Plasma acyl ghrelin and nonesterified fatty acids are the best predictors for hunger status in pregnant gilts¹

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ABSTRACT: Sows are usually restricted fed during pregnancy to maximize their reproductive efficiency, which may predispose sows to a state of hunger. However, an objective measurement of hunger status has not been established. In the present study, we examined the correlation of plasma hormones and NEFA and selected the best predictors for hunger status using pregnant gilts. Three different levels of feed intake (0.5, 1.0 and 2.0 × maintenance energy intake [0.5M, 1.0M and 2.0M, respectively]) were imposed from Day 28 to 34 of gestation to create different hunger statuses in pregnant gilts. Plasma hormones related to energy homeostasis and NEFA were analyzed to quantify their response to different levels of feed intake. A total of 18 gilts (197.53 ± 6.41 kg) were allotted to 1 of 3 dietary treatments using a completely randomized design. Results showed that BW change, ADG, and G:F from Day 28 to 34 of gestation were higher ($P < 0.01$) for gilts on the 2.0M feeding level than for gilts on the 0.5M feeding level. Plasma acyl ghrelin concentrations showed a relatively flat pattern during the 24-h period. Plasma acyl ghrelin and NEFA concentrations

and areas under the curve (AUC) were greater ($P < 0.05$) in gilts on the 0.5M level of feed intake than in those on the 2.0M level of feed intake. No differences were observed among the 3 feeding levels in terms of plasma glucagon-like peptide 1 and leptin concentrations. Additionally, consumption time for 1.82 kg feed on Day 35 of gestation was longer ($P < 0.01$) in gilts fed the 2.0M level of feed intake from Day 28 to 34 of gestation than in those on the 0.5M level of feed intake. Simple linear regression results showed that the AUC of acyl ghrelin was the best predictor for consumption time ($R^2 = 0.82$), whereas the AUC of NEFA was the best predictor for BW ($R^2 = 0.55$) or backfat change ($R^2 = 0.42$) from Day 28 to 34 of gestation. In conclusion, our data suggested that a relative flat pattern existed in pregnant gilts in terms of the diurnal plasma profile of acyl ghrelin and that the level of feed intake of pregnant gilts was negatively correlated with plasma concentrations of acyl ghrelin and NEFA, which, in turn, were negatively associated with feed consumption time. The AUC of acyl ghrelin and NEFA seemed to be the best predictors for hunger status of pregnant gilts.

Key words: acyl ghrelin, consumption time, feed intake, nonesterified fatty acid, pregnant gilt

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INTRODUCTION

Numerous studies have shown that ghrelin may serve as both a short-term and a long-term indicator

of energy homeostasis. It has been well documented that ghrelin secretion is upregulated in conditions of negative energy balance, such as fasting and anorexia nervosa, and downregulated in the case of positive energy balance, such as feeding and obesity (Ariyasu et al., 2001; Toshinai et al., 2001; Tschöp et al., 2001a; Wren et al., 2001).

The diurnal profile of ghrelin concentrations has been investigated in nonpregnant rodents (Tschöp et al., 2000; Sánchez et al., 2004), growing pigs (Reynolds et al., 2010), and humans (Ariyasu et al., 2001; Cummings et al., 2001; Tschöp et al., 2001a; Liu et al., 2008). These studies clearly demonstrate

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that ghrelin concentration increases before feeding and declines after feeding, indicating that ghrelin may function as a physiological meal initiator. However, to the best of our knowledge, we are not aware of any study investigating the diurnal profile of acyl ghrelin concentrations in pregnant sows. Additionally, it has been reported that ghrelin plays important roles in fertilization, implantation, and embryo/fetal development (Fuglsang, 2007; Luque et al., 2014). Therefore, the first objective of this study was to characterize the diurnal plasma profile of acyl ghrelin concentrations in pregnant gilts.

Leptin and insulin are proportional to animal body fat mass, which plays a critical role in long-term food regulation and energy homeostasis (Polonsky et al., 1988; Zhang et al., 1994). However, intraventricular administration of glucagon-like peptide 1 (GLP-1) can reduce short-term but not long-term food intake or BW in both lean and obese rats (Donahey et al., 1998), indicating that GLP-1 may exhibit its function in the short-term regulation of food intake and energy homeostasis. Additionally, NEFA is an indicator of fat mobilization, and its concentration would increase in the negative energy balance (Weldon et al., 1994). Therefore, the second objective of this study was to examine the correlations among the plasma hormones and NEFA and select the best predictors for hunger status or energy homeostasis in pregnant gilts.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of University of Minnesota approved the experimental protocol of this study.

Animals and Management

The present experiment was conducted at the University of Minnesota's Southern Research and Outreach Center in Waseca, MN. Eighteen Large White × Danish Landrace crossbred gilts (parity 0; TOPIGS 20 [Topigs Norsvin Canada Inc., Winnipeg, MB, Canada]) were used in this study and kept in individual stalls (2.1 m length by 0.6 m width by 0.6 m height) throughout gestation. All gilts were fitted with cephalic vein catheters according to procedures described by Moehn et al. (2004). Briefly, a catheter was tunneled under the skin from the incision site to a point of exit on the shoulder, where an access port was connected with the catheter. After surgery, gilts were treated with analgesic and antibiotics and allowed 2 wk for recovery. Then, all gilts were fed a small portion of feed mixed with 6.8 mL Matrix (15 mg altrenogest; Merck & Co., Inc., Kenilworth, NJ) each day; after finishing the small portion of feed, the rest of the feed was delivered to

the gilts. The Matrix feeding regime was imposed for 2 wk to synchronize estrus in the experimental gilts. After withdrawing Matrix, all gilts came to estrus in 1 wk. All gilts were bred twice via AI on 2 consecutive days. On Day 35 after AI, all gilts were confirmed pregnant with an ultrasound detection machine (Preg-Alert Pro; Renco Corp., Minneapolis, MN).

