h feeding regime which cumulatively resulted in greatest 1-h total FAA. This is the first study to report the effect of feeding time on 1-h total FAA, 24 -h feeding and total activity pattern of pregnant sows and there is no data to the best of our knowledge to compare our results with.

## **CONCLUSION**

In conclusion, feeding sows once daily at 0730 h appears to minimize sows' FAA, daily feeding and total activity, but similar cortisol response relative to pregnant sows fed daily at 1130 and 1530h. Additionally, feeding sow daily at 1130 h resulted in greater FAA, feed and total activities but reduced cortisol concentration, suggesting that elevated sow activity might not necessary indicate activation of hypothalamic-pituitary-adrenal axis.

Under iso-caloric intake per kilogram live metabolic weight, 2× daily feeding appears to be the threshold that reduces sows' FAA, total and feeding activities, and activation of the HPA axis and exhibit the potential to improve sow welfare in relation 1× and 3× daily feeding regimes.

***Limitation of study***

The study had one limitation due to housing constraint. Animals on different feeding frequency regimes and feeding time schedules could not be housed in different rooms within the barn. The influence of some sows feeding and others not feeding could not be delineated. However, to reduce this expected impact on our results, we allowed 21 days adaptation to the feeding regimes and schedules. This limitation may highlight the need for further research.

Table 4. 1 Composition and nutrient analysis of gestation diet, as fed basis

Ingredients, %	Gestation diet
Yellow dent corn	65.35
Soybean meal, dehulled, solvent extracted	10.00
Corn DDGS <sup>1</sup> , > 6 and < 9% oil	20.00
Choice white grease	1.50
Dicalcium phosphate	1.20
Limestone, ground	1.00
Sodium chloride	0.35
L-Lys-HCL	0.10
Swine breeder premix <sup>2,3</sup> (EB Plus)	0.50
<b>Total</b>	<b>100.00</b>
<b>Analyzed composition</b>	
DM, %	89.34
GE, kcal/g	4,431
CP, %	15.70
NDF, %	13.30
ADF, %	4.80
<b>Calculated nutrient composition</b>	
SID Metabolizable energy (ME), kcal/kg	3,379
SID Lys, %	0.58
SID Met, %	0.26
SID Met+Cys, %	0.48
SID Thr, %	0.45
SID Trp, %	0.12
Total available P	0.61
Ca, %	0.70
Crude fat, %	6.30
SID Lys/ME, g/Mcal	1.71

<sup>1</sup>Dried distiller's grains with solubles.

<sup>2</sup>Mineral and vitamin mixture supplied per kilogram of diets: 15 mg of Cu (as CuSO<sub>4</sub>); 124 gm of Fe (as FeSO<sub>4</sub>·7H<sub>2</sub>O); 40 mg of Mn (as MnO); 124.7 mg of Zn (as ZnO); 2.2 mg of I (as Ca(IO<sub>3</sub>)<sub>2</sub>); 0.30 mg of Se (as Na<sub>2</sub>SeO<sub>3</sub>); 11000 IU of vitamin A; 2750 IU of vitamin D<sub>3</sub>; 55 IU of vitamin E; 4.4 mg of vitamin K<sub>3</sub>; 1.1 mg of thiamine; 9.9 mg of riboflavin; 55 mg of nicotinic acid; 33 mg of d-pantothenic acid; 2.2 mg of pyroxidine; 0.06 mg of vitamin B<sub>12</sub>; 2.6 mg of folic acid; and 0.22 mg of biotin, and 1.92 mg of Na.

<sup>3</sup>Swine breeder premix was supplied by Agric-Nutrition Services, INC. Shakopee, MN.

Table 4. 2 Ethogram of sows' behavioral activity

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1. Total activity	The number of 5 second periods that the Solution detected sow movement.
2. Total feed activity	The number of 5 second periods that the Solution detected feeding behavior (eating and/or sham chewing).
3. Total feed anticipatory activity	The sum of all feeding activity 1-h prior to each feeding time (de Godoy et al., 2015).

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Table 4. 3 Cortisol concentration (ng/ml), cortisol AUC, and probability values of pregnant sows subjected to different feeding times under iso-caloric intake (Least squares means).

Item	Time of day							Total AUC, ng*h/ml <sup>4</sup>
	0630 h	0830 h	1030 h	1230 h	1430 h	1630 h	1830 h	
Trt. 1 ( 0730 h) <sup>1</sup>	0.663	0.698	0.465	0.319	0.372	0.345	0.484	316.41 <sup>ab</sup>
Trt. 2 (1130 h) <sup>2</sup>	0.686	0.390	0.423	0.302	0.324	0.281	0.471	272.15 <sup>b</sup>
Trt. 3 (1530 h) <sup>3</sup>	0.682	0.388	0.459	0.357	0.564	0.450	0.394	349.10 <sup>a</sup>
SEM	0.09	0.08	0.09	0.07	0.08	0.08	0.09	57.21

<sup>1</sup>Sows received their daily gestation ration once at 0730 am

<sup>2</sup>Sows received their daily gestation ration once at 1130 am

<sup>3</sup>Sows received their daily gestation ration once at 1530 pm

<sup>4</sup>Total area under the curve (AUC) was calculated using the trapezoidal summation method

<sup>a,b</sup>Least squares means in each column followed by different superscripts significantly differ ( $P \leq 0.05$ ) (Tukey Kramer adjusted).

Table 4. 4 Effect of feeding frequency on cortisol concentration (ng/ml), cortisol AUC, and probability values of pregnant sows under iso-caloric intake (Least squares means)

Item	Time of day							Total AUC, ng*h/ml <sup>4</sup>
	0630 h	0830 h	1030 h	1230 h	1430 h	1630 h	1830 h	
Trt.1 (1×) <sup>1</sup>	0.663	0.698	0.457	0.319	0.372	0.345	0.484	326.02 <sup>b</sup>
Trt.4 (2×) <sup>2</sup>	0.523	0.412	0.313	0.191	0.335	0.264	0.458	246.52 <sup>a</sup>
Trt.5 (3×) <sup>3</sup>	0.743	0.502	0.560	0.346	0.430	0.388	0.593	348.42 <sup>b</sup>
SEM	0.07	0.08	0.07	0.07	0.07	0.08	0.08	23.52

<sup>1</sup>Sows received their daily gestation ration once at 0730 am.

<sup>2</sup>Sows daily gestation ration was split into 2 and each portion offered at 0730 am and 1530 h.

<sup>3</sup>Sows daily gestation ration was split into 3 and each portion offered at 0730 am, 1130 am and 1530 h.

<sup>4</sup>Total area under the curve (AUC) was calculated using the trapezoidal summation method.

<sup>a,b</sup>Least squares means in each column of feeding frequency followed by different superscripts significantly differ ( $P \leq 0.05$ ) (Tukey Kramer adjusted).

Table 4. 5 Basal, pre- and post-prandial cortisol concentrations, and AUC with reference to feeding time (Least squares means)<sup>1</sup>.

Variable	Treatment			SEM	P - value
	T1 <sup>2</sup>	T2 <sup>3</sup>	T3 <sup>4</sup>		
Time 0 (baseline)	0.647	0.712	0.673	0.14	0.873
1 hr. before feeding	0.653	0.422	0.564	0.09	0.135
1 hr. after feeding	0.698 <sup>a</sup>	0.302 <sup>b</sup>	0.450 <sup>ab</sup>	0.08	0.017
3 hr. after feeding	0.447	0.324	0.382	0.07	0.429
5 hr. after feeding	0.312	0.289	* <sup>5</sup>	0.08	0.744
AUC <sup>6</sup> , ng*min/ml	108.41 <sup>a</sup>	68.65 <sup>b</sup>	83.62 <sup>ab</sup>	13.24	0.036
AUC <sup>7</sup> , ng*min/ml	156.33 <sup>a</sup>	98.36 <sup>b</sup>	* <sup>5</sup>	20.16	0.020

<sup>1</sup>Total area under the curve (AUC) was calculated using the trapezoidal summation method

<sup>2</sup>Sows received their daily gestation ration once daily at 0730 h.

<sup>3</sup>Sows received their daily gestation ration once daily at 1130 h.

<sup>4</sup>Sows received their daily gestation ration once daily at 1530 h.

<sup>5</sup>Sow group fed at 1530 h could not be sampled 5 hours after feeding.

<sup>6</sup>AUC from time 0 to 3 hours after feeding

<sup>7</sup>AUC from time 0 to 5 hours after feeding

<sup>ab</sup>Least squares means within a row with uncommon superscript significantly differ ( $P \leq 0.05$ ) (Tukey Kramer adjusted).



Table 4. 6 Basal, post-prandial cortisol concentrations, and AUC with reference to feeding frequency (Least squares means)<sup>1</sup>

Variable	Treatment			SEM	P - value
	T1(1×) <sup>2</sup>	T4(2×) <sup>3</sup>	T5(3×) <sup>4</sup>		
Time 0 (baseline)	0.655	0.530	0.758	0.11	0.276
1 hr. after feeding	0.685	0.425	0.520	0.13	0.083
3 hr. after feeding	0.447 <sup>ab</sup>	0.313 <sup>a</sup>	0.560 <sup>b</sup>	0.06	0.043
5 hr. after feeding	0.315	0.196	0.343	0.06	0.128
AUC <sup>5</sup> , ng*min/ml	108.27 <sup>a</sup>	72.73 <sup>b</sup>	102.82 <sup>ab</sup>	13.53	0.030
AUC <sup>6</sup> , ng*min/ml	154.16 <sup>a</sup>	103.07 <sup>b</sup>	156.74 <sup>a</sup>	15.61	0.005

<sup>1</sup>Total area under the curve (AUC) was calculated using the trapezoidal summation method.

<sup>2</sup>Sows received their daily gestation ration once at 0730 h.

<sup>3</sup>Sows daily gestation ration was split into 2 and each portion offered at 0730 and 1530 h.

<sup>4</sup>Sows daily gestation ration was split into 3 and each portion offered at 0730, 1130 and 1530 h

<sup>5</sup>AUC from time 0 to 3 hours after first partial or full feeding.

<sup>6</sup>AUC from time 0 to 5 hours after first partial or full feeding.

<sup>ab</sup>Least squares means within a row uncommon superscript significantly differ ( $P \leq 0.05$ ) (Tukey Kramer adjusted).

Table 4. 7 Pregnant sows activity counts per 15 minutes and AUC in response to feeding time (Least squares means)<sup>1</sup>

Variable	Treatment			SEM	P-value
	T1 <sup>2</sup>	T2 <sup>3</sup>	T3 <sup>4</sup>		
Total activity	3.00 <sup>a</sup>	3.29 <sup>b</sup>	3.09 <sup>a</sup>	0.04	< 0.001
Total feeding activity	2.40 <sup>a</sup>	2.70 <sup>b</sup>	2.53 <sup>ab</sup>	0.06	< 0.001
Total Feed anticipatory activity <sup>5</sup>	2.98 <sup>a</sup>	3.57 <sup>b</sup>	3.32 <sup>c</sup>	0.02	< 0.001
Total activity AUC <sup>6</sup> , Counts*24-h	6.53 <sup>a</sup>	6.83 <sup>b</sup>	6.70 <sup>c</sup>	0.01	< 0.001
Total Feeding activity AUC <sup>6</sup> , Counts*24-h	6.26 <sup>a</sup>	6.42 <sup>b</sup>	6.35 <sup>c</sup>	0.02	< 0.001

<sup>1</sup>Data were analyzed and presented using the logarithmic transformation ( $\log_{10}(x + 0.5) + 0.5$ ).

<sup>2</sup>Sows received their daily gestation ration once at 0730 am.

<sup>3</sup>Sows received their daily gestation ration once at 1130 am.

<sup>4</sup>Sows received their daily gestation ration once at 1530 pm.

<sup>5</sup>Total feed anticipatory activity was the sum of every 15 minute feeding activity 1-h pre-prandial.

<sup>6</sup>Total area under the curve (AUC) was calculated using the trapezoidal summation method.

<sup>abc</sup>Least squares means within a row for feeding time with uncommon superscript letters significantly differ ( $P \leq 0.05$ ) (Tukey Kramer adjusted).

Table 4. 8 Pregnant sows activity counts per 15 minutes and AUC in response to feeding frequency (Least squares means)<sup>1</sup>

Variable	Treatment			SEM	P-value
	T1 (1×) <sup>2</sup>	T4 (2×) <sup>3</sup>	T5 (3×) <sup>4</sup>		
Total activity	3.00 <sup>a</sup>	2.66 <sup>b</sup>	3.01 <sup>a</sup>	0.05	< 0.001
Total feeding activity	2.40 <sup>a</sup>	2.00 <sup>b</sup>	2.40 <sup>a</sup>	0.07	< 0.001
Total Feed anticipatory activity <sup>5</sup>	2.98 <sup>b</sup>	2.58 <sup>a</sup>	3.22 <sup>c</sup>	0.03	< 0.001
Total activity AUC <sup>6</sup> , Counts*24-h	6.53 <sup>b</sup>	6.23 <sup>a</sup>	6.57 <sup>b</sup>	0.01	< 0.001
Total feeding activity AUC <sup>6</sup> , Counts*24-h	6.26 <sup>a</sup>	5.69 <sup>b</sup>	6.16 <sup>a</sup>	0.05	< 0.001

<sup>1</sup>Data were analyzed and presented using the logarithmic transformation ( $\log_{10}(x + 0.5) + 0.5$ ).

<sup>2</sup>Sows received their daily gestation ration once at 0730 h.

<sup>3</sup>Sows daily gestation ration was split into 2 and each portion offered at 0730 and 1530 h.

<sup>4</sup>Sows daily gestation ration was split into 3 and each portion offered at 0730, 1130 and 1530 h.

<sup>5</sup>Total feed anticipatory activity was recorded 1-h pre-prandial for each feeding occasion

<sup>6</sup>Total area under the curve (AUC) was calculated using the trapezoidal summation method.

<sup>abc</sup>Least squares means within a row with uncommon superscript significantly differ ( $P \leq 0.05$ ) (Tukey Kramer adjusted).

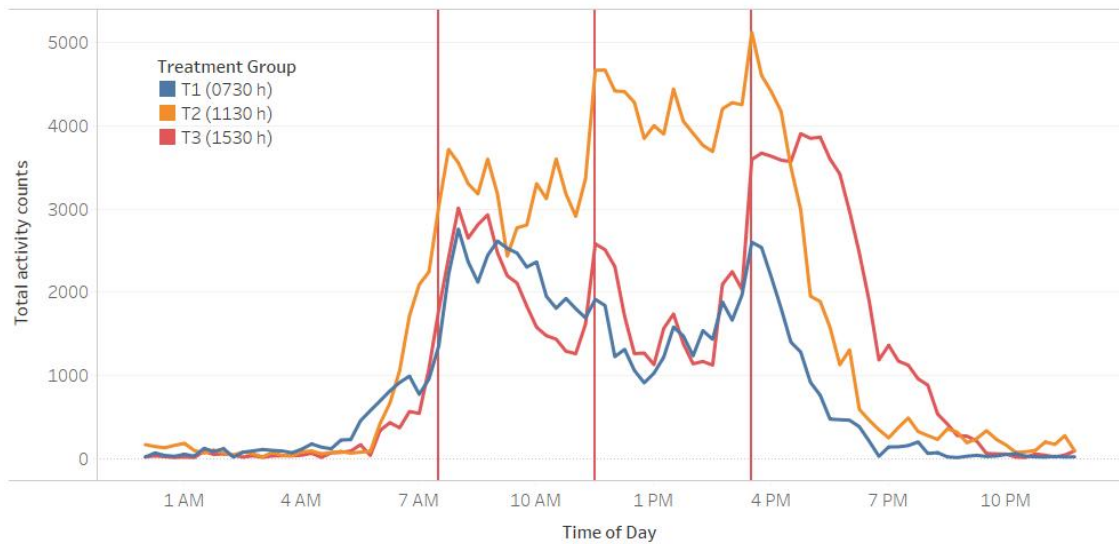


Figure 4. 1 Temporal pattern of pregnant sow’s total activity with respect to feeding time measured 24-h daily for 7 days under limit-fed conditions.

The graph represents sows fed once daily at 0730 (T1, blue curve), 1130 (T2, red curve), and 1530 h (T3, orange curve). The vertical red lines indicate feeding times for each treatment group. Light were turned on at 0730 h and switched off at 1630 h daily.

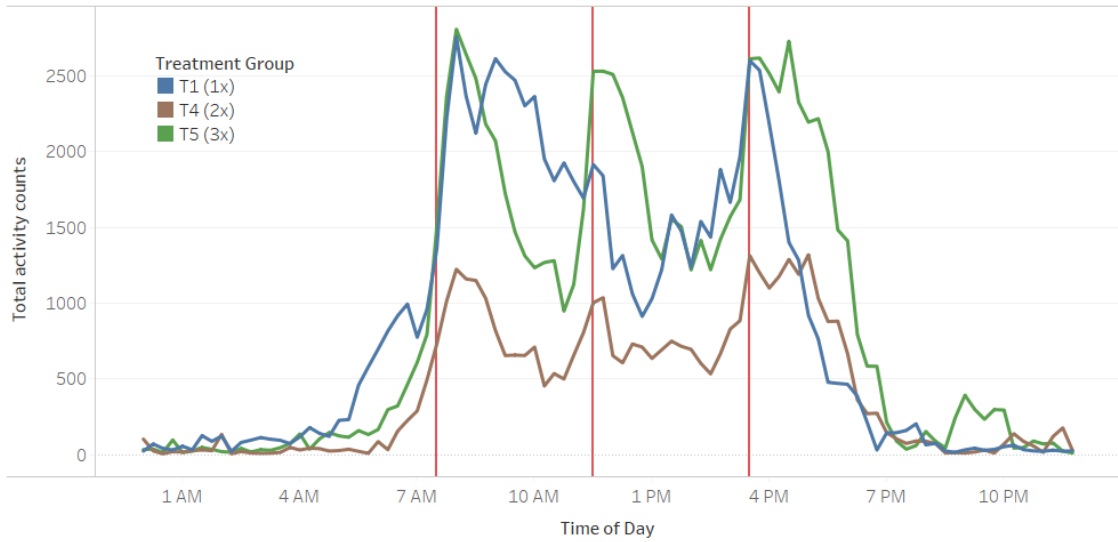


Figure 4. 2 Temporal pattern of pregnant sow’s total activity measured over a 24-h period over 7 days on different feeding frequency regimes under limit-fed conditions.

The graph represents sows fed 1× daily at 0730 (T1, blue curve), 2× daily at 0730 and 1530 h (T4, brown curve), and 3× daily at 0730, 1130, and 1530 h (T5, red green). The vertical red lines indicate feeding times for each treatment group. Light were turned on at 0730 h and switched off at 1630 h daily.

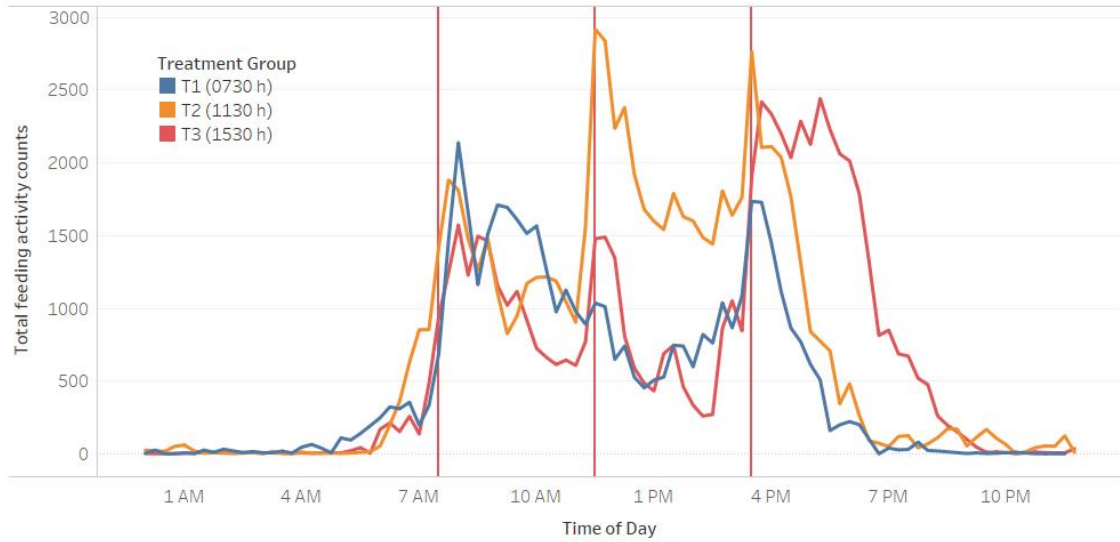


Figure 4. 3 Temporal pattern of 24-h feeding activity profile of pregnant sows fed at different times of the day for 7 days under limit-fed conditions.

The graph represents sows fed once daily at 0730 h (T1, blue curve), 1130 (T2, red curve), and 1530 h (T3, orange curve). Light were turned on at 0730 h and switched off at 1630 h daily. The vertical red lines indicate feeding times for each treatment group.

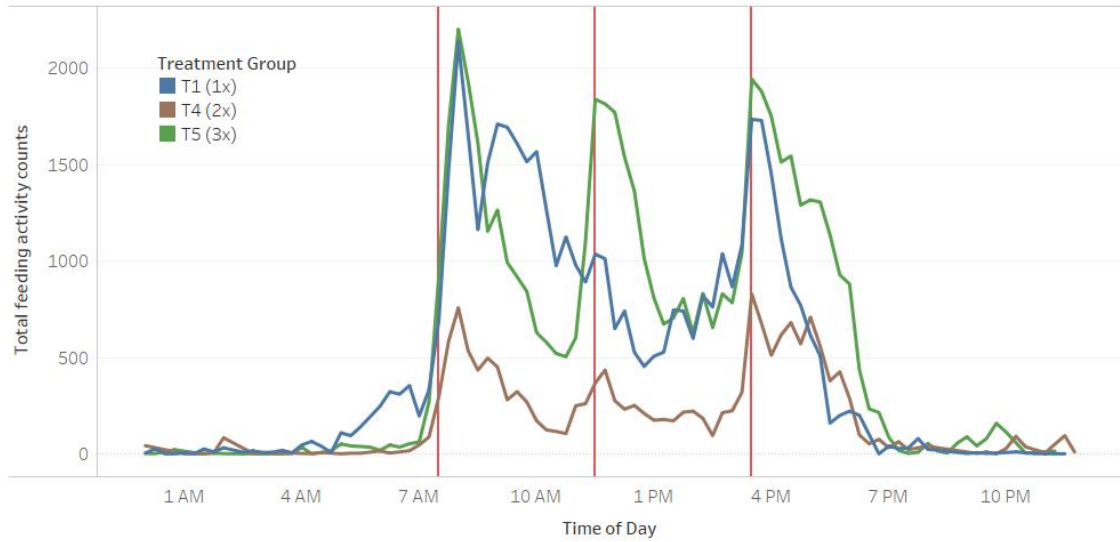


Figure 4. 4 Temporal pattern of pregnant sow’s feeding activity measured over a 24-h period over 7 days on different feeding frequencies under limit-fed conditions.

The graph represents sows fed 1×daily at 0730 (T1, blue curve), 2× daily at 0730 and 1530 h (T4, brown curve), and 3× daily at 0730, 1130, and 1530 h (T5, green curve). The vertical red lines indicate feeding times for each treatment group. Light were turned on at 0730 h and switched off at 1630 h daily.

## **Chapter 5. Quantitative evaluation of hunger in pregnant sows: metabolic and hormonal responses to feeding time and frequency under limit-fed condition**

### **SUMMARY**

The effects of feeding frequency and feeding time on glycemic response and endocrine status was studied in pregnant sows in a state of positive energy balance. The objectives were to determine the pattern of metabolite and hormonal response and indirectly quantify perceived hunger in pregnant sows under limit-fed conditions. Thirty sows [(Landrace × Yorkshire); BW =  $211.86 \pm 1.29$  kg; parity =  $3.80 \pm 0.16$ ; backfat (BF)  $13.50 \pm 0.25$  mm] were blocked by parity, farrowing date, and randomly assigned to 1 of 5 treatments in a randomized complete block design in stalls. Treatments include sows fed corn-soybean meal-based diet once at [0730 (Control, T1), 1130 (T2), or 1530 h (T3)], twice [half ration at 0730 and 1530 h (T4)], or thrice [one third portion at 0730, 1130, and 1530 h (T5)], with daily feed quantity kept at  $1.25 \times$  maintenance energy intake ( $100 \times (\text{BW})^{0.75}$ ) kcal ME/d. Sows received feed which providing  $6935$  kcal ME day<sup>-1</sup> from d30 - 60 of gestation. The gestation diet contained SID Lys:ME of  $1.71$  g/Mcal. On d 51 of gestation, sows were fitted with ear vein catheters with a nonsurgical procedure using a 12-gauge needle. Feeding time or frequency did not affect fasting concentrations of glucose, insulin, ghrelin, and total PYY ( $P > 0.100$ ). The control sows (1× daily) had greater 23-h mean glucose concentration than sows fed 3× daily ( $P = 0.002$ ). Sows on 2× daily feeding regime had similar mean glucose concentrations as sows either fed 1× daily ( $P = 0.117$ ) or 3× daily ( $P = 0.568$ ). The overall geometric mean of active ghrelin concentration for sows fed 3× daily tended to be lower ( $P = 0.063$ ) compared with control sows and significantly lower ( $P = 0.018$ ) than sows fed 2× daily. Provision of similar amount of



energy at different times of the day resulted in comparable glucose, active ghrelin, and PYY total AUC ( $P > 0.100$ ). Insulin 30 minutes post-prandial response to identical meals was greater ( $P < 0.05$ ) at 0730 and 1130 h than 1530 h. Attenuated insulin response in sows on 1530 h feeding schedule resulted in (31.7%;  $P = 0.083$ ) and (13.8%;  $P = 0.546$ ) greater 23-h insulin AUC for sows fed daily at 1130 and 0730 h, respectively. Consumption of 3 small meals (2311 kcal/meal) in 8 h resulted in 9.0% reduction in 23-h glucose total AUC compared with pregnant sows fed 1× daily. Sows fed 3× daily had 42.5% greater 23-h insulin total AUC, 27.4% reduced in active ghrelin total AUC, and 14.2% greater PYY total AUC but these variables did not differ with sows fed 1× daily. Active ghrelin total AUC correlated positively with PYY total AUC during pregnancy ( $r = 0.50$ ,  $P = 0.028$ ). Feeding time had no effect on glucose, active ghrelin, and total PYY 23-AUC. In conclusion, feeding time to elicit maximum insulin response are 0730 and 1130 but not at 1530 h. It is suggested that hunger in pregnant sows occurs at active ghrelin/total PYY ratio of  $5.06 \pm 0.20$  and at a mean plasma glucose concentration of  $70.37 \pm 1.39$  mg/dL and below.

**Key words:** Pregnant sows, feeding frequency, feeding time, insulin, total peptide YY, active ghrelin.

## INTRODUCTION

Dietary energy intake levels have significant effects on sows' reproductive performance (Hoving et al., 2011; Che et al., 2015). Dyck et al. (1980) demonstrated that embryonic survival decreased from 82.8% to 71.9% when quantity of feed fed increased above daily requirement from 1.5 kg to 3.0 kg daily. As such, pregnant sows are restrictively fed 40 to 60 % of their ad-libitum feed intake during gestation (Meunier-Salaün et al., 2001). The

feeding practice, however, may allow gestation sows to experience hunger most part of the day as evidenced by increased activity (foraging behavior) and motivation for food (Meunier-Salaün et al., 2001; D'Eath et al., 2009). Efforts to reducing hunger in gestation sows are geared toward dietary manipulations such as dilution of feed with fiber and provision of the feed *ad-libitum* (Sandilands et al., 2006). However, the benefits of high fiber in pregnant sows diets to reduce hunger and improved welfare is equivocal (Meunier-Salaün et al., 2001; Jensen et al., 2012). But the quantitative diet restriction which results in perceived hunger and apparent negative animal welfare has received less attention in pregnant sows.

Feeding behavior is closely related to hunger drive (Sugino et al., 2002). Provision of feed within a narrow time window each day leads to significant changes in physiology and behavior (Johnston, 2014). Also, increasing feeding frequency may directly affect hormones, gastric stretch, emptying, and impact hunger and satiety (Speechly and Buffenstein, 1999). However, without holding total caloric intake constant, the usefulness of meal frequency and meal-timing data can be limited in evaluating hunger (Kulovitz et al., 2014). Two main hormones extensively studied and known to have opposite effect on appetite are ghrelin and peptide YY (PYY) (Schwarz et al., 2011; Odunsi et al., 2009). Ghrelin can affect hunger sensation (Martínez et al., 2014). Postprandial suppression of ghrelin is determinant of insulin secretion since ghrelin and insulin concentrations are inversely related (Flanagan et al., 2003). In turn, glucose is a major regulator of insulin secretion (Aronoff et al., 2004). Therefore, glucose and insulin have suppressive effects on ghrelin and are important for ghrelin regulation (Flanagan et al., 2003; Vatansever-Ozen, 2011). It was hypothesized that changes in the timing and frequency of feeding

would affect levels of hunger without altering the quantity of feed provided per live metabolic weight. The objectives of this study were to determine the pattern of glucose, insulin, active ghrelin, total PYY responses to time and feeding frequency under normal production system and indirectly quantify hunger in pregnant sows. Finally, ghrelin/PYY ratio was used as an index of relative hormonal exposure to predict at what times hunger in pregnant sows' or desire to feed is greatest.