Dietary Treatments, Experimental Design, and Data Collection

Gilts were fed once a day at 0730 h during the gestation period. From breeding to Day 27 of gestation, all gilts were fed the same amount of food (1.82 kg). On Day 27 of gestation, gilts (197.53 ± 6.41 kg) were randomly allotted to 1 of 3 dietary treatments. The experiment started on Day 28 and continued to Day 35 of gestation (Fig. 1). From Day 28 to 34 of gestation, gilts were fed 1 of 3 different levels of feed intake based on maintenance energy intake ($100 \times \text{BW}^{0.75}$ kcal ME/d; NRC, 2012): 1) $0.5 \times$ maintenance energy intake (**0.5M**), 2) $1.0 \times$ maintenance energy intake (**1.0M**), and 3) $2.0 \times$ maintenance energy intake (**2.0M**). The gestation diet (Table 1) met or exceeded the NRC (2012) requirements for swine.

Gilt BW was recorded and backfat (**BF**) thickness was measured using an ultrasonic detection machine (Preg-Alert Pro) on both the left and right sides at the P2 position (6.5 cm from the dorsal midline at the level of the last rib) on Day 28 and 35 of gestation for calculation of the BW and BF changes during this period. A feed consumption test was conducted to record the consumption time of the same amount of feed (1.82 kg) for each gilt on d 35 of gestation (Fig. 1).

Chemical Analysis

All analyses were conducted in duplicate. The gestation diet was analyzed for DM (method 934.01; AOAC, 2006) and CP (method 984.13; AOAC, 2006) at the University of Minnesota's Southern Research and Outreach Center. Concentrations of Ca (method 958.01; AOAC, 2006), P (method 958.01; AOAC, 2006), and AA (method 982.30; AOAC, 2006) were analyzed at the Experiment Station Chemical Laboratories (University of Missouri, Columbia, MO).

Blood Collection and Plasma Analysis

From Day 28 to 34 of gestation, the first 6 d was considered a period of adaptation to the diets and the blood collection was conducted on the seventh day. Blood samples for all gilts were collected via cephalic vein catheters right before feeding (0 h), then every 15 min for 2 h,

Figure 1. Schematic representation of the experimental process. 0.5M, 1.0M, and 2.0M = 0.5, 1.0, and 2.0 $\times$ maintenance energy intake, respectively.

and then every 2 h until the completion of 24 h. Before blood collection, 50 μ L dipeptidyl peptidase-IV inhibitor (5 mM; EMD Millipore, Billerica, MA) and 180 μ L 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (100 mM; Sigma-Aldrich Corp., St. Louis, MO) were added to the blood collection tubes. Blood samples (6 mL) were added to the chilled collection tubes containing K_3EDTA (15% solution, 0.08 mL/tube) as an anticoagulant, and the contents were gently mixed to make sure the added chemicals were equally distributed in the blood. After gentle mixing, the tubes were centrifuged at $699 \times g$ for 15 min at 4°C. After centrifugation, each plasma sample was carefully transferred to 6 Self-Lock Eppendorf microtubes (Eppendorf, Hamburg, Germany), with 0.5 mL in each microtube. In the microtube for acyl ghrelin analysis, 10 μ L of 6 N HCl was added to acidify the plasma to provide additional protection. The plasma samples were snap frozen in liquid nitrogen and then stored at -80°C until analysis for acyl ghrelin, GLP-1, leptin, insulin, and NEFA. Acyl ghrelin concentrations in plasma sampled at every 2-h time point were measured, whereas blood samples collected at specific time points were analyzed for GLP-1 (0, 0.5 and 1 h after the meal), leptin (0, 4, and 8 h after the meal), insulin (0, 15 min, 30 min, 45 min, 60 min, 75 min, 90 min, 2 h, and 4 h after the meal), and NEFA (0, 4, and 8 h after the meal).

Commercial ELISA kits were used for analysis of plasma acyl ghrelin (EZRGRA-90K; EMD Millipore) and leptin (BG-POR11464; Novatein Biosciences, Woburn, MA). Plasma GLP-1 (7-36 amide) was measured using a commercial fluorescent immunoassay kit (FEK-028-11; Phoenix Pharmaceuticals, Inc., Burlingame, CA). Acyl ghrelin, leptin, and GLP-1 assay kits were not validated in our lab for porcine plasma acyl ghrelin, leptin, and GLP-1 analysis, respectively. Plasma insulin was analyzed using a commercial RIA kit (PI-12K; EMD

Table 1. Composition and nutrient content of experimental diets in gestation (as-fed basis)

Item	Gestation
Ingredient, %	
Corn	65.23
Soybean meal	10.00
cDDGS ¹	20.00
Choice white grease	1.50
Limestone	1.00
Dicalcium phosphate	1.20
Lysine HCl (78%)	0.10
Salt	0.35
Premix ²	0.50
Tylan ³	0.13
Energy and nutrient composition, %	
ME, MJ/kg ⁴	13.8
CP ⁵	15.70
Total Ca ⁵	0.75
STTD ⁶ P ⁴	0.35
SID ⁷ Lys ⁴	0.57
SID Met + Cys ⁴	0.52
SID Thr ⁴	0.44
SID Trp ⁴	0.12

¹cDDGS = corn distiller's dried grains with solubles (containing 27.3% CP, 5.47% ether extract, 0.05% Ca, 0.94% P, and 0.77% Lys).