## **MATERIALS AND METHODS**

### ***Animals, Housing, and Management***

The study was conducted at the swine unit of University of Minnesota Southern Research and Outreach Center, Waseca, MN. University of Minnesota Institutional Animal Care and Use Committee approved all protocols used in the study (IACUC number 171011961).

Sows were kept in individual conventional gestation stalls with fully slatted floors measuring (2.1 m × 0.59 m × 0.97 m) under temperature-controlled environment (21 – 23 °C) on a 9-h light and 15-h of dark schedule, with light on at 0730 h and turned off at 1630 h. All sows exhibiting estrous were recorded and artificially inseminated twice, 24 h apart using fresh diluted semen from Duroc boar (Compart Boar Store, Nicollet, MN). The conception rate was 93% for the farrowing group sampled. On day 30 of gestation and before feeding, initial BW and BF thickness were recorded using an ultrasound machine (Lean-Meater, Renco Corp., Minneapolis, MN). Measurements were taken at the last rib about 5 cm lateral from the dorsal midline on both left and right sides using oil as coupling fluid and the two readings were averaged. Sows that returned to estrous were excluded from the experiment after pregnancy check on d 35 postcoitum with the aid of Ultrasonic

pregnancy checker (Classic medical supply, Inc., Florida, FL). Sows had free access to water through nipple drinker fitted to each stall. Feed offered to sows was restricted to 2 kg from weaning through breeding to 30 d which is considered optimal for sows and gilt during that phase.

### ***Experimental Design, Dietary Treatments, and Feed Line Calibration***

Thirty multiparous sows (TN 70; Landrace X Yorkshire, Topigs Norsvin USA, Burnsville, MN) breed with average BW =  $211.86 \pm 1.29$  kg and parity =  $3.80 \pm 0.16$  were blocked by parity, breeding date and balanced for BW and randomly allocated to 1 of 5 treatments with 6 replicates per treatment in complete randomized design. All pregnant sows received a common corn-soybean meal-based diet from d 30 to 60 of gestation. Nutrients met or exceeded NRC (2012) nutrient requirements for gestation sows. The diet and chemical composition of the diet is presented in Table 1. Experimental treatments were imposed from 30 d of gestation and 21 d acclimation to the new feeding regime or schedule was provided. Body weights on day 30, was used to adjust the amount of feed fed between d 30 and 60 of gestation. To standardize ME intake per kilogram live BW<sup>0.75</sup>, the daily quantity of feed fed was scaled to the BW<sup>0.75</sup> live weight (Le Naou et al., 2014) and fed at 1.25 times (Prunier and Quesnel, 2000) the maintenance requirements for sows ( $100 \times \text{BW}^{0.75}$  kcal ME/d; NRC, 2012). On average, sows received 6935 kcal ME/d from d 30 to 60. To provide a daily energy intake, sows received on average 2.21 kg from days 30 to 60 of gestation. Sows were fed individually by raising the feeder ball valve of an Accu-Drop feed dispenser (AP Systems, Assumption, IL) to drop the required amounts of feed into the feeding troughs. The Accu-Drop feed dispensers were calibrated at the d 30 at various set points and related the volume of Feed Dispenser, Y (cm<sup>3</sup>) to kilogram weight

of feed (x) delivered as:  $Y = 5.4864x + 1.9087$ ;  $R^2 = 0.9892$ . The required daily feed allowance was provided once daily at: 0730 (Control, T1), 1130 h, (T2), 1530 h (T3), twice [half ration at 0730 and 1530 h (T4)], or thrice [a third portion at 0730, 1130, and 1530 h (T5)].

### ***Catheterization Procedure***

On d 51 of gestation, 30 focal sows (from  $N = 60$  sows) were fitted with a temporary ear vein catheters into the Auricularis lateralis with a nonsurgical procedure using a 12-gauge needle (Newman et al., 2014). Briefly, the sow's ear was shaved and washed with antiseptic solution (Hibiscrub, ICI, Macclesfield, UK) and cleaned with 70% surgical alcohol. The sows were snare restrained and a rubber band placed around the base of the ear to expose the ear vein. A 12 gauge by 50 mm teflon catheter [Cathlon IV (4424), Critikon, Broadlands, Berkshire, UK] was inserted in the preferred vein close to base of the ear. After catheter insertion into the vein, the rubber band was cut and sterile gauze was used to cover the ear. About 10 ml of blood was drawn to test for correct positioning and functionality of the catheters. The entire catheters were flushed with 10 ml physiological saline solution (154 mM NaCl) containing 250 IU of heparin/ml and closed by means of a plug. The area surrounding the site of catheter insertion was cleaned with 70% surgical alcohol. Approximately 75 cm of plastic tubing (internal diameter, 0.76 mm; outside diameter, 2.29 mm; Norton, Akron, OH) was attached to the end of the catheters by means of a two-way stopcock to extend the catheters and to facilitate sampling with minimal disturbance to animals. The ends of the plastic tubes were locked with a rubber infusion plug through which samples were drawn. The external portion of the tubing was locked and wrapped in a zip lock bag and attached to the back of the sows using cement

glue. After the catheterization procedure, the sows were moved to their respective stalls by creating empty spaces between stall to prevent sow to sow contact.

### ***Blood Collection and Sample Preparation***

Blood collection was done on d 52 of gestation (after 21 d adaptation period) pre- and post-prandial from 0700 to 0500 h without imposing stress on the sows for plasma glucose, insulin, total PYY, and acyl-ghrelin concentrations. To obtain a fixed reference point for the measurements of postprandial changes in metabolites and hormones, the feeding period was restricted to 15 minutes after feed delivery. Blood samples were drawn at 0700 h, before feeding (fasting), 0715; Post-prandial at: 0800, 0830, 0900, 1100, 1130, 1200, 1300, 1400, 1500, 1600 1700, 1900, 2100, 2300, 0100, 0300, and 0500 h. Time 0730, 1130, 1530 h were the feeding times. Catheters were flushed following each blood-letting with 10 mL of physiological saline solution (154 m M NaCl) and tubing sealed to keep it patent between sampling. The blood samples (4 to 5 mL) were collected on ice with heparinized syringes into 8-mL chilled polypropylene vacutainer tubes spray-coated with 1.8mg K<sub>3</sub>EDTA as anticoagulant containing serine protease inhibitor 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF), (Sigma-Aldrich, St. Louis, MO). Tubes were gently inverted few times to mix the contents and immediately centrifuged for 15 min at  $2,500 \times g$  at 4°C. The supernatant was aliquoted into four Eppendorf storage tubes designated for glucose, insulin, acyl-ghrelin, and PYY analysis. Plasma samples designated for active ghrelin analysis were acidified with 50 µL of 0.05 N HCl. Based on ghrelin assay manufacturer recommendations (EMD Millipore, Billerica, MA), 10 µL of AEBSF (1 mg/mL) were added to 1,000 µL of all plasma

samples. Because HCl lowers the pH and AEBSF inhibits serine esterases and proteases, the *n*-octanoic acid bound to the third serine residue of ghrelin was maintained (Stockwell-Goering et al., 2015). All samples were stored at  $-80^{\circ}\text{C}$  until analyzed for glucose, insulin, total PYY and active ghrelin. At the end of blood sampling, catheters were flushed with 10 mL of 10 U/mL heparinized saline to replace the fluid loss and prevent clotting prior to their removal.

### ***Chemical Analysis***

Feed samples were analyzed for DM, GE, CP, NDF, and ADF. Dry matter content of feeds was determined by the oven-drying method (method 934.01; AOAC, 2006). Gross energy was determined by basic bomb calorimeter (IKA Werke GmbH and Co. KG, Staufen, Germany) with benzoic acid in the samples being used as calibration. The CP content ( $\text{N} \times 6.25$ ) in the basal diet was determined using Kjeldahl method (method 984.13, AOAC, 2006; Kjeltec 2300 Analyzer, Foss, Höganäs, Sweden). Determination of crude fat was by ether extract (method 920.39; AOAC, 2006) using an ANKOM XT15 extraction system (ANKOM Technology, Macedon, NY). The NDF and ADF content were determined using filter bag technique (ANKOM 2000 fiber analyzer, method 12 and 13; ANKOM Technology, Macedon, NY) (methods 973.18 and 973.19; AOAC, 2006). To determine the total ash content, samples of basal diet were ashed in a high temperature muffle furnace at  $600^{\circ}\text{C}$  for 6 h (Isotemp Muffle Furnace, Thermo Fisher Scientific Inc., Hampton, NH).

### ***Biochemical and Hormonal Analysis***

All samples of one subject were run in duplicate in a single assay. Plasma glucose was analyzed by enzymatic spectrophotometric analysis using Glucose oxidase- peroxidase reagent (Glucose Autokit, Wako Diagnostics, Richmond, VA) previously described (Burdick et al., 2014). Plasma glucose was quantitated on Olympus AU 2700 analyzer (Beckman Coulter, Inc., Brea, CA). This assay kit has previously been validated for use in porcine plasma (Schweer et al., 2017). The inter-assay and intra-assay coefficients of variation were 8.2% and 2.2%, respectively. Insulin was quantified using radioimmunoassay (RIA) kit (HI-14K; EMD Millipore, Billerica, MA) and performed in the laboratory of Veterinary College, Cornell University (Endocrinology Animal Health Diagnostic Center, Ithaca, New York). This assay kit has previously been validated for use in porcine plasma (Løvendahl and Purup, 2002). The intra-assay and inter-assay variation of 3.90% and 8.0%, respectively. Plasma active ghrelin and total PYY were quantified using (EMD Millipore Rat/Mouse Ghrelin (Active) ELISA Kit, Millipore Corporation, Billerica, MA, USA) and Human PYY total ELISA Kits (EMD Millipore PYY Total ELISA Kit, Millipore Corporation, Billerica, MA, USA), respectively, at Veterinary Diagnostic Laboratory (University of Minnesota, St. Paul, MN). All ELISAs were performed according to manufacturer's protocols. Parallelism and linearity of the active ghrelin and total PYY were ensured using 4 dilutions of a pooled porcine plasma sample which resulted in concentrations ranging from 40.2 to 417.1 pg/mL and 103.1 to 638.3 pg/mL, respectively. Linearity and recovery for active ghrelin and total PYY were 82.9 to 106.5% and 81.1 to 104.2%, respectively, relative to the expected concentrations. The intra-assay coefficients of variations for active ghrelin and total PYY were 5.5 and



2.02, and inter-assay variation were 12.8 and 11.74, respectively. The sensitivity of the assay was 8.0 and 6.5 pg·mL<sup>-1</sup> for active ghrelin and total PYY, respectively. All ELISA plates were read using a Synergy H1 system (BioTek Instruments, Inc., Winooski, VT).

### ***Statistical Analysis***

Normality and homogeneity of residual data sets were examined graphically and verified using the quantile-quantile plot (Q-Q plot) with PROC UNIVARIATE of SAS 9.4 (SAS Inst. Inc., Cary, NC). Fasting acyl-ghrelin, PYY, insulin, and glucose concentrations represented measurement at 30 minutes before feeding for each treatment. Glucose, insulin, acyl-ghrelin, and PYY data were analyzed using the PROC MIXED procedure of SAS 9.4 as repeated measures in time either from baseline to 3.5-h post-prandial or from baseline to 21 or 23-h post-prandial. The main effects of treatment (feeding time or feeding frequency), time and treatment × time interactions were tested. Random effect of sow was included in the model. Autoregressive process of first order (Littell et al., 1998) was used to model repeated observation within sow as covariation structure for glucose and acyl-ghrelin, while heterogenous process of first order was used for insulin and PYY variables. The selection of the covariation structure was based on Akaike information criterion (AIC). Significant means were separated for treatment using the PDIFF statement in the PROC MIXED model after detecting a significant treatment effect. Where treatment and time interaction was statistically significant ( $P < 0.05$ ), within-time treatment effects were evaluated.

Changes from baseline to 30 minutes or 60 minutes postprandial in metabolite and hormone concentrations were evaluated using one sided PROC paired T-test of SAS. Individual sow data were averaged for each time point for graphical presentation. Area

under the curve [(4-h, from fasting to 3.5-h post-prandial and 21 or 23-h, from fasting to 20.5 or 22.5-h post-prandial)] for glucose, insulin, PYY, and acyl-ghrelin were calculated using the trapezoidal method. A log transformation was used to stabilize the variance in ghrelin, PYY, and their respective AUC data. The AUC data were analyzed using PROC GLIMMIX of SAS. Back transformed geometric mean with ( $\pm 95\%$  confidence intervals) for plasma ghrelin and PYY concentrations over time and AUC are presented. Correlation coefficients between fasting metabolic and hormones and total AUC were computed using the PROC CORR procedure of SAS and considered significantly different from 0 at  $P \leq 0.05$ . Treatment least squares means within feeding time or feeding frequency were compared to each other and Tukey adjustment was used to control for experiment-wise error. Each pregnant sow was the experimental unit. All statistical test with  $P \leq 0.05$  was considered significant whereas  $0.05 < P \leq 0.10$  was considered tendency toward significance.

## RESULTS

### *Pre- and post-prandial glucose, insulin, and active ghrelin pattern in response to feeding frequency*

Plasmatic levels of glucose, insulin, back transformed geometric means with 95% confidence interval of ghrelin and PYY concentrations, and total AUC in response to feeding frequency are provided in Table 2 and 3. Feeding frequency by time interaction was not significant for any response variable measured ( $P > 0.10$ ) except total PYY variable ( $P = 0.0001$ ). Independent of feeding regime, the maximum mean active ghrelin to total PYY ratio observed within the day was  $5.06 \pm 0.2$  at glucose concentration of  $70.37 \pm 1.39$  mg/dL (Figures 9 and 10). Time had significant effect on glucose, insulin,

PYY, and ghrelin in response to feeding frequency (Figure 3, 4, 6, and 8;  $P \leq 0.001$ ). The glycemic response to feeding frequency showed that sows fed 3× daily had lower overall mean glucose concentration (73.89 vs. 67.96 mg/dL;  $P = 0.002$ ) compared with control sows fed 1× daily. Sows on 2× daily feeding regime had similar mean glucose concentrations compared with sows either fed 1× daily (69.86 vs. 67.96 mg/dL;  $P = 0.117$ ) or 3× daily (69.86 vs. 73.89;  $P = 0.568$ ). The overall geometric mean of active ghrelin [262.7 (95% CI: 291.7 - 236.6)] for sows fed 3× daily tended to be lower ( $P = 0.063$ ) relative to the control sows [383.0 (95% CI: 435.4 - 336.9)] and significantly lower ( $P = 0.018$ ) compared with sows fed 2× daily [(415.3 (95% CI: 472.2 - 365.3))] respectively. Feeding sows 1× and 2× daily resulted in similar mean active ghrelin response ( $P = 0.896$ ). Overall mean response of insulin ( $P = 0.155$ ) and PYY ( $P = 0.798$ ) to feeding frequency were not different.