²Supplied the following nutrients per kilogram of diets: 12,114 IU vitamin A, 2,753 IU vitamin D, 66 IU vitamin E, 4.4 mg vitamin K, 1 mg thiamine, 10 mg riboflavin, 55 mg niacin, 33 mg pantothenic acid, 2.2 mg pyridoxine, 1.6 mg folic acid, 0.06 mg vitamin B₁₂, 0.5 mg I from ethylenediamine dihydriodide, 0.3 mg Se from sodium selenite, 548 mg choline from choline chloride, and metal polysaccharide complexes of zinc sulfate (125 mg Zn), iron sulfate (125 mg Fe), manganese sulfate (40 mg Mn), and copper sulfate (15 mg Cu).

³Tylan 40 (Tylosin phosphate 40; Elanco Animal Health, Indianapolis, IN).

⁴Calculated values according to NRC (2012).

⁵Analyzed values.

⁶STTD = standardized total tract digestible.

⁷SID = standardized ileal digestible.

Table 2. Effects of different levels of feed intake from Day 28 to 34 of gestation on growth performance of gilts

Items	Level of feed intake ¹			SEM	P-value
	0.5M	1.0M	2.0M		
Number of gilts	6	6	6		
BW change, ² kg	-6.14 ^a	-1.82 ^a	5.61 ^b	1.18	<0.01
BF change, ³ mm	-0.75 ^a	-0.38 ^a	0.25 ^b	0.27	<0.01
ADG, ⁴ kg/d	-0.88 ^a	-0.36 ^a	0.80 ^b	0.17	<0.01
Feed intake, ⁵ kg	5.65 ^a	11.30 ^b	21.95 ^c	0.43	<0.01
G:F, ⁶ kg/kg	-1.09 ^a	-0.21 ^b	0.26 ^c	0.13	<0.01

^{a-c}Least squares means within a row with different superscripts differ ($P < 0.01$).

¹0.5M, 1.0M, and 2.0M = 0.5, 1.0, and 2.0 × maintenance energy intake ($100 \times \text{BW}^{0.75}$ kcal ME/d), respectively.

²The BW change of gilts from Day 28 to 34 of gestation.

³BF = backfat. This represents the BF change of gilts from Day 28 to 34 of gestation.

⁴The ADG of gilts from Day 28 to 34 of gestation.

⁵The total feed intake consumed by gilts from Day 28 to 34 of gestation.

⁶The G:F of gilts from Day 28 to 34 of gestation.

Millipore), which was previously validated by Whisnant and Harrell (2002). Plasma NEFA was analyzed using the enzymatic colorimetric method (NEFA HR[2]; Wako Chemicals USA, Inc., Richmond, VA), which was validated by Govoni et al. (2007). The intra-assay CV for the assays were 5.1, 4.5, 2.0, 7.5, and 4% for acyl ghrelin, GLP-1, leptin, insulin, and NEFA, respectively.

Statistical Analysis

SAS 9.4 (SAS Inst. Inc., Cary, NC) was used for all data analysis. The individual gilt served as the experimental unit. The LSMEANS statement was used to calculate the least squares means. Tukey–Kramer adjustment was used for multiple comparisons of the least squares means. Pooled SEM was calculated for each measurement. A probability of $P \leq 0.05$ was considered significant, and $0.05 < P < 0.1$ was declared a trend.

The GLIMMIX procedure was used for the data analysis. Gilt BW change, BF change, ADG, ADFI, and G:F from Day 28 to 34 of gestation were analyzed in the default normal linear regression model. Plasma hormones and NEFA concentrations analyzed at different time points were considered repeated measurements. Different covariance structure candidate models were examined, and the best fit model was selected for each measurement based on Akaike information criterion and Bayesian information criterion values. The best covariance structure models selected for acyl ghrelin, GLP-1, leptin, insulin, and NEFA were Toeplitz, Toeplitz, compound symmetry, first-order autoregressive, and first-order antedependence, respectively. The total area under the curve (AUC) for each plasma parameter was calculated using the trapezoidal method (Yeh and Kwan, 1978)

and analyzed using the default linear regression model. Specifically, the area of each segment can be calculated by multiplying the average concentration by the segment width. The total AUC from the first to last time points can then be calculated by adding together the areas.

The CORR procedure was used to examine correlation coefficients among feed consumption time and BW and BF changes from Day 28 to 34 of gestation and plasma parameters. Consumption time and BW and BF changes from Day 28 to 34 of gestation were considered dependent variables. If the plasma parameters were significantly correlated or tended to be correlated with the dependent variables, simple linear regression models were built using the REG procedure.