Fasting concentrations of glucose and PYY did not differ in response to feeding frequency ( $P > 0.10$ ). Compared with sows fed 1× ( $P = 0.054$ ) and 2× daily ( $P = 0.062$ ), pregnant sows on 3× daily feeding schedule tended to have greater fasting insulin levels. On the contrary, a tendency for lower fasting ghrelin concentrations was observed ( $P = 0.072$ ) in sows fed 3× daily relative to sows fed 2× daily but similar to sows on 1× daily feeding schedule ( $P > 0.10$ ). Feeding sows 1× or 2× daily resulted in similar fasting insulin ( $P = 0.990$ ) and ghrelin levels ( $P = 0.537$ ). Change in glucose concentration from fasting level to 30 or 60 minutes post-prandial was not significant with respect to feeding frequency ( $P > 0.10$ ). Sows fed 1× ( $P = 0.018$ ) and 3× daily ( $P = 0.032$ ) had significantly higher insulin concentration at fasting compared with 30 minutes post-prandial. Sows on different feeding frequency regimes had reduced ghrelin concentration 60 minutes after feeding ( $P$

$\leq 0.005$ ). Sows fed 2× daily ( $P = 0.009$ ) and 3× daily ( $P = 0.010$ ) had increased PYY concentration 30 minutes post-prandial from fasting level but this increase was not profound in sows fed 1× daily ( $P > 0.10$ ).

The 4-h glucose, insulin, and PYY AUC were not different among treatments ( $P > 0.10$ ). The 23-h glucose AUC was 9% lower for sows fed 3× daily compared with control sows ( $P = 0.023$ ). Sows fed 2× daily tended to have 7.8% lower 22-h glucose AUC relative to the control sows ( $P = 0.093$ ). Sows on either 2× or 3× daily feeding regime had similar 22-h glucose ( $P = 0.919$ ) and insulin ( $P = 0.509$ ) AUC. Sows fed 3× daily had tendency of 42.5% greater 22-h insulin AUC compared with control sows ( $P = 0.069$ ). The control sows and sows fed 2× daily had similar 22-h insulin ( $P = 0.541$ ) and active ghrelin ( $P = 0.981$ ) AUC. There was tendency of reduced 4-h active ghrelin AUC in sows fed 3× daily relative to sows fed 2× daily ( $P = 0.079$ ) but similar to control sows ( $P = 0.119$ ). Although not significant, sows on 3× daily feeding schedule had lower 22-h active ghrelin AUC compared with control sows ( $P = 0.265$ ) and sows fed 2× daily ( $P = 0.351$ ). The 22-h PYY AUC was not different among treatments ( $P > 0.10$ ).

***Pre- and post-prandial glucose, insulin, and active ghrelin pattern in response to feeding time***

Plasma glucose, insulin, back transformed geometric means with 95% confidence interval of ghrelin and PYY concentrations, and total AUC in response to feeding time are provided in Table 4 and 5. No significant treatment by time interaction was observed for glucose, insulin, and PYY variables ( $P > 0.10$ ) except active ghrelin ( $P < 0.005$ ), (Figures 1, 2, 5, and 7), respectively. Except for glucose ( $P = 0.261$ ), time had significant effect on insulin, PYY, and ghrelin from fasting level to 23-h post-prandial ( $P \leq 0.012$ ). Pregnant

sows fed at different times of the day had similar fasting levels of glucose, insulin, ghrelin, and PYY ( $P > 0.10$ ). Feeding sows at 0730 and 1130 h daily did not elicit glucose, insulin, and ghrelin response at 30, 90, and 210 minutes post-prandial ( $P > 0.10$ ). Reduction and trend towards lower glucose concentration in 1530 h fed sows at 90 minutes compared with sow groups fed at 0730 ( $P = 0.014$ ) and 1130 h ( $P = 0.058$ ), respectively, was not expected. Except for sow group fed at 1530 h that exhibited a trend ( $P = 0.053$ ) in 30 minutes postprandial geometric mean increase from fasting levels, sows fed at 0730 ( $P = 0.584$ ) and 1130 h ( $P = 0.274$ ) did not differ in PYY concentration.

As expected, feeding induce insulin increase was observed at 60 minutes post-prandial with control sows having greater insulin concentration than 1130 ( $P = 0.008$ ) and 1530 h ( $P = 0.0003$ ) fed sows (Figure, 2). Similar response was noted at 1200 ( $P = 0.012$ ) and 1300 h ( $P = 0.017$ ) with 1130 h fed sows having greater insulin response than sows on 1530 h feeding regime. At 1600 h no significant feeding induced insulin response was noted for sows fed daily at 1530 h compared with sows fed at 0730 and 1130 h ( $P > 0.10$ ). Fasting ghrelin concentration tended to be lower 30 minutes post-prandial in sows fed at 1130 h ( $P = 0.065$ ) but did not significantly reduced in treatment groups fed 0730 and 1530 h ( $P > 0.10$ ). Glucose post-prandial increased from fasting level was not different for different feeding times ( $P > 0.10$ ). Sows fed daily at 0730 and 1130 h had increased ( $P = 0.018$ ) and propensity to increase ( $P = 0.088$ ) in insulin concentration from fasting to 30 minutes post-prandial, respectively, but not in sows fed daily at 1530 h. Neither the 4-h nor 23-h glucose AUC was affected by feeding time ( $P > 0.10$ ). Although not significant, the 4-h insulin AUC was lower in sows fed at 1530 h than in sows fed daily at 0730 (62.97 vs 108  $\mu\text{U}\cdot\text{h}/\text{mL}$ ;  $P = 0.303$ ) and 1130 h (62.97 vs 132.2  $\mu\text{U}\cdot\text{h}/\text{mL}$ ;  $P =$

0.178). Consequently, the insulin 23-h AUC for sows on 1530 feeding schedule tended to be lower compared with sows fed daily at 1130 h (272.6 vs 398.8  $\mu\text{U}\cdot\text{h}/\text{mL}$ ;  $P = 0.083$ ) and numerically reduced relative to sows fed daily at 0730 h (272.6 vs 316.4  $\mu\text{U}\cdot\text{h}/\text{mL}$ ;  $P = 0.546$ ). Sows receiving their daily feed at 0730 and 1130 h had similar 23-h insulin AUC ( $P = 0.276$ ). Provision of equal amount of energy per kilogram live  $\text{BW}^{0.75}$  at different time of the day resulted in similar 4-h and 23-h active ghrelin and total PYY total AUC ( $P > 0.10$ ).

#### ***Correlations of Metabolite, Hormones and AUC data***

Fasting plasma active ghrelin levels correlated positively with fasting plasma total PYY levels of pregnant sows ( $r = 0.57$ ,  $P = 0.010$ ). Similarly, active ghrelin total AUC correlated positively with PYY total AUC ( $r = 0.50$ ,  $P = 0.028$ ). Other correlations among glucose, insulin, active ghrelin, and total PYY variables of pregnant sows was significant ( $P > 0.10$ ).

## **DISCUSSION**

The present study examined the effect of feeding frequency (1 $\times$ , 2 $\times$ , and 3 $\times$ ) and time of feeding (0730, 1130, and 1520 h) on glucose, insulin, active ghrelin, and total PYY pattern to indirectly quantify perceived hunger in gestation sow under normal production conditions. As such sows were not fed the traditional way. Typically, gestations sow's nutrient requirements are estimated based on ME and fiber content of the diet, length of gestation, parity, expected litter size and birth weight, anticipated maternal weight gain, and body condition (NRC, 2012). Therefore, feeding levels are adjusted to attain a targeted body condition score (BCS) of 3 (scale: 1 = emaciated and 5 = overly fat) at farrowing (Li et al., 2014). The implication is that thin sows feed allowance is increased while fat or

obese sows daily ration is reduced. Each sow is however, different in terms of assessment of hunger since they have different maintenance requirement per kilogram BW<sup>0.75</sup>. Sows were fed 125% their maintenance energy requirement of NRC, (2012) to arrive at feeding level similar to their normal gestation feed intake.

### ***Effect of Feeding Time and Frequency on Plasma Glucose and insulin Responses***

Time of feeding did not show a discernable pattern or treatment effect on mean glucose concentration, fasting glucose concentration, 4-h, and 23-h glucose AUC when sows were fed similar amount of energy per kilogram live metabolic weight. Glucose concentrations are tightly regulated in healthy animals and controlled by a variety of homeostatic mechanisms (Vahl et al., 2014; Bourgot et al., 2018). During the activity or feeding period blood glucose is mainly of dietary origin and during the resting or starvation period glucose is recruited from the liver to maintain blood glucose levels within a relatively narrow range (Kalsbeek et al., 2014). Therefore, it was not surprising that glucose concentrations, 4-h, and 23-h glucose AUC were less subject to significant differences among feeding times as opposed to substantial response found for insulin. Although, glucose did not show significant differences with feeding time, substantial changes in 23-h mean glucose concentration and AUC occurred with feeding frequency indicating that glucose concentrations can be altered through feed pattern. The 23-h mean concentration of glucose and AUC fell by 5.9 mg/mL and 153.8 mg\*h/mL, respectively, when feeding frequency of iso-caloric diet increased from 1× to 3× in sows. Our primary assumption was that consumption of more frequent meals (3× daily) within the day would result in higher blood glucose concentrations over the course of the day than the consumption of 1

meal (1×) to induce greater satiety since inhibition of glucose metabolism with the glucose analog 2-deoxy-D-glucose (glucoprivic feeding) increases food intake in animals (Smith and Epstein, 1969) and increases hunger sensations and food intake in humans (Thompson and Campbell, 1977). However, it was theorized that tissues of meal-fed animals utilize glucose more rapidly and the enhanced glucose utilization allows for considerable portions of glucose to be converted to storage forms (Leveille, 1970; Adams and Morgan, 1981). This may account for the reduced glucose concentration in sows fed 3× daily.

However, effects of feeding frequency on glucose concentrations have been inconsistent in different studies. Consistent with our result, Carlson et al. (2007) reported lower mean glucose concentration and 22.3% lower glucose AUC in healthy, normal-weight middle-aged men and women who had 3 meals a day over those who had 1 meal daily of iso-energetic diet even though insulin AUC did not differ. Similarly, Leidy et al. (2010) observed a 30% reduction in glucose AUC with meal frequency in human. Also, feeding frequency reduced glucose concentration in male rats relative to ad-libitum fed rats (Vahl et al., 2014). Conversely, feeding pregnant sows 2× daily on barley based diet (84.5% barley and 12% soybean meal containing 48.5% C.P) elicited greater mean glucose concentration of  $71.4 \pm 1.7$  mg/dL than sows fed 1× daily ( $67.4 \pm 1.9$  mg/dL) during second trimester of gestation (d 37 to 74) but the authors did not evaluate the AUC (Williams et al., 1979). Additionally, the 24 h AUC for glucose secretion profile was significantly greater in lean healthy males on 14× daily feeding regime relative to those on 3× daily feeding (Munsters and Saris, 2012). A research report indicated no significant differences in glucose concentration with feeding frequency in human and animal



experiments as reported by Raynor et al. (2015). Discrepancies in results could be attributed to differences in dietary composition.

Different feeding strategies affect insulin secretion patterns and concentrations (Scrimgeour et al., 2008). When pigs were entrained to receive their ration either once or twice daily, insulin secretion pattern responded to the period of feeding, with significant increases occurring approximately 1 h post-prandial (Scrimgeour et al., 2008). Likewise, insulin concentration from fasting level was increased in our study 30 minutes post-prandial in response to feeding with significant increases occurring in sows fed at 0730 and 1130 h. This post-prandial increase in insulin concentration is in line with the findings of Martínez et al. (2014) in pregnant sows. Insulin is an important metabolic hormone, the concentrations of which usually increase post-prandial to provide energy to cells (Vieira et al., 2010). One of the most striking observations of this study was that feeding pregnant sows at different time of day stimulated insulin secretion, which resulted in a distinct spike following each feeding at 0730 and 1130 h, whereas plasma insulin concentration was attenuated in sows fed once daily at 1530 h. Furthermore, sows on different feeding frequency regimes elicited a response at 0730 and 1130 h but sows that received full, half or a third portion of their daily ration at 1530 h had attenuated insulin response. These results suggest that feeding sows twice daily at 0730 and 1130 h may be beneficial to optimize insulin response. Consequently, both the 4-h and 23-h insulin AUC was lower for sows fed daily at 1530 h compared with sows on 0730 and 1130 h feeding regime. This means that feeding time is a major determinant of insulin response rather than feeding pattern or frequency.

The focus of this study was not on the separate effects of feeding frequency or feeding time. Nevertheless, both factors seem to interact regarding insulin secretion because by controlling feeding time and feeding frequency, we created two completely different insulin secretion patterns (Figures 2 and 4). Virtually all life forms (prokaryotes to eukaryotes) have circadian oscillators due to regular 24-hourly rotation of the earth. Therefore, feeding at inappropriate time may result in circadian misalignment, or diminished circadian oscillations (Bechtold and Loudon, 2013). In line with our findings, the postprandial insulin response was greater after morning feeding than after afternoon feeding, as reported earlier in human (Kalsbeek et al., 2014; Kessler et al., 2017); growing pigs (Malmlöf et al. 1990), and sows (Valros et al. 2003) using equal food or feed portions at each feeding occasion. It was speculated that the elevated morning insulin response could be attributable to longer fasting period before the morning feeding (Wientjes, 2013). Our data provide evidence that time of feeding influences insulin response to a greater extent than fasting periods between meals since sows fed 1× daily at 0730, 1130, and 1530 h had equal fasting periods between meals. Conversely, Plat et al. (1996) explained that insulin responses are higher in the morning to offset the catabolic effect of cortisol, which levels are elevated in the morning. Additionally, a decrease in pancreatic  $\beta$ -cell responsiveness to glucose in human at dinner time (Morrison et al., 2015) or circadian rhythm of insulin secretion (Boden et al., 1996) have also been reported. Finally, the concentrations of incretins such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which stimulates insulin secretion have greater concentration in the morning than afternoon in human (Lindgren et al., 2009). Thus, the attenuated postprandial insulin responses at 1530 h compared to the morning, as

observed in our study with reference to either feeding time or feeding frequency, could be mediated by several mechanisms.

Feeding sows 2× and 3× daily resulted in 27.3% and 42.5% greater 22-h insulin AUC, respectively, compared with control sows. Surprisingly, increase in insulin AUC was accompanied by a reduction in glucose AUC. Glucose is a major but not the sole regulator of insulin secretion (Aronoff et al., 2004). The incretin effect of food ingestion caused a more potent release of insulin than glucose infused intravenously (Aronoff et al., 2004). Additionally, insulin is secreted if the blood glucose concentration is greater than 60 mg/dL (Gerich, 1993). Sows on 3× daily feeding regime had mean glucose concentration of 67.96 mg/dl. We speculate that each feeding occasion induced an incretin effect of GLP-1 and GIP release which act as stimulants for insulin secretion since they are elevated during the postprandial period (Takahashi et al., 2018). Additionally, the glucose which was released at each feeding occasion may increase the glucose concentration above the threshold of 60 mg/dL to induce greater insulin response relative to control sows fed 1× daily.

#### ***Effect of Feeding Time and Frequency on Plasma Ghrelin and Peptide YY Responses***

Although not significant, sows fed 2× and 3× daily had 3.9% and 27.4% lower active ghrelin total AUC compared with the control sows (1×). Similar reduction in ghrelin AUC was reported in sheep on meal frequency (Solomon et al., 2008). In the short term regulation of food intake, gastric sensation, emptying and volume are some physiological factors associated with postprandial fullness and the desire to eat (Delgado-Aros et al., 2004). The distention of gastric wall activates stretch receptors and mechanoreceptors inhibiting the ingestion of food (Havel, 2001). It is speculated that sows fed multiple

times had suppressive effect on ghrelin by spreading the energy and the nutrient loads within the day relative to sows fed once daily. Overall, sows on 3× daily feeding regime although not significant, had reduced active ghrelin 23-h AUC, increased insulin 23-h AUC, and lower glucose 23-h total AUC compared with sows fed 1× daily. In support of this observation, Flanagan et al. (2003) reported that postprandial suppression of ghrelin is determinant of insulin secretion since ghrelin and insulin concentrations are inversely related. Additionally, similar result but opposite effect was found by other researchers in human study. Schwarz et al. (2011) reported that ghrelin administration in healthy human decreased insulin levels causing an elevation in blood glucose concentrations.

However, the lack of significant effect of insulin on ghrelin could be ascribed to the fact that same calories per metabolic weight were consumed. It was documented that the insulin-ghrelin relationship is less apparent with increase meal frequently with the same caloric consumption. The lack of inverse relationship with meal frequency on iso-energetic diet may be related to less insulin-related fluctuations in ghrelin leading to less pre-prandial increase in ghrelin and hence less gastric sensations or “hunger” (Lucidi et al., 2002; Schwarz et al., 2011). Additionally, the effect of feeding frequency regimes on total active ghrelin AUC of pregnant sows resonates with findings in human studies. In over-weight or obese (Allirot et al., 2013; Leidy et al., 2010) or lean (Allirot et al., 2013; Scrimgeour et al., 2008) individuals subjected to 8 week hypocaloric intervention, ghrelin AUC was not different between meal frequency regimes. Furthermore, post-prandial ghrelin reduction depends on nutrient content but not the volume of a meal (Tschöp et al., 2000; Erdmann et al., 2003).

Feeding time did not significantly influence total active ghrelin AUC although sows fed daily at 1130 and 1530 h had 34.9% and 12.4% greater AUC, respectively, relative to the controls sows fed at 0730 h. This suggests that feeding sows once daily at 0730 h may be beneficial relative to offering feed at 1130 and 1530 h. There is no data that has investigated the effect of feeding time on active ghrelin response and cannot compare the current results with. However, the lower active ghrelin levels at 0830, 1200 and 1500 h in pregnant sows fed once daily at 0730, 1130, and 1530 h respectively, could be related to the high insulin concentration at those time points. Lucidi et al. (2002) reported that postprandial hyperinsulinemia accounts for the reduction in plasma ghrelin that occurs during meal intake.

Diurnal variation of ghrelin in human studies revealed a nocturnal rise in plasma ghrelin concentration between 0000 and 0600 lean individuals (Blundell et al., 2010) but factors that modulate ghrelin secretion during such times other than meal-related events is not clear (Spiegel et al., 2011). By feeding sows at different time of the day, this nocturnal increase in plasma ghrelin concentration was masked. However, the nocturnal increase in plasma ghrelin concentration was found in sows on feeding frequency regime at 0100 but it was not profound. Active ghrelin has a half-life of 9 to 13 minutes (Akamizu et al., 2004). Because we sampled our sows every 1 or 2 hours from 0000 h, substantial changes in concentration of the active ghrelin could have been missed within the sampling intervals. The present data suggest that feeding time and frequency in sows per kilogram live metabolic weight and under normal production condition does not appear to have superior effects on modulation of ghrelin release. In sows, ghrelin concentration is reported to increase in response to energy insufficiency (Bradford and Allen, 2008; Wertz-

Lutz et al., 2010). This means that energy status of our sows was adequate since they were fed 25% above their maintenance energy requirements. Therefore, given similar energy intake above sows maintenance requirement within 24 h we did not expect to see any differences with feeding frequency or feeding time.

Neither feeding time nor frequency affected PYY levels and AUC during pregnancy even though sows fed 2× and 3× daily had 12.5% and 12.4 % greater total AUC of PYY, respectively, relative to the control sows. Similar to our observation, PYY levels did not change in human during the first trimester of pregnancy (Valsamakis et al., 2014). However, the marginal increase in the AUC of sows fed 2× and 3× daily could be due to production of short-chain fatty acid (SCFA). Fermentation of dietary fiber in the small intestine and colon produces SCFA, such as acetate, propionate, and butyrate, which are known to affect PYY release. It was reported that rectal and ileal infusions of SCFA in pigs, humans, rodents (Ingerslev et al., 2017) and oral propionate or butyrate administration in mice stimulated PYY release (Lin et al., 2012). Increased feeding frequency of concentrate diet tended to increase acetate to propionate ratio in cows (French and Kennelly, 1990) but no difference was obtained in sows fed either 1× or 2× (Jensen, 2012). We speculate that the increase in PYY AUC in sows on feeding frequency regimes could be due to increase production of SCFA which are known to activate the G-protein-coupled free fatty acid receptors 3 and 2 (FFAR3 and FFAR2) to increase PYY secretion (Brown et al., 2003).

Contrary to our expectations, we found an elevated and relatively stable concentration of circulating PYY in our pregnant sows fed once daily at 1130 and 1530 h compared with control sows fed once daily at 0730 h. Although not significant, feeding pregnant sows

daily at 1130 and 1530 h resulted in 34.5% and 20.9% increase in total AUC, respectively, relative to the control sows. However, the relatively high levels of PYY with respect to feeding time also mirrored active ghrelin concentration which was not expected since ghrelin and PYY reciprocally inhibit each other (Hill et al., 2012). Lack of reciprocal association could be due to physiological status (pregnancy) of the sows. Pregnancy is characterized by a series of metabolic changes that promote adipose tissue accretion to meet increased metabolic demand (Tovar et al., 2004). Metabolic adaptation during pregnancy and caloric restriction is complex (Hall et al., 2015). Such metabolic changes resulted in several orexigenic and anorexigenic signals in rats (Reis et al., 2002) and human (García et al., 2003).

In line with our findings, a marked increase in circulating PYY levels was found in pregnant rats and women and it was proposed that gestation may be associated with a PYY state of resistance through alteration of Y2 receptor (Y2R), which mediates the effect of PYY<sub>3-36</sub> (García et al., 2000; Valsamakis et al., 2010). However, chronic food restriction completely prevented the pregnancy-associated increase in circulating PYY levels in pregnant rats (Tovar et al., 2004). Therefore, it is possible that the increased plasma PYY concentrations during pregnancy may represent a physiological adaptation to the positive energy balance during pregnancy. Hence, we speculate that lack of reciprocal inhibition of the two hormones could be attributed to the fact our sows were not nutritionally compromised. Additionally, we did not compare the active form of peptide tyrosine tyrosine (PYY<sub>3-36</sub>) with the active form of ghrelin.

The present results demonstrate that active ghrelin and total PYY responses to different feeding frequency regimens are quite different (Fig. 6, Fig. 8), but such feeding patterns

have no effect on total AUC when pregnant sows are fed similar amount of energy based on their live kilogram metabolic weight (Table 3).

***Plasma Active Ghrelin and PYY Ratio as Index of Hormonal Exposure and their correlations during pregnancy***

Ghrelin can affect hunger sensations (Erdmann et al., 2007), increased appetite and therefore feeding initiation (Martínez et al., 2014). To determine when changes in ghrelin and PYY occurred throughout the day in response to feeding time or frequency; ghrelin-to-PYY ratio at each time point was used as an index of relative exposure of each hormone. Hill et al. (2013) theorized that if the ratio increases throughout the day, it could be interpreted in 1 of 4 ways: 1) increases in the ghrelin concentration is far greater than corresponding increases in the PYY concentration, suggesting that hunger or desire to feed prevails throughout the day; 2) decreases in the PYY concentration occurred simultaneously with increases in the ghrelin concentration, 3) at any PYY concentration, the ghrelin level was increasing, or 4) at any ghrelin concentration, the PYY level was decreasing. The opposite explanations could be used for decreases in the ratio. The ghrelin/PYY ratio observed independent of feeding time or feeding regime revealed two distinct feeding peaks within the daytime suggesting that influence of ghrelin on physiological processes may be greater at those time point (figures 9 and 10). If ghrelin secretion is regulated by hunger drive or considered as physiological meal initiator, then the pattern suggest that twice daily feeding at 0730 and between 0100 and 1530 h could be desirable to meet physiological drive to feed and it is tempting to speculate that this occurs at active ghrelin/total PYY ratio of  $5.06 \pm 0.20$  and at a mean plasma glucose concentration of  $70.37 \pm 1.39$  mg/dL and below. Pregnant sows fasting active ghrelin



concentration and AUC correlated positively with fasting total PYY levels and AUC, respectively. This suggests a possible parallelism of their secretion during normal pregnancy.

## CONCLUSION

In conclusion, consumption of 3 small meals (2311 kcal/meal) in 8 h resulted in 9.0% reduction in 23-h glucose total AUC compared with pregnant sows fed 1× daily. Sows fed 3× daily had 42.5% greater 23-h insulin total AUC, 27.4% reduced in active ghrelin total AUC, and 14.2% greater PYY total AUC but these variables did not differ with sows fed 1× daily. Active ghrelin total AUC correlated positively with PYY total AUC during pregnancy. Plasma insulin response appears to be a function of frequency of feeding and time of the day at which calories are ingested. Time of feeding to elicit maximum insulin responses are 0730 and 1130 h but not at 1530 h. Therefore, feeding pregnant sows twice daily at 0730 and 1130 h may elicit maximum insulin response, reduce active ghrelin concentration, reduce hunger, and could improve sow's welfare as oppose to feeding pregnant sows once daily. It is suggested that hunger in pregnant sows occurs at active ghrelin/total PYY ratio of  $5.06 \pm 0.20$  and at a mean plasma glucose concentration of  $70.37 \pm 1.39$  mg/dL and below.

### *Limitations of study*

This study has some limitations. First, the sampling intensity was not the same as we could not obtain blood samples 1 hour post-prandial for sows fed daily at 1130 and 1530 h compared with sows fed daily at 0730 h. Secondly, even though blood samples were successfully drawn for all the 6 sows in treatment 5, our sample size varied in other treatments due to sows flipping their ears to remove catheters, scratching themselves

against the pen to destroy catheters, and blocking of catheters to prevent blood sampling at the designated time points. On average, sows, n = 4, T1; n = 2, T2; n = 4, T3; n = 4, T4 and n = 6, T5 were sampled for 23 h. The reduced sample size severely limits the statistical assessment and the robustness of any conclusion. A larger sample size (through repeated sampling) would have provided a better insight over the sampling period. Therefore, our conclusions with respect to imposed treatments are indicative rather than conclusive.

Table 5. 1 Composition and nutrient analysis of gestation diet, as fed basis.

Ingredients, %	Gestation diet
Yellow dent corn	65.35
Soybean meal, dehulled, solvent extracted	10.00
Corn DDGS <sup>1</sup> , > 6 and < 9% oil	20.00
Choice white grease	1.50
Di-calcium phosphate	1.20
Limestone, ground	1.00
Sodium chloride	0.35
L-Lys-HCL	0.10
Swine breeder premix <sup>2,3</sup> (EB Plus)	0.50
<b>Total</b>	<b>100.00</b>
<b>Analyzed composition</b>	
DM, %	89.34
GE, kcal/g	4,431
CP, %	15.70
NDF, %	13.30
ADF, %	4.80
<b>Calculated nutrient composition</b>	
SID Metabolizable energy (ME), kcal/kg	3,379
SID Lys, %	0.58
SID Met, %	0.26
SID Met+Cys, %	0.48
SID Thr, %	0.45
SID Trp, %	0.12
Total available P	0.61
Ca, %	0.70
Crude fat, %	6.30
SID Lys/ME, g/Mcal	1.71

<sup>1</sup>Dried distiller's grains with solubles.

<sup>2</sup>Mineral and vitamin mixture supplied per kilogram of diets: 15 mg of Cu (as CuSO<sub>4</sub>); 124 gm of Fe (as FeSO<sub>4</sub>·7H<sub>2</sub>O); 40 mg of Mn (as MnO); 124.7 mg of Zn (as ZnO); 2.2 mg of I (as Ca(IO<sub>3</sub>)<sub>2</sub>); 0.30 mg of Se (as Na<sub>2</sub>SeO<sub>3</sub>); 11000 IU of vitamin A; 2750 IU of vitamin D<sub>3</sub>; 55 IU of vitamin E; 4.4 mg of vitamin K<sub>3</sub>; 1.1 mg of thiamine; 9.9 mg of riboflavin; 55 mg of nicotinic acid; 33 mg of d-pantothenic acid; 2.2 mg of pyridoxine; 0.06 mg of vitamin B<sub>12</sub>; 2.6 mg of folic acid; and 0.22 mg of biotin, and 1.92 mg of Na.

<sup>3</sup>Swine breeder premix was supplied by Agric-Nutrition Services, INC. Shakopee, MN.

Table 5. 2 Fasting and post-prandial glucose and insulin concentrations and their respective area under the curve in response to feeding frequency.