RESULTS

Gilt Performance

The results of different levels of feed intake from Day 28 to 34 of gestation on gilt performance are presented in Table 2. Body weight change, BF change, ADG, ADFI, and G:F were greater ($P < 0.01$) for gilts on the 2.0M level of feed intake than for gilts on the 0.5M and 1.0M levels of feed intake. There were no differences ($P > 0.10$) between gilts on the 0.5M and 1.0M levels of feed intake in terms of BW change, BF change, and ADG. In contrast, ADFI and G:F were all greater ($P < 0.01$) for gilts on the 1.0M level of feed intake than for gilts on the 0.5M level of feed intake.

Plasma Hormones

Plasma acyl ghrelin concentrations of gilts with each level of feed intake did not decrease after eating and were maintained at a constant level except for an abrupt increase at 10 h after eating during the 24-h sample collection period (Table 3; Fig. 2). However, gilts on the 0.5M level of feed intake had greater ($P < 0.05$) acyl ghrelin concentration at the majority of time points and AUC than those on the 2.0M level of feed intake.

Plasma insulin concentrations right before feeding (0 h) were similar ($P = 0.80$) among gilts on the 3 levels of feed intake (Table 4; Fig. 3). However, gilts on the 1.0M level of feed intake tended ($P = 0.07$) to have higher insulin concentration at 45 min after eating compared with gilts on the 0.5M level of feed intake. Gilts on the 2.0M level of feed intake had a greater ($P < 0.01$) insulin concentration at 60 min after eating than those on the 0.5M and 1.0M levels of feed intake as well as at 75 min after eating than those on the 0.5M level of feed intake. Additionally, insulin concentrations decreased to the similar levels among 3 levels of feed intake at 90 min ($P = 0.60$) and 2 h ($P = 0.30$) after eating. However,

Table 3. Effect of different levels of feed intake from Day 28 to 34 of gestation on the diurnal change of plasma concentrations of acyl ghrelin (pg/mL)

Level of feed intake ¹	Time point													AUC ²
	0 h	2 h	4 h	6 h	8 h	10 h	12 h	14 h	16 h	18 h	20 h	22 h	24 h	
0.5M	211.32	262.11	206.56	227.49	238.02	296.33	205.78	201.37	247.24	169.45	183.54	172.97	169.16	5,162.35
1.0M	174.37	137.82	137.82	178.81	178.33	233.88	171.67	180.61	154.54	126.08	154.48	172.05	159.09	4,040.69
2.0M	87.64	128.52	106.52	141.66	133.53	215.55	106.23	132.21	151.30	98.70	130.77	132.94	121.46	3,251.14
SEM	19.69	33.60	23.43	29.28	21.19	13.30	24.17	31.58	21.42	17.64	15.40	10.24	38.45	411.60
P-value	<0.01	0.01	0.01	0.10	<0.01	<0.01	0.01	0.24	<0.01	0.01	0.04	<0.01	0.61	0.02

¹0.5M, 1.0M, and 2.0M = 0.5, 1.0, and 2.0 × maintenance energy intake (100 × BW^{0.75} kcal ME/d), respectively.

²AUC = area under the curve. This represents the total AUC for the plasma acyl ghrelin concentrations from 0 to 24 h after the meal.

Figure 2. Effect of different levels of feed intake from Day 28 to 34 of gestation on the diurnal change of plasma acyl ghrelin concentrations (pg/mL) in pregnant gilts. Gilts were fed once daily at 0730 h; 0 h represents right before feeding and the other time points represent the hours after feeding. 0.5M, 1.0M, and 2.0M = 0.5, 1.0, and 2.0 × maintenance energy intake, respectively. Trt = treatment.**Table 4.** Effect of different levels of feed intake from Day 28 to 34 of gestation on the postprandial plasma concentrations of insulin (microunits/mL)

Level of feed intake ¹	Time point									AUC ²
	0 h	15 min	30 min	45 min	60 min	75 min	90 min	2 h	4 h	
0.5M	8.85	11.42	24.00	39.17	26.65	22.83	26.37	17.55	11.47	75.41
1.0M	8.17	17.28	31.70	78.35	50.25	47.40	39.00	20.02	19.68	116.60
2.0M	9.60	21.48	25.00	48.16	72.62	59.63	37.52	25.70	18.03	122.31
SEM	1.52	3.80	6.19	13.41	8.80	10.76	9.59	3.77	2.28	13.75
P-value	0.80	0.18	0.63	0.07	<0.01	0.05	0.60	0.30	0.03	0.06

¹0.5M, 1.0M, and 2.0M = 0.5, 1.0, and 2.0 × maintenance energy intake (100 × BW^{0.75} kcal ME/d), respectively.

²AUC = area under the curve. This represents the total AUC for the plasma insulin concentration from 0 to 4 h after the meal.

Figure 3. Effect of different levels of feed intake from Day 28 to 34 of gestation on the postprandial plasma insulin concentrations (microunits [μ U]/mL) in pregnant gilts. Gilts were fed once daily at 0730 h; 0 h represents right before feeding and the other time points represent the hours after feeding. 0.5M, 1.0M, and 2.0M = 0.5, 1.0, and 2.0 $\times$ maintenance energy intake, respectively. Trt = treatment.

plasma insulin concentrations were greater for gilts on the 1.0M and 2.0M levels of feed intake at 4 h after eating than for those on the 0.5M level of feed intake. Furthermore, insulin concentrations reached the maximum of 39.17 and 78.35 microunits/mL at 45 min after eating for gilts on the 0.5M and 1.0M levels of feed intake, respectively, whereas the insulin concentration reached a maximum of 72.62 microunits/mL at 60 min after eating for gilts on the 2.0M level of feed intake. The AUC of plasma insulin concentrations tended ($P = 0.06$) to be greater for the 1.0M and 2.0M levels of feed intake than for the 0.5M level of feed intake.

Plasma concentrations of GLP-1 right before feeding (0 h) and the AUC of GLP-1 were not different ($P > 0.10$; Table 5) among the 3 levels of feed intake, but gilts on the 2.0M level of feed intake tended to have higher levels of GLP-1 at 0.5 ($P = 0.07$) and 1 h ($P = 0.08$) after eating than gilts on the 0.5M and 1.0M levels of feed intake. No differences ($P > 0.10$; Table 5) were found among gilts on the 3 levels of feed intake in terms of plasma concentrations of leptin at different time points after eating and the AUC. However, the fasting NEFA concentration was greater ($P < 0.01$; Table 5) in gilts on the 0.5M level of feed intake than in those on the 1.0M and 2.0M levels of feed intake. Additionally, the fasting NEFA concentration was also greater ($P < 0.01$) in gilts on the 1.0M level of feed intake than in their counterparts on the 2.0M level of feed intake. Furthermore, NEFA concentrations at 4

and 8 h after eating and the AUC were greater ($P < 0.01$) in gilts fed the 0.5M level of feed intake than in those fed the 1.0M and 2.0M levels of feed intake.

Consumption Time and Pearson Correlations

After the period of imposing dietary treatments (from Day 28 to 34 of gestation), the feed consumption test revealed that gilts on the 2.0M level of feed intake took more ($P < 0.01$; Fig. 4) time to finish the 1.82 kg feed than gilts on the 0.5M level of feed intake at d 35 of gestation. Results of the Pearson correlation showed that consumption time of 1.82 kg feed at d 35 of gestation was strongly and negatively correlated with the AUC of acyl ghrelin ($r = -0.91$, $P < 0.01$; Table 6) and NEFA ($r = -0.71$, $P < 0.01$; Table 6). The acyl ghrelin AUC was positively correlated with the NEFA AUC ($r = 0.72$, $P < 0.01$).

Results of the Pearson correlation also revealed that BW change from Day 28 to 34 of gestation was inversely ($r = -0.77$, $P < 0.01$; Table 7) correlated with the AUC of NEFA, whereas it tended to be negatively correlated with the AUC of acyl ghrelin ($r = -0.50$, $P = 0.08$) and positively correlated with insulin ($P = 0.48$ and $P = 0.09$, respectively). In terms of BF change from Day 28 to 34 of gestation, it was inversely correlated with the AUC of both acyl ghrelin ($r = -0.64$, $P < 0.05$) and NEFA ($r = -0.69$, $P < 0.01$). Additionally, the AUC of GLP-1 tended ($r = 0.49$, $P = 0.09$) to be positively correlated with BF change from Day 28 to 34 of gestation.

Table 5. Effect of different levels of feed intake from Day 28 to 34 of gestation on plasma concentrations of glucagon-like peptide 1 (GLP-1; pg/mL), leptin (ng/mL), and NEFA (mmol/L)

Level of feed intake ¹	GLP-1				Leptin				NEFA			
	0 h	0.5 h	1 h	AUC ²	0 h	4 h	8 h	AUC ³	0 h	4 h	8 h	AUC ⁴
0.5M	174.13	207.47	218.52	201.90	0.90	1.43	0.96	9.42	0.47	0.29	0.17	2.43
1.0M	186.15	242.38	245.16	229.02	0.86	1.50	0.89	9.50	0.21	0.09	0.04	0.87
2.0M	279.44	351.44	355.95	334.57	1.09	1.55	1.04	10.46	0.13	0.09	0.03	0.68
SEM	49.43	49.43	49.43	52.77	0.09	0.09	0.09	0.62	0.03	0.02	0.02	0.15
<i>P</i> -value	0.19	0.07	0.08	0.14	0.18	0.64	0.54	0.41	<0.01	<0.01	<0.01	<0.01

¹0.5M, 1.0M, and 2.0M = 0.5, 1.0, and 2.0 × maintenance energy intake (100 × BW^{0.75} kcal ME/d), respectively.

²AUC = area under the curve. This represents the total AUC for plasma GLP-1 concentrations from 0 to 1 h after the meal.

³This represents the total AUC for plasma leptin concentrations from 0 to 8 h after the meal.

⁴This represents the total AUC for plasma NEFA concentrations from 0 to 8 h after the meal.

Simple linear regression models selected for prediction of feed consumption time, BW change, or BF change in pregnant gilts are shown in Table 8. If feed consumption time was considered a dependent variable, the AUC of acyl ghrelin was the best predictor based on R^2 and root mean square error. For predicting changes of gilt BW or BF during the period of Day 28 to 34 of gestation, the AUC of NEFA was the best predictor.