Item	Metabolite or hormone concentrations						Total AUC <sup>1</sup>		P-values <sup>2</sup>		
	Trt <sup>3</sup> .	Fasting	Post-prandial				4-h	23-h	Trt.	Time	Trt.*Time
Glucose (mg/dl)	1× <sup>4</sup>	72.27	84.84	77.50	68.84	67.26	252.54	1707.31 <sup>a</sup>	0.003	<0.001	0.803
	2× <sup>5</sup>	70.49	74.09	84.28	72.40	74.04	261.35	1574.08 <sup>ab</sup>			
	3× <sup>6</sup>	66.02	76.56	77.78	71.13	59.89	242.47	1553.51 <sup>b</sup>			
	SE	2.11	5.81	3.32	3.94	5.68	7.03	36.65			
Insulin (μU /mL)	1×	6.06 <sup>x</sup>	39.16 <sup>y</sup>	44.02	35.31	12.85	100.09	316.37	0.155	<0.001	0.397
	2×	5.94	26.17	41.62	59.30	11.19	120.70	304.68			
	3×	8.10 <sup>x</sup>	61.73 <sup>y</sup>	43.58	23.02	15.29	98.74	385.59			
	SE	0.58	18.67	12.85	15.11	1.95	22.33	49.44			

<sup>1</sup>Area under the curve (AUC) of glucose (mg\*h/dL) and insulin (μU\*h/mL) were determined by the trapezoidal method.

<sup>2</sup>P values for effects of feeding frequency, time, and feeding frequency × time interaction from fasting level to 21.5 or 22.5-h post-prandial.

<sup>3</sup>Treatment. <sup>4</sup>Sows received full daily ration of feed once at 0730 h (n = 4). <sup>5</sup>Sows were fed 2× daily (n = 4), half ration at 0730 and 1530 h. <sup>6</sup>Sows were fed 3× daily (n = 6), (a third portion of ration at 0730, 1130, and 1530 h).

<sup>x,y</sup>Within a row, least squares means with uncommon superscript letters differ from baseline (0700 h) ( $P \leq 0.05$ ).

<sup>a,b</sup>Within a column, least squares means with uncommon superscript letters differ between feeding frequencies ( $P \leq 0.05$ ; Tukey-Kramer test).

Table 5. 3 Fasting and post-prandial active ghrelin and total PYY concentrations and their respective area under the curve in response to feeding frequency.

Item	Hormone concentrations						Total AUC <sup>1</sup>		P-values <sup>2</sup>		
	Trt <sup>3</sup> .	Fasting	Post-prandial				4-h	23-h	Trt.	Time	Trt.*Time
Active ghrelin <sup>7</sup> (pg/mL)	1× <sup>4</sup>	676.9 <sup>x</sup>	602.3	146.8 <sup>y</sup>	411.4	522.5	1978.8	8806.4	0.011	<0.001	0.184
		(917.3 - 499.5)	(816.2 - 444.4)	(198.9 - 108.3)	(557.6 - 303.6)	(708.1 - 385.6)	(2335.1 - 1676.8)	(10232.5 - 7579.1)			
	2× <sup>5</sup>	944.5 <sup>x</sup>	411.3	297.0 <sup>y</sup>	424.5	525.9	2085.9	8462.5			
		(1280.0 - 696.9)	(557.4 - 303.5)	(402.4 - 219.1)	(575.3 - 313.3)	(712.7 - 388.1)	(2461.6 - 1767.6)	(9832.9 - 7283.2)			
	3× <sup>6</sup>	473.9 <sup>x</sup>	337.5	85.0 <sup>y</sup>	204.5	303.6	1241.9	6391.5			
		(607.4 - 369.7)	(432.6 - 263.3)	(109.0 - 66.3)	(262.1 - 159.6)	(389.1 - 236.9)	(1421.7 - 1084.9)	(7224.7 - 5654.3)			
Total PYY <sup>7</sup> (pg/mL)	1×	146.8	159.7 <sup>b</sup>	-	183.7	138.2	659.5	2912.7	0.798	<0.001	<0.001
		(179.6 - 112.0)	(195.4 - 130.5)	-	(224.7 - 150.1)	(169.1 - 112.9)	(749.8 - 580)	(3272.6 - 2592.4)			
	2×	137.4 <sup>x</sup>	261.9 <sup>ay</sup>	-	126.4	176.9	722.9	3330.4			
		(168.1 - 112.3)	(320.4 - 214.0)	-	(154.7 - 103.3)	(216.5 - 144.6)	(822.0 - 635.8)	(3741.9 - 2964.2)			
	3×	90.5 <sup>x</sup>	151.1 <sup>by</sup>	-	146.2	136.7	563.1	3325.1			
		(106.6 - 76.7)	(178.2 - 128.2)	-	(172.4 - 124)	(161.2 - 115.9)	(625.3 - 507.1)	(3656.8 - 3023.4)			

<sup>1</sup>Area under the curve of active ghrelin (pg\*h/dL) and total PYY (pg\*h/mL) were determined by the trapezoidal method.

<sup>2</sup>P values for effects of feeding frequency, time, and feeding frequency × time interaction from fasting level to 21.5 or 22.5-h post-prandial.

<sup>3</sup>Treatment. <sup>4</sup>Sows received full daily ration of feed once at 0730 h (n = 4). <sup>5</sup>Sows were fed 2× daily (n = 4), half ration at 0730 and 1530 h. <sup>6</sup>Sows were fed 3× daily (n = 6), a third portion of ration at 0730, 1130, and 1530 h.

<sup>7</sup>Values are geometric mean (upper and lower 95% confidence interval).

<sup>x,y</sup>Within a row, geometric mean with uncommon superscript letters differ from baseline (0700 h) ( $P \leq 0.05$ ).

<sup>a,b</sup>Within a column, geometric mean with uncommon superscript letters differ between feeding frequencies ( $P \leq 0.05$ ; Tukey-Kramer test).

Table 5. 4 Fasting and post-prandial glucose and insulin concentrations and their respective area under the curve in response to feeding time.

Item	Metabolite or hormone concentrations					Total AUC <sup>1</sup>		<i>P</i> -values <sup>2</sup>		
	Trt <sup>3</sup> .	Fasting -30 min	Post-prandial			4-h	23-h	Trt.	Time	Trt.*Time
			+30 min	+90 min	+210 min					
Glucose (mg/dl)	0730 <sup>4</sup> h	72.27	84.84	68.84 <sup>a</sup>	67.26	291.49	1707.31	0.571	0.009	0.138
	1130 <sup>5</sup> h	78.29	81.37	67.43 <sup>ab</sup>	85.95	307.62	1636.51			
	1530 <sup>6</sup> h	71.65	83.85	56.29 <sup>b</sup>	90.05	294.15	1717.28			
	SE	3.69	5.85	2.58	10.32	9.55	53.82			
Insulin ( $\mu$ U /mL)	0730 h	6.06 <sup>x</sup>	39.16 <sup>y</sup>	35.31	12.85	108.00	316.37	0.116	<0.001	0.210
	1130 h	10.28	48.35	42.11	15.50	132.15	398.82			
	1530 h	11.16	20.05	16.70	12.31	62.97	272.56			
	SE	2.22	11.75	8.76	2.59	22.49	32.18			

<sup>1</sup>Area under the curve (AUC) of glucose (mg\*h/dL) and insulin ( $\mu$ U\*h/mL) were determined by the trapezoidal method.

<sup>2</sup>*P* values for effects of feeding time, time, and feeding time  $\times$  time interaction from fasting level to 21.5 or 22.5 hours post-prandial.

<sup>3</sup>Treatment. <sup>4</sup>Sows received full daily ration of feed at 0730 h, (n = 4).

<sup>5</sup>Received full daily ration of feed at 1130 h, (n = 2). <sup>6</sup>Sows received full daily ration of feed at 1530 h, (n = 4).

<sup>x,y</sup>Within a row, least squares means with uncommon superscript letters differ from baseline (0700 h) ( $P \leq 0.05$ ).

<sup>a,b</sup>Within a column, least squares means with uncommon superscript letters were differ between feeding times ( $P \leq 0.05$ ; Tukey-Kramer test).

Table 5. 5 Fasting and post-prandial active ghrelin and total PYY concentrations and their respective area under the curve in response to feeding time.

Item	Hormone concentrations					Total AUC <sup>1</sup>		<i>P</i> -values <sup>2</sup>		
	Trt <sup>3</sup> .	Fasting -30 min	Post-prandial			4-h	23-h	Trt.	Time	Trt.*Time
		+30 min	+90 min	+210 min						
Active <sup>7</sup> ghrelin (pg/mL)	0730 <sup>4</sup> h	676.9 (961.6 - 476.4)	602.3 (855.7 - 423.9)	411.4 (584.5 - 289.6)	522.5 (742.3 - 367.8)	2683.5 (3348.7 - 2150.5)	8806.4 (10680.7 - 7261.1)	0.786	0.012	0.005
	1130 <sup>5</sup> h	1067.8 <sup>x</sup> (1754.7 - 649.8)	316.8 <sup>y</sup> (520.6 - 192.8)	929.4 (1527.2 - 565.6)	663.4 (1090.2 - 403.7)	3629.1 (4963.6 - 2653.4)	13530.1 (17774.6 - 10299.1)			
	1530 <sup>6</sup> h	695.8 (988.6 - 489.8)	785.8 (1116.3 - 553.1)	327.1 (464.7 - 230.3)	323.7 (459.8 - 227.8)	2824.2 (3524.3 - 2263.2)	9894.6 (12000.5 - 8158.3)			
	0730 h	146.8 (171.1 - 125.9)	159.7 <sup>a</sup> (186.2 - 137)	183.7 (214.1 - 157.5)	138.2 (161.1 - 118.5)	659.5 (750.5 - 579.5)	2912.7 (3305.5 - 2566.6)			
Total PYY <sup>7</sup> (pg/mL)	1130 h	196.1 (243.7 - 157.8)	249.5 <sup>b</sup> (309.9 - 200.8)	187.4 (232.8 - 150.8)	199.1 (247.3 - 160.2)	828.3 (994.5 - 689.9)	4449.4 (5321.0 - 3720.6)	0.358	0.248	0.147
	1530 h	156.4 <sup>x</sup> (182.3 - 134.1)	213.5b <sup>y</sup> (248.9 - 183.1)	169.8 (198.0 - 145.6)	235.2 (274.3 - 201.7)	788.7 (897.5 - - 693)	3682.1 (4178.7 - 3244.6)			

<sup>1</sup>Area under the curve (AUC) of active ghrelin (pg\*h/dL) and total PYY (pg\*h/mL) were determined by the trapezoidal method.

<sup>2</sup>*P* values for effects of feeding time, time, and feeding time × time interaction from baseline to 21.5 or 22.5 hours post-prandial.

<sup>3</sup>Treatment. <sup>4</sup>Sows received full daily ration of feed at 0730 h, (n = 4).



<sup>5</sup>Received full daily ration of feed at 1130 h, (n = 2). <sup>6</sup>Sows received full daily ration of feed at 1530 h, (n = 4).

<sup>7</sup>Values are geometric mean (upper and lower 95% confidence interval).

<sup>x,y</sup>Within a row, geometric mean with uncommon superscript letters differ from baseline (0700 h) ( $P \leq 0.05$ ).

<sup>a,b</sup>Within a column, geometric mean with uncommon superscript letters were differ between feeding times ( $P \leq 0.05$ ; Tukey-Kramer test).

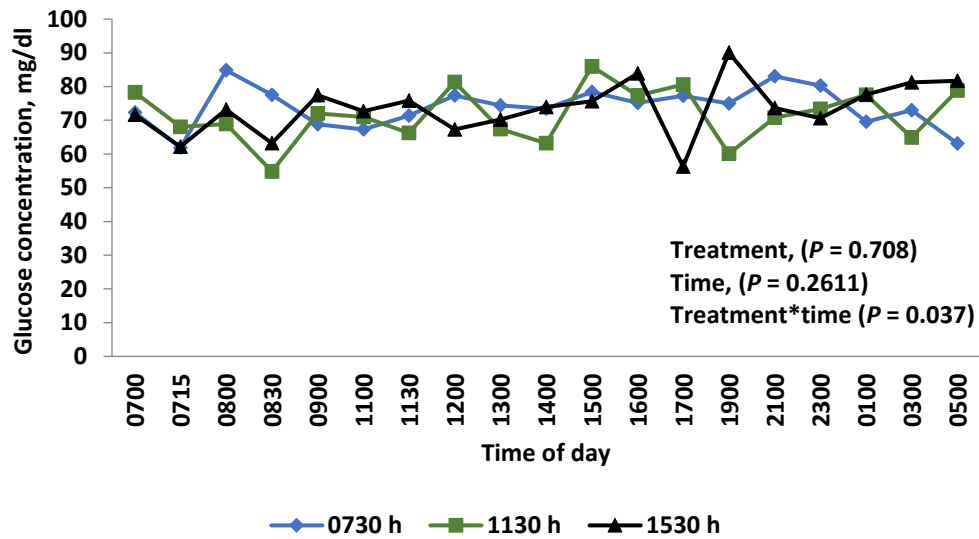


Figure 5. 1 Pattern of plasma glucose concentrations in pregnant sows subjected to the three different feeding times.

Sows on limit-fed regimen were fed at 0730, 1130, and 1530 h daily to meet approximately 125% of their daily ME maintenance requirements. Maximal SEM were 4.6, 7.8, and 5.5 for sows fed at 0730,  $n = 4$ ; 1130,  $n = 2$ ; and 1530 h,  $n = 4$ , respectively.

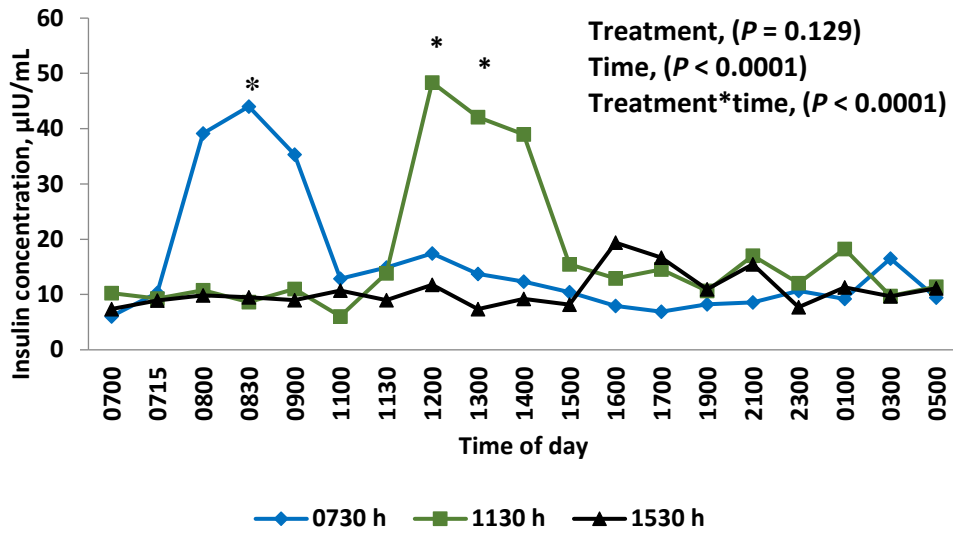


Figure 5. 2 Pattern of plasma insulin concentrations in pregnant sows subjected to the three different feeding times.

Sows on limit-fed regimen were fed at 0730, 1130, and 1530 h daily to meet approximately 125% of their daily ME maintenance requirements. Maximal SEM were 7.9, 6.2, and 4.4 for sows fed at 0730,  $n = 4$ ; 1130,  $n = 2$ ; and 1530 h,  $n = 4$ , respectively. Asterisks indicate time points where statistically difference were detected between two extreme data points,  $*P < 0.05$ .

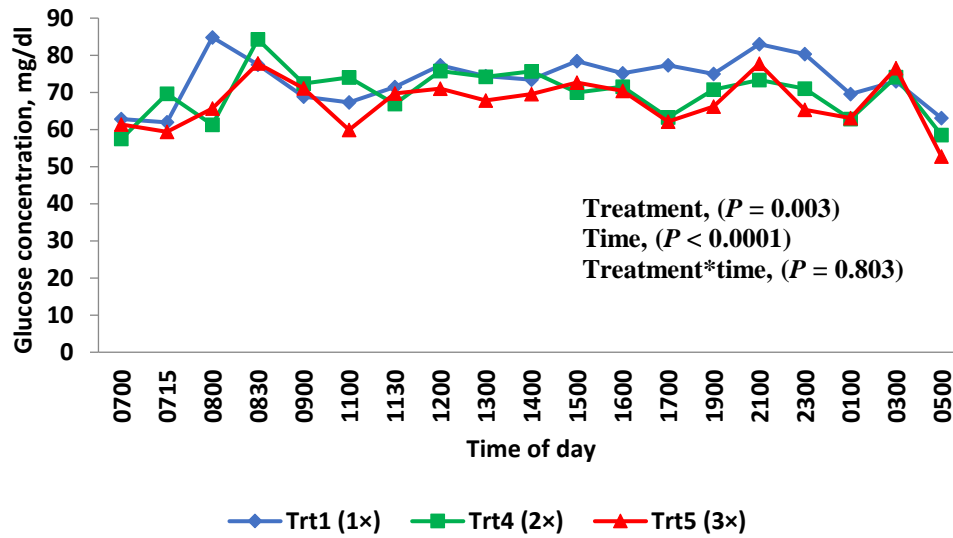


Figure 5. 3 Pattern of plasma glucose concentrations in pregnant sows subjected to the three different feeding regimes.

Sows on limit-fed regimen were fed 1×, 2×, and 3× daily to meet approximately 125% of their daily ME maintenance requirements. Maximal SEM were 4.6, 5.3, and 3.8 for sows fed at 0730,  $n = 4$ ; 1130,  $n = 4$ ; and 1530 h,  $n = 6$ , respectively.

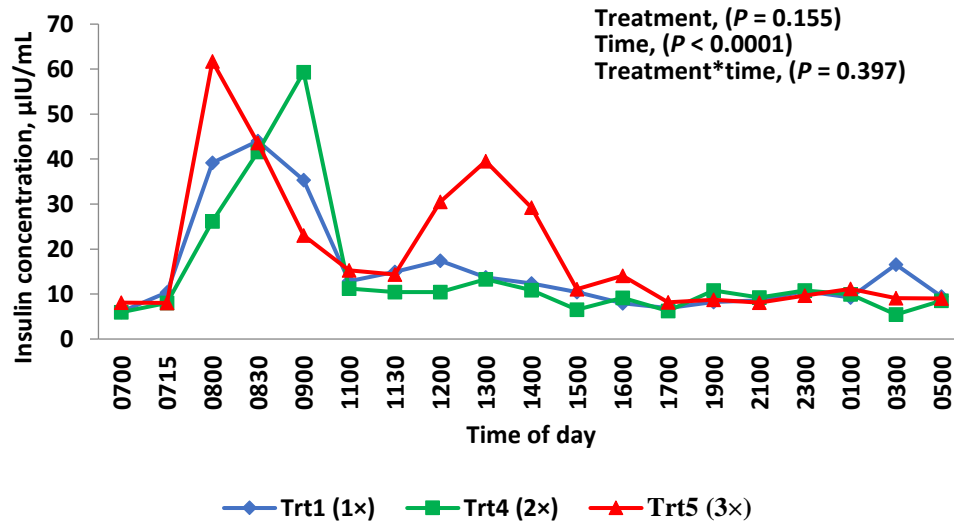


Figure 5. 4 Pattern of plasma insulin concentrations in pregnant sows subjected to the three different feeding regimens.

Sows on limit-fed regime were fed 1×, 2×, and 3× daily to meet approximately 125% of their daily ME maintenance requirements. Maximal SEM were 7.9, 9.2, and 6.5 for sows fed at 0730,  $n = 4$ ; 1130,  $n = 4$ ; and 1530 h,  $n = 6$ , respectively.

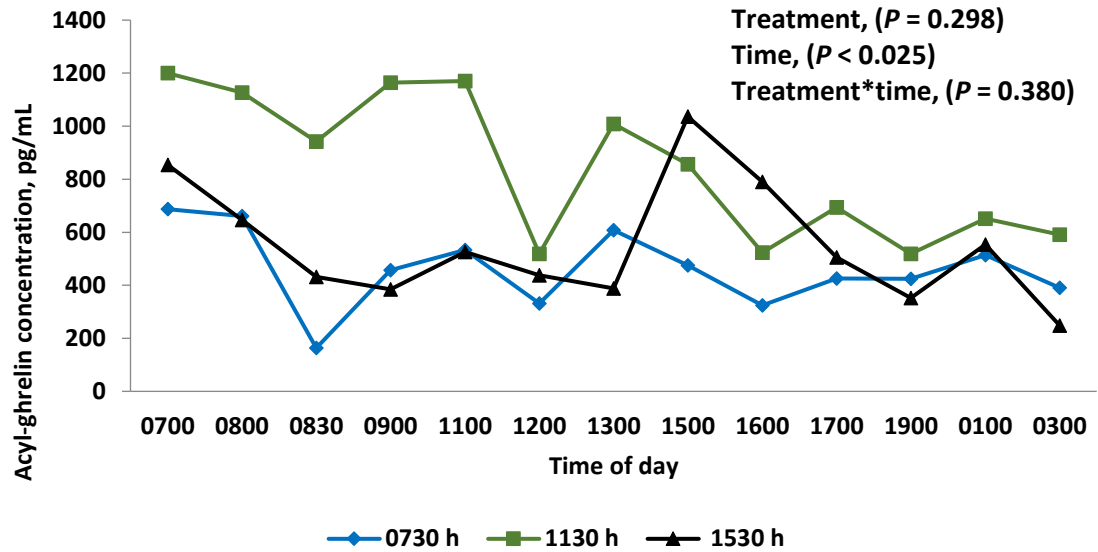


Figure 5. 5 Pattern of plasma acyl-ghrelin levels in pregnant sows subjected to the three different feeding times.

Sows on limit-fed regime were fed at 0730, 1130, and 1530 h daily to meet approximately 125% of their daily ME maintenance requirements. Maximal SEM on log scale were 2.4, 2.5, and 2.9 for sows fed at 0730,  $n = 4$ ; 1130,  $n = 2$ ; and 1530 h,  $n = 4$ , respectively.

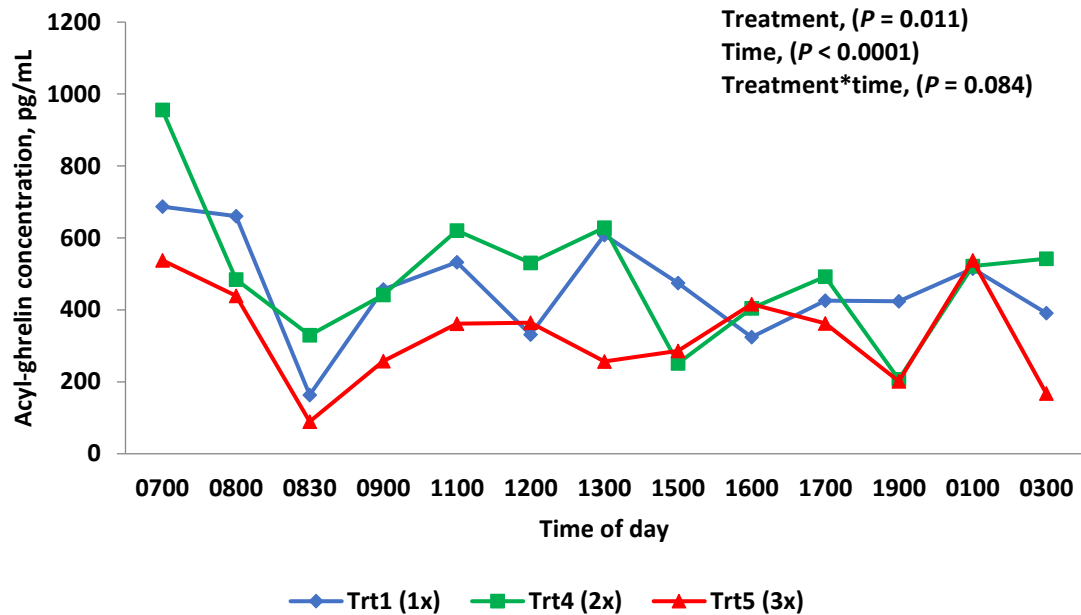


Figure 5. 6 Pattern of plasma ghrelin levels in pregnant sows subjected to the three different feeding regimens.

Sows on limit-fed regime were fed 1×, 2×, and 3× daily to meet approximately 125% of their daily ME maintenance requirements. Maximal SEM on log scale were 2.3, 2.4, and 2.7 for sows fed at 0730,  $n = 4$ ; 1130,  $n = 4$ ; and 1530 h,  $n = 6$ , respectively.

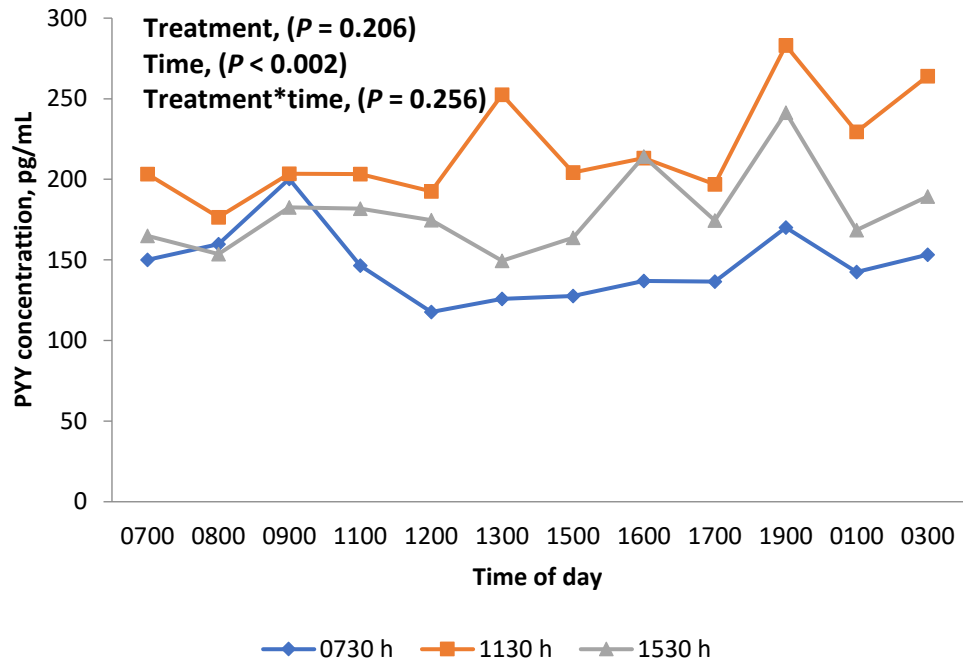


Figure 5. 7 Pattern of plasma PYY levels in pregnant sows subjected to the three different feeding times.

Sows on limit-fed regime were fed at 0730, 1130, and 1530 h daily to meet approximately 125% of their daily ME maintenance requirements. Maximal SEM were 47.4, 62.9, and 45.9 for sows fed at 0730,  $n = 4$ ; 1130,  $n = 2$ ; and 1530 h,  $n = 4$ , respectively.



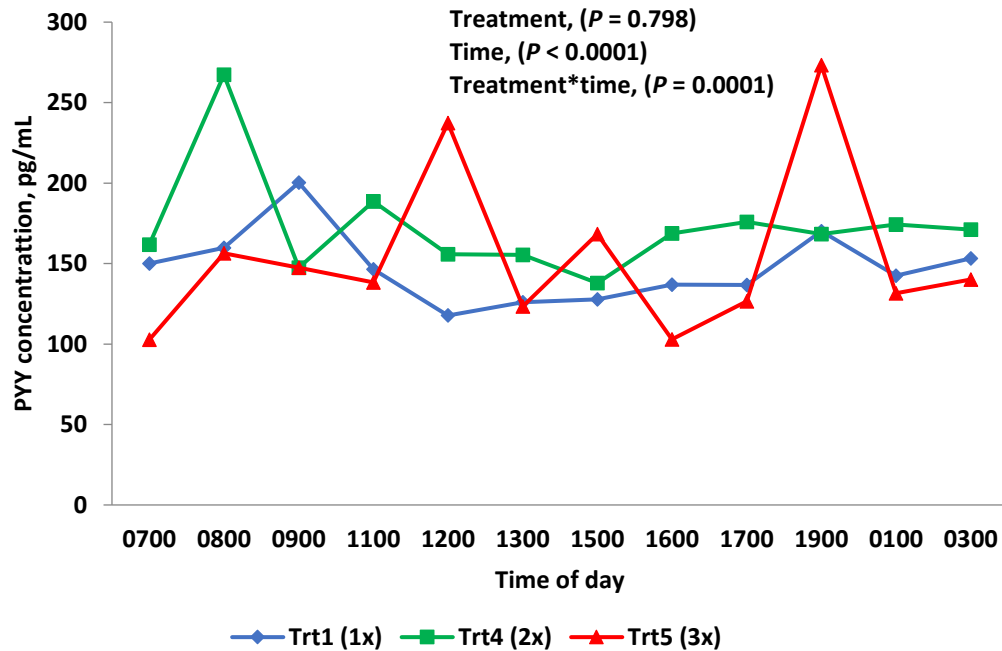


Figure 5. 8 Pattern of plasma PYY levels in pregnant sows subjected to the three different feeding regimens.

Sows on limit-fed regime were fed 1 $\times$ , 2 $\times$ , and 3 $\times$  daily to meet approximately 125% of their daily ME maintenance requirements. Maximal SEM were 61.6, 61.7, and 60.5 for sows fed at 0730,  $n = 4$ ; 1130,  $n = 4$ ; and 1530 h,  $n = 6$ , respectively.