DISCUSSION

The present study investigated the impact of feed intake during gestation on energy homeostasis and hunger status as measured by the hormones involved in feed intake regulation and the consumption time of a certain amount of feed in pregnant gilts.

Acyl Ghrelin

Several studies on rodents (Tschöp et al., 2000; Sánchez et al., 2004), growing pigs (Reynolds et al., 2010), and humans (Ariyasu et al., 2001; Cummings et al., 2001; Tschöp et al., 2001a; Liu et al., 2008) have supported the notion that ghrelin is a physiological meal initiator, with concentrations rising before feeding and declining after feeding. However, our experiment demonstrated that plasma acyl ghrelin concentrations did not increase before eating or decrease after eating, which was similar to previous reports on plasma acyl ghrelin in finishing pigs fed ad libitum (Reynolds et al., 2010) and total ghrelin in finishing pigs fed ad libitum once or twice a day (Scrimgeour et al., 2008). However, when the finishing pigs were restricted fed, plasma acyl ghrelin rose 2 h before feeding and declined to nadir

Figure 4. Effect of different levels of feed intake from Day 28 to 34 of gestation on the consumption time (min) of gilts offered 1.82 kg of feed on d 35 of gestation. Values were least squares means of 6 animals per level of feed intake, with their SE represented by vertical bars. A significant difference ($P < 0.01$) exists between the 0.5 × maintenance energy intake (0.5M) and 2.0 × maintenance energy intake (2.0M) levels of feed intake. 1.0M = 1.0 × maintenance energy intake. ^{a,b}Values with different superscripts differ ($P < 0.05$).

Table 6. Pearson correlation matrix among consumption time, plasma hormones, and NEFA concentrations

	Consumption time ¹	Ghrelin_AUC ²	GLP-1_AUC ²	Leptin_AUC ²	Insulin_AUC ²	NEFA_AUC ²
Consumption time	1.00					
Ghrelin_AUC	-0.91**	1.00				
GLP-1_AUC	0.32	-0.64*	1.00			
Leptin_AUC	-0.15	-0.07	0.60*	1.00		
Insulin_AUC	0.43	-0.45	0.38	0.25	1.00	
NEFA_AUC	-0.71**	0.72**	-0.55*	-0.04*	-0.68	1.00

¹The feed consumption time when all gilts were offered 1.82 kg of feed on Day 35 of gestation.

²Ghrelin_AUC, GLP-1_AUC, Leptin_AUC, Insulin_AUC, and NEFA_AUC = the total area under the curve calculated for acyl ghrelin, glucagon-like peptide 1, leptin, insulin, and NEFA, respectively.

*0.01 < *P* < 0.05; ***P* < 0.01.

in 1 h (Reynolds et al., 2010). Additionally, Govoni et al. (2005) reported that plasma acyl ghrelin showed a significant reduction only after 72 h of fasting in prepuberal gilts, whereas Salfen et al. (2003) reported a significant ghrelin increase after 36 to 48 h of food deprivation in weanling pigs. It seemed that gilts were less sensitive to fasting in terms of plasma acyl ghrelin concentrations compared with weanling and finishing pigs. Furthermore, Barretero-Hernandez et al. (2010) observed that preprandial plasma total ghrelin concentrations were 35.9% greater than postprandial concentrations in finishing pigs; however, feed restriction for 5 wk did not change the plasma total ghrelin concentration, indicating that the length of time and severity of feed restriction imposed in that study might not have been sufficient to elicit changes in total ghrelin concentrations. In studies conducted by Barretero-Hernandez et al. (2010) and Scrimgeour et al. (2008), plasma total ghrelin concentrations were analyzed, whereas other authors analyzed acyl ghrelin. It can be speculated that difference types of ghrelin (total ghrelin or acyl ghrelin) analysis and the methodologies used to analyze the ghrelin can contribute to the variation among studies. Therefore, the discrepancy among studies may be attributed to different experimental conditions, such as different physiological stages of the pigs, the blood sampling procedure, the feeding regime, and the type of ghrelin analysis.

In the current study, diurnal plasma acyl ghrelin concentrations were determined in pregnant gilts. To the best of our knowledge, this was the first study investigating the diurnal profile of acyl ghrelin concentrations in pregnant sows. It was reported that plasma acyl ghrelin concentrations were the highest on Day 30 of gestation in pregnant sows (Govoni et al., 2007); however, only 1 blood sample was collected for the selected days during gestation in that study; therefore, the change of diurnal acyl ghrelin concentrations were not known.

Interestingly, an abrupt increase of acyl ghrelin concentrations was observed at 10 h after eating, which may be related to the regular light management in the research facility. It was reported that sleeping enhanced nocturnal

plasma ghrelin concentration in healthy human subjects and that the nocturnal increase was blunted during sleep deprivation (Dzaja et al., 2004). The nocturnal increase of acyl ghrelin concentrations was also observed by Liu et al. (2008). It was possible that in the current experiment, the abrupt increase of acyl ghrelin concentrations was attributed to sleep, due to the fact that the light in the facility was turned off around 9 h after the meal.

Even though the acyl ghrelin concentrations did not vary before and after the meal, acyl ghrelin concentrations were higher in the gilts on the lower feed intake than in the gilts on the higher feed intake, which is in agreement with a previous report on changes of plasma concentrations of total ghrelin as affected by amount of feed intake in pigs (Scrimgeour et al., 2008). Data gleaned over the last decade demonstrated that the plasma ghrelin concentration was elevated under conditions of negative energy balance such as starvation and anorexia, whereas it declined under conditions of positive energy balance such as feeding and obesity (Ariyasu et al., 2001; Tschöp et al., 2001a; Govoni et al., 2005). In keeping with this, it was reported that fasting plasma ghrelin was negatively correlated with body mass index (Tschöp et al., 2001b). The results of the aforementioned literature were related to observations in nonpregnant animals or humans. In our current experiment, however, the animals were in the pregnant status, which may yield different results. Our current experiment also showed that the AUC of acyl ghrelin was negatively correlated with BF change. Cools et al. (2013) found no association between ghrelin and BF thickness in sows during the peripartum period. Nevertheless, sow blood was collected only once a day for analysis of ghrelin in their study. In agreement with the findings of the present experiment, Gualillo et al. (2002) demonstrated that plasma ghrelin levels and gastric ghrelin mRNA expression were upregulated during undernutrition in pregnant rats. The fact that plasma ghrelin levels were elevated during undernutrition was further supported by the evidence that intravenous administration of ghrelin resulted in the inhibition of thyroid stimulating hormone (Wren et al.,

Table 7. Pearson correlation matrix among BW and backfat change from Day 28 to 34 of gestation, plasma hormones, and NEFA concentrations

	BW change ¹	BF change ²	Ghrelin_AUC ³	GLP-1_AUC ³	Leptin_AUC ³	Insulin_AUC ³	NEFA_AUC ³
BW change	1.00						
BF change	0.84***	1.00					
Ghrelin_AUC	-0.50*	-0.64**	1.00				
GLP-1_AUC	0.27	0.49*	-0.64**	1.00			
Leptin_AUC	-0.01	0.24	-0.07	0.60**	1.00		
Insulin_AUC	0.48*	0.52*	-0.45	0.38	0.25	1.00	
NEFA_AUC	-0.77***	-0.69***	0.72***	-0.55**	-0.04	-0.68**	1.00

¹The BW change of gilts from Day 28 to 34 of gestation.

²BF = backfat. This represents the BF change of gilts from Day 28 to 34 of gestation.

³Ghrelin_AUC, GLP-1_AUC, Leptin_AUC, Insulin_AUC, and NEFA_AUC = the total area under the curve calculated for acyl ghrelin, glucagon-like peptide 1, leptin, insulin, and NEFA, respectively.

*0.01 < *P* < 0.05; ***P* < 0.01.

2000), which may be responsible for the reduction of energy expenditure in times of limited nutrition. It was possible that ghrelin may function as a signal for energy insufficiency during pregnancy, acting as an inhibitory factor to avoid excessive metabolic drain. Collectively, these findings confirmed that acyl ghrelin may serve as a long-term physiological indicator of energy homeostasis in both nonpregnant and pregnant animals and humans.

Insulin

Our present study showed that plasma insulin concentrations responded differently among gilts fed 3 different levels of feed intake. An early study conducted in sheep showed that ingestion of a low amount of food was followed by a rapid increase in plasma insulin concentrations, with maximum values being reached 1 to 3 h after feeding, whereas ingestion of a high amount of food resulted in a higher insulin concentration for longer duration (Bassett, 1974). It was also demonstrated that a maximum concentration of insulin was reached at 60 min after a carbohydrate-rich meal (Erdmann et al., 2003). In agreement with the results reported in the 2 aforementioned reports, the current experiment showed that plasma insulin concentrations responded differently among gilts fed 3 different levels of feed intake. For the gilts fed the lowest level of feed intake, insulin concentrations rapidly reached the maximum level, with attenuated values, whereas for gilts fed the highest level of feed intake, the maximum insulin concentration was postponed, with a higher value, compared with the lowest level of feed intake.

Glucagon-Like Peptide 1

Studies have demonstrated that central GLP-1 administration reduces short-term food intake in rats (Turton et al., 1996; Tang-Christensen et al., 2001)

and humans (Näslund et al., 1999). However, long-term food intake or BW was not affected by intravenous GLP-1 administration in both lean and obese rats (Donahey et al., 1998). Additionally, peripheral administration of GLP-1 elicits satiety in both normal-weight (Gutzwiller et al., 1999) and obese individuals (Näslund et al., 1999). In the current study, even though no significant differences were found among the 3 different levels of feed intake, plasma GLP-1 concentrations increased in a dose-dependent manner in response to different levels of feed intake, indicating that gilts on the higher level of feed intake were more satiated than gilts on the lower energy level.

Nonesterified Fatty Acids

Nonesterified fatty acid is an indicator of fat mobilization, and its concentration would be increased when the energy balance is negative (Weldon et al., 1994). It has been shown that plasma NEFA concentrations were higher in pigs fed once daily compared with pigs fed twice daily or ad libitum (Scrimgeour et al., 2008), which may be driven by different feed intake among these 3 feeding regimes, and therefore, different levels of energy homeostasis were created. In the study conducted by Govoni et al. (2005), plasma NEFA concentrations increased during fasting and reached the highest concentrations at 72 h after fasting; however, plasma NEFA concentrations returned to basal levels within 6 h on refeeding, which indicated that NEFA was a good indicator for short-term energy homeostasis. Additionally, NEFA concentrations continued to increase during lactation, reaching the maximum levels on Day 21 of lactation (Govoni et al., 2007), which suggested that NEFA was also involved in the long-term regulation of energy homeostasis.