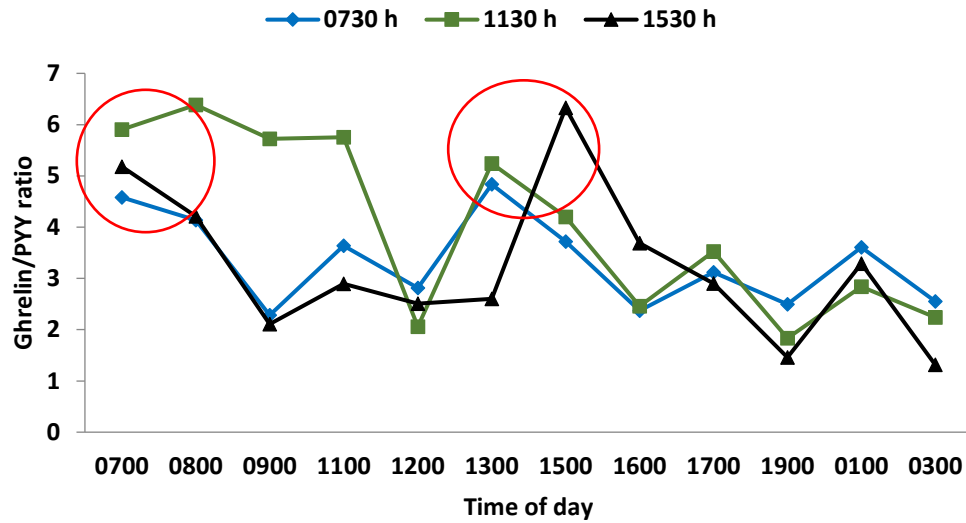


Figure 5. 9 Pattern of plasma ghrelin/PYY in pregnant sows subjected to the three different feeding times.

Sows on limit-fed regime were fed at 0730, 1130, and 1530 h daily to meet approximately 125% of their daily ME maintenance requirements. The circles indicate the point of highest ratio for each treatment. Maximal SEM were 4.4, 1.6, and 1.2 for sows fed at 0730,  $n = 4$ ; 1130,  $n = 2$ ; and 1530 h,  $n = 4$ , respectively.

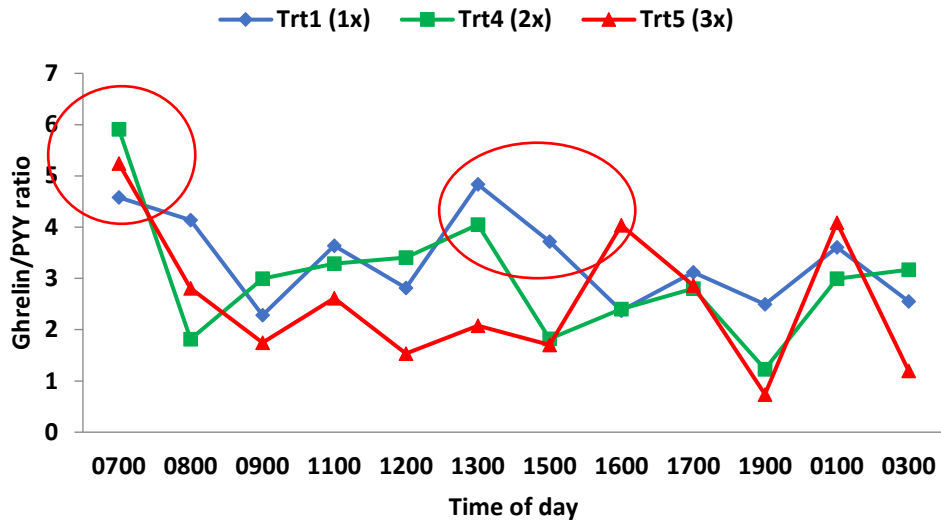


Figure 5. 10 Pattern of plasma ghrelin/PYY ratio in pregnant sows subjected to the three different feeding regimens.

Sows on limit-fed regime were fed 1×, 2×, and 3× daily to meet approximately 125% of their daily ME maintenance requirements. The circles indicate the point of highest ratio for each treatment. Maximal SEM were 4.4, 1.2, and 0.9 for sows fed at 0730,  $n = 4$ ; 1130,  $n = 4$ ; and 1530 h,  $n = 6$ , respectively.

## Chapter 6. Overall Summary and Implications

### BACKGROUND OF STUDY

Pregnant sows are restricted-fed to enhance optimal reproductive performance and longevity in commercial production (Brouns and Edwards, 1994). Quantitative restriction of feed is associated with signs of hunger. Dilution of feed with fiber to reduce hunger in pregnant sows has provided equivocal results (Jensen et al., 2015). An unresolved issue in the nutrition of swine is understanding of perceived hunger in pregnant sows under limit fed conditions. Strategies to evaluate hunger in swine include physiological (Doyle et al., 2015), behavioral (Rushen et al., 2011), and motivational (Jensen et al., 2012) measurements. Provision of feed within a narrow time window each day led to significant changes in physiology and behavior (Johnston, 2014). Additionally, feed anticipatory activity occurs when animals are entrained to receive feed at specific time frame, a behavior suggesting hunger, and motivation to eat (Merkestein et al., 2012). It was suggested that increasing meal frequency may directly affect hormones, gastric stretch, and gastric emptying, which impact hunger and satiety (Speechly and Buffenstein, 1999). Feeding behavior can be measured by feeding time, meal duration, meal frequency, feeding rate, and rumination time (Bargo et al., 2018). However, without holding total caloric intake constant, the usefulness of meal frequency and meal-timing data can be limited (Kulovitz et al., 2014). In this dissertation, the effects of changing the timing of the same amount of energy per kilogram live BW<sup>0.75</sup> presentation or splitting the same amount of energy and fed at different times were used as a model to understand hunger, cortisol, and performance response in pregnant sows under limit-fed conditions.

## **Research Question**

How does feeding frequency or time of feed ingestion affect sow and litter performance, cortisol and sow behavior, and perceived hunger based on iso-caloric intake per kilogram live metabolic weight under normal production conditions?

## **HYPOTHESIS**

It is hypothesized that changes in the timing and frequency of feeding will affect hunger, sow behavior, cortisol, sow and litter performance without altering the quantity of feed provided per kilogram live metabolic weight under normal production conditions.

## **MAIN OBJECTIVES**

The objectives were to determine the effects of feeding time and frequency on physiological and behavioral responses to hunger under normal production condition and investigate impact on sow and litter performance.

### **The specific objectives were:**

- (i) To determine the effects of feeding time and frequency on sow and reproductive performance under iso-caloric intake. (Chapter 2 and 3)
- (ii) To indirectly quantify stress and sow behavioral activities associated with feeding time and frequency under iso-caloric intake (Chapter 4)
- (iii) To investigate the effects of feeding time and frequency on plasma insulin, ghrelin and Peptide YY and glucose patterns and indirectly quantify hunger in pregnant sows. (Chapter 5)
- (iv) Use ghrelin/PYY ratio as an index of relative hormonal exposure to predict at what times hunger in pregnant sows or desire to feed is greatest. (Chapter 5)

## APPROACH

The study was carried out to indirectly quantify hunger in gestation sow under normal production conditions and evaluate its impact sow and litter performance as such sows were not fed the traditional way. Typically, gestation sows' nutrient requirements are estimated based on ME and fiber content of the diet, length of gestation, parity, expected litter size and birth weight, anticipated maternal weight gain, and body condition (NRC, 2012). Therefore, feeding levels are adjusted to attain a targeted body condition score (BCS) of 3 (scale: 1 = emaciated and 5 = overly fat) at farrowing (Li et al., 2014). The implication is that thin sows feed allowance is increased while fat or obese sows daily ration is reduced. Each sow is however different in terms of their maintenance requirement per kilogram  $BW^{0.75}$ . A procedure is required to standardize their energy intake based on maintenance requirement and caloric density of the feed to understand hunger in sows. For the first time, to standardize ME intake per kilogram live  $BW^{0.75}$ , the daily quantity of feed fed was scaled to the  $BW^{0.75}$  live weight (Le Naou et al., 2014) and fed at 1.25 times (Prunier and Quesnel, 2000) the maintenance requirements for sows ( $100 \text{ kcal} \times BW^{0.75}$ ) specified by NRC, (2012). Sows were fed individually by raising the feeder ball valve of an Accu-Drop Feed Dispenser (AP Systems, Assumption, IL) to drop the required amounts of feed into the feeding troughs. The Accu-Drop feed dispensers were calibrated at the d 30, 60, and 90 at various set points and related the volume of Feed Dispenser, Y ( $\text{cm}^3$ ) to kilogram weight of feed (x) delivered as  $Y = 5.4864x + 1.9087$ ;  $R^2 = 0.9892$ . The required daily full allowance of feed was provided once a day at: [0730 (Control, T1), 1130 (T2), or 1530 h (T3)]; twice [half ration each at 0730 and 1530 h (T4)], or thrice [a third portion of ration allotment at 0730, 1130, and 1530 h (T5)].

## **FINDINGS FROM VARIOUS STUDIES**

In chapter 2, the effect of different feeding times given similar energy intake per kilogram live BW<sup>0.75</sup> during gestation on sow's performance was investigated. The study showed that feeding pregnant sows once daily at 0730 and 1130 h did not change sow BW, BF, and reproductive parameters. However, provision of total daily energy intake once daily at 1530 h altered energy and nutrient metabolism to beneficially increase BF gained during gestation, tended to improve the number of piglets weaned, and supported normal reproductive performance in sows compared with conventional feeding time. The result implies that linking nutritional management techniques to time of feeding may enhance BF gains and number of piglets weaned during gestation and lactation, respectively.

In chapter 3, the objective of the study was to investigate the effect of daily feeding frequency (1×, 2×, or 3×) and sow parity based on same rate of maintenance energy intake during gestation on sow performance. The study indicated that feeding gestation sows 2× daily tended to increase BF depth from days 30 to 109 of gestation and reduced the number of stillborn compared with control sows. Sows fed 2× and 3× daily during gestation exhibited a potential to improve the number of piglets weaned compared with sows 1× daily. This implies that increasing gestation sow feeding frequency from 1× daily to 2× and 3× daily may reduce the number of stillborn and increase litter size at weaning although most of the reproductive traits were not affected. Parity (P6+) sows had greatest BW gain during lactation but greater number of stillborn, 72 h, and pre-weaning mortalities relative to younger sows even though litter size was equalized during cross-fostering. This implies that cross-fostering to balance the number of piglets nursed by a sow may be counter-productive at weaning. Cross-fostering may work with younger parity

sows but not older or advanced parities or litter standardization practiced instead of litter size equalization as fostering strategy to improve production efficiency.

In chapter 4, the effects of feeding time and frequency on behavior and cortisol responses in pregnant sows under iso-caloric conditions were investigated. The control sows (0730 h) had reduced total and feeding activity AUC relative to sows on 1130 and 1530 h feeding schedules. Sows on 1130 h feeding schedule had greater FAA, 24-h total activity count, total and feeding activity AUC compared with sows fed daily at 1530 h. The results showed that feeding sows once daily at 0730 h appears to minimize sows' feed anticipatory activities (FAA), daily feeding and total activity, but similar cortisol response relative to pregnant sows fed daily at 1130 and 1530h. Additionally, feeding sow daily at 1130 h resulted in greater FAA, feed and total activities but reduced cortisol concentration, suggesting that elevated sow activity might not necessary indicate activation of hypothalamic-pituitary-adrenal axis.

Under iso-caloric condition per kilogram live metabolic weight, sows fed 2× daily tended to have lower and reduced 12-h cortisol AUC compared with control sows (1× daily) and sows on 3× daily feeding regime, respectively. Additionally, feed FAA, 24-h total, and feeding activity were reduced with sows fed 2× daily compared with control sows and sows fed 3× daily. Hence, sows on 2× daily feeding regime had lower 24-h total and feeding activities AUC compared with both sows fed 1× and 3× daily, respectively. This indicates that 2× daily feeding appears to be the threshold that reduces sows' total activity, feeding activity, and activation of the HPA axis and exhibit the potential to improve sow welfare in relation 1× and 3× daily feeding regimes.



In chapter 5, the objectives were to determine the pattern of metabolite and hormonal response and indirectly quantify perceived hunger in pregnant sows under limit-fed conditions. The study showed that consumption of 3 small meals in 8 h resulted in 9.0% reduction in 23-h glucose total AUC, 42.5% greater 23-h insulin total AUC, 27.4% reduction in active ghrelin total AUC, and 14.2% greater PYY total AUC compared with pregnant sows fed 1× daily. Feeding time to elicit maximum insulin response is 0730 and 1130 but not at 1530 h. This implies that feeding sows 1× daily at 1530 h may reduce insulin response. Plasma insulin response appears to be a function of frequency of feeding and time of the day at which calories are ingested. Active ghrelin total AUC correlated positively with PYY total AUC during pregnancy. Feeding time had no effect on glucose, active ghrelin, and total PYY 23-AUC. The pattern of ghrelin/PYY ratio suggests that the 0730 h and between 0100 and 1530 h might be the time of day where the physiological drive to feed may predominate relative to other times of the day. It is tempting to conclude that hunger in pregnant sows occurs at active ghrelin/total PYY ratio of  $5.06 \pm 0.20$  and at a mean plasma glucose concentration of  $70.37 \pm 1.39$  and below.

## **IMPLICATIONS OF STUDY**

The practical implication of this study is that in the electronic feed station all sows should be fed well before 1530 h to maximize insulin response. Feeding frequency within an appropriate time can maximize insulin response. Importantly, this research demonstrates a simple management technique to develop a swine model of insulin resistance or low insulin sensitivity by feeding sows once daily at 1530 h. Additionally, this study shows that although limited-fed gestation sows may experience hunger, it does not compromise their welfare, as indicated by their body weight gain during gestation, reproductive

performance, and the HPA axis response to stress. Feeding pregnant sows 2× daily exhibited a potential to improve sow and reproductive performance, reduced cortisol and sow activity but provide comparable metabolic and hormonal response to sows on 1× or 3× daily feeding regime. It is recommended that pregnant sows should be fed twice daily to reduce the number of stillborn and improve sow and reproductive performance, reduce cortisol, and enhance sow welfare in the swine industry under limit-fed condition.

### ***Future Research***

1. The original sample size of six sows per treatment was reduced due to practical limitations. Future studies should consider batch sampling to increase the sample size to improve the statistical assessment and the robustness of conclusions drawn.
2. Defining the concentration of cortisol in saliva that indicates when the welfare of the sows is detrimentally affected will be important to establish?
3. Investigate whether sows in electronic feed station access the electronic feeder in a particular order each day to determine if they feed at similar or different times of the day?

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