In the current study, gilts on the lower level of feed intake had higher plasma NEFA concentration than gilts

Table 8. Simple linear relationships between consumption time and plasma parameters and between gilt BW or BF change from Day 28 to 34 of gestation and plasma parameters

Equation number	Equation	R^2	Root MSE ¹
1	Consumption time ² = $40.38 - 2.88 \times 10^{-2} \times \text{Ghrelin_AUC}^3$	0.82	1.52
2	Consumption time = $32.31 - 2.81 \times \text{NEFA_AUC}^3$	0.46	2.59
3	BW change ⁴ = $10.40 - 2.67 \times 10^{-2} \times \text{Ghrelin_AUC}^3$	0.18	5.38
4	BW change = $-8.77 - 0.07 \times \text{Insulin_AUC}^3$	0.16	5.43
5	BW change = $6.27 + 5.14 \times \text{NEFA_AUC}^3$	0.55	3.97
6	BF change ⁵ = $2.47 - 5.48 \times 10^{-4} \times \text{Ghrelin_AUC}^3$	0.36	0.76
7	BF change = $-1.23 - 5.80 \times 10^{-3} \times \text{GLP-1_AUC}^3$	0.17	0.87
8	BF change = $-1.19 - 0.01 \times \text{Insulin_AUC}^3$	0.21	0.85
9	BF change = $1.19 - 0.73 \times \text{NEFA_AUC}^3$	0.42	0.72

¹MSE = mean square error.

²The feed consumption time when all gilts were offered 1.82 kg of feed on Day 35 of gestation.

³Ghrelin_AUC, GLP-1_AUC, Leptin_AUC, Insulin_AUC, and NEFA_AUC = the total area under the curve calculated for acyl ghrelin, glucagon-like peptide 1, leptin, insulin, and NEFA, respectively.

⁴The BW change of gilts from Day 28 to 34 of gestation.

⁵BF = backfat. This represents the BF change of gilts from Day 28 to 34 of gestation.

on the higher level of feed intake, indicating that gilts eating less were exposed to a negative energy balance and therefore mobilized more fat reserves to meet the daily energy requirement, which was further supported by the evidence that gilts on the 0.5M and 1.0M levels of feed intake lost BF from Day 28 to 34 of gestation.

In the present study, plasma NEFA concentrations were positively correlated with plasma acyl ghrelin concentrations. The relationship between plasma NEFA and plasma ghrelin concentrations were also reported in growing pigs in response to a 54-h fast (Inoue et al., 2005) and reported in growing pigs fed once daily (Scrimgeour et al., 2008). In contrast, Govoni et al. (2007) reported that plasma acyl ghrelin concentrations were not associated with plasma NEFA concentrations. The discrepancy among different studies could be attributed to different experimental procedures. For example, in the study conducted by Govoni et al. (2007), 1 blood sample was collected for each selected day during gestation and lactation, and the relationship between acyl ghrelin and NEFA was examined for these 2 measurements collected throughout gestation and lactation, whereas other studies examined only the relationship between plasma acyl ghrelin and NEFA concentrations for these 2 measurements collected at different time points within a day.

Predictors of Hunger Status

Visual analog scales are widely used in human research to evaluate appetite and mood (Parker et al., 2004; Krishnan et al., 2016). However, this approach could not be applied to animals due to the fact that animal cannot express their magnitude of feeling. In the current experiment, we evaluated the effect of different

levels of feed intake from Day 28 to 34 of gestation on the consumption time of the same amount feed (1.82 kg) on d 35 of gestation. We noted that gilts on the 0.5M level of feed intake consumed feed much faster than gilts on the 2.0M level of feed intake, suggesting that gilts on the 0.5M level of feed intake were hungrier than gilts on the 2.0M level of feed intake. Therefore, we proposed the idea of using consumption time as the dependent variable to examine its relationship with plasma hormones related to energy homeostasis. The results indicated that the AUC of acyl ghrelin was the best single predictor for consumption time. Similarly, it was reported that plasma concentrations of acyl ghrelin were negatively correlated with meal length and positively associated with the number of meals for growing-finishing pigs (Lents et al., 2016). Additionally, BW and body adiposity are 2 important indicators for body energy homeostasis (Keesey and Powley, 2008). It was thought that BW and BF changes from Day 28 to 34 of gestation may indicate the status of energy homeostasis of gilts. Using these 2 parameters as the dependent variables, the results showed that the AUC of NEFA was the best single predictor.

Conclusion

Our data suggested that feed intake of pregnant gilts was negatively correlated with plasma concentrations of acyl ghrelin and NEFA, which, in turn, were negatively related to feed consumption time. There were no apparent preprandial and postprandial changes in plasma concentrations of acyl ghrelin during the period of early pregnancy in gilts. The AUC of acyl ghrelin and NEFA seemed to be the best predictors for hunger status of pregnant gilts.

